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EVALUATION OF ANTIBACTERIAL EFFECT OF SILVER DIAMINE FLUORIDE AND SODIUM FLUORIDE VARNISH ON BACTERIAL BIOFILM (AN IN VITRO STUDY)

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ABSTRACT

Objectives: The present in vitro study was carried to compare the antibacterial effect of silver diamine fluoride (SDF 38%) and sodium fluoride varnish (NaF 5%) on inhibition of bacterial biofilm formation. **subjects and methods**: a total number of 48 biofilms were classified into three main groups (16 for each) and treated with. Group A: SDF 38% and Group B: NaF Varnish 5% for 1 minute only per day for three consecutive days. Group C: left untreated (control group). After treatment, fresh media was added to biofilms that were grown at 1st day and at 3rd day. Streptococcus mutans (S. mutans) and Lactobacillus acidophilus (L. acidophilus) were grown anaerobically. Then bacterial count was calculated by colony forming unit (CFU) per mL at 1st and 3rd day. **Results:** There was a statistically significant difference in the count of S. mutans and L. acidophilus between all groups as indicated by the One-way ANOVA test in which the highest bacterial count was found in Group C group followed by Group B while the least bacterial count was found in Group A. Thus, results recorded SDF 38 % showed a higher reduction than NaF 5% according to S. mutans and L acidophilus at 1st and 3rd day respectively. **Conclusion**: SDF is a non-invasive and simple approach for inhibiting S. mutans and L acidophilus growth that causing dental caries.

KEYWORDS: Silver Diamine Fluoride, Sodium Fluoride Varnish, Bacterial Biofilm, Streptococcus mutans and L. Acidophilus.

INTRODUCTION

Dental caries is the most common chronic disease of childhood [(1)]. The percentage of children found to be caries-free about primary teeth was 7% however, this proportion would increase to 15.6% if the enamel lesions were excluded [(2)]. Dental caries is a multifactorial chronic bacterial disease that causes demineralization and destruction of the hard tissues usually by the production of acid by bacterial fermentation of the food debris accumulated on the tooth surface [(3)]. It was reported that microorganisms believed to lead to the occurrence of caries within the oral flora, Streptococcus mutans (S. mutans) and Lactobacillus acidophilus (L. acidophilus) play major roles in this process. S. mutans is the main factor that initiates caries and is a very important factor of enamel decay. The bacteria of the genus L. acidophilus are important in further caries development, especially in the dentin [(4)].

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Caries progression occurs by simultaneous demineralization of enamel and dentin and degradation of the organic matrix. Once the caries lesion is developed, treatment options include restorative and non-restorative measures [5]. Nonrestorative approaches may be invasive, such as preventive resin restorations; or noninvasive, such as SDF, fluoride therapy, or sealants [5].

One of the most effective methods of preventing caries progression is application of fluoride. The anticaries activity of fluoride-containing products can be attributed to inhibition of the demineralization processes, enhancement of enamel remineralization, and effects on the biological activities of cariogenic microorganisms [6]. It has been proposed that SDF contains fluorides and silver in combination. Low quantities of fluoride can prevent some bacterial strains from producing and metabolically active in biofilm[7,8]. Fluoride suppresses the formation of cariogenic bacteria in tooth plaque at high concentrations[9]. One way to prevent biofilm is to attach its ions to the components of the bacterial cell and to affect enzymes like enolase and proton-extruding adenosine triphosphatase [10]. Another mechanism is the inhibition of the carbohydrate metabolism of acidogenic oral bacteria [11,12]. Without removing intact tooth structure, SDF gives the chance to arrest or stop the spread of caries lesions. SDF also seems to remineralize the dentin [13].

NaF varnish is one of the most used fluoride products for promoting the remineralization of dental hard tissues. NaF varnish at 5% (containing 22,600 ppm fluoride) is known to be effective in preventing dental caries. The Cochrane Review reported that it reduced 37% of early childhood caries (ECC) development in young children. [14] The American Dental Association recommended that fluoride varnish should be applied to high-risk patients for caries prevention at 3- to 6-month intervals.[15] The present invitro study was carried to compare the antibacterial effect of silver diamine fluoride (SDF 38%) and sodium fluoride varnish (NaF 5%) on inhibition of bacterial biofilm formation.

SUBJECTS AND METHODS

Study design: Prospective laboratory study.

Study setting: The sample consisted of 48 biofilms formed in two 24 well plate by using two types of bacteria. Streptococcus mutans and Lactobacillus acidophilus under anaerobic conditions at the microbiological lab, faculty of Medicine, Al-Azhar University, Girls, Cairo.

Sample size:

Sample size calculation based on mean streptococcus mutans colonies among studied groups SD & NAF retrieved from previous study (development between different sealant material retrieved from previous research [19]. Using G*power version 3.0.10 to calculate sample size based on effect size =1.22, 2-tailed test, α error =0.05 and power = 90.0%, the total calculated sample size were 16 in each group (three groups).

Ethical considerations:

The research protocol was ethically accepted with code: 566/2667 at 30/10/2020 by committee of faculty of Dental Medicine, Boys, Cairo, Al- Azhar University.

Source of reference bacterial strains:

S. mutans: ATCC 25175

L. acidophilus: ATCC 4356

Prepared at Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Egypt .

Media used:

1. Brain heart infusion broth and agar (BHI) (HIMEDIA, India):

S. mutans were grown in BHI broth and strains were maintained on BHI agar plates .

2. De Man, Rogosa and Sharpe broth and agar (MRS) (HIMEDIA, India):

L. acidophilus were grown in L-MRS liquid culture and maintained on L-MRS agar plates.

Grouping:

Total number of 48 biofilms were classified into three main groups (n=16).

Group A: The 16 biofilms were treated with SDF 38% for 1 minute only per day for three consecutive days and biofilms were washed 3 times with fresh media.

Group B: The 16 biofilms were treated with Sodium Fluoride Varnish 5% for 1 minute only per day for three consecutive days and biofilms were washed 3 times with fresh media.

Group C: The 16 biofilms were left untreated (control group).

After treatment, fresh media was added to biofilms that were grown at 1st day and at 3rd day.

Growth of Bacterial Strains:

Streptococcus mutans and Lactobacillus acidophilus were grown on brain heart infusion media (BHI) and De Man, Rogosa and Sharpe (MRS) media respectively anaerobically in an atmosphere consisting of 85% N2 10% CO2 and 5% H2 at 37 ºC in an anaerobic chamber (Coy Manufacturing). Overnight cultures of the cariogenic oral bacteria were grown in biofilm media consisting of 1 part BHI broth, 1 part L-MRS broth, and 1% sucrose. To form biofilms for treatment, two 24 well plate was coated with saliva to allow the bacteria to attach to the salivary proteins.

To the coated wells, bacterial sample will be added and incubated after 48 hours of growth, the biofilms will be treated with 38% silver diamine fluoride (SDF) and 5% sodium fluoride (NaF) or left untreated (control).

Observation:

Colonies appeared on the plates after 36-48 hours of anaerobic growth. Colonies were counted by CFU per mL .

Statistical analysis

Data was analyzed using SPSS (statistical package for social sciences) version 22. The appropriate statistical test was applied according to data type with the following suggested tests: One-way ANOVA test was used to compare between the three groups. the post-Tukey's test was used.

RESULTS

***Streptococcus mutans* (CFU/ml) count**

There was a statistically significant difference in *Streptococcus mutans* count (CFU/ml) between the different tested groups) as indicated by the One-way ANOVA test. the first day, the higher *Streptococcus mutans* (CFU/ml) count of (343.8±40.3) was recorded in the untreated group (group III), followed by the NaF-treated group (group II) with a *Streptococcus mutans* (CFU/ml) count of (102.5±11.3). While the lower *Streptococcus mutans* (CFU/ml) count of (61.3±10.9) was recorded in SDF treated group (group I). the third day, the higher *Streptococcus mutans* (CFU/ml) count of (552.5±32.6) was recorded in the untreated group (group III), followed by the NaF-treated group (group II) with a *Streptococcus mutans* (CFU/ml) count of (138.8±8.1). While the lower *Streptococcus mutans* (CFU/ml) count of (88.8±8.1) was recorded in SDF treated group (group I).

***L. Acidophilus* count (CFU/ml) count**

There was a statistically significant difference in *L. Acidophilus* count (CFU/ml) between the different tested groups as indicated by the One-way ANOVA test. the first day, the higher *L. Acidophilus* (CFU/ml) count of (272.5±33.4) was recorded in the untreated group (group III), followed by the NaF-treated group (group II) with a *L. Acidophilus* (CFU/ml) count of (70.00 ±8.9). While the lower *L. Acidophilus* (CFU/ml) count of (30.00 ±8.9) was recorded in SDF treated group (group I). the third day, the higher *L. Acidophilus* (CFU/ml) count of (551.3±28.9) was recorded in the untreated group (group III), followed by the NaF-treated group (group II) with an *L. Acidophilus* (CFU/ml) count of (103.8±10.2). While the lower *L. Acidophilus* (CFU/ml) count of (65.00±5.2) was recorded in SDF treated group (group I).

TABLE (1) *S. mutans* count (CFU/ml) and *L. Acidophilus* count (CFU/ml) along with the study on the first and third day.

Bacterial number	Time	Control	SDF 38 %	NaF 5 %	P-value
***Streptococcus mutans* count (CFU/ml)**	**The first day**	343.8±40.3^{A}	61.3±10.9^{B}	102.5±11.3^{C}	<0.00001*
	P0	P1< 0.00001*, p2< 0.00001*, p3=0.008 *			
	The third day	552.5±**32.6**^{A}	88.8± 8.1 ^{B}	138.8± 8.1 ^{C}	<0.00001*
		P1< 0.00001*, p2< 0.00001*, p3< 0.001*			
***L. Acidophilus mutans* count (CFU/ml)**	**The first day**	272.5 ±33.4^{A}	30.00 ±8.9^{B}	70.00 ±8.9^{C}	<0.00001*
		P1< 0.00001* , p2< 0.00001* , p3=0.575			
	The third day	551.3 ±28.9^{A}	65.00 ± 5.2^{B}	103.8 ± 10.2^{C}	<0.00001*
		P1< 0.00001*, p2< 0.00001*, p3< 0.001*			

**; significant at P < 0.05.*

**; Different capital letters mean statistically significant. (in the same row)*

P1: Between control and SDF. *P2: Between control and NaF.* *P3: Between SDF and NaF.*

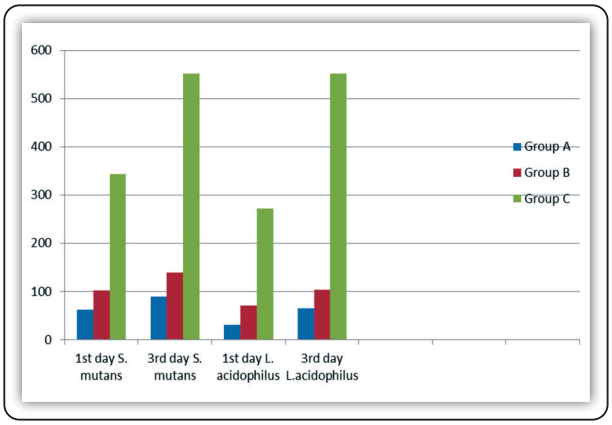

FIG (1) Diagram showing the reduction percentage in the S. mutans count and L. acidophilus count after SDF and NaF treatment on the first and third days.

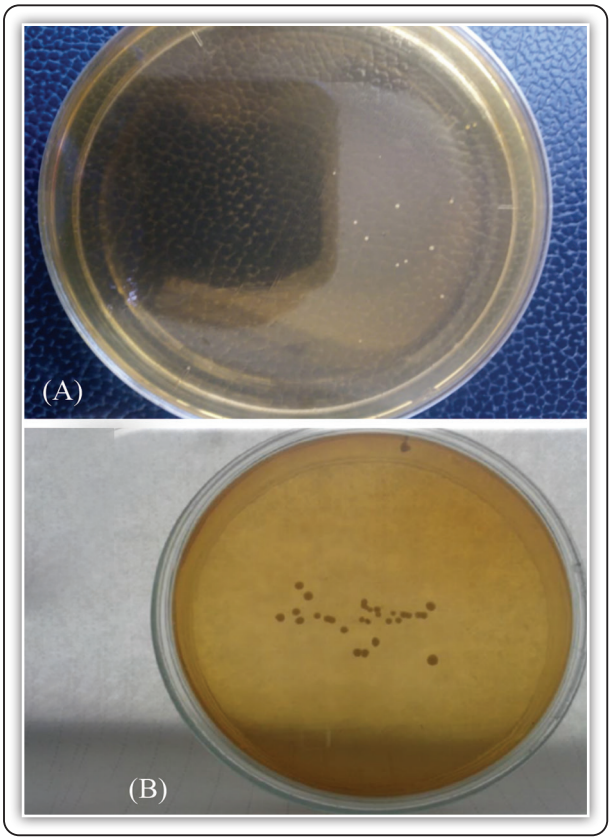

FIG (2) A, Growth of S. Mutans colonies on BHI agar plate. B, Growth of L. acidophilus colonies on MRS agar plate.

DISCUSSION

The first aim of this study was to develop a reliable model to mimic an in vivo biofilm [19]. The accumulation of oral biofilm can lead to oral diseases such as dental caries or periodontitis [20]. The demineralization of the tooth surface and the development of dental caries are caused by the acid produced by the oral biofilm through the metabolism of sugar, which leads to a subsequent drop in the ambient pH value [21]. The two most significant bacteria linked to dentine caries are S. mutans and L. acidophilus [22]. It is necessary to suppress the bacteria that create acid if the closed cycle of this process is to be stopped. Silver diamine fluoride (SDF) has been widely used to prevent and arrest caries [23]. SDF has an antibacterial function because both the silver ions and the fluoride contained in SDF appear to have the ability to inhibit the formation of cariogenic biofilm [24]. Fluoride can inhibit the production and metabolic activity of certain strains of bacteria within biofilm at low concentrations [25]. This study used 38% SDF because it is the most commonly used concentration [26].

On the first and third day, the treated samples showed a statically significant decrease in ***Streptococcus mutans*** (CFU/ml) count after SDF and NaF treatment. SDF treated group recorded lower count than and NaF. This study demonstrated SDF-inhibited cariogenic biofilm formation. The inhibition was obvious in third day after SDF application based on a very low CFU. In agree with our results, Ollie et al [27] investigated bacterial growth inhibition of 38% silver diamine fluoride (SDF) solution and 5% sodium fluoride (NaF) varnish on artificial dentine caries lesions. SDF exerted stronger inhibition of biofilm growth than SDF with NaF. According to Mei et al., [28] Silver diamine fluoride (SDF) has been clinically successful in halting dentin caries; hence, this study sought to determine how it works. On dentin carious lesions, 38% SDF suppresses the growth of multispecies cariogenic biofilms [29]. SDF is regarded as a useful tool for halting caries as a result. The research on the processes used by silver diamine fluoride (SDF) to stop caries was reviewed by Zhao et al. in their study [30]. To cariogenic bacteria, primarily Streptococcus mutans, they discovered that SDF was bactericidal. The growth of cariogenic bacteria is inhibited by SDF, a bactericidal drug.

On the first and third day, the treated samples showed a statically significant decrease in *L. Acidophilus* (CFU/ml) count after SDF and NaF treatment. SDF treated group recorded lower count than and NaF. Similarly, the application of 38% SDF on cariogenic biofilms consisting of S. mutans, S. sorbrinus, L. acidophilus, Lactobacillus rhamnosus, and A. naeslundii, could inhibit the growth of this mixed-species biofilm [31].

The present results revealed significant differences between the control and the treatment groups (SDF and NaF), and the number of viable microorganisms was reduced in the samples treated with the antibacterial agents. The differences in the observed antibacterial activities between the control and tested material groups suggested that the number of viable microorganisms was affected by the use of different antibacterial agents. The highest antibacterial activity was observed in the SDF group. These results were consistent with those of Nicholas L. Luke2018, Virginia Commonwealth University [19].

Data from our present study on viable cell counts are consistent with the results of these aforementioned studies, demonstrating decreased CFU in the SDF group under both aerobic and anaerobic culture conditions. The impact of silver diamine fluoride on oral biofilm was recognized by Zhang et al. [32] SDF's efficacy in caries arrest can be attributed to the fact that it prevented the growth of cariogenic bacteria such Streptococcus mutans, Lactobacillus acidophilus, Streptococcus sobrinus, Lactobacillus rhamnosus, Actinomyces naeslundii, and Enterococcus faecalis. Mei et al., [33] investigated the use of 38% silver diamine fluoride (SDF) as a treatment for preventing

secondary caries. the teeth were soaked in a 5% sucrose solution containing *Streptococcus mutans* and *Lactobacillus acidophilus* for 28 days. Conditioning with 38% SDF can increase resistance of restorations to secondary caries.

CONCLUSION

Based on the present study, SDF and NaF varnish had a significant antibacterial effect against S. mutans and lactobacillus bacteria. SDF is a non-invasive, simple and low-cost approach to arresting dental caries and more effective than NaF varnish in arresting dental caries.

However, SDF produces a black coating that may not be clinically acceptable so further studies are needed to seek a more acceptable, successful and biocompatible material for remineralization propose.

REFERENCES

1. Bach K and Manton D. Early childhood caries New Zealand perspective. J Primary Health Care 2014;6(2):169-74.
2. Baginska J, Rodakowska E and Milewski R. Dental caries in primary and permanent molars in7-8-year-old schoolchildren evaluated with Caries assessment spectrum and treatment (CAST) index. BMC Oral Health 2014;2: 14-74.
3. Vos T, Flaxman A and Naghavi M. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;15: 2163-96.
4. Karaolanoglu S and Akgul H. The association between the DMFs index and levels of salivary S. mutans and L. acidophilus of subjects living in ErzurumTurkey. J Dent Sci 2010;5(2):70-4.
5. Fontana M, Gonzalez-Cabezas C. Evidence-Based Dentistry Caries Risk Assessment and Disease Management. Dent Clin North Am 2019;63(1):119-28.
6. Ccahuana-Vásquez RA, Cury JA , S. mutans biofilm model to evaluate antimicrobial substances and enamel demineralization. Braz Oral Res 2010;24: 135-41.
7. Chu, C.H.; Lo, E.C.M. Promoting caries arrest in children with silver diamine fluoride: A review. Oral Health Prev. Dent. 2008; 6:315–21.
8. Nassar, H.M.; Gregory, R.L. Biofilm sensitivity of seven Streptococcus mutans strains to different fluoride levels. J. Oral Microbiol.2017;9:1328265.
9. Pandit, S.; Kim, J.E.; Jung, K.H.; Chang, K.W.; Jeon, J.G. Effect of sodium fluoride on the virulence factors and composition of Streptococcus mutans biofilms. Arch. Oral Biol. 2011;56:643–49.
10. Chu, C.H.; Mei, L.; Seneviratne, C.J.; Lo, E.C.M. Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomyces naeslundii biofilms. Int. J. Paediatr. Dent. 2012; 22: 2–10 .
11. Fakhruddin, K.S.; Egusa, H.; Ngo, H.C.; Panduwawala, C.; Pesee, S.; Venkatachalam, T.; Samaranayake, L.P. Silver diamine fluoride (SDF) used in childhood caries management has potent antifungal activity against oral Candida species. BMC Microbiol. 2020;20:95.
12. Yu, O.Y.; Zhao, I.S.; Mei, M.L.; Lo, E.C.M.; Chu, C.H. Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions. J. Dent. 2018; 78: 65–71.
13. Jiang M, Wong MCM, Chu CH, Dai L, Lo ECM. Effects of restoring SDF-treated and untreated dentine caries lesions on parental satisfaction and oral health related quality of life of preschool children. J Dent 2019;88:103171.
14. Marinho VC, Worthington HV, Walsh T, Clarkson J. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2013;7:2279.
15. American Dental Association Council on Scientific Affairs. Professionally applied topical fluoride: evidence–based clinical recommendations. J Am Dent Assoc. 2006;137:1151–9.
16. Luke , L. N. A comparison of the antimicrobial efficacy of silver diamine fluoride and silver nitrate : an in vitro study. Virginia Common wealth University2018;1:7- 9.
17. 20.Takahashi N, Washio J, Mayanagi G. Metabolomic approach to oral biofilm characterization—a future direction of biofilm research. Journal of Oral Biosciences. 2012 Aug 1;54(3):138-43.
18. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. Journal of dental research. 2011 Mar;90(3):294-303.
19. Chu CH. Treatment of early childhood caries: a review and case report. Gen Dent. 2000; 48:142-8.
20. Chu, C.H.; Lo, E.C.M. Promoting caries arrest in children with silver diamine fluoride: A review. Oral Health Prev. Dent. 2008, 6, 315–321.

21. Mei, M.L.; Li, Q.L.; Chu, C.H.; Lo, E.C.M.; Samaranayake, L.P. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. Ann. Clin. Microbiol. Antimicrob. 2013, 12, 4.

22. Nassar, H.M.; Gregory, R.L. Biofilm sensitivity of seven Streptococcus mutans strains to different fluoride levels. J. Oral Microbiol. 2017, 9, 1328265.

23. 26. Mei ML, Chu CH, Lo EC, Samaranayake LP. Fluoride and silver concentrations of silver diammine fluoride solutions for dental use. Int J Paediatr Dent. 2013 Jul;23(4):279-85.

24. Ollie YY, Zhao IS, Mei ML, Lo EC, Chu CH. Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions. Journal of Dentistry. 2018 Nov 1;78:65-71.

25. Mei ML, Li QL, Chu CH, Lo EM, Samaranayake LP. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. Annals of clinical microbiology and antimicrobials. 2013 Jan;12(1):1-7.

26. Ishiguro T, Mayanagi G, Azumi M, Otani H, Fukushima A, Sasaki K, Takahashi N. Sodium fluoride and silver diamine fluoride-coated tooth surfaces inhibit bacterial acid production at the bacteria/tooth interface. J Dent. 2019 May;84:30-35.

27. Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML, Lo EC, Chu CH. Mechanisms of silver diamine fluoride on arresting caries: a literature review. International dental journal. 2018 Apr 1;68(2):67-76.

28. Mei ML, Li QL, Chu CH, Lo EC, Samaranayake LP. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. Ann Clin Microbiol Antimicrob. 2013 Feb 26;12:4.

29. Zhang J, Got SR, Yin IX, Lo EC, Chu CH. A Concise Review of Silver Diamine Fluoride on Oral Biofilm. Applied Sciences. 2021 Apr 4;11(7):3232.

30. Mei ML, Zhao IS, Ito L, Lo EC, Chu CH. Prevention of secondary caries by silver diamine fluoride. International dental journal. 2016 Apr 1;66(2):71-7.