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ANTI-BACTERIAL EFFECT OF PIT AND FISSURE SEALANT TREATED WITH NANO- SILVER

Mohamed Essam Abou Ammo [1*], Ibrahim FaroukBarakat [2], Hamdy Mahmoud Badreldin [3]

ABSTRACT

Objectives: the objective of this study is to evaluate effect of pits and fissure sealant on streptococcus bacteria microbiologically. **Subjects and methods:** forty patients participated in the study. Group I (control group): Clinpro™ sealant was applied to twenty primary molars of random selected patient. Group II (test group with Clinpro™ sealant coated by Nano silver) Clinpro™ sealant mixed with Nano silver was applied to twenty primary molars of random selected patient. Treatment protocol for both groups include Isolation of the Tooth, Acid Etching, Sealant Placement and Curing, and Sealant evaluation after placement. Microbiological assessment for quantification of the *S. mutant's* colonies. **Results**: Streptococcus count /level: At the baseline (Before), After one week, after one month, and After 3 months the difference was statistically insignificant. Streptococcus Levels percent reduction: From baseline to 1 week, the mean percent reduction of streptococcus levels was 36.2±7.2 for Group I and was 40.6±8.5 for Group II. This was statistically significant with p value = 0.087. **Conclusion:** Clinpro sealant alone or mixed with Nano silver was effective in reducing *S. mutans* biofilm formation.

KEYWORDS: Pits and fissure sealants, Clinpro, Nano silver, S. mutans.

INTRODUCTION

Dental caries still the disease number one affecting children all over the world. Although the incidence of dental caries in children has decreased recently in many nations, it nevertheless remains a significant public health problem. 80% of dental cavities in permanent teeth are caused by children and teens between the ages of 5 and 17, who make up around 25% of the population [(1)]. The majority of these goods attempt to remove S. mutans using either therapeutic or preventative methods once the carious lesion has healed. These resources include mouthwashes, fluoride varnish, fluoride foods, toothpaste, gels, laser treatment, and the use of pit and fissure sealants [(2, 3–7)].

Fissure sealants are a dental treatment that prevents tooth decay. It is frequently used in young children who are more prone to tooth decay and is implanted frequently as soon as the adult molar teeth erupt [(8, 9)].

It has been established that silver nitrate and silver diamine fluoride have bactericidal effects on S. mutans. However, their use has been restricted due to the tooth coloring caused by high concentrations of silver precipitation, which can also cause pulp irritation due to excessive silver ion penetration into the dentin [(10, 11).] Although the particular methods by which particulate silver destroys bacteria and fungus are unknown, various speculative ways in

1. Masters Candidate, Dentist at Egyptian Ministry of Health.
2. Professor, Head of Pedodontics & Oral Health Dept, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University.
3. Lecturer in Pedodontics & Oral Health Department, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University.

• ***Corresponding author*:** mohamedabuamo5@gmail.com

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which it may do so have been proposed [12], One of them is the electrostatic attachment of silver ions to the anionic components of microbe membranes, which appears to cause cell content leakage, the loss of cell motility (in motile bacteria), and cell death. Additionally, it appears that Ag + ions are harmful, poisoning metabolic enzymes and impeding pathways for transporting electrons. Additionally, they appear to deactivate bacterial DNA and RNA through as-yet-undiscovered processes [13, 14].In this study we aim to make combination between Nano-silver and pit and fissure sealant to evaluate anti-bacterial effect of Nano-silver on *S. mutans* biofilms.

SUBJECTS AND METHODS

Selection of patients:

Patient selection was done at the clinic of pediatric and public health department, Faculty of Dental medicine Azhar University, ranging from 6 to 14 years old children with initial diagnosis free of carious with deep pit and fissure that need preventive measurement to save it from decay due to dental caries, both male and female healthy patients.

Inclusion criteria

Parents and patient's acceptance and cooperation. Patient age from 6 to 14 years' old. Young permanent molars. Vital teeth free from caries and any morphological disturbance.

Exclusion criteria

Uncooperative child. Children whom parents refused to participate in the study Children with systemic disease that can affect his immunity or cooperation. Mobile tooth indicated for extraction.

Patients consent: Following initial examination and acceptance, a written informed consent was taken from parents after detailed explanation of the procedure and length of the treatment with emphasis on the possible outcome.

Grouping of patients:

The study included forty patients. The ethical committee of Azhar University's department of dentistry medicine accepted the protocol for this investigation with approval code (539/3037), which was planned as a randomised controlled clinical trial with a maximum follow-up of three months. A drawing was conducted using sealed envelopes to determine which category a particular tooth would be put in, as follows: Group I (control group): Clinpro™ sealant was applied to twenty primary molars of random selected patient. Group II (test group with Clinpro™ sealant coated by Nano silver) Clinpro™ sealant mixed with Nano silver was applied to twenty primary molars of random selected patient.

ClinproTM sealant mixed with Nano silver Preparation[1-5]

A total of 7.7 µg of silver nano-powder was weighted using an electronic sensitive balance on a sterilized mixing pad. According to Pal et al[5]., silver nanoparticles were created by a chemical reduction process. By microwave irradiating silver nitrate (AgNO3) solution in ethanolic medium and utilising PVP as a stabilising agent, silver nanoparticles were created. Ethanol was observed to act as a reducing agent in the presence of microwave SNPs Gel: 2 gm of PVP 30K (Loba CHIME, india) was sprinkled gently and gradually over the solution of Silver NPs 400 ppm under mild temperature with vigorous stirring to get homogenous gel. The entire content of one syringe of ClinproTM sealant (1.2 ml) was extracted from its container in a clean dry mixing capsule at x ray box Then, it was mixed with silver nano particles using an amalgamator for 10 seconds to obtain a homogenous mix . Silver nanoparticle-added ClinproTM Sealant was reloaded immediately to the original syringe in a dark x ray container. fig(1,a-f).

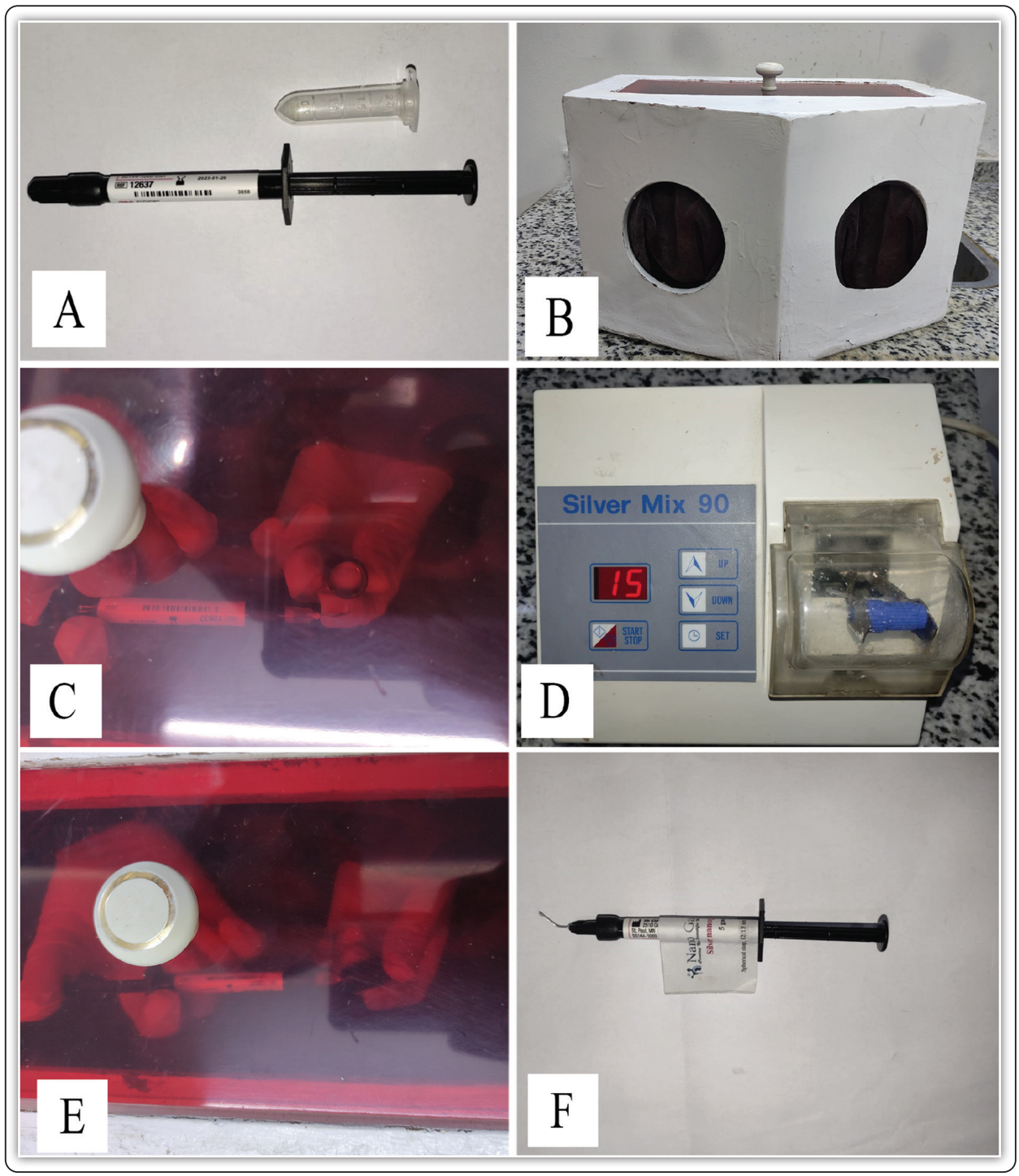

FIG (1) Photograph showing (a) nano silver in capsule after reduction by ethanol and clinpro pit and fissure sealant,(b) x ray dark container, (c) extraction of nano silver and entire content of clinpro sealant at sterilized amalgam capsule, (d) amalgamator using to get homogenous mix,(e) reload of the mix at dark container, (f) nano silver mixed with clinpro pit and fissure sealant.

Treatment protocol for both groups :

Isolation of the Tooth

Acid Etching: 37% phosphoric acid (gel) applied either directly with special application tips or with a small disposable brush.

Sealant Placement and Curing: Clinpro pit and fissure sealant kit used with their own dispensers and instructions.

Visible Light Cured Sealant: 20 seconds: exposure to visible light by (Coxo db-686 latte light cure device).

Sealant evaluation after placement: The operator inspected the sealant for any holes, bubbles, or insufficient material after curing it and before removing the isolation material. In an effort to remove the sealant, the explorer used to verify sealant retention.

Microbiological assessment:

First visit procedures: Swap was taken from the occlusal tooth surface of the patient before any procedures and saliva samples was plated on culture plates comprising mitis salivary agar culture media for quantification of the *S. mutant's* colonies to determine the numbers of streptococcus mutants in the oral cavity. The molars were isolated by rubber dam before etching; the molars were etched for 15 sec. to 30 sec. by acid H then rinsed by water [6]. The occlusion was checked after application to avoid any high spots.

Second visit procedures: After one-week swap was taken by sterile swap from the occlusal tooth surface to assess the numbers of streptococcus mutants of the selected tooth.

Third visit procedures: After one month the previous procedures were done to evaluate the retention, secondary caries and streptococcus mutants count in the oral biofilm.

Fourth visit procedures: After two months of the previous procedures were done to evaluate the retention, secondary caries and streptococcus mutants count in the oral biofilm.

RESULTS

Streptococcus count /level

The mean and standard deviation of Streptococcus count /level of both groups are presented in table (1). **At the baseline (Before),** the mean level of streptococcus was 1056.7±229.8 for Group I and was 1153.5±189.4 for Group II. This was statistically insignificant with p value = 0.154.

After one week, the mean level of streptococcus was 678.5±174.9 for Group I and was 687.7±158 for Group II. This was statistically insignificant with p value = 0.862.

After one month, the mean level of streptococcus was 450±207 for Group I and was 428.3±229.2 for Group II. This was statistically insignificant with p value = 0.755

After 3 months, the mean level of streptococcus was 242.2±136.3 for Group I and was 213.9±139.6 for Group II. This was statistically insignificant with p value = 0.522

Comparing mean level over time in each single group was statistically significant (p<0.001) and pairwise comparisons revealed that all time groups are statistically significant from each other's

TABLE (1) Mean and SD of Streptococcus levels at different time points in the tested groups

	Group I (Clinpro)		Group II (Clinpro / Nano silver)		P value 1
	Mean	**SD**	**Mean**	**SD**	
Streptococcus					
Before	1056.7	229.8	1153.5	189.4	0.154
1Week	678.5	174.9	687.7	158	0.862
1Month	450	207	428.3	229.2	0.755
3Months*	242.2	136.3	213.9	139.6	0.522
P value2	**<0.001**		**<0.001**		

P≤0.05 is statically significant; P1: for comparison between 2 groups by independent t test. P2: for comparison over time in each group separately by repeated measure ANOVA followed by Bonferroni post hoc test.

**: analysis was repeated by Non parametric test to ensure robustness of results.*

Streptococcus Levels percent reduction

The mean and standard deviation of Streptococcus count /level percent reduction of both groups are presented in table (2). **From baseline to 3 months,** the mean percent reduction of streptococcus levels was 76.9±12.4 for Group I and was 81.3±12.6 for Group II. This was statistically insignificant with p value = 0.270.

From baseline to 1 month, the mean percent reduction of streptococcus levels was 57.6±18.9for Group I and was 63.3±17.5 for Group II. This was statistically insignificant with p value = 0.323.

From baseline to 1 week, the mean percent reduction of streptococcus levels was 36.2±7.2 for Group I and was 40.6±8.5 for Group II. This was statistically significant with p value = 0.087.

TABLE (2) Mean and SD of Streptococcus levels percent reduction between the tested groups

	Group I (Clinpro)		**Group II** (Clinpro / Nano silver)		**P value**
	Mean	**SD**	**Mean**	**SD**	
Streptococcus Percent reduction					
Before To 3 Months	76.9	12.4	81.3	12.6	0.270
Before To 1 Months	57.6	18.9	63.3	17.5	0.323
Before To 1 Week	36.2	7.2	40.6	8.5	0.087

P≤0.05 is statically significant; analysis done by independent t test

DISCUSSION

Fissure sealants are frequently utilized as a prophylactic strategy in various nations. Fissure sealants have significantly lowered the incidence of tooth cavities. Pit and fissure sealants, which are mechanically retained resins applied to the enamel's surface to cover the tooth's anatomical "defects," have been successful in preventing dental decay in molars. However, failure rates have been estimated to range from 5% to 10% annually [15].

Due to their ability to stick to other plaque bacteria as well as the enamel's salivary pellicle, S. mutans play a significant part in the aetiology of dental caries. Strong acid makers like S. mutans provide an acidic environment that increases the risk of cavities. Usually, 6 to 24 months after S. mutans first appears in tooth cavities, caries develops. It has been demonstrated that S. mutans is susceptible to the bactericidal effects of silver nitrate and silver diamine fluoride. However, because to tooth pigmentation brought on by high concentrations of silver precipitation, which can also induce pulp irritation due to excessive silver ion penetration into the dentin, their usage has been restricted[10,11]. In the current study, we assessed whether adding NNPs to pit and fissure sealant would boost their preventive benefits by getting rid of the primary dental caries-causing bacteria, S. mutans. In this investigation, forty patients took part (24 males, 16 females). They were split into two equal groups at random; Clinpro sealant is in Group I. (Control group) Team II: (test group with Clinpro TM sealant coated by Nano silver).

The starting point (Before), after a week, a month, and three months, there was no statistically significant difference in the mean streptococcus levels between Group I and Group II. It was statistically significant (p 0.001) to compare each group's mean level with time. The sealant provided an indirect extra caries-preventive benefit by reducing S. mutans, which in turn removed the decalcification it had previously produced.

Recent research has revealed that silver nanoparticles (NNPs) exhibit bactericidal and bacteriostatic effects on a variety of viruses and microorganism species. In earlier research, we demonstrated that NNPs specifically target Streptococcus mutans, the main bacterium linked to tooth decay[16]. According to research, using NNPs with sizes of 40 and 60nm, respectively, the least inhibitory concentration of NNP against Streptococcus mutans is 4.86g/ml, while the minimum bacteriostatic

concentration is 6.25g/ml[17]. When these microbiological features are introduced to Gantrez, a toothpaste adhesive that doesn't discolour the tooth structure, they persist [17,18]. With nanoparticles between 50 and 100nm in size, NNP toxicity has also been avoided in periodontal tissue with minimal alterations to cell viability [19].

This is agreed upon by Espinosa-Cristbal, L. F., Martnez-Castaón, etc. Silver nanoparticles are powerful bactericidal agents, as shown by their antibacterial action on Streptococcus mutants, and their antimicrobial activity are controlled by their sizes and concentrations [20]. In agreement with Nandita Waikhom, et al., "Antimicrobial effectiveness of nano silver fluoride varnish in reducing Streptococcus mutans in saliva and plaque biofilm when compared with Chlorhexidine and Sodium Fluoride Varnishes," which indicates a decrease in s.mutans due to the antibacterial effect of nano-silver [21].

CONCLUSION

Clinpro sealant alone or mixed with Nano silver was effective in reducing *S. mutans* biofilm formation. Future studies are needed to assess how adding NNPs to various resins, glass-ionomer cements, and varnishes affects their ability to kill bacteria.

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