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INFLUENCE OF DIFFERENT RESTORATIVE MATERIALS ON THE SENSITIVITY OF *STREPTOCOCCUS MUTANS* BIOFILM TO DIFFERENT ANTIMICROBIAL MOUTHRINSES *IN-VITRO*

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Objectives: *The aim of this study was to assess the antibacterial effects of chlorhexidine gluconate, sodium fluoride, and sodium fluoride-xylitol combination mouthrinses on S. mutans biofilm in-vitro, and most significantly, we aimed to compare different restorative materials regarding the sensitivity of S. mutans biofilms formed on their surfaces to the antibacterial effects of the tested mouthrinses.* ***Results:*** *Chlorhexidine gluconate 0.12%, sodium fluoride 0.05%, and sodium fluoride 0.05% - xylitol 25% combination exhibited statistically significant antibacterial effects on S. mutans biofilm. The addition of xylitol 25% to sodium fluoride 0.05% mouthrinse was associated with higher antibacterial effects compared with sodium fluoride alone. S. mutans biofilms formed on GIC specimens exhibited the highest sensitivity to antibacterial treatments, whereas biofilms formed on stainless steel specimens showed the lowest sensitivity.* ***Conclusion:*** *S. mutans biofilms formed on different restorative materials exhibited different sensitivity levels to antimicrobial mouthrinses.*

INTRODUCTION

Restorative care involves restoration of carious teeth with dental materials such as amalgams, composite resins, or glass inomer-cements. However, in the case of large multi-surface carious lesions, stainless steel crowns are usually used[1]. Despite the huge advantages associated with restorative treatment, these materials still have some drawbacks; mainly secondary caries vulnerability[2].

The adhesion of oral bacteria, especially *Streptococcus mutans* (*S. mutans*), to teeth and restorative materials and the consequent biofilm formation on these surfaces is the main cause of plaque related diseases and restorative treatment failure[3]. While primary caries cases are responsible for less than half of the dental restorations produced annually, most of the restorations are applied for the replacement of old restorations, and the majority of these require replacement because of secondary caries[1]. *In-vivo* and *in-vitro* studies have demonstrated that *S. mutans* is strongly associated with caries development as it's often isolated from plaque samples of either natural or restorative surfaces during early stages of caries[4].

Several conventional restorative materials have been proposed to have antibacterial and anti-biofilm properties, such as amalgams and glass inomer cements, mainly due to the release of metallic ions and fluoride respectively. On the other hand, composite resins were found to lack any antimicrobial effect; in fact these fillings were shown to promote bacterial growth and biofilm formation, especially for *S. mutans*[5].

One of the convenient approaches for reducing biofilm formation on the surfaces of natural teeth and restorative materials is the use of antimicrobial mouthrinses[3]. Various

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antimicrobial agents may appear to be convenient for plaque control. However, only few were found to be clinically efficient. That's because many antimicrobial agents lack substantivity and efficacy against oral bacteria[6].

Chlorhexidine is a bisbiguanide compound with cationic properties. It has a wide spectrum including most of the Gram positive and Gram negative bacteria, bacterial spores, lipophilic viruses, as well as yeasts etc. Several studies have reported the ability of chlorhexidine to suppress *S. mutans* count in saliva and dental plaque, thus limiting the incidence of dental caries[7]. Chlorhexidine is considered the golden standard against which the efficacies of other antimicrobial agents are compared. However, the use of chlorhexidine is associated with a common side effect; which is brownish staining of teeth, as well as restorations, and tongue[7].

Various studies have stated that fluoride decreases the occurrence of dental caries and slows down or even reverses the progression of existing lesions mainly by reducing the rate of enamel demineralization and increasing the rate of re-mineralization. Fluoride can also influence bacterial metabolism directly. However, the significance of this mechanism is still disputed[8].

Xylitol is a naturally occurring five-carbon sugar polyol that has been permitted by several countries to be used in foods, pharmaceuticals, and oral health products, mainly in chewing gums, toothpastes, and mouthwashes[9]. The anti caries effect of xylitol has been claimed to be based on the reduction of *S. mutans* levels in plaque and saliva by disturbing bacterial energy production processes, thereby leading to "futile energy cycle" and cell death eventually[10].

In this *in-vitro* experimental study, we aimed to assess the antibacterial effects of chlorhexidine gluconate, sodium fluoride, and sodium fluoride-xylitol combination mouthrinses on *S. mutans* biofilm, and most importantly, we aimed to compare different restorative materials regarding the sensitivity of *S. mutans* biofilms formed on their surfaces to the antibacterial effects of the tested mouthrinses.

MATERIALS AND METHODS

Bacterial Samples Collection

Samples were collected from children visiting the pediatric clinic in the faculty of dentistry in Damascus University. Sterile cotton swabs were used to collect samples. Afterwards, the swabs were immediately immersed in nutrient broth and incubated for 24 hrs at 37°C.

Isolation and identification of *Streptococcus mutans*

Each sample was streaked on Mitis Salivarius Agar plate (HiMedia, India) and incubated in a microaerophilic environment using candle jar method for 48 hrs at 37°C. Morphologically different colonies were re-grown separately on Mitis Salivarius Agar for another 48 hrs (candle jar, 37°C) in order to obtain pure cultures. Gram stain and biochemical tests (mannitol fermentation, sorbitol fermentation, arginine hydrolysis, and NaCl 6.5% tolerance) were used to identify *Streptococcus mutans* isolates. All of the biochemical tests were carried out manually using phenol red broth (Liofilchem, Italy) for mannitol and sorbitol fermentation tests, nutrient broth (Abtek, UK) + 6.5% NaCl for NaCl 6.5% tolerance test. Todd Hewitt broth (Abtek, UK) and Moeller's decarboxylase broth (Abtek, UK) were used for arginine hydrolysis test. The scheme of the biochemical reactions used to identify *S. mutans* is elucidated in figure 1. Ten clinical isolates were identified and preserved at +4°C on Tryptic Soya Agar slants (TSA, HiMedia, India) and periodically transferred to fresh media.

Enamel and Dental restorative specimens' preparation

In this *in-vitro* experimental study, specimens of four commercial dental restorative materials (resin composite, glass-inomer cement, amalgam, and stainless steel crowns) in addition to enamel as a control were tested. Table 1 shows the tested materials. Disks were fabricated with 5 mm diameter and 2 mm thickness as follows: First, Enamel specimens were carved out of intact premolars with no caries, or restorations, which had been extracted due to orthodontic treatment plans.

Table 1: Restorative materials used in this study.

Restorative material	Material type
EcoSphere shape (DMG®)	Resin composite
Medicem (PROMEDICA®)	Glass-inomer cement
Non gamma 2 alloy (BMS DENTAL®)	Amalgam
Kids crown®	Stainless steel crown

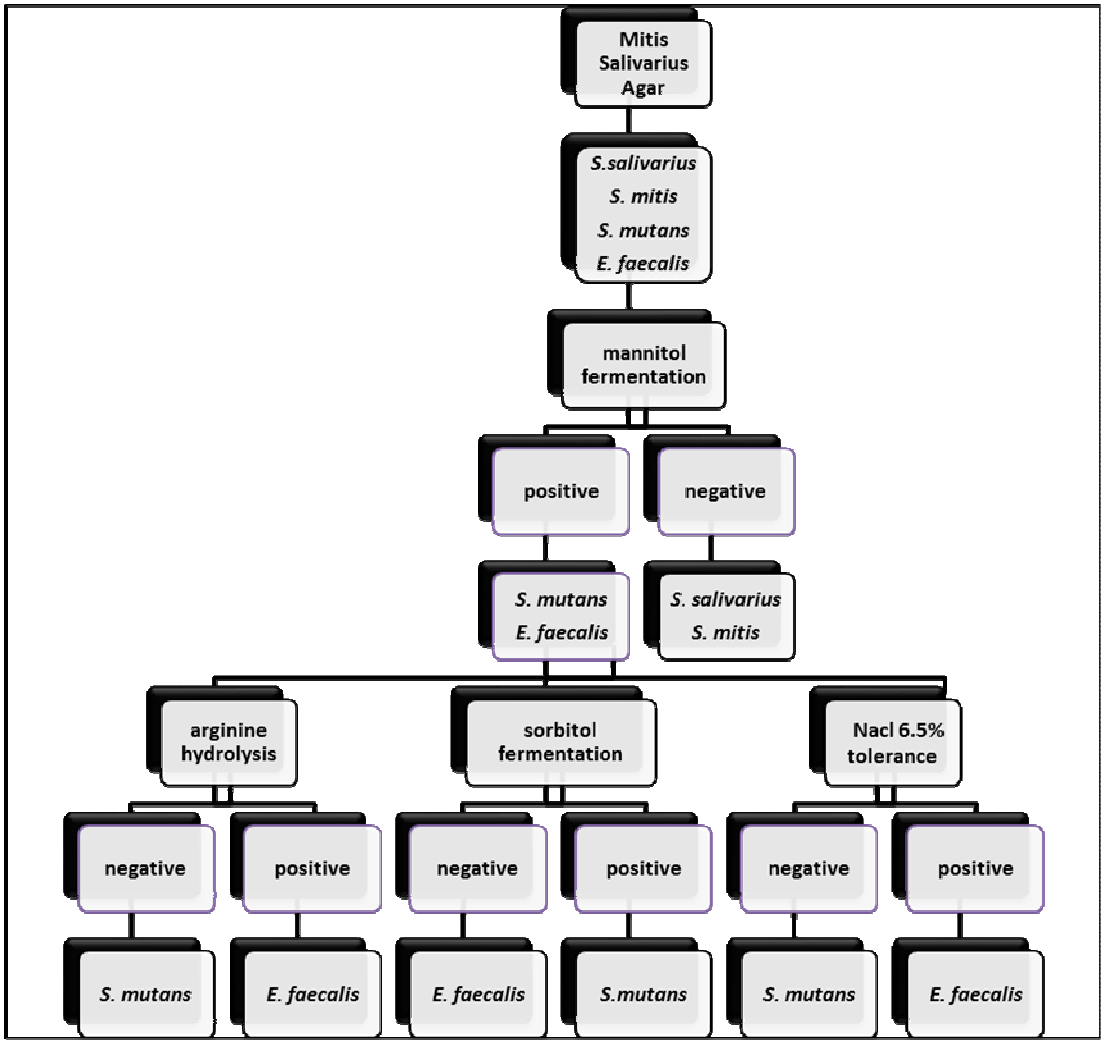

Fig. 1: The scheme of the biochemical reactions used to identify *S. mutans*.

Then, enamel blocks were used to form identical holes with the above mentioned dimensions in a rubber silicon mold. The silicon mold was used to produce disk shaped specimens of amalgam, GIC, and resin composite according to manufacturer's instructions for each material. Resin composite specimens were light cured for 40 seconds on each side and all of the disks were finished following the clinically recommended methods. Stainless steel specimens were carved out of prefabricated stainless steel crowns corresponding to the same surface area of the disks (70.65 mm^2). All specimens were sterilized by autoclaving for 15 min at 121°C[11&12].

Saliva Coating of the Specimens

Unstimulated saliva from one healthy donor was collected over the period of several days. Subsequently, the saliva samples were pooled and centrifuged (30 min; 4°C; 2500 rpm). The supernatant was transported into new sterile tubes and heated in a water bath (60°C, 30 min), re-centrifuged (30 min, 4°C; 2500 rpm), and stored at -20°C[13]. 100 µL of saliva was plated onto nutrient agar and incubated for 48 hrs, no bacterial growth was observed indicating the elimination of bacteria that could disturb the biofilm formation test. The sterile disks were placed in sterile eppendorf tubes containing 100 µL of saliva for 2 hrs to allow salivary pellicle formation[14].

Biofilm formation assay

A bacterial suspension was prepared from each of the ten clinical isolates of *S. mutans* by harvesting the colonies from nutrient agar plates previously inoculated and incubated aerobically for 24 hrs at 37°C. Subsequently, these colonies were suspended in sterile saline. The turbidity of the bacterial suspension was adjusted to 0.5 Mcfarland ($1.5x10^8$ CFU/ml= $1.5x10^7$ CFU/100µl).

After the formation of the salivary pellicle, the specimens were transported to new eppendorf tubes containing 100 µl of the bacterial suspension and incubated for 24 hrs at 37°C to allow the formation of *S. mutans* biofilm on the surface of the specimens. Afterwards, the specimens were gently rinsed with sterile saline to remove unattached cells; subsequently the specimens were vortexed in sterile tubes containing 1.5 ml of sterile saline for two minutes[15]. Serial dilutions were made from each of these tubes and 0.1 ml of each dilution was cultured on the surface of nutrient agar and incubated at 37°C for 24 hrs. Afterwards, colonies were counted by unaided vision and the number of *S. mutans* cells adhered on the surface of the restorative materials was expressed in CFU/ml and then converted to CFU/mm^2 (adherent cells on the specimens).

Evaluation of the anti-biofilm activity

First, biofilms of *S. mutans* isolates were formed on the surface of the specimens following the same procedures carried out previously. After biofilm formation, and rinsing of the specimens to remove unattached cells, these specimens were transferred into new tubes containing 100 µl of one of the three tested mouthrinses, and were set for 1 minute. Afterwards, the specimens were rinsed gently with sterile saline to remove the traces of the antibacterial mouthrinse. The specimens were then vortexed in sterile tubes containing1.5 ml of sterile saline for two minutes[16&17]. Serial dilutions were made from each of these tubes and 0.1 ml of each dilution was cultured on the surface of nutrient agar and incubated at 37°C for 24 hrs. Colonies were then counted by unaided vision and the number of *S. mutans* cells still adhered on the surface of the specimens after the application of the antibacterial treatment was expressed in CFU/ml then converted to CFU/mm^2 and compared with the base level of adhesion before the application of the mouthrinses.

Statistical Analysis

All experiments were conducted twice; Wilcoxon test was used to assess the effectiveness of the tested mouthrinses on *S. mutans* biofilms, and paired sample t-test was used to compare the tested restorative materials regarding the sensitivity of *S. mutans* biofilms formed on their surfaces to the antimicrobial treatments. The significance level was set at 0.05.

RESULTS AND DISCUSSION

Results

Table 2 and figure 2 show the numbers of *S. mutans* cells adhered to the surface of each of the restorative materials before the application of any antimicrobial treatment, and after the application of chlorhexidine gluconate 0.12%, sodium fluoride 0.05%, and sodium fluoride 0.05% - xylitol 25% combination respectively.

All of the three antibacterial mouthrinses tested significantly reduced the count of *S. mutans* in the biofilms formed on the surfaces of all of the tested restorative materials ($p< 0.05$).

Table 3 shows the mean decrease in the number of *S. mutans* in the biofilms formed on the surfaces of the tested restorative materials after the application of the tested antibacterial mouthrinses on these materials. Comparing

Table 2: Number (log_{10}.CFU/mm^2) of *S. mutans* cells adhered to the surface of the tested restorative materials.

Clinical isolate		Stainless steel	Amlagam	GIC	Composite resin	Enamel
S1	Base level of adhesion	3	3.6	4.6	5	4.6
	After application of CHX 0.12%	0.9	1	1.3	3.3	1.6
	After application of NaF 0.05%	2.7	1.7	2.6	3.6	2.6
	After application of NaF 0.05%+xylitol 25%	2.6	1	1	3	2.6
S2	Base level of adhesion	1.3	3	3.7	4	4
	After application of CHX 0.12%	0	0	0	1	1.3
	After application of NaF 0.05%	1.3	2.6	2.3	2.7	3
	After application of NaF 0.05%+xylitol 25%	1.3	2	1.9	2.7	2.7
S3	Base level of adhesion	2.3	3.3	4	4	4
	After application of CHX 0.12%	1.3	0.3	1.6	3	2.6
	After application of NaF 0.05%	1.3	2.7	1.3	2.3	3.6
	After application of NaF 0.05%+xylitol 25%	1.3	1.6	1.3	2.3	2.6
S4	Base level of adhesion	1.6	3.3	3.9	3.9	4
	After application of CHX 0.12%	0.9	0	0	1.6	1.6
	After application of NaF 0.05%	1.3	2	2.6	3.6	2.6
	After application of NaF 0.05%+xylitol 25%	1.3	1.3	1.3	2	2.3
S5	Base level of adhesion	1.6	2.7	3.6	3.7	4.3
	After application of CHX 0.12%	0.9	0	1.3	0.7	1
	After application of NaF 0.05%	1.6	1.6	2.6	2.6	2.6
	After application of NaF 0.05%+xylitol 25%	1.6	1	1.3	2.6	2.6
S6	Base level of adhesion	2.7	3.6	4.7	4.9	4.6
	After application of CHX 0.12%	1.3	0	0	3	1.6
	After application of NaF 0.05%	2	2.6	2	3.7	2.6
	After application of NaF 0.05%+xylitol 25%	2	2.3	1	3.7	2.6
S7	Base level of adhesion	1.6	2.7	3.3	3.7	3.6
	After application of CHX 0.12%	1.3	0.3	0.3	2.6	1.6
	After application of NaF 0.05%	1.6	1	2.7	3.6	3.6
	After application of NaF 0.05%+xylitol 25%	1.6	1	1.3	3.6	2.6

Clinical isolate		Stainless steel	Amlagam	GIC	Composite resin	Enamel
S8	Base level of adhesion	2.7	3.6	4.3	4.9	4.6
	After application of CHX 0.12%	1.3	0	0	0.6	1.6
	After application of NaF 0.05%	2.7	1.6	3.7	3.7	3.7
	After application of NaF 0.05%+xylitol 25%	2.3	1.3	2.6	3.7	2.6
S9	Base level of adhesion	2.9	3.7	4.6	4.9	4.7
	After application of CHX 0.12%	1	0	0	0.6	1.3
	After application of NaF 0.05%	2.3	2	2.6	3.6	3.6
	After application of NaF 0.05%+xylitol 25%	1.6	1.6	2.6	3.6	3.6
S10	Base level of adhesion	1.3	2.7	3.6	3.7	3.9
	After application of CHX 0.12%	0.7	0	0	1.7	2.6
	After application of NaF 0.05%	1.3	2	2.6	3.6	3.7
	After application of NaF 0.05%+xylitol 25%	1.3	2	2.6	3.6	3.6
Aver-age	Base level of adhesion	2.1 ± 0.68	3.2 ± 0.4	4.03 ± 0.49	4.2 ± 0.57	4.2 ± 0.38
	After application of CHX 0.12%	1 ± 0.40	0.1 ± 0.32	0.45 ± 0.66	1.8 ± 1.08	1.6 ± 0.52
	After application of NaF 0.05%	1.81 ± 0.57	1.98 ± 0.53	2.5 ± 0.60	3.3 ± 0.53	3.16 ± 0.52
	After application of NaF 0.05%+xylitol 25%	1.72 ± 0.64	1.51 ± 0.47	1.69 ± 0.67	3.08 ± 0.64	2.78 ± 0.44

CHX= chlorhexidine gluconate, NaF= sodium fluoride.

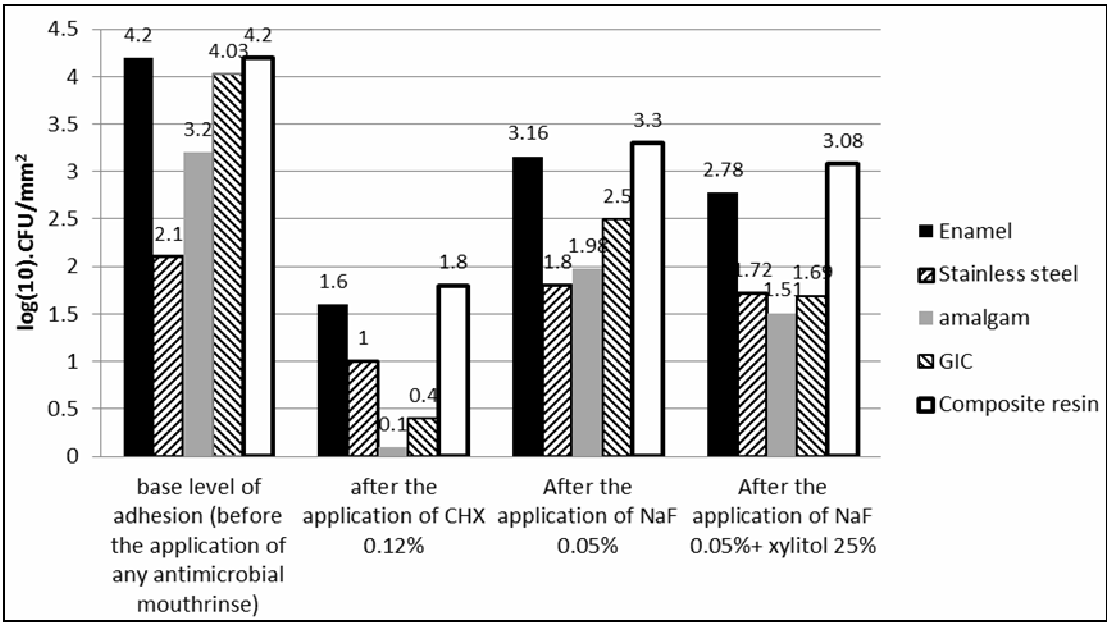

Fig. 2: Number of *S. mutans* cells (log_{10}.CFU/mm^2) adhered onto the surface of tested restorative materials.

Table 3: The average decrease in the number of *S. mutans* in the biofilms formed on the surfaces of the tested restorative materials after the application of the tested antibacterial mouthrinses on these materials.

	Stainless steel	Amalgam	GIC	Composite resin	Enamel
CHX 0.12%	1.14 ± 0.58	3.06 ± 0.46	3.58 ± 0.84	2.46 ± 1.17	2.55 ± 1.05
NaF 0.05%	0.29 ± 0.36	1.24 ± 0.56	1.53 ± 0.78	0.97 ± 0.57	1.07 ± 0.71
NaF 0.05%-xylitol 25%	0.41 ± 0.46	1.71 ± 0.58	2.34 ± 0.84	1.19 ± 0.65	1.45 ± 0.54

these values indicates that chlorhexidine gluconate 0.12% has the most effective antibacterial properties. On the other hand comparing sodium fluoride 0.05% with sodium fluoride 0.05% - xylitol 25% combination indicates that the addition of xylitol increases the antimicrobial effect of sodium fluoride, prominently for GIC and amalgam specimens.

Comparing the tested restorative materials regarding the sensitivity of *S. mutans* biofilms formed on their surfaces to antimicrobial treatments indicates that in the case of chlorhexidine gluconate and sodium fluoride-xylitol combination, *S. mutans* biofilms formed on the surface of GIC specimens were significantly the most susceptible to the antibacterial effects of these two mouthrinses, while biofilms formed on the surface of stainless steel specimens were the least susceptible ($p< 0.05$). In the case of sodium fluoride 0.05% however, biofilms formed on GIC specimens were the most susceptible to the antimicrobial effects of sodium fluoride followed by the biofilms formed on amalgam specimens without statistical significant between these two restorative materials ($p> 0.05$). On the other hand, biofilms formed on the surface of stainless steel specimens showed the lowest level of susceptibility to sodium fluoride ($p< 0.05$).

Discussion

The *in-vitro* approach used in this study has the benefit of ensuring standardized test conditions, and therefore high reproducibility. On the other hand, the used *in-vitro* system integrated enamel, saliva, and S. *mutans* to mimic some *in-vivo* features. Nevertheless the batch culture technique used in this study has some disadvantages; as in contrast to the situation in the oral cavity, products of bacterial metabolism accumulate in batch cultures.

All of the test specimens were incubated with saliva for 2 hrs prior to incubation with the bacterial suspension, as the salivary pellicle may influence microbial adhesion and metabolic activity significantly. This time of incubation was chosen based on previous studies that showed that the pellicle reaches its maximum thickness after 2 hrs of saliva exposition[14].

Chlorhexidine gluconate has been demonstrated to be the most effective antibacterial agent in several studies like a study conducted by Poggio *et al.*[12] and a study conducted by Auschill *et al.*[18]. This fact was also confirmed by the results of the present study, as chlorhexidine significantly reduced the count of *S. mutans* in biofilms formed by all of *S. mutans* clinical isolates on different dental restorative materials.

Very few data are available in literature about the specific effect of antibacterial mouthrinses on bacterial biofilms formed on different dental restorative materials[3&19]. This study demonstrated that biofilms of *S.mutans* formed on various surfaces showed significant differences in the sensitivity to antimicrobial treatments. This result highlights the importance of choosing the relevant surface when testing the anti-biofilm effects of a given mouthrinse.

The results of the current study reported that biofilms formed on the surface of glass-inomer cement specimens were the most susceptible to chlorhexidine, and on the other hand, biofilms formed on the surface of stainless steel specimens exhibited the lowest sensitivity to the antimicrobial treatment. A

possible explanation for this phenomenon could be the differences in chlorhexidine adsorption rates onto different materials, as the surface of glass-inomer cement is relatively rougher and may adsorbs chlorhexidine to a larger extant compared with the smooth surface of stainless steel specimens, add to that the probable synergetic effect of chlorhexidine with the fluoride released from glass-inomer cement.

Another explanation for this phenomenon may be attributed to the Microbiology-related corrosion of stainless steel, which can be defined as the deterioration of the metal surface due to the direct or indirect activity of microorganisms[20]. Bacteria in the biofilm decrease the pH by generating acidic metabolites; subsequently the surface oxides of the dental alloys dissolve, leading to decline in the corrosion resistance of the metal[21]. However, the altered surfaces of dental alloys can accelerate the expression of bacterial virulence genes and biofilm formation as a result. In this regard, Zhang *et al.* proposed that corroded alloy surfaces may upregulate gene expression of the glucosyltransferases B, C, D, glucan-binding proteins B, fructosyltransferase, and lactate dehydrogenase in *S. mutans*[5]. Thus, the increased accumulation of extracellular matrix producing a thick biofilm could play a critical role in the protection of *S. mutans* from the antibacterial activity of chlorhexidine.

Fluoride on the other hand, is one of the most important agents for controlling dental caries. The main proved mechanism of action of fluoride in caries management is by increasing mineral uptake by enamel and decreasing demineralization. However, another reported mode of action is by affecting bacterial metabolism[22]. In this regard, our study is in agreement with the results of many previous studies that demonstrated the antibacterial and antibiofilm properties of fluoride salts like the study of karami *et al.*[23], and the *in-vivo* study conducted by Sajadi *et al.*[24]. The effects of fluoride on streptococci are attributed in part to the inhibition of enolase, which is one of the glycolytic system enzymes, as this inhibition reduces the intracellular level of phosphoenolpyruvate (PEP), leading to decrease in bacterial sugar uptake through PEP-dependent phosphotransferase system (PEP-PTS). Furthermore, fluoride can inhibit bacterial proton-translocating ATPase that is partly responsible for the proton fluxion out of the cells, resulting in acidification of the intracellular PH. The dissociation of unionized hydrofluoric acid into H^+ and F^- in the cells also promotes intracellular acidification[9]. The results of these events together lead eventually to reduction in the bacterial metabolic activity.

As in the case of chlorhexidine, biofilms of *S.mutans* formed on various restorative surfaces showed significant differences in the sensitivity to the antibacterial properties of sodium fluoride, as biofilms formed on the surface of glass-inomer cement specimens were the most susceptible to sodium fluoride, followed by amalgam with no statistical significant between the two materials, while biofilms formed on the surface of stainless steel specimens exhibited the lowest sensitivity to this antimicrobial treatment. This result can be also explained by the same reasons mentioned in the case of chlorhexidine, relating to the different levels of antibacterial agent adsorption onto the surface of different dental materials, biocorrosion of the metal alloy in stainless steel leading to thicker more resistant biofilm, in addition to the probable additive effect of the external fluoride derived from the mouthwash to the fluoride that is naturally released from glass inomer cement specimens.

Considering the significant role of *S. mutans* in the development of dental caries, and because of the side effects of chlorhexidine, in addition to the limited antimicrobial efficacy of sodium fluoride compared with chlorhexidine as noticed in the current study, we tried to investigate the therapeutic effects of xylitol and sodium fluoride combination mouthwash on *S. mutans* biofilm in an attempt to meet the optimum criteria of an efficient mouthwash with sweet taste.

The results of the current study reported that sodium fluoride 0.05% - xylitol 25% combination mouthrinse significantly decreased the count of *S. mutans* in biofilms formed on the surfaces of all of the tested restorative materials confirming the effective antibacterial properties of this combination. These properties may be explained by the results of previous studies that investigated the intracellular glycolytic intermediates, and reported that xylitol inhibits the upper part of the glycolytic pathway, while fluoride inhibits

the lower part of it[9]. Our results are in accordance with a study performed by Goncalves *et al.*[25], in which they reported a reduction of salivary *S. mutans* after using 0.05% NaF solution containing xylitol. On the other hand, this result is in disagreement with the findings of Giertsen *et al.*[26], in which, they concluded that using 0.025% NaF + 20% xylitol did not affect the levels of mutans streptococci in dental plaque and saliva, this disagreement may be attributed mainly to the different concentrations of fluoride and xylitol used.

Moreover, the result of this study also demonstrated once again that biofilms of *S.mutans* formed on various surfaces showed significant differences in sensitivity to sodium fluoride - xylitol combination mouthwash, as biofilms formed on the surface of glass inomer cement specimens were the most susceptible to this antibacterial treatment, while biofilms formed on the surfaces of stainless steel specimens exhibited the lowest level of sensitivity. Beside the different levels of mouthrinse adsorption between the tested materials, and biocorrosion of the stainless steel specimens, this result can be also attributed to the synergetic effect of xylitol and fluoride, taking into account the fluoride released from the glass inomer cement specimens as well.

Comparing the antibacterial effects of the three tested mouthrinses indicates that chlorhexidine gluconate 0.12% was the most effective antibacterial mouthrinse on *S. mutans* biofilms formed on all of the tested restorative materials. In this regard, many previous studies have also confirmed that chlorhexidine has the best anti-plaque properties, and that it is still considered the golden standard against which the efficacies of other antimicrobial agents are compared[7]. This is in accordance with the findings of Nassar *et al.*[22] in which they concluded that the antibacterial properties of chlorhexidine are superior to those of sodium fluoride. However, the results of the current study disagreed with the results of a study conducted by Zajkani *et al.*[27] in which they reported no statistically significant differences between the antibacterial effects of chlorhexidine, and sodium fluoride-xylitol mouthrinses, this disagreement may be attributed to the different concentrations of chlorhexidine, fluoride and xylitol concentrations used.

On the other hand, comparing sodium fluoride 0.05% - xylitol 25% combination mouthrinse with sodium fluoride 0.05% alone have shown that the addition of xylitol 25% to sodium fluoride mouthrinse was associated with increased antibacterial effects, prominently on the biofilms formed on GIC and amalgam specimens. This may be explained by the possible synergistic effects of sodium fluoride and xylitol in inhibiting bacterial glycolytic pathways as mentioned earlier.

Conclusion

Within the limitation of the current study we concluded that:

- Chlorhexidine gluconate 0.12%, sodium fluoride 0.05%, and sodium fluoride 0.05% - xylitol 25% combination exhibited statistically significant antibacterial effects on *S. mutans* biofilm.
- Chlorhexidine mouthrinse exhibited the highest antibacterial efficacy in reducing *S. mutans* count in the biofilm.
- The addition of xylitol 25% to sodium fluoride 0.05% mouthrinse increased the antibacterial effects of this mouthrinse.
- Most importantly; *S. mutans* biofilms formed on the surfaces of different restorative materials exhibited different sensitivity levels to the tested mouthrinses, as biofilms formed on GIC specimens showed the highest sensitivity, while biofilms formed on stainless steel specimens exhibited the lowest susceptibility to antimicrobial treatments.

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تأثير المواد الترميمية المختلفة على حساسية بيوفيلم العقديات الطافرة لتأثير الغسولات المضادة للجراثيم فى المختبر

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الأهداف: هدفت هذه الدراسة إلى تقييم الغسولات الفموية المكونة من الكلورهيكزيدين غلوكونات ، أو فلوريد الصوديوم ، أو مزيج فلوريد الصوديوم مع الزايليتول من حيث تأثيرها المضاد للجراثيم على بيوفيلم العقديات الطافرة. كما هدفت بشكل رئيسي إلى مقارنة مختلف المواد الترميمية من حيث حساسية بيوفيلم العقديات الطافرة المتشكل على سطوحها للتأثير المضاد للجراثيم للغسولات المدروسة.

المواد والطرائق: أستخدمت عشر عزلات سريرية من العقديات الطافرة لتشكيل البيوفيلم على سطوح عينات من أربع مواد ترميمية تجارية وهي (حشوات الكومبوزيت الراتنجية ، وحشوات الغلاس إينومير ، والأملغام ، وتيجان الستانلس ستيل) ، وأستعملت عينات من الميناء كشاهد. تم تقييم التصاق كل من هذه العزلات السريرية على سطح كل من العينات باستخدام طريقة عد العيوش وتم اتخاذ هذه القيم كقيم مرجعية للالتصاق ، حيث قورنت بها قيم التصاق هذه العزلات السريرية بعد تطبيق الغسولات المضادة للجراثيم المدروسة (الكلورهيكزيدين غلوكونات ٠,١٢% ، وفلوريد الصوديوم ٠,٠٥% ، ومزيج فلوريد الصوديوم ٠,٠٥% مع الزايليتول ٢٥%).

النتائج: أبدى كل من محلول الكلورهيكزيدين غلوكونات ٠,١٢% ، ومحلول فلوريد الصوديوم ٠,٠٥% ، وكذلك مزيج فلوريد الصوديوم ٠,٠٥% مع الزايليتول ٢٥% تأثيراً مضاداً للجراثيم ذو أهمية إحصائية على بيوفيلم العقديات الطافرة. كما ترافقت إضافة الزايليتول بنسبة ٢٥% إلى غسول فلوريد الصوديوم ٠,٠٥% مع زيادة في التأثير المضاد للجراثيم لهذا الغسول ، وذلك بشكل أكبر بالنسبة لكل من عينات الغلاس إينومير ، والأملغام. أبدى بيوفيلم العقديات الطافرة المتشكل على سطح عينات الغلاس إينومير الحساسية الأكبر للتأثير المضاد للجراثيم للغسولات المدروسة ، في حين ترافق البيوفيلم المتشكل على سطح عينات الستانلس ستيل مع الحساسية الأقل.

الخلاصة: أبدى بيوفيلم العقديات الطافرة المتشكل على سطوح مواد ترميمية مختلفة حساسية متباينة للغسولات المضادة للجراثيم.