International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Evaluation of Antibacterial Activity of Adhesive Systems Reinforced by Fluoroapatite or Calcium Fluoride (In Vitro Study)

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Abstract: ***Objectives: The purpose of this study was to evaluate and test the antibacterial activity of ideal ratio addition of Fluoroapatite or calcium fluoride to the Total etch and Self etch adhesive systemsand were compared to the control non-fluoridated adhesives against selected microorganisms which were Streptococcus mutans and Lactobacilli. Methods: Total etch adhesive system (Tetric® N-Bond) and Self etch adhesive system (Tetric® N-Bond Self-Etch) were used with ratio addition of Fluoroapatite or calcium fluoride that didn't affect the shear bond strength of adhesive systems. Ten discs for each of six groups (TE, TE +FA 3%, TE + CaF_2 5%, SE, SE + FA 1% andSE + CaF_2 7%) were prepared, 5 discs of each group used for each microorganism were used to assess the antimicrobial effect of the fluoridated adhesives in vitro by measuring the inhibition zone by direct contact against selected microorganisms and comparing it with control groups. Data were statistically-analyzed using ANOVA, LSD and T-tests. Results: The result showed that, there was a significant difference between Fluoridated and control groups in antibacterial activity at P≤0.01 level. Conclusions: All Fluoridated adhesives have antibacterial effects against streptococcus mutans and Lactobacilli.***

Keywords: antibacterial, Fluoroapatite

1. Introduction

Dental caries is a biofilm-related oral diseaseand related to the increased consumption of dietary sugar. If dental biofilms are permitted to stay on the tooth surfaces with the continuous consumption of sugar, acidogenic and aciduric bacteria, as members of dental biofilms, will form an organic acid as a result of metabolism the sugar. Dental carieswill be initiated as a result of dissolution of the tooth surfaces due to the low pH environment in the biofilmmatrix. In spite of additional acidogenic and aciduric bacteria included, Streptococcus *mutans* is considered amongst the most significant microorganisms in the etiology of caries and has been utilized in many caries studies[1,2,3].

Streptococcusmutans are thought to be the fundamental etiological microorganisms in caries disease which are the most important factor in initiation and progression of caries lesions, with lactobacilli and other bacteria contributing in the disease progression. Rarely, some different microorganisms have been considered as initiator microorganisms [4,5].

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Histological and bacteriologic researches have demonstrated that only a few percentages of the teeth after cavity preparation are sterile. The residual bacteria left in dentin in the cavity preparation can maintain their activity for a long time and multiply within the smear layer even if there is a good seal from the oral cavity as shown in a number of studies. This can be a bacterial toxins source which may diffuse and cause recurrence of the caries [6].

Protection of dental-pulp complex and antibacterial activity are desired properties of restorative materials, especially for self-etching adhesives, which partially remove the smear layer and residual bacteria may remain on cavity walls at the interface, increasing the risk of secondary caries [7].

Secondary caries formation can be inhibited by the fluoride-containing adhesive systems as indicated by previous studies,this is possible because the fluoride ions can penetrate into the dentin after being released within the hybrid layer [8].

Hamilton et al. in**1996**reported that, the fluoride has many mechanisms for it's anticariogenic effects which includes demineralization reduction, remineralization enhancement, pellicle interference and interference of plaque formation and inhibition of the microbial growth and metabolism[9]. The aim of the study was to evaluate the antibacterial effect of reinforcement of bonding adhesive systems with Fluoroapatite or Calcium Fluoride without affecting the shear bond strength of bonding adhesive systems

2. Material and Method

In this study the best ratio addition (that didn't affect the shear bond strength) of Fluoroapatite or Calcium fluoride to Tetric® N-Bond and Tetric® N-Bond Self-Etch adhesive which was 3% of Fluoroapatite or 5% of Calcium fluoride to Tetric® N-Bond adhesive, while the best ratio addition for Tetric® N-Bond Self-Etch adhesive were 1% of Fluoroapatite or 7% of Calcium fluoride were done to study their antibacterial activity against Streptococcus mutans and Lactobacilli microorganisms. Ten adhesive disc specimens (fluoridated and control non-fluoridated of an inner diameter of 8 mm and a depth of 1.0 mm) for each group were prepared, 5 discs of each group used for each microorganism. A custom-made silicone molds with an inner diameter of 8 mm and a depth of 1.0 mm were used to prepare specimens. Adhesive solution was placed in a silicone mold with a glass slide to cover the top surface, then light-cured for 40 s from the top side using a visible light curing unit (SDI, Japan) [10]. Each specimen was removed

from its mold and additionally light-cured for 20 s from the bottom side.

The medium used for antibacterial activity test were Mueller-Hinton agar for Streptococcus mutans and Chocolate blood agarfor Lactobacilli.

The selective medium for the cultivation of Streptococcus mutans was Mitis Salivarius Bacitracin Agar (MSB) and for Lactobacilli was BD LBS Agar (also known as Rogosa Agar).

The microorganisms cultured directly from swabs that were taken from volunteer patients that attend the Medical Center in Baghdad. The swab rolled over a small area of the surface at the edge on MSB agar and BD LBS Agar; then streaked for isolation from this inoculated area.

For MSB agar, the plates were incubated anaerobically utilizing a gas pack for 48 hours at 37 °C then aerobically for 24 hours at room temperature while for BD LBS Agar, the plates were incubated anaerobically for three days at 37 °C.

For many types of antimicrobial susceptibility testing, a standard inoculum of bacteria must be used. The number of bacteria in a liquid medium can be determined by visually comparing the turbidity of the liquid medium to a standard that represents a known number of bacteria in suspension. Standard inoculums were also prepared for each bacterial strain which fit to 0.5 McFarland Nephelometer Standard (A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4)).

50 µL from each bacterial strain were taken separately using a micropipette and added to petri dishes containing Muller-Hinton agar (for Streptococcus mutans) and Chocolate blood agar (for Lactobacilli) and streaked using a glass rod.

The prepared disc specimens (Fluoridated and control groups) were applied directly on the Muller-Hinton agar and Chocolate blood agar using a tweezers and adapted well on the agar surfaces. Then, the lid of the petri dish was closed. Anaerobic incubation was done using an anaerobic jar with gas packs.

After 24 hours of incubation, the Chocolate blood agar plates were removed from the incubator and examined for the inhibition zone around each disc and after 48 hours of incubation the Muller-Hinton agar plates were removed from the incubator and examined and measured the inhibition zone using a ruler around each disc.The measurement of inhibition zonedone by two microbiologists separately.

3. Results

The mean, maximum, minimum, standard deviation, and standard error values of the inhibition zone for the Streptococcus Mutans and Lactobacilli microorganisms results for Total Etch groups and for Self-Etch groups are represented in tables 1&2.

The results of this study showed that the TE+CaF_2 5% exhibited high percentage of reduction of the Streptococcus Mutans microorganisms among Total Etch groups and SE+FA 1% among Self-Etch groups as shown in figure [1].

For Lactobacilli microorganisms the control group shows the least inhibition zone among the Total Etch groups with the same mean values of inhibition zones for TE+FA 3% and TE+CaF_2 5%. While for the Self-Etch groups, the SE+CaF_2 7% group shows the highest mean value of inhibition zone against Lactobacilli microorganisms as shown in figure [2].

To see whether the difference in mean values among the groups is significant or not, statistical analysis using ANOVA and LSD tests were applied.

ANOVA test for Streptococcus Mutans microorganisms among Total Etch groups and Self-Etch groups shows a significant difference at $P<0.05$ level as shown in tables 3&4.

For comparison between fluoridated and non-fluoridated groups of Total Etch effects against Streptococcus Mutans microorganisms, LSD test done. the results showed that there was a highly significant difference between control group and TE+CaF_2 5% and there was a non-significant difference between TE+FA 3% group and TE+CaF_2 5% group as shown in table (5).

LSD test shows that there was a non-significant difference between SE+FA 1% and SE+CaF_2 7% in the inhibition zones against Streptococcus Mutans microorganisms while there was a significant difference between control and other groups as shown in table (6).

For Lactobacilli microorganisms, ANOVA test between Total Etch groups shows a significant difference at $P<0.05$ level, and a highly significant difference at $P<0.05$ level between Self-Etch groups as shown in tables 7&8.

For comparison between groups of Total Etch effects against Lactobacillimicroorganisms, LSD test shows that there was a significant difference between Total Etch groups as shown in table (9).

In the Self-Etch groups, LSD test shows that there was a non-significant difference between SE+FA 1% and SE+CaF_2 7% in the inhibition zones against Lactobacillimicroorganisms while there was a highly significant difference between control group and SE+CaF_2 7% group and a significant difference between control group and SE+FA 1%group as shown in table (10).

T-test was done to compare between control groups and between Fluoroapatite addition groups and Calcium Fluoride addition groups against Streptococcus Mutans microorganisms and against Lactobacilli microorganisms.

For Streptococcus Mutans microorganisms, there was a highly significant difference between Self-Etch control group and Total Etch control group. Also, there was a highly significant difference between TE+CaF2 5%group and

SE+CaF2 7% group, and significant difference between TE+FA 3%group and SE+FA 1% group as shown in table (11).

ForLactobacillimicroorganisms, there was a highly significant difference at P<0.001 level between TE+FA 3% group and SE+FA 1% group and there was a significant difference between other groups as shown in table (12).

4. Discussion

In spite of the deficiency of the Fluoride is not the cause of dental caries but this ion is the main therapeutic material that efficiently controls the progression of dental caries [(11)].

In addition to the demineralization inhibition action of the Fluoride by binding Calcium and Phosphate that dissolved as a result of dissolution of apatite by acid attack and prevent of leaching of the mineral constituents away of tooth structure through the great affinity of Fluoride to the Calcium and Phosphate that leads to formation of Fluoroapatite which is a more resistance to acid attack, in addition to that the Fluoride may has antimicrobial effects such as it is effect on adhesion of bacteria which is important to the colonize of bacteria to the tooth[(12)], also the Fluoride cause modification of bacterial metabolism which is essential for acid production by bacteria and also the Fluoride can interfere with enzyme enolase which leads to inhibit the process of glycolysis and also effect the intra and extra cellular polysaccharides[(13,14)].

Many researchers reported that even with low amount levels of Fluoride, the bacterial growth can be reduced and the acid production by streptococcus mutans can be inhibited through the enough formation of hydrogen Fluoride when the PH falls and this hydrogen Fluoride when formed enough can inhibits acid production by bacteria [(15,16,17,18)].

In this study the antibacterial effects of Fluoridated adhesive were compared to non-Fluoridated adhesives against the most etiological dental caries microorganisms (streptococcus mutans and Lactobacilli by measuring the inhibition zone caused by adhesive whether Fluoridated or not Fluoridated against these selected microorganisms.

This study showed that Fluoridated adhesive has antibacterial effects against streptococcus mutans and Lactobacilli.

The Fluoridated Self Etch adhesive groups showed the largest inhibition zone against streptococcus mutans and Lactobacilli and this may be due to large amount of Fluoride liberation and released because there was an acid-base reaction which leads to Fluoride release and the largest release of Fluoride caused largest inhibition zone against streptococcus mutans and Lactobacilli and this in agreement with many studies such as**Moura et al.**and **Pandit et al.**[(19,20)] who showed that when Fluoride concentration increased the proportion of these microorganisms decreased.

The Fluoridated Total Etch adhesive groups showed the least inhibition zone amongst the Fluoridated adhesives and this may be due to release of little amount of Fluoride because the Fluoride release needs water to be released from any resin based restorative materials and this little released of Fluoride caused small inhibition zone against streptococcus mutans and Lactobacilli; any how even little amount of Fluoride can cause reduced bacterial growth and gives antibacterial effect and this in agreement with **Pandit et al.**[(20)].

In the clinical situation the presence of dentinal fluids may cause high release of Fluoride which will transported to the gap and exerting it is antibacterial action.

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Table 1: Descriptive statistics of inhibition zones of adhesive groups for Streptococcus Mutans.

	TE	TE+FA 3%	TE+CaF 5%	SE	SE+FA 1%	SE+CaF 7%
Mean	9.6	11.6	13	14.6	21.2	19.2
SD	1.140	1.1401	1.224	0.894	4.4384	1.303
SE	0.511	0.511	0.549	0.401	1.990	0.584
Min	8	10	11	14	17	18
Max	11	13	14	16	28	21

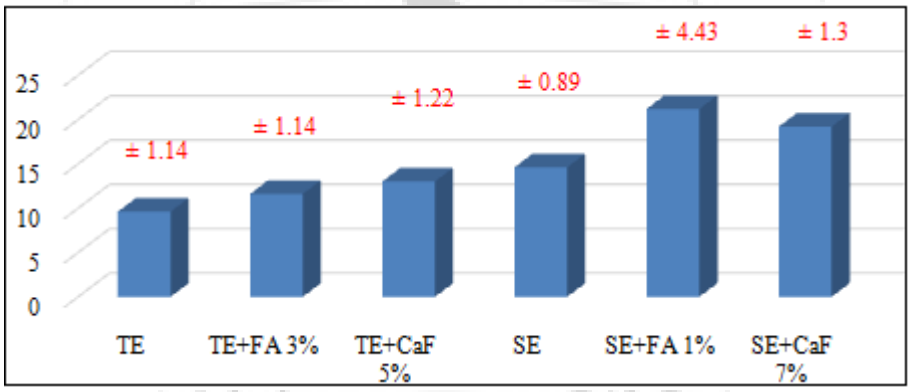

Figure 1: Mean inhibition zone values of adhesive groups against Streptococcus Mutansmicro organisms.

Table 2: Descriptive statistics of inhibition zones of adhesive groups for Lactobacilli

	TE	TE+FA 3%	TE+CaF 5%	SE	SE+FA 1%	SE+CaF 7%
Mean	10.6	13.6	13.6	14	18.2	19.2
SD	1.6733	1.1401	0.547	0.707	1.483	1.923
SE	0.7503	0.5112	0.245	0.317	0.665	0.862
Min	8	12	13	13	16	17
Max	12	15	14	15	20	22

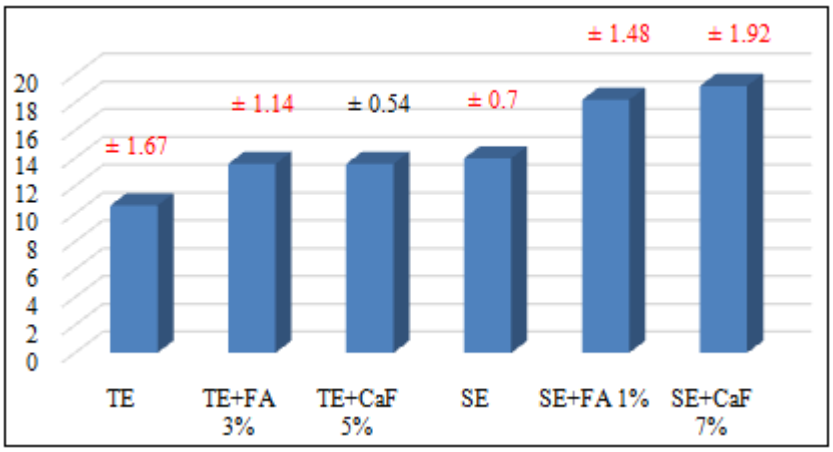

Figure 2: Mean inhibition zone values of adhesive groups against Lactobacilli microorganisms

Table 3: ANOVA test of inhibition zones for Total Etch groups against Streptococcus Mutans microorganisms

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.200	2	14.600	10.683	.002
Within Groups	16.400	12	1.367		
Total	45.600	14			

Table 4: ANOVA test of inhibition zones for Self-Etch groups against Streptococcus Mutans microorganisms

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	114.533	2	57.267	7.739	.007
Within Groups	88.800	12	7.400		
Total	203.333	14			

Table 5: LSD test of the Total Etch groups against Streptococcus Mutans microorganisms

	P-value	Sig
TE& TE+FA 3%	0.019	S
TE& TE+CaF2 5%	P<0.01	HS
TE+FA 3%& TE+CaF2 5%	0.083	NS

Table 6: LSD test of the Self-Etch groups against Streptococcus Mutans microorganisms

	P-value	Sig
SE& SE+FA 1%	0.002	S
SE& SE+CaF2 7%	0.02	S
SE+FA 1%& SE+CaF2 7%	0.268	NS

Table 7: ANOVA test of inhibition zones for Total Etch groups against Lactobacilli microorganisms

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30.000	2	15.000	10.227	.003
Within Groups	17.600	12	1.467		
Total	47.600	14			

Table 8: ANOVA test of inhibition zones for Self-Etch groups against Lactobacilli microorganisms.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.133	2	38.067	17.844	.000
Within Groups	25.600	12	2.133		
Total	101.733	14			

Table 9: LSD test of the Total Etch groups against Lactobacilli microorganisms

	P-value	Sig
TE& TE+FA 3%	0.002	S
TE& TE+CaF2 5%	0.002	S
TE+FA 3%& TE+CaF2 5%	1.00	S

Table 10: LSD test of the Self-Etch groups against Lactobacilli microorganisms

	P-value	Sig
SE& SE+FA 1%	0.001	S
SE& SE+CaF2 7%	P<0.01	HS
SE+FA 1%& SE+CaF2 7%	0.300	NS

Table 11: T-test between groups against Streptococcus Mutans microorganisms

	t-test	P-value	Sig
TE& SE	15.811	P<0.001	HS
TE+FA 3%& SE+FA 1%	6.249	0.003	S
TE+CaF2 5%& SE+CaF2 7%	16.590	P<0.001	HS

Table 12: T-test between groups against Lactobacillimicro organisms

	t-test	P-value	Sig
TE& SE	6.668	0.003	S
TE+FA 3%& SE+FA 1%	18.779	P<0.001	HS
TE+CaF2 5%& SE+CaF2 7%	8.257	0.001	S