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Evaluation of Antibacterial and Mechanical Properties of Three Different Pit and Fissure Sealant Materials

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***Abstract**:*
***Background:** Newer technologies and the development of pit and fissure sealants have shifted the treatment philosophy from 'drill and fill' to that of 'seal and heal'*
***Aim**: Assess and compare antibacterial activity and mechanical properties of three different pit and fissure sealants.*
***Materials and Method**: Fluoride releasing resin based sealant (Clinpro), surface prereacted glass filler containing sealant (Beautisealant) and amorphous calcium phosphate containing sealant (Aegis) were used in this study. Their antibacterial activity against Streptococcus mutans was assessed using agar diffusion test and their mechanical properties (shear bond strength, diametrical tensile strength and microhardness) were evaluated. One way ANOVA and post hoc tests were used appropriately for statistical analysis (SPSS version 22).*
***Result**: There was statistically significant difference between all groups regarding antibacterial activity and microhardness. No significant difference between Clinpro and Aegis regarding shear bond strength and diametrical tensile strength was observed.*
***Conclusion**: Clinpro showed highest antibacterial activity and diametrical tensile strength but lowest microhardness value. Beautisealant had highest shear bond strength. Aegis showed best microhardness and diametrical tensile strength valued.*
***Keyword**: Aegis, Beautisealant, Clinpro, Fissure sealant, Agar diffusion test.*

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I. Introduction

Dental caries is considered the most common bacterial disease affecting oral cavity. It is caused by an ecological shift in the composition and activity of the bacterial biofilm following prolonged exposure to fermentable carbohydrates, leading to a break in the balance between demineralization and remineralization[1,2].Pit and fissure caries account for 80% of total caries experience. The morphology of pits and fissures provides an environment for plaque and bacterial retention. Moreover, enamel is thinner in pits and fissures, which allows accelerated demineralization. The high susceptibility of pits and fissures to caries provides the rationale for protection of these areas[3-6].

In the recent era of preventive dentistry, pit and fissure sealants are considered an ideal occlusal preventive measure. Caries preventive effect of sealant materials relies on the creation of effective mechanical obstacle to the leakage of nutrients to cariogenic microorganisms in deeper parts of pits and fissures[3].Fluoride releasing resin based sealant is a bioactive sealant that has a dual role of actions in caries prevention. It not only mechanically seals pits and fissures but also has an anticariogenic properties[1,3,7].

Recently, a functional surface pre-reacted glass filler (S_PRG) containing fissure sealant has developed. The S_PRG filler releases several types of ions such as fluoride, strontium, aluminum, sodium, borate and silicate into either normal saliva or lactic acid conditions. Strontium and fluoride have been shown to improve the acid resistance of teeth by converting hydroxyapatite into strontium apatite and fluoroapatite. Furthermore, S-PRG filler exhibits antibacterial activity[8-10].

A recently introduced amorphous calcium phosphate (ACP) containing fissure sealant has been marketed with claimed anti-cariogenic properties and remineralization potential[11-13]. The premise behind this sealant type is that, at low pH value, ACP is capable of breaking down and releasing saturating levels of calcium and phosphate ions. These concentrations are conducive to the formation of hydroxyapatite (HAP). Therefore, ACP containing fissure sealant is considered a smart bioactive material[12,13].

The clinical durability of fissure sealants depends upon their mechanical properties to resist not only permanent indentation of the material itself but also dislodging forces acting within oral cavity. Moreover, the antibacterial effects of different sealant materials have been proved to enhance the caries preventive effect of diverse pit and fissure sealants [1,3,9-11]. For this reasons, the purpose of this study was to compare between fluoride-releasing resin based sealant, S-PRG containing sealant and ACP containing sealant regarding antibacterial activity and mechanical properties.

II. Materials and Methods

Study design: This study was carried out as an experimental study.

Study setting: Evaluation of mechanical properties was performed at Biomaterial Department, Faculty of Dentistry, Tanta University. Bacteriological assessment was performed at Microbiology Department, Faculty of Medicine, Mansoura University.

Materials: The fluoride releasing Clinprosealant*, the BeautiSealant** containing S-PRG filler and the Aegis*** containing ACP were selected for this study.

1. Agar diffusion test

a. Preparation of sealant blocks and tested microorganism

From each sealant material, approximately12mg were applied to 6 mm paper disks and immediately polymerized according to the manufacturer's instructions. Ten test specimens were prepared from each sealant type.Streptococcus mutans was grown in 5 mL brain heart infusion broth (BHI) for 24 hr at 37C. The turbidity of each bacterial suspension was adjusted to 0.6 McFaralndstandard.

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b. Agar diffusion test technique

Mitis-Salivarius agar plates were inoculated with 200 µL of Streptococcus mutans strain. Sealant specimens were immediately placed on freshly inoculated plates and incubated for 48 hours. The bacterial inhibition zone halo was measured using digital caliper and expressed in millimeters by subtracting the sealant diameter from the average zone of inhibition.

2. Shear bond strength assessment

A. Preparation of sealant specimen

Thirty caries free human premolars, extracted for orthodontic purposes were collected. Teeth were cleaned using a low speed micromotor to remove smear layer and then were stored in distilled water until use.Premolars were embedded in chemically cured acrylic resin.The teeth were randomly divided into three equal groups.

Caries free middle segment of proximal surfaces was chosen for preparation of sealant core.Aplastic ring of 3mm internal diameter and 3 mm height was placed over the enamel perpendicular to the proximal surface of teeth. The remaining surface was covered by adhesive tape.

The enamel surface of the concise area within the ring of each tooth of group 1 and 3 was etched according to the manufacturer's instructions with 37% phosphoric acid for 30 seconds, then copiously washed by water and air dried until a chalky –white enamel surface.The enamel surface of the concise area within the ring surfaces of each tooth of group 2 was treated with Beautisealant primer and allowed to set for 5 seconds. Then, the adhesive was dried into a smooth glossy film using a gentle stream of air.

The respective sealant of each group was applied into plastic ring and cured with light source according to the manufacturer's instructions. After curing, the plastic ring was cut with scalpel blade.

B. Shear bond strength assessment

Shear bond strength was measured using universal testing machine. Shearing force was applied perpendicular to the prepared sealant surface with testing chisel at the crosshead speed of 0.5mm|min (Figure 1).The load of failure was divided by bonding area to express the bond strength in MPa.The fractured surfaces of the debonded samples were inspected under binocular stereomicroscope at 40x magnification to determine the mode of failure for each specimen.

Figure 1: Shearing forces applied perpendicular to Interface

3. Diametrical tensile strength assessment

According to American Dental Association no.27 62, sealants were placed in the plastic mould (4mm diameter, 6mm height) in 2 mm increment and cured on both sides to ensure complete curing. The cylindrical specimens were finished using carborundum disks and stored in distilled water for 24 hours. Diametrical tensile strength was measured using universal testing machine by directing the force against lateral surface of specimen with cross head speed 1mm\min until fracture occurred (Figure 2).

Figure 2: Forces directed against lateral surface

3. Microhardness assessment

Sealant specimens were prepared in a round stainless steel mould of 8mm diameter and 2mm thickness.Sealant was cured with light source according to the manufacturer’s instruction.Surface Micro-hardness of the specimens was determined using Digital Display Vickers Micro-hardness Tester (Figure 3). A load of 200g was applied to the surface of the specimens for 10 seconds.

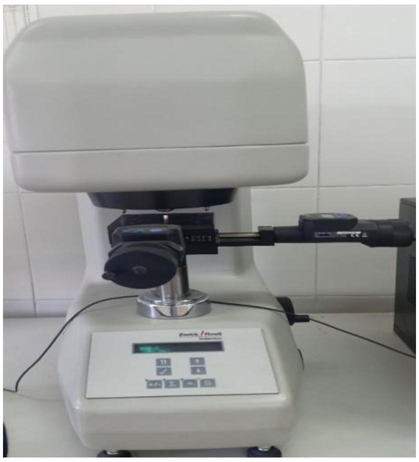

Figure 3: Vickermicrohardness tester.

Statistical analysis

For the purpose of statistical analysis,the data obtained was fed into computer and analyzed using statistical package of social science (SPSS) version 22.0 Analysis of variance (ANOVA) and post hoc test were performed.

III. Results

1- Agar diffusion test

The comparison of inhibition zones in the different study groups is shown in table 2 and figure 5. ANOVA and Tukeypost hoc tests reveal a statistically significant difference between all groups (Table 2, Figure 4).

Table 1: Inhibition zone of Streptococcus mutans among different groups.

Inhibition Zone mm)	Mean ± SD	F	P- value	Tukey'spost hoc test	
				Group comparison	P- value
Group I	4.938 ± 0.129	392.43	<0.0001*	Gp I vsGp II	< 0.001*
Group II	4.012 ± 0.109			Gp I vsGp III	< 0.001*
Group III	2.022 ± 0.237			Gp II vsGp III	< 0.001*

* mean statistical significant among group

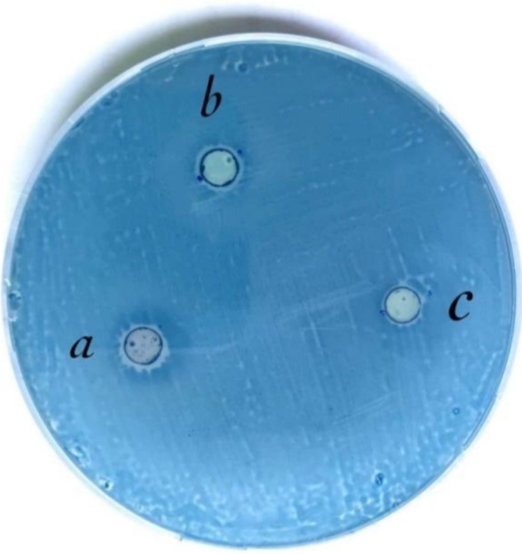

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Figure 4: Inhibition zone around sealant disks. a: group I sealant, b: group II sealant and c: group III sealant

2 - Shear bond strength

The mean shear bond strength of group I, II, and III are 13.928 MPa, 17.947 MPa and 11.749 MPa respectively. ANOVA and Tukey,s post hoc testsreveal a statistically significant difference between group I & group II and between group II & group III. On the other hand, the difference between group I & group III did not reach the level of statistical significance (Figure 5). Stereomicroscope imaging showed that the mode of failure in group I and II was a mixed failure, whereas, a cohesive failure was reported in group III specimens (Figure 8).

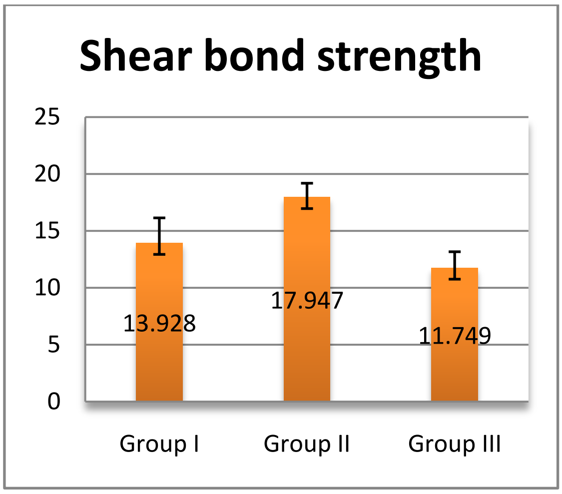

Figure 5: Comparison of shear bond strength.

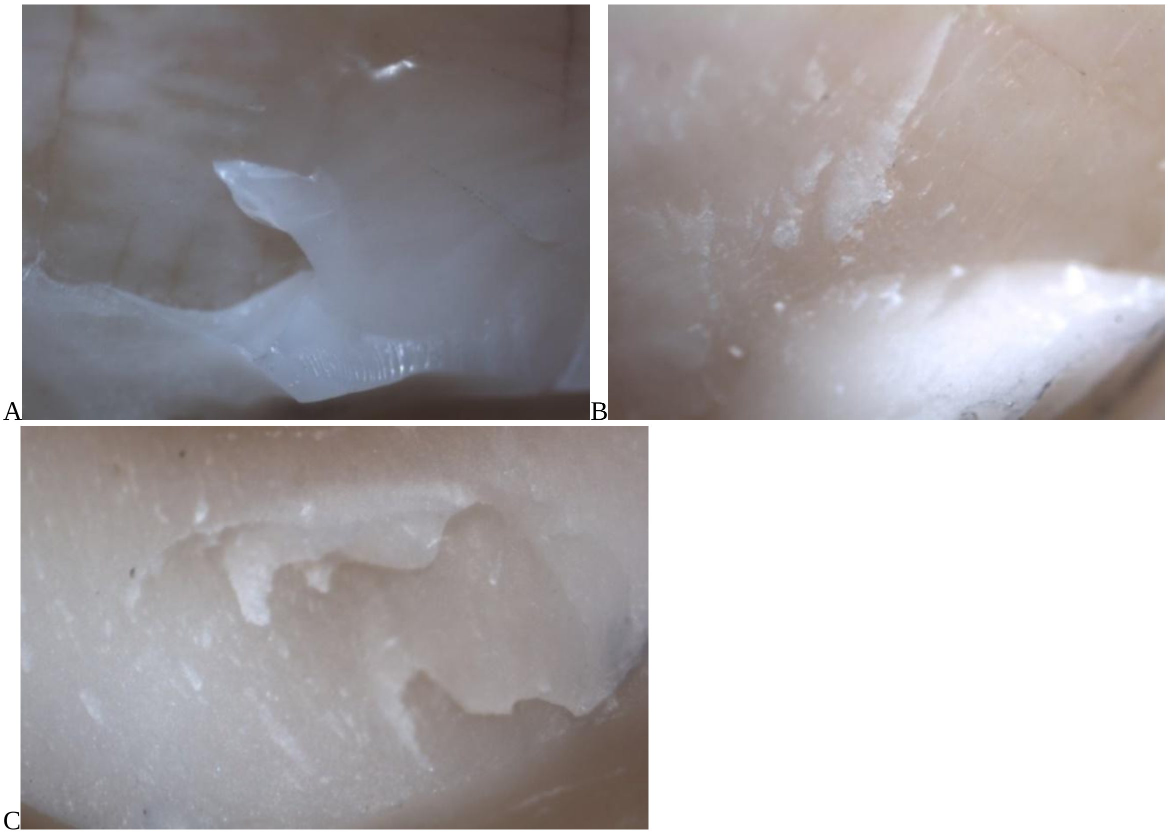

Figure 6: A, Mixed mode of failure of group I. B, Mixed mode of failure of group II. C, Cohesive mode of failure of group III under stereomicroscope.

3- Diametrical tensile Strength

Results indicate that the mean diametrical tensile strength of group I, II and III are 41.78 MPa, 26.91MPa and 42.05 MPa Respectively.Tukey Post hoc analysis reveals a significant difference only between group I & group II and between group II & group III (Figure 7).

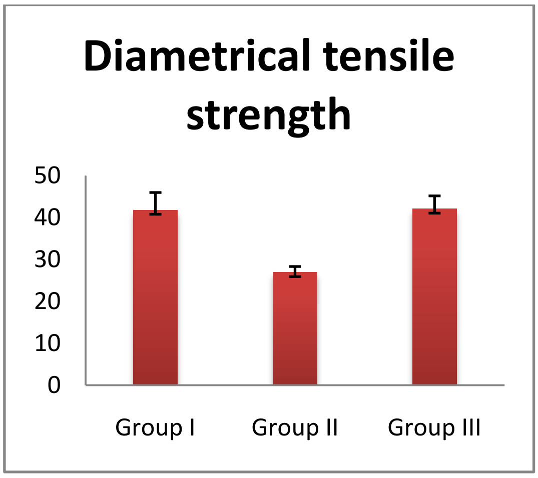

Figure 7: Diametrical tensile strength of different groups.

4-Microhardness

Results reveal that the mean microhardness of group I, II and III was 21.73 Kg\mm2, 32.99 Kg\mm2 and 113.06Kg\mm2 respectively. ANOVA and Tukey's post hoc tests reveal a statistically significant difference between all group (Figure 10).

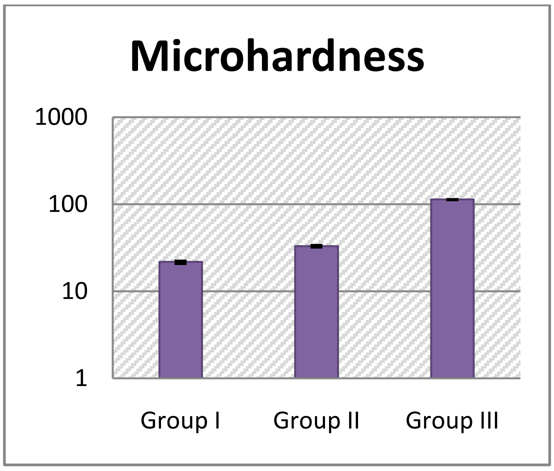

Figure 10: Microhardness of different groups.

V. Discussion

The concept of using pit and fissure sealants has been widely recognized as an effective preventive measure. Sealantscan prevent the development of caries, or its progression, by preserving the pit and fissure system from the effects of the cariogenic dental plaque[14].As microleakage of sealant cannot be avoided, various bacteriological studies reported that some cariogenic bacteria could persist on the occlusal surface of sealed teeth. Therefore, the antibacterial properties of the sealant materials may enhance their caries preventive effect[15]. Thus, this study was carried out to explore the antibacterial properties of three different fissure sealants.

In the current study, antibacterial evaluation was done using agar diffusion test (ADT) as it provides rapid results of the antimicrobial agent's effects and a better understanding of their impact on the viability and cell damage of the tested microorganism. Additionally, it is the most suitable method for evaluating antibacterial effect of sealants which result from ions diffusion into the agar. ADT was performed using Streptococcus mutans as the primary etiological agent of dental caries and a frequent caries lesion isolate. Mitissalivarius agar was also utilized as a selective enriched medium of this cariogenic bacterium. Since S.mutans is naturally resistant to bacitracin, mitissalivarius agar was modified by adding 0.2% bacitracin to prevent microbial contamination during the experiment.

In the current study, the agar diffusion test showed that all tested sealant materials have antibacterial effects against streptococcus mutans. Moreover, Clinpro has a significantly higher antibacterial effect compared to Beautisealant and Aegis. This result comes in line with ***Naorungroj et al., 2010*** who reported that Clinpro had contact antibacterial effect in the agar diffusion assay and its activity against streptococcus mutans retained over time[16].

The antibacterial effect of Clinpro sealant is mainly attributed to the fluoride release. Bacterial inhibition by fluoride is a result of diffusion of hydrogen fluoride into bacterial culture resulting in deterioration of bacterial metabolism. Fluoride release, also, inhibits the glycolytic enzyme enolase and proton-extruding ATPase, as well as the bacterial colonization.

Moreover, the results of the present study agree with ***Ferreira et al., 2013*** who reported that the biofilm formed on Aegis had a significantly lower Streptococcus mutans counts than enamel biofilm[17]. The antibacterial activity of Aegis may be explained by its ability to release calcium and phosphate that neutralizes acid solution and elevate pH that make environment unfavorable for bacterial growth.

Also, the present results parallel that of ***An et al., 2015*** who demonstrated that the sealant containing S-PRG filler had a less powerful anti-bacterial property and increased biofilm formation capacity compared to the fluoride releasing Clinpro sealant[18].The antibacterial activity of Beautisealantis primarily due to the release of inorganic elements, such as strontium, boron, aluminum and fluoride from glass filler.

Additionally, the result of the current study coincides with the result ***of Shinonaga et al., 2018*** who reported that Beautisealant was able to reduce number of Streptococcus mutans using bioluminescence method[19]. On the other hand, beautisealant had a less potent anti-bacterial property compared with apatite ionomer containing sealant.

The premise behind evaluating shear bond strength is that the retention of fissure sealants is a micromechanical process established by the infiltration and subsequent polymerization of the sealant resin tags into the etched enamel. Therefore, shear bond strength provides a clue about the retention rate and accordingly the clinical success of sealant.

The mean shear bond strength of Clinpro, Beautisealant and Aegis were found to be 13.9MPa, 17.94MPa and 11.75MPa respectively.In the present study, shear bond strength of Clinpro was not significantly different from that of Aegis. This result comes in line with the result of ***Utneja et al., 2018*** who reported that there was no statistically significant difference of the microshear bond strength between Clinpro and Aegis[20].

Additionally, shear bond strength of Beautisealant was significantly higher than that of Clinpro and Aegis. The current finding contradicts with the result of ***An et al., 2015***[21]. The reason for the higher shear bond strength of Beautisealant compared to Clinpro and Aegis is mainly due to its advanced adhesive technology and presence of inorganic fillers in its composition. The use of Beautisealant primer containing phosphoric and carbonic acid monomer could improve sealant flowability by making enamel surface hydrophobic. Moreover, it compensates the effect of adding large amount of filler which increase sealant viscosity.

On the other hand, the low shear bond strength of Aegis noted in the present study could be attributed to the inherent material weakness caused by the addition of ACP fillers. Moreover, the bonding mechanism may be impaired due to the retention of calcium phosphate ions on the tooth surface.

In the current study, the diametrical tensile strength was evaluated because the clinical utility of a sealant material depends on its ability to endure the tensile stresses and strains induced during mastication[22].

In the present study, the mean diametrical tensile strength (DTS) of Clinpro was found to be 41.78MPa. This result is in line with that of ***Shanmugaavel et al., 2015*** who reported similar value 43.37 MPa[22].Moreover, the mean diametrical tensile strength of Beautisealant, reported in the present work, was 26.91MPa. The current result coincides with ***Kaga et al, 2011*** who reported that diametrical tensile strength of Beautisealant was 24.3MPa[9]. Up till now, there are no in vitro studies comparing diametrical tensile strength of Clinpro, Beautisealant and Aegis.

On the other hand, the microhardness of different sealants was evaluated in the current study because the sealant material hardness correlates well with its compressive strength, resistance to intraoral softening, and degree of conversion which ultimately determine the longevity of sealant. Additionally, sufficient hardness ensures that the sealants are resistant to in-service scratching from mastication and abrasion[13].

In the present work, the microhardness of Aegis was superior as compared to those of Clinpro and Beautiselant. The present finding coincides with the results of ***Zawaideh et al., 2016*** who reported that Aegis had higher microhardness value than fluoride releasing resin based sealant[13]. On the other hand, microhardness of Beautisealant was higher than Clinpro. The differences in microhardness values of different sealant materials may be correlated to variations in the filler content of the diverse materials.

One of the limitations of the present investigation is its in vitro nature where the results may not necessarily be the same as those that would be obtained in the oral environment. In oral cavity, sealant materials, contrary to in vitro circumstances, are subjected to chemical, thermal, and mechanical challenges. Therefore, more research is needed to prove the in vivo clinical reliability of these newer sealant products.

IV. Conclusion

Based on this study's results, the following conclusions can be obtained:

1. All of the tested materials are capable of inhibiting Streptococcus mutans growth . The fluoride releasing resin based sealant has a higher antibacterial effect than sealant containing S-PRG filler and amorphous calcium phosphate containing sealant.
2. PRG filler containing fissure sealant has the highest shear bond strength value compared to that of fluoride releasing resin based sealant and amorphous calcium phosphate containing sealant. On the other hand, the diametrical tensile strength of PRG filler containing fissure sealant is lower than that of fluoride releasing resin based sealant and amorphous calcium phosphate containing sealant.
3. Amorphous calcium phosphate containing fissure sealant shows a superior surface micro hardness characteristics compared to fluoride releasing resin based sealant and PRG filler containing fissure sealant.

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