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EVALUATION OF REMINERALIZING ABILITY AND MECHANICAL PROPERTIES OF TWO FLUORIDE RECHARGEABLE FISSURE SEALANTS IN PRIMARY MOLARS: AN IN VITRO STUDY

Aya Saad Hussien*, Salwa Mohamed Awad ** *and* Ashraf Yassin Alhosainy***

ABSTRACT

Aim: This study was conducted to assess the remineralizing ability, microleakage and flexural strength of resin-based and bioactive-based sealants in primary teeth.

Methods: Thirty-two exfoliated second primary molars were prepared and sealed with one of the following materials: BeautiSealant and Helioseal F plus. Sixteen samples were subjected to a pH-cycling model (7 days of demineralization–remineralization cycles). Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) were used to assess the remineralizing abilities before and after the studied sealants applied. The other (16) samples were subjected to a thermocycling model to assess the microleakage after sealants application. Flexural strength for each sealant material was also assessed following the ISO 4049 protocols using a Universal Testing Machine. Data were collected, tabulated then statistically analyzed using the Statistical Package of Social Science (SPSS) program Windows (Standard version 22).

Results: After sealants application, the mean weight percentages of calcium (32.4,32.8), phosphate (15.7,16.6) and fluoride (13.6,14.02) showed a significant increase for Helioseal F and BeautiSealant respectively compared with baseline values. The mean Calcium/ Phosphate ratio (Ca/P) for Helioseal F plus was 2.163 and 1.796 for BeautiSealant after sealant application with no statistical significance difference was found. On the other hand, Helioseal F plus showed higher significance difference than BeautiSealant in flexural strength test.

Conclusion: The bioactive giomer glass ionomer-based sealants could be used successfully as alternative to resin-based sealants as they showed comparable remineralizing ability and mechanical properties.

KEYWORDS: Bioactive sealants, BeautiSealant, Helioseal F plus, Primary teeth.

* Clinical Demonstrator of Pediatric Dentistry, Faculty of Dentistry, Mansoura University
** Professor of Pediatric Dentistry, Faculty of Dentistry, Mansoura University
*** Lecturer of Pediatric Dentistry, Faculty of Dentistry, Mansoura University

INTRODUCTION

Several studies found that the prevalence of occlusal caries in primary dentition was high and when caries confined to enamel only, sealing of the pits and fissures is advocated.[1-3] Recent literature data have shown that progression of non-cavitated occlusal caries in primary teeth can be arrested by sealing pits and fissures with a resin-based sealant as recorded in permanent teeth.[4]

Pit and fissure sealants are considered as a primary preventive measure; it is one of the most effective and least invasive means available to ensure the complete protection of the occlusal surfaces from dental caries.[5] There is a great evidence that sealants can inhibit the progression of non-cavitated carious lesions.[6]There are many options of commercially available sealant materials, with the most widely used ones being resin-based sealants (RBS) and glass ionomer sealants (GIS).[7]

Glass ionomer sealants have been recognized for their clinical characteristics, as they have been used in the prevention of dental caries. However, it has some disadvantages such as poor hydrolytic stability, low flexure, and toughness and, most importantly, the short-term release of fluoride.[8, 9] On the other hand, conventional resin-based fissure sealants are not self-adhering so that, the surface area of teeth must be modified or etched by acids, in addition to, its application is very sensitive, microleakage, time-consuming and fracture toughness.[10]

Currently, resin-based sealants are undergoing increased and alert development to improve their properties, overcome the drawbacks of the conventional materials and to enhance the performance of such preventive procedures. Researchers have experimented new bioactive resin-based materials that may play a role in preventing the process. The newly developed flowable materials are reported to have superior mechanical properties.[11]

Bioactive materials provide benefits regarding protection against caries through fluoride release, the capacity to release a protective amount of fluoride is dependent on the hydric degradation of the incorporated bioactive glass.[12, 13] These bioactive materials can intervene and stop the progression of carious lesions and allow the damaged tissues to heal when utilized in an early stage of the disease. Such materials have the ability to release, absorb and re-release calcium, phosphate and fluoride, which will act as a reservoir of ions when the demineralization process initiates and can reverse it.[14, 15]

Giomers represent new development in the hybrid material category and consist in a stable glass-ionomer phase on a glass core resulted from an acid-base reaction between fluoridated glass and poly-carboxylic acid, in the presence of water developed as ("Pre-Reacted Glass ionomer filler" or PRG).[12] Appling PRG-technology to the filler in resin based composite materials gives them bioactive properties through fluoride release and recharge, similar to traditional glass-ionomers, but maintaining at the same time physical and esthetical properties of the composites.[16-18]

A few research have studied the performance of bioactive resin-based sealants specially in primary teeth. Therefore, this study aimed to assess the remineralizing ability, microleakage and flexural strength of two fluoride rechargeable fissure sealants in primary teeth.

MATERIAL AND METHODS

The present study was designed as an experimental in-vitro model to assess and compare the remineralizing ability and mechanical properties of a resin-based sealant (Helioseal F plus, Ivoclar Vivadent AG, Schaan, Liechtenstein) and a bioactive glass sealant (BeautiSealant, SHOFU, Kyoto, Japan) in primary molars.

Ethical consideration:

Ethical approval from ethical committee of scientific research of Faculty of Dentistry, Mansoura

University, *(The code number: **M18020822**)* was obtained before starting the study.

Sample size calculation

Sample size was calculated using G*power sample size calculator program (3.1.9.6) for Mac. At power equal 95%, α equal 0.05 and effect size (2.1) based on the anticipated mean values of mineral released from the two bioactive sealants according to **Fita k *et.al*** [19] and **Ibrahim MS *et.al***[20]. Statistical t test for the difference between two independent means of two groups calculation was used, and the minimal total sample size was (12 teeth), which was increased to (32 teeth) to increase the statistical power. For measurement of the flexural strength the minimal sample size was (12 specimens) which increased to (14 specimens) to increase the power.

Sample selection:

Thirty-two sound maxillary or mandibular second primary molars were collected from the Pediatric Dentistry Department Clinic, Faculty of Dentistry, Mansoura University. The collected teeth were extracted during their shedding period, for orthodontics reasons, and/or over retention. The selected molars with deep pits and fissures preserved in (0.1 %) thymol before their usage. [21] Molars with visible or detectable caries at any surface, restoration, occlusal stain or cracks or white spot lesion were excluded: [22-24]

Study design:

The collected molars were randomly allocated into two equal groups (n= 16) according to the type of dental fissure sealant that was used.

1. Group I: Helioseal F Plus
2. Group II: BeautiSealant

Clinical procedure:

Collected teeth were cleaned with prophylaxis pumice to remove debris before mounting in a cylindrical acrylic block. Dental fissure sealants for the two groups were applied following the manufacturer's instructions, then molars were stored in artificial saliva.

1. Measurements of remineralizing ability:

After sealant application, a pH-cycling model were used to mimic the loss and gain of minerals in the oral environment. Sixteen molars were alternated between the demineralization and remineralization cycles for 7 days.[20] Samples were air-dried then immersed in 30 mL (per sample) demineralization solution at pH 4.7 for 6 hrs. at room temperature. Samples were taken out from demineralization solution then rinsed with distilled water, dried and immersed in 15 mL (per sample) artificial saliva at pH 7 for 18 hrs. at room temperature.[20, 25]

The weight percentages of calcium (Ca), phosphate (P) and fluoride (Fl) analysis by (SEM-EDX) was performed twice: once at the base line (before pH cycle) and then 7 days later (after the pH cycle).

2. Measurement of microleakage:

The other (16) molars with fissure sealants were kept in artificial saliva for 24 hours before thermo-cycling. The samples placed into thin lace-like fabrics with different colors then subjected to 500 thermal cycles between 5°C and 55°C. Duration of exposure at each temperature was 20 seconds.[26]

After thermocycling, all surfaces of (16) molars were coated with two layers of nail varnish except for 1.5mm from the sealant margins. Then immersed in a 1% solution of methylene blue for 24 hrs, rinsed with distilled water, dried, and sectioned longitudinally in a bucco-lingual direction using a water-cooled diamond disk. The extent of dye penetration at sealant/enamel interface was assessed using the light stereomicroscope (OLYMPUS SZ II. Olympus optical Co. Tokyo, Japan) under x60 magnification.

A ranked scale described by **Övrebö RC and Raadal M** [27] was used to score dye penetration. Both sections were scored and the section with the greatest dye infiltration was considered as the score of dye penetration for that tooth. (**Table1**)

TABLE (1) The rank scale for dye penetration score:

Score	Result
0	No dye penetration
1	Dye penetration limited to the outer half of the sealant
2	Leakage up to the inner half of the sealant
3	Dye penetration extending to the underlying fissure

3. Measurement of flexural strength:

Seven specimens for each sealant type were fabricated in stainless steel mold size (2 X 2 X 25 mm). A glass slap and polyester strip were placed below the mold then the sealant was injected into that mold, another polyester strip was placed on the top and pressed using a glass slap. The sealant material was light cured from both sides for 20 sec. The specimens were stored in the incubator at 37 C° for 24 hrs.[20] A three-point bending test was conducted to measure the flexural strength of the specimens using a Universal Testing Machine. Each specimen was fixed on a metal fixture with 20 mm span. Compressive load was applied to the center of the specimen with a cross head speed of 1 mm/min till fracture. The flexural strength was calculated in Megapascals (MPs) using computer software (Bluehill, Instron, England).[20, 28]

Statistical analysis:

Data were collected, tabulated then statistically analyzed using the Statistical Package of Social Science (SPSS) program Windows (Standard version 22). Data was tested for its normality by Shapiro–Wilk test. Paired t-test was used to compare between the two means of the same group at different evaluation time. Unpaired t-test was performed to compare between the means of two different groups at the same evaluation time. Mann-Whitney test was conducted to compare between the means of two groups with unnormal distributed data. P level less than or equal 0.05 was considered significant.

RESULTS

Regarding the measurement of remineralizing ability of the fissure sealants used in this study, EDX reports of all samples before and after sealants application and the levels of calcium, phosphate and fluoride measured as weight % were evaluated. High statistically significant (p = 0.000) increase in the mineral content of the three minerals after sealants application. No statistically significant difference was found among the two sealants groups at baseline or after sealants application regarding the weight % of the three measured minerals. **Figure (1)**

The calcium/ phosphate ratio mean values before sealants application were (2.027) and (1.972) for Helioseal F plus and BeautiSealant groups respectively. No statistically significant difference was found between the mean values at baseline (P = 0.186). Similarly, after sealants application no

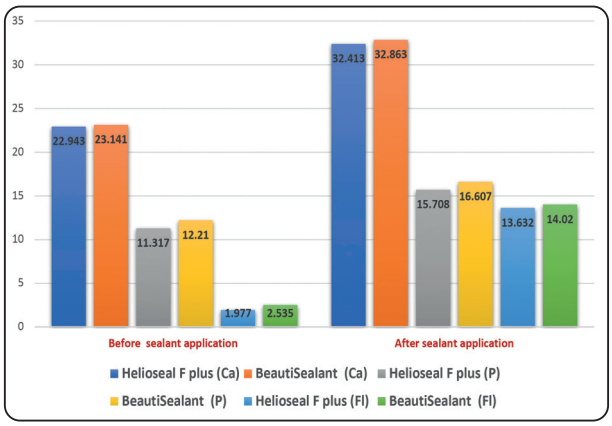

Fig. (1) Comparing the mean calcium, phosphate and fluoride weight % before and after different fissure sealants application in the two groups.

statistically significant difference (P = 0.114) was found between the mean values of Ca/p ratio (2.163) and (1.796) for Helioseal F plus and BeautiSealant groups respectively. For each sealant group, comparing the mean value of Ca/p ratio at baseline and after sealant application showed no statistically significant difference (p = 0.393) for Helioseal F plus group and (P = 0.240) for BeautiSealant group. **Table (2)**

TABLE (2) The mean and standard deviation of the calcium / phosphate ratio (Ca/p ratio) before and after different fissure sealants application in the two groups.

Ca/p ratio	Baseline	After application	Paired t test (p value)
	Mean ± SD	Mean ± SD	
Helioseal F plus	2.027 ± 0.243	2.163±0.288	0.910 (0.393)
BeautiSealant	1.972 ± 0.140	1.796±0.402	1.285 (0.240)
Unpaired t test (p value)	1.391 (0.186)	1.687 (0.114)	

Concerning the microleakage measurement, molars sealed with Helioseal F plus, score (0) constituted the highest percentage (62.5%) as five teeth scored (0) followed by score (1) as three teeth (37.5%) scored (1) with no appearance of score (2) or (3) in this group. On the other hand, only one tooth (12.5%) sealed with BeautiSealant showed score (3) and three teeth took score (0). The majority of teeth in this group scored (1) with no appearance of score (2) in this group. No statistically significant difference was found between the two sealants regarding the microleakage score percentages (X^2 = 1.643, P = 0.4400). **Table (3)**

The mean values of the microleakage results for the two groups were presented in **Table (4)**, the mean microleakage score for Helioseal F plus group was (0.375 ± 0.517) and (0.875 ± 0.991) for BeautiSealant with no statistically significant difference between them (U = 22.500, P = 0.263).

TABLE (3) The number and percentages of microleakage scores of Helioseal F plus and BeautiSealant

Sealant type	Microleakage scores			
	Score 0 N (%)	Score 1 N (%)	Score 2 N (%)	Score 3 N (%)
Helioseal F plus	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)
BeautiSealant	3 (37.5%)	4 (50%)	0 (0%)	1 (12.5%)
Total	8 (50%)	7 (43.75%)	0 (0%)	1 (6.25%)
X^2 (p value)	1.643 (0.440)			

TABLE (4) The mean values of the microleakage scores of Helioseal F plus and BeautiSealant

	Mean ± SD	Min – Max	Mean Rank	Median
Helioseal F plus	0.375±0.517	0.00 – 1.00	7.31	0
BeautiSealant	0.875±0.991	0.00 – 3.00	9.69	1
Mann Whitney U (p value)		22.500 (0.263)		

Table (5), showing the mean and standard deviation values of flexural strength for the tested sealants materials. Helioseal F plus sealant showed higher mean value of flexural strength (82.562 ± 3.513) than that for BeautiSealant (77.374 ± 3.977) which was significant (P = 0.02).

TABLE (5) The mean and standard deviation of flexural strength for Helioseal F plus and BeautiSealant fissure sealants materials.

Sealant type	Flexural strength	
	Mean ± SD	Min - Max
Helioseal F plus	82.562 ± 3.513	77.45 – 88.73
BeautiSealant	77.374 ± 3.977	71.03 – 83.76
Unpaired t test (p value)	2.587 (0.02)*	

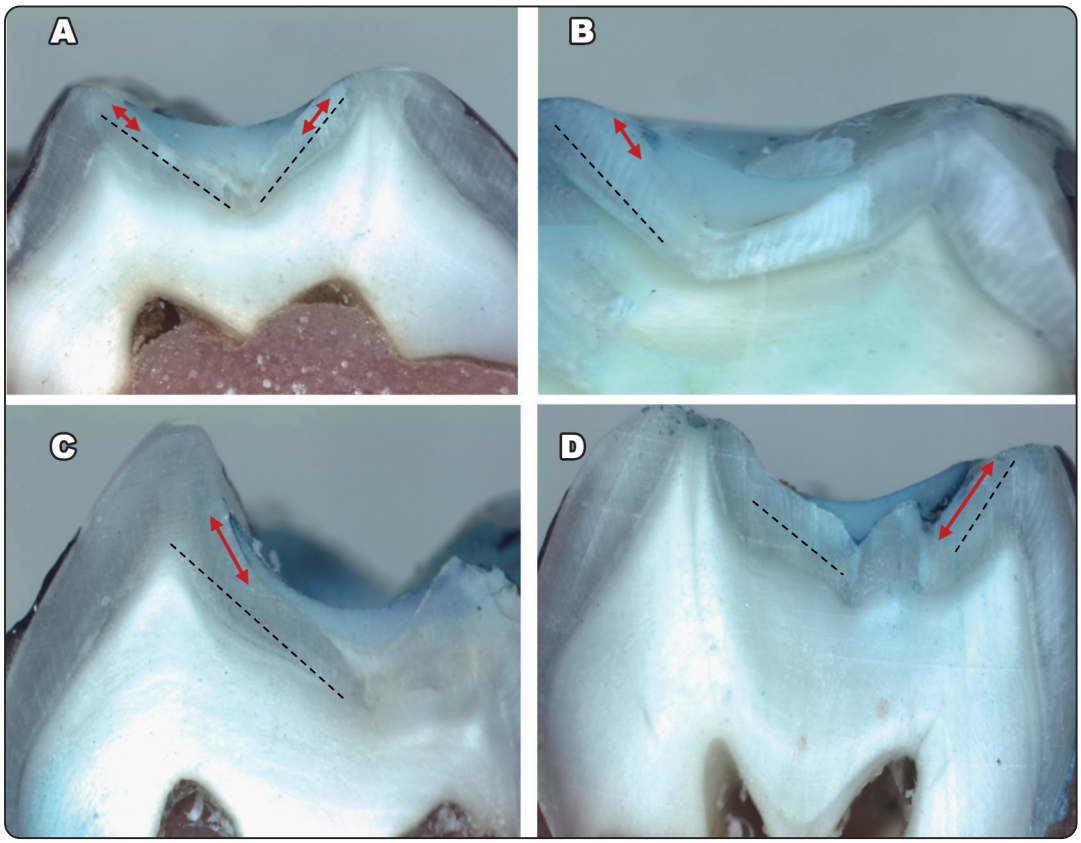

Fig. (2) Microleakage scores of Helioseal F plus: Fig (A and B) showing score (1) penetration of dye to the outer half. Microleakage scores of BeautiSealant: Fig (C); showing score (1) penetration of dye to the outer half. Fig (D) showing score (3) penetration of dye to the underlying fissure.

DISCUSSION

Pit and fissure sealant has become an indispensable necessity for children and adolescents.[29] The use of sealants undoubtedly contributes to reducing the incidence of dental caries, there is a new revolutionary set of sealants that are releasing fluoride cross the whole world.[30,31] Unique bioactive rechargeable sealants include unique capabilities, including the ability to release and recharge fluoride in the mouth.[32] The present study was conducted to compare between a novel bioactive giomer material (Beautisealant) and unique Bis-GMA-free sealant material (Helioseal F Plus) in primary teeth.

The present study measured the release of ions from two bioactive fissure sealants as an invitro model due to its stability, low cost, and capacity to decrease the influence of confounding circumstances.

In the present study, second primary molars were selected because their surfaces are broader and wider than first molars. Primary second molars were chosen either maxillary or mandibular teeth, equally and randomly distributed on the two tested sealants materials. To provide more accurate results without enamel weakening or alteration due to the presence of caries, molars were chosen with sound occlusal surfaces.[2,33,34]

The pH cycling model was used in present study to simulate the periodic changes in pH that occur in the oral environment and are similar to the caries process.[35,36] The pH cycle model for primary teeth should be shorter than for permanent teeth

because primary teeth have more imperfections in the hydroxyapatite crystals than permanent enamel.[37-39] Most of studies on permanent teeth use 10 days pH cycling; however, this model, if used on primary sections, produces lesions that are too extensive for evaluation. In the present study, 7 days pH cycling model was used to get the maximum benefit without causing harm to primary teeth.[40, 41]

The present study used microleakage test to assess marginal and internal adaptability of fissure sealant. The microleakage test was done by dye penetration method with methylene blue dye.[42] The methylene blue dye has several features as it's cost-effective, easy to handle, has a high degree of staining, and has a smaller molecular weight than bacterial toxins.[43, 44]

The ISO 40494 three-point bending test is used to determine the flexural properties of dental materials. This ISO test standard requires a beam specimen of 25 x 2 x 2 mm for the three- point bending test.[45]

In the current study, changes in mineral content of enamel of pits and fissures after sealants application were assessed, and surface alterations on enamel were examined using EDX, which are regarded as the most accurate techniques for evaluating the remineralizing ability.[46] EDX is a chemical elemental microanalysis technique which was widely used in measuring mineral content at the ultrastructural level in several studies.[47] It was used in this study to assess the remineralization ability of both materials on the demineralized enamel surface and to compare their effects on the enamel surface.

EDX recorded changes in Ca, P, F, and the Ca/P ratio as indicators of the mineral recharging ability of the materials under consideration, and baseline data were taken to improve the precision of the results. Investigations proved that bioactive sealant could regain the mineral content into the artificially decalcified enamel surface. The bioactivity of sealant material provides the possibility of high Ca and P concentrations and releasing of fluoride within the demineralized enamel to a higher level than that existing in normal oral fluids allowing for a great potential to enhance remineralization. This is according to the study conducted by **Ibrahim MS *et.al* (2021)**[20] **and Salma RS *et.al* (2022).**[48]

Regarding the results of the present study, EDX findings recorded higher calcium and phosphorus deposition in all the enamel specimens from the baseline and after the two sealants application. By comparing Ca and P weight percentages between after Helioseal F and BeautiSealant, they were nearly the same for the two sealants (32.413 and 32.863 for Ca) and (15.708 and 16.607 for P) respectively, with no statistically significant difference between the two groups. This result comes in accordance with other invitro study performed by **Ibrahim MS *et.al* (2021)**,[20] and **Klaophimai A *et.al* (2021)**,[49] who assessed ions-releasing ability of different bioactive sealants on specimens of the materials. BeautiSealant represented higher ions release than resin-based sealant. The high level of ions released recorded by the present study may be explained by bioactivity of the material that might have enabled the increase in Ca and P concentrations and the release of fluoride within enamel to a higher level than that existing in normal oral fluids allowing for a great potential to enhance remineralization.[50]

The results of the current study regarding microleakage, revealed no statistically significant difference between the two sealants used. The mean microleakage score for BeautiSealant was (0.875) and (0.375) for Helioseal F plus. This finding came in agreement with several studies that showed that there's no difference between the use of resin-based and giomer-based sealants in the sealing ability.[41-43] The differences in the microleakage score between the previous studies can explained using different criteria in each study and the different type of teeth selected in them.

Regarding flexural strength test in the present study, the results revealed that Helioseal F plus

has high value of flexural strength compared to BeautiSealant. The mean value of flexural strength recorded for Helioseal F plus group was (82.56). These results came in conformity with the results of **Osorio E *et.al* (2006)**,[51] who evaluated the in vitro performance of simplified formulation of photocuring resin used as dental sealants and two commercially available sealants, one of them (Helioseal).The mean value of flexural strength by three-point bending test in Helioseal was (80.86). The Helioseal sealants differ from one another in terms of their delivery form and their viscosity, color, and fluoride content. However, they all show the same high clinical performance. Helioseal differs from Helioseal F and Helioseal F plus as it is delivered in a bottle, doesn't contain fillers or fluoride, but the mechanical properties are very similar.

Thunyakitpisal P *et.al* (2016),[52] investigated the light-activated pit and fissure resin-based sealant for flexural strength by comparing between resin-based sealants. The mean value of flexural strength in Helioseal by three-point bending test was (81.45) and in Helioseal Clear was (79.78) which is nearly close to the results of the present study.

The mean value of flexural strength in BeautiSealant reported in the present study was (77.37). These results came in accordance with the results of **Ibrahim MS *et.al* (2021)**,[20] who compared flexural strength between different bioactive pit and fissure sealants, the BeautiSealant mean value of flexural strength by three-point bending test was (78.4). On the other hand, **Panpisut P *et.al* (2022)**,[53] when assessed the mechanical properties of ion-releasing dental bioactive sealants, the mean flexural strength value of BeautiSealant was (92) measured by biaxial bending test. The difference of mean flexural strength values between the studies may be related to the different tests used to measure it. Despite the biaxial flexural strength test is considered as an analogue of three-point bending test, using a different type of test can affect the measured values. The ISO three-point bending test is more accurate and reliable than the biaxial bending test.[54]

Lastly, in this study by comparing the two sealants' materials, bioactive glass sealant showed a comparable recharging ability and marginal seal with resin-based sealant.

CONCLUSION

The bioactive giomer glass ionomer-based sealants could be used successfully as alternative to resin-based sealants as they showed comparable remineralizing ability and mechanical properties.

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