ORIGINAL RESEARCH

Effect of Aging on the Microhardness of Different Resin-based Fluoride-releasing Fissure Sealants: An *In Vitro* Study

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Abstract

Aim: The aim of the present study was to evaluate the effect of aging on the microhardness score [Vickers hardness number (VHN)] of different resin-based fluoride-releasing sealants compared to non-fluoride resin-based sealants.

Materials and methods: A total of 48 extracted sound molars that were sectioned mesiodistally were used. In the current study, four types of sealants were compared: Group A, a resin-based non-fluoride-releasing pit and fissure sealant (Eco-S sealant) served as a control. Groups B, C, and D received resin-based fluoride-releasing pit and fissure sealants Helioseal F, Fissurit F, and Embrace™ WetBond, respectively. Subsequently, each group was further divided into immediate and aged subgroups. The samples had been evaluated regarding their microhardness using a pyramidal diamond indenter of a Vickers hardness test at two-time intervals: Immediately and after the aging process through thermocycling.

Results: There were no statistically significant differences between mean VHN and material types ($p = 0.72$). Aging appeared to significantly increase the mean VHN ($p = 0.001$). The interaction model between material type and time factor showed that the effect of aging differs by the material type, where the VHN of the Embrace™ group increased significantly after aging from 24.33 ± 5.60 to 31.70 ± 3.59 ($p = 0.001$).

Conclusion: While there were no significant differences in the microhardness of commonly used fluoride-releasing fissure sealants, time appears to significantly increase the mean microhardness score (VHN), especially in the Embrace™ group.

Clinical significance: Embrace™ WetBond fissure sealant showed a significant improvement in the mean microhardness score (VHN) with time. However, clinical studies with long-term follow-up are needed to confirm our results.

Keywords: Aging, Fissure sealant, Microhardness, Resin-based fluoride-releasing sealants.

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Introduction

Dental caries remain a major global public health concerns[1] with harmful consequences if left untreated, especially in the pediatric population.[2,3] Most dental caries in children older than 6 years of age are confined to plaque retentive occlusal surfaces of first, followed by second, permanent molars.[4,5] The introduction of fissure sealants more than half a century ago to prevent caries in pits and fissures of occlusal surfaces is now considered one of the most effective measures for managing initial caries lesions, especially in high-risk children.[6]

A large body of clinical evidence attests to the effectiveness of fissure sealants in preventing caries,[7,8] especially during the eruption of the first permanent molars[9] and primary molars.[10] Furthermore, fissure sealants have been shown to arrest non-cavitated (incipient enamel) lesions on occlusal surfaces of molars.[6,11]

The most common sealants include glass ionomers and composite-based sealants.[12] Recent technological advances in the development of filled and unfilled resin materials with the addition of bioactive fluoride have further expanded the range of application selection of fissure sealant materials. In addition, resin-based sealants offer higher mechanical properties, including retention, fracture, and wear resistance, compared to other types of fissure sealants,[13] and are, therefore, considered as the gold standard preventive measure by the American Dental Association.[14] The clinical success and durability of fissure sealants depend on several factors, including chemical composition, physical and mechanical properties, and the oral environment. In recent years, fillers have improved the mechanical properties, especially wear resistance and hardness of fissure sealants, to withstand the occlusal forces, thermal, and pH changes in the oral environment.[15] The content and the size of the fillers can affect the fracture resistance and are also shown to influence the viscosity of the sealants,[16] which are critical for the infiltration of the material into occlusal pits and fissures.[17] The wear resistance of filled and unfilled resin-based sealants is also affected by the degree of polymerization, which contributes to the ultimate long-term clinical performance of these matrix-based fissure sealants.[15]

There are many resin-based fissure sealants developed and introduced into the consumer market to overcome technique sensitivity due to moisture contamination of the majority of sealants and improve wear resistance while increasing anticaries behavior by the addition of fluoride. At the same time, there has been an increasing number of experimental studies to assess the mechanical and anticaries properties of resin-based fissure sealants.[11,18–20]

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However, differences in polymerization at different time intervals between bioactive fluoride resin-based sealants and traditional non-fluoride-releasing fissure sealants have not yet been evaluated. There is also limited evidence for the effect of aging of various resin-based pit and fissure sealants.[21]

Therefore, this study aimed to compare the microhardness immediately and after the aging of three commercially available fluoride-releasing fissure sealants *in vitro*. These sealants have different chemical compositions (Table 1), namely Helioseal F™ (HF: Ivoclar Vivadent, Liechtenstein), Fissurit F™ (FF: VOCO, Germany), and the relatively newer sealant Embrace™ WetBond™ (EWB: Pulpdent, USA). A non-fluoride-containing sealant (Eco-S: Vericom Co, Korea) was used as a control. The null hypothesis was there is no detectable difference in the microhardness of the fissure sealant materials before and after aging.

MATERIALS AND METHODS

Sample Collection and Ethical Approval

Forty-eight sound molar teeth were used in this study. The teeth were caries-free and were obtained from healthy adult patients aged 18–45 years with the patients' informed consent under a protocol reviewed and approved by the Institutional Review Board (IRB) at King Saud University (Riyadh, Saudi Arabia) and the College of Dentistry Research Center (CDRC) of King Saud University (Riyadh, Saudi Arabia). These patients were referred to the Oral and Maxillofacial Clinic of the Dental University Hospital at King Saud University to extract one or more teeth as part of their regular dental management. All teeth were stored in distilled water at 37°C and used within a week of extraction. Any teeth with caries lesions or enamel defects were excluded from the study.

Study Design

The four tested sealant materials were assigned to four different groups: Group A was allocated for Eco-S sealant (Vericom Co, Korea), a resin-based non-fluoride-releasing fissure sealant and designated as the control. Groups B, C, and D were designated as the resin-based fluoride-releasing fissure sealants, Helioseal F (Ivoclar Vivadent AG, Liechtenstein), Fissurit F (VOCO GmbH, Germany), and Embrace™ WetBond (PULPDENT Corporation, USA), respectively. Details of the composition of each sealant material are described in Table 1. Each of the four groups was further divided into immediate and aging subgroups (Fig. 1).

Specimen Preparation

For each of the four groups, 12 extracted sound molar teeth were assigned. Each tooth was sectioned mesiodistally into two halves using a slow-speed water-cooled diamond blade (MetLab Technologies, Limited, London, UK). One half was assigned to the immediate subgroup, and the other half was assigned to the aged subgroup. A total of 12 sections were used in each subgroup for each sealant material (Fig. 1). A class V rectangular slot of 3 × 2 × 1 mm dimension in each tooth section was prepared on the buccal or lingual surface. Slots were acid-etched with 35% phosphoric acid for 15 seconds and then rinsed with water. The samples were gently air-dried to remove excess water without over drying the exposed enamel within the slots. Each sealant material was then placed according to its manufacturer's instructions. The curing of the sealant materials was

Table 1: Summary of dental fissure sealants available commercially tested in this study

Brand name	*Manufacturer*	*Composition*
Helioseal F	Ivoclar Vivadent, Schaan, Lichtenstein	Matrix: Bis-GMA, UDMA, TEGDMA Filler: 20% wt. fluorosilicate glass, 21.5% wt. silicon dioxide Fluoride: Yes
Fissurit F	Voco, Cuxhaven, Germany	Matrix: Bis-GMA, hexandioldimethacrylate, 7,7,9-trimethyl-4,13-dioxo-3,14dioxa-5,12-diazahexandecan-1,16-diyldimethacrylate Filler: 9.5% wt. silicon dioxide Fluoride: Yes
Embrace WetBond	Pulpdent Corporation, Watertown, Massachusetts, USA	Matrix: Urethane dimethacrylate (UDMA) Filler: 43% wt. mixture of hydrophilic and hydrophobic materials Fluoride: Yes
Eco-S	Vericom Co., Ltd., Korea	Matrix: Bisphenol A glycidyl dimethacrylate (Bis-GMA), triethyleneglycol dimethacrylate Filler: Fumed silica Fluoride: No

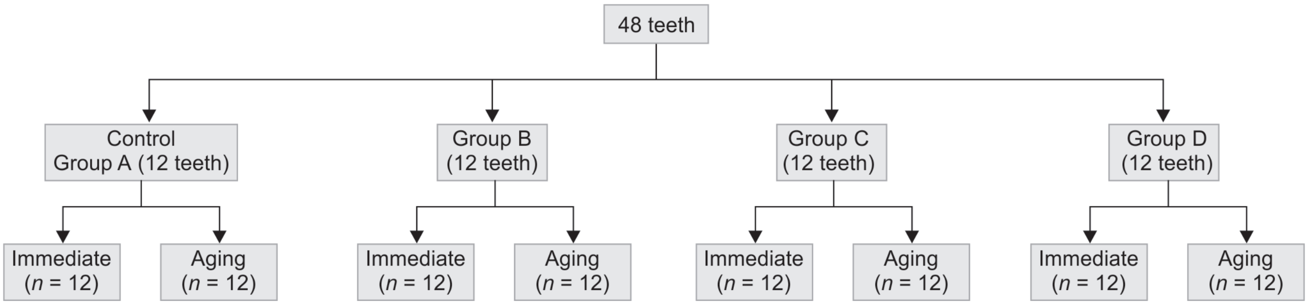

Fig. 1: Distribution of samples between the groups

activated using the Elipar™ S10 LED curing light (3M ESPE, St. Paul, Minnesota, USA) (1200 mW/cm^2 intensity) with a wavelength of 430 and 480 nm for 40 seconds. All sections were stored for 24 hours in distilled water at 37°C. The first half was tested immediately from each tooth, and the other half were tested after aging.

Thermocycling

The samples assigned for the aging subgroup were subjected to the aging process using a thermocycling machine (SD Mechatronik Thermocycler, USA). The thermocycling was run for 1,500 cycles in water baths set at 5 and 55°C for 15 seconds at each temperature and 10 seconds dwell time between the two baths.

Microhardness

Vickers hardness test was used to measure the microhardness of the different sealant materials immediately and after aging. A microhardness tester (Micromet 2103, Buehler, Lake Bluff, Illinois, USA) with pyramidal diamond indenter at 50 g load was used to detect the Vickers hardness of the sealant surface. Each sample was measured at three different surface points located at the middle and the end of the sealant material with at least a 500 µm distance between each point (Fig. 2). The Vickers hardness number (VHN) (kg/mm^2) was recorded for each point.

Statistical Analysis

The effect of aging on the microhardness of different sealant materials was analyzed using a two-way analysis of variance (ANOVA). Additionally, a one-way ANOVA was used to detect the difference between each sealant material at each time point. An independent *t*-test was used to determine the effect of aging for each sealant material. The level of significance was set at less than 0.05 (*p*-value). All statistical analyses were performed using SPSS statistical software for windows (version 23; SPSS Inc., Chicago, Illinois, USA).

Fig. 2: The pyramidal diamond indentation on Eco-S sealant material

RESULTS

The one-way ANOVA results for the immediate and aging groups for each material are presented in Table 2. The immediate VHN was the highest in the Helioseal F group (27.62 ± 5.83) followed by Eco-S (27.33 ± 2.31), Fissurit F (25.91 ± 3.55), and Embrace WetBond (24.33 ± 5.60). On the contrary, the VHN after aging was the highest in the Embrace WetBond group (31.70 ± 3.59), followed by Helioseal F (29.75 ± 3.30), Fissurit F (28.43 ± 5.21), and Eco-S (28.21 ± 5.62). However, there was no statistically significant difference in the VHN between sealant materials at both times.

Two-way ANOVA revealed no statistically significant difference in the mean VHN among the material groups ($p = 0.72$). On the contrary, there was a significant association between mean VHN and time factor ($p = 0.001$). Aging appeared to significantly increase the mean VHN. However, the interaction model showed that the effect of aging differs by the material type, where it was highly noticeable in the Embrace WetBond group (Fig. 3).

An independent *t*-test was performed for each material between the immediate and aging groups (Table 3). There was an increase in the mean VHN with aging across all the groups. However, the only statistically significant difference was found in the Embrace WetBond group, where the immediate VHN was 24.33 ± 5.60 and the aged VHN was 31.70 ± 3.59 at $p = 0.001$.

DISCUSSION

There is limited evidence on the effect of aging on the extent of curing represented by Vickers microhardness among different types of the fluoride-releasing pit and fissure sealants.[21] Therefore, the purpose of the present study was to evaluate microhardness at different time intervals for comparative analysis of various fluoride-releasing resin-based sealants and a non-fluoride resin-based sealant.

Microhardness is considered an indirect measure of the degree of conversion (DC). DC is the magnitude of a polymer formed by the conversion of the monomer after irradiation.[22,23] The most upper and lower layers of a sealant placed on the occlusal tooth surface are critical.[22] Any low DC in those layers may result in deficiency in

Table 2: Association between VHN and material type

Time	*Material*	*N*	*Mean (VHN) ± Std. deviation*	*ANOVA p value*
Immediate	Eco-S	12	27.33 ± 2.31	0.28
	Helioseal F	12	27.62 ± 5.83	
	Fissurit F	12	25.91 ± 3.55	
	Embrace WetBond	12	24.33 ± 5.60	
Aging	Eco-S	12	28.21 ± 5.62	0.22
	Helioseal F	12	29.75 ± 3.30	
	Fissurit F	12	28.43 ± 5.21	
	Embrace WetBond	12	31.70 ± 3.59	

Table 3: Association between VHN and time factor

Material	*Time*	*N*	*Mean (VHN) ± Std. deviation*	*p value*
Eco-S	Immediate	12	27.33 ± 2.31	0.62
	Aging	12	28.21 ± 5.62	
Helioseal F	Immediate	12	27.62 ± 5.83	0.28
	Aging	12	29.75 ± 3.30	
Fissurit F	Immediate	12	25.91 ± 3.55	0.19
	Aging	12	28.43 ± 5.21	
Embrace WetBond	Immediate	12	24.33 ± 5.60	0.001
	Aging	12	31.70 ± 3.59	

Fig. 3: The effect of aging on VHN stratified by the sealant material

marginal integrity, which will increase solubility, in turn permitting the proliferation of cariogenic bacteria with ultimate clinical failure of the sealant.[22,24]

Although a difference in microhardness between fluoride-releasing sealants and the control group was anticipated, the results attained reflected otherwise. This could be due to the use of Eco-S as a control group, compared to previous studies that used Delton clear and Helioseal clear as a non-fluoride resin-based releasing sealant.[25] Both of these materials have shown a lower mean of VHN compared to the VHN score of the Eco-S group in our study.

Although the Fissurit F sealant did not reach the highest VHN compared to other sealants, our score is higher than the Mazaheri et al. study, where they found a mean microhardness of 15.96 ± 4.27 for Fissurit F fluoride-releasing sealant.[26] The reason for this lower value compared to our study findings could be due to their smaller sample size (nine samples). Other possibilities include differences in methodological design and outcome assessment tools.

Another study was published in 2019 to test the microhardness of Embrace WetBond and Helioseal F. The study showed a mean of 33 for WetBond and 26 for Helioseal F.[27] Luckily, Helioseal F has a very close mean VHN score to what we can find in the literature. On the contrary, for Embrace WetBond, there was a large difference between the literature means and the mean value reported in this study, where the result mean was much lower. With this in mind, the difference could be attributed to the immediate testing of the sample in Diener et al. study.[27]

In this study, all the materials went through the aging process using a thermocycling machine for 1,500 cycles with a dual time of 10 seconds. It had revealed no significant difference between the samples in the immediate group and the aged group except for the Embrace WetBond group. There was a significant difference between the immediate and aged sample means in the latter group, with the microhardness increased with time. This could be explained by the Embrace WetBond sealant material's chemical composition, which is different from other sealants. The material contains high filler content with the urethane dimethacrylate (UDMA) matrix (Table 1). All other sealants have minimal fillers with the Bis-GMA matrix. Both functional monomers have a high affinity toward water during the initial setting, resulting in water sorption.[28] However, Bis-GMA showed higher hydrophilicity due to the presence of hydroxyl functional group when compared to the urethane group.[29] The absorbed water is presented either as unbound water that fills the nanopores between the polymer chains or as bound water that is attached to polymer chains by van der Waals forces or hydrogen bonds. Hydrolytic degradation occurs at the interface between the filler particles and the matrix polymer during the aging process. The presence of high filler content resulted in the reduction of such degradation and improved the material hardness.[30] This could explain the increase in the VHN for the Embrace WetBond group.

Several experimental and clinical studies have compared some of the mechanical properties of Embrace WetBond to other fissure sealants and have shown superior physical characteristics in addition to its lesser sensitivity to moisture,[31,32] while other studies have shown similar clinical success rates.[33] In a recent clinical study, Embrace WetBond was found to exhibit lower wear and tear with greater marginal integrity (83%), retention (97%), and superior marginal adaptation (93%) compared to other resin-based sealants.[31] In another clinical study comparing Embrace WetBond to Helioseal F sealant, there was no difference between the two sealants in terms of retention, marginal adaptation, or caries development.[33]

An *in vitro* study by Zinelis et al. aimed to assess the microhardness at two different time intervals (at set and following 48 hours) of Embrace WetBond and Helioseal F. It was concluded that a statistical difference could be identified in terms of time, but no significant difference in terms of comparison of mechanical properties between the materials.[27] It appears that there was a significant impact of the aging variable in the literature for Embrace WetBond material, and this result coincides with what we found in our study. Nonetheless, Helioseal F showed a significant difference between the set and the aged group, which contradicts our results. The difference in results could be explained by the small sample size used by Zinelis et al., which was only six samples, and the difference in the aging process, load, and dwell time for the Vickers hardness test.[27] On the contrary, Thunyakitpisal et al. found no significant difference in the mean of microhardness of Helioseal F.[25] The study used 10 samples kept at room temperature for 24 hours before evaluating their microhardness. Their conclusion is comparable with the results found in our study for Helioseal F sealant material. Furthermore, an *in vitro* study by Kim et al., using a 10-g load indenter, found that Vickers hardness for Fissurit F sealant was reduced significantly after storage.[34] This contradiction could be related to storing the samples in a dry container for a long time and the difference of the load chosen (10 g) for the Vickers hardness test.

A limitation of this study is that the sealant material was tested after it was applied on a smooth surface. This was to reduce the variations between samples. In the clinical situation, the sealants are applied in different types of fissures that have varying depths. This might affect the DC for the sealant materials and might behave differently than the current study results. The other limitation of the current research is that only resin-based sealant materials were used. It could be valuable to test the effect of aging on the microhardness of other sealant materials, such as glass ionomer-based sealant or flowable resin composite.

Conclusion

While there were no significant differences in the microhardness of commonly used fluoride-releasing fissure sealants, time appears to significantly increase the mean microhardness score (VHN), especially in the Embrace™ group. However, due to the presence of many variances that may contribute to the results in real clinical situations, such as the masticatory force, thermal variation, and intraoral chemical environments that may affect the specific materials, further *in vivo* studies are now needed.

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