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Comparative evaluation of viscosity, volumetric shrinkage, microleakage, penetration depth and marginal adaptation of two hydrophilic and a contemporary hydrophobic pit and fissure sealant- A multiparametric in vitro study

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Abstract

Aim: To evaluate and compare the viscosity, volumetric shrinkage, microleakage, penetration depth and marginal adaptation of Embrace Wetbond^{TM} hydrophilic pit and fissure sealant (EWS), Ultraseal XT Hydro^{TM} (UXHS) hydrophilic sealant, under dry and moist surface condition, and Clinpro^{TM} (CL) hydrophobic sealant.

Materials and Methods: The relative viscosity, in Centipose, of the test sealants was assessed using a Brookfield viscometer. The volumetric shrinkage percentage was determined using a Digital analytical balance. A total of 50 selected teeth were prepared for microleakage, penetration depth & marginal adaptation testing. The tooth specimens were randomly divided into 5 groups (n=10): I A: EWS - Dry; I B: EWS – Moist; II A: UXHS- Dry & II B; UXHS – Moist and III: Clinpro-Dry (Control). The microleakage was assessed using 1% methylene blue dye penetration method. The sealant penetration depth and marginal adaptation were determined using grade scales by viewing under 40X stereomicroscope. To find the significant difference in the multivariate analysis the Kruskal Wallis test followed by the Mann-Whitney were used.

Results: There was no statistically significant difference in the mean viscosity values. UXHS demonstrated significantly lower volumetric shrinkage. UXHS-Dry demonstrated the least microleakage. UXHS-Moist showed the maximum penetration into the fissure system. UXHS-Dry demonstrated the significantly better marginal adaptation.

Conclusion/Clinical significance: UXHS demonstrated significantly less microleakage and better marginal adaptation in comparison with EWS and CL. No significant difference were observed in sealant penetration and sealant viscosity. UXHS demonstrated significantly lower volumetric shrinkage percentage.

Keywords: Hydrophilic, hydrophobic, marginal adaptation, microleakage, penetration depth, pit and fissure sealant, viscosity, volumetric shrinkage.

Introduction

Pit and fissure sealing is an effective, minimally invasive, primary preventive measure, offering complete protection to the occlusal surfaces of caries- susceptible teeth from dental caries.[1] The preventive benefits of these sealants rely on their long-term retention[2], ability of sealants to penetrate and thoroughly fill the pit and fissure system, and good marginal adaptation.[3] The penetration of sealant materials into fissures may in turn be affected by the viscosity of sealants.[4,5] Additionally, microleakage that can potentially result from polymerization shrinkage, could lead to caries developing below the sealant material. [5] Polymerisation shrinkage often causes adhesion failure, subsequent formation of microgaps[6] and loss of the marginal integrity of the filling, compromising the mechanical and chemical long-term stability of the restoration.[7] Tremendous improvements in pit and fissure sealant technology have led to the development of novel materials with enormous potential. One recent innovation has been the development of two moisture tolerant hydrophilic sealants, Embrace Wetbond™ (EWS) and UltraSeal XT Hydro™ (UXHS).

Materials and Methods

A comparative evaluation of the viscosity, volumetric shrinkage, microleakage and penetration depth of Embrace Wetbond™ hydrophilic pit and fissure sealant (EWS), Ultraseal XT Hydro™ hydrophilic sealant (UXHS) and Clinpro™ (CL) hydrophobic sealant was undertaken in this in vitro study. The materials used in the present study are listed below [Table 1].

Viscosity

The relative viscosities, in Centipose (cP), of the three pit and fissure sealants EWS, UXHS and CL were assessed in triplicate at room temperature (27^0C) in a Brookfield viscometer (DV-E Viscometer) calibrated at 100 revolutions per minute using an S-18 spindle. In the present study, the viscometer used, required a minimum volume of 5 ml test specimen. To achieve this, 0.5 ml of each sealant was first diluted in 4.5 ml of the diluent, methyl methacrylate monomer and the relative viscosities of the three sealants were obtained.

Polymerisation Shrinkage

The volumetric shrinkage percentage (% Shrinkage) of EWS, UXHS and CL was determined by measuring the difference in specific gravity between uncured and cured test specimens, in air and water, in accordance with a modified version of ASTM method D792 "Specific Gravity and Density of Plastics by Displacement"[11, 12], using a Digital analytical balance in a temperature-controlled room and protected from air drafts and electrostatic influences.

Specific gravity of unpolymerized pit and fissure sealant test specimens

The weight of the empty Eppendorf tube was measured using the Digital Analytical Balance. Then, 0.6 ml of the test specimen was taken in an Eppendorf tube and weighed using the Digital Analytical Balance. Equal volume of distilled water (DW) was taken in the same Eppendorf tube and the weight was again measured. The weight of the sealant and equal volume of distilled water was computed by deducting the weight of the empty Eppendorf tube from the weight obtained. The Specific gravity was computed using the following formula:

Specific gravity of the Sealant = Weight of the sealant /Weight of equal volume of DW.[11]

Specific gravity of polymerized pit and fissure sealant test specimens

To measure the specific gravity of the polymerized resin, six cylindrical specimens were made in a customised Teflon mould (7mm diameter/2mm height) and the upper surface of the specimens was covered with an OHP sheet to avoid an oxygen inhibition layer. Photoactivation was carried out using the established protocol following the Manufacturer's instructions. Specimens were weighed after 15 min of dry and dark storage ensuring minimum exposure to light.[13] The specimens were weighed in air and in water, and the specific gravity was computed using the following equation:[11, 12]

$$\text{SP gr} = a/(a+w-b)$$

Where a= weight of the disk in air in grams (g), b= weight of the disk and wire in water in grams (g), w=weight of the wire in water in grams (g).

The percentage of volume shrinkage after polymerization was calculated from the specific gravities according to the equation:[12]

$$\%\ \text{Shrinkage} = 1 - \left(\frac{SP\ gr\ (uncured)}{SP\ gr\ (cured)}\right) \times 100$$

Microleakage and penetration ability and marginal adaptation

A total of 50 caries free, intact unrestored, extracted human permanent molars teeth with U, V, type of fissure anatomy were selected after examining under 10X stereomicroscope, and prepared for microleakage, penetration depth and marginal adaptation testing. The tooth specimens were randomly divided into 5 groups (n=10) as follows: Group I A: EWS - Dry; Group I B: EWS – Moist; Group II A: UXHS- Dry and Group II B; UXHS – Moist and Group III: Clinpro Sealant on dry enamel (Control). After cleaning the occlusal surfaces using a brush with fluoride-free pumice in a low-speed hand-piece, debris remaining in the pits and fissures was removed using an explorer. The occlusal surfaces were etched & rinsed as per the Manufacturer's instructions. Dry enamel was prepared by drying the tooth with oil-free air for 10 seconds until a frosty white appearance was achieved. Moist enamel was prepared by lightly drying the tooth with oil-free air for 1 second and removing the excessive moisture with a cotton pellet to achieve a shiny appearance. After sealant application, there was a waiting period of 20 seconds to allow the sealant to penetrate sufficiently into the pits and fissures. The teeth were then light-cured using an LED light-curing unit as per Manufacturer's instructions.

The sealed teeth were stored in distilled water at 37℃ for 24 hours. After removal of the remaining moisture using gauze, the apices were sealed with a resin-modified glass ionomer and the tooth surface was coated with two layers of nail varnish except for an area of 1mm from sealed occlusal surface. All teeth were immersed in 1% methylene blue solution for 24 hours. After 24 hrs the tooth specimens were rinsed with distilled water and cleaned using a soft bristled tooth brush. Prior to testing, the cleaned teeth were stored in distilled water at room temperature and were subjected to testing within four weeks of storage. For testing the tooth specimens were mounted on acrylic blocks using a customized metallic mould (2±0.1 cm diameter and 1±0.1 cm height). The mounted tooth specimens were then sectioned twice, in a longitudinal direction, bucco-lingually, using a low speed diamond disc resulting in four test specimens per tooth. The test specimens were stored dry in test tubes. The investigator was blinded with regards to the test groups at this point to avoid bias during testing.

Scoring of microleakage and penetration ability and marginal adaptation

Each sample (four surfaces per tooth) was observed with a Stereomicroscope at 40X magnification (Nanatom technologies, Bangalore) and was photographed using a digital camera (Pixel Inc, Korea).

The microleakage, penetration depth and marginal adaptation of the sealant were graded according to the following criteria:

The microleakage of the sealant material:[14, 15]

0 = No dye penetration

1 = Dye penetration limited to the outer half of the sealant

2 = Dye penetration extending to the inner half of the sealant

3 = D ye penetration extending to the underlying fissure

The penetration ability of the sealant material:[15, 16]

1 = Sealant penetrated to 1/3 the total length of the fissures.

2 = Sealant penetrated to ½ the total length of the fissures.

3 = Sealant penetrated to total length of the fissures.

Marginal adaptation of the sealant material:[16]

1 = Smooth adaptation, Sealant flows with enamel and no ledges.

2 = Sealant is not well adapted. Ledges may be present.

Statistical analysis

The collected data was analysed with IBM.SPSS statistics software 23.0 Version. To describe about the data descriptive statistics, mean & standard deviation were used. To find the significant difference in the multivariate analysis the Kruskal Wallis test followed by the Mann-Whitney were used. In both the above statistical tools the probability value 0.05 has been considered as significant level.

Results

EWS demonstrated the highest relative mean viscosity value and CL and UXHS demonstrated equal mean relative viscosity values, which was not statistically significant ($p = 0.066$) [Table 2]. There was statistically a highly significant difference between the mean volumetric shrinkage values of EWS and UXHS, UXHS and CL and EWS and CL respectively ($p \leq 0.01$). UXHS demonstrated significantly lower volumetric shrinkage in comparison to EWS ($p = 0.004$) and CL ($p=0.004$) while EWS demonstrated significantly lower volumetric shrinkage in comparison with CL ($p=0.004$) [Table 3]. UXHS-Dry demonstrated the least microleakage followed by UXHS-Moist, CL, EWS-Dry and EWS-Moist. There was statistically a highly significant difference in the overall mean microleakage scores of EWS- Dry, EWS- Moist, UXHS- Dry, UXHS- Moist and CL ($p =0.0005$). Both EWS and UXHS demonstrated higher mean microleakage scores under moist surface condition compared to dry surface condition, but the difference was not statistically significant [Table 4] UXHS-Moist showed the maximum penetration into the fissure system followed by UXHS-Dry, CL, EWS-Dry, and EWS-Moist. However, the difference in the overall mean penetration depth scores of EWS, UXHS, CL was not statistically significant ($p =0.215$) [Table 5]. UXHS-Dry demonstrated the best marginal adaptation followed by UXHS-Moist, CL, EWS-Dry and EWS-Moist. There was a highly statistically significant difference in the overall mean marginal adaptation scores of EWS, UXHS and CL ($p =0.001$). No significant difference was observed, for the different surface conditions, i.e. between EWS-Dry and EWS- Moist and UXHS- Dry and UXHS- Moist respectively [Table 6].

Microleakage and penetration showed a highly significant negative correlation between them, while a highly significant positive correlation was shown for microleakage and adaptation. Penetration depth and marginal adaptation showed a significant negative correlation between them [Table 7].

Discussion

Tooth surfaces with pits and fissures are particularly susceptible to the development of dental caries, in both the primary and the permanent dentition. Absence of post eruptive maturation and contact with the opposing arch, lack of salivary access as a result of surface tension, which reduces the effectiveness of fluoride and prevents remineralization[17,18], favours the development of carious lesions in these sites. Occlusal pits and fissures vary in shape, but are generally narrow and tortuous, and this morphology renders pits and fissures inaccessible to mechanical means of debridement. Additionally, the close proximity of the fissure base to the dentino-enamel junction and remnants of debris and pellicle in the fissures increase caries susceptibility of fissures by many folds.[19]

The ideal time for sealant placement is soon after tooth eruption, as newly erupted teeth are less mineralized and more susceptible to acid attack than teeth exposed to saliva for several years.[20] The risk for caries peaks within two-four years after tooth eruption and declines thereafter.[18, 21, 22]The contact of teeth with the distal marginal ridge of gingiva during the period of eruption can result in the contamination of the occlusal surface by moisture or saliva[21] as the oral cavity is a 100 percent humid environment, without the use of rubber dam, complete isolation and moisture control is difficult to achieve. However, the application of rubber dam is very cumbersome in young individuals.[23]

To overcome challenges in the placement of conventional resin-based pit and fissure sealants such as patient co-operation, moisture control and retention research has focused on improving the clinical technique, the delivery system and the chemical makeup of sealant materials. One recent innovation has been the development moisture tolerant hydrophilic sealants.

Embrace Wetbond^{TM} (EWS) is a new generation moisture tolerant, resin-based sealant that can bond to slightly moist teeth, both chemically and micromechanically. According to the manufacturer, it incorporates di-, tri, and multifunctional acrylate monomers into a hydrophilic, resin acid integrating network (R.A.I.N.).[8] It is hydro-balanced, water activated, pH controlled, water miscible[8], self-priming, self-adhesive and reportedly less technique sensitive.[7, 18, 24] It contains 36.6% filler particles, which are activated by moisture.[25, 26] EWS does not contain Bisphenol A or Bis-GMA and is fluoride releasing.[8, 25, 27]

UltraSeal XT® hydro™ (UXHS) is another hydrophilic resin sealant. According to the manufacturer, it is self-adhesive, light-curable, methacrylate-based, radiopaque, fluoride-releasing resin based pit and fissure sealant [9, 28], and contains diurethane dimethacrylate, triethylene glycol dimethacrylate, and methacrylic acid.[9] It is reportedly stronger and more wear resistant as it has 53 wt% mixture of inorganic filler particles. UXHS is applied using a syringe and Inspiral Brush tip.[9] (Ultradent Products). The manufacturer has suggested that the spiral brush action of the Inspiral™ Brush tip, causes shear thinning of the thixotropic UXHS sealant. The material after curing is hydrophobic unlike other hydrophilic sealants.[9, 28]

The preventive benefits of sealants rely on their long-term retention [2], ability of sealants to penetrate and to thoroughly fill morphological surface defects, good marginal adaptation[3], low sorption and solubility and cariostatic action.[19] Another key consideration for the success of a pit and fissure sealant is adequate adhesion which is dependent on the penetration of the material into the previously etched system of fissures.[5] The ability of the sealant to penetrate into the fissures influences long-term retention of the sealant and is dependent on the type of the fissures, deposition of the material, and the physico-chemical characteristics of the sealer resin used. The penetration of sealant materials into fissures is also affected by the viscosity of sealants with low viscosity sealants having been reported to show higher penetration than sealants with high viscosity. Poor sealing ability can lead to microleakage that could in turn lead to caries developing below the sealant material.[5,17,29-31] Microleakage could potentially result from polymerization shrinkage.[3,7,17,32]

Various studies have evaluated the effect of filler content of the sealant on viscosity, volumetric shrinkage, microleakage and penetration depth.[14, 29, 30, 33-36] Only a few studies have compared the two hydrophilic sealants, EWS and UXHS. Moreover, the reports have been controversial.[5, 37, 38] Furthermore, there appear to be no studies that have compared the polymerization shrinkage of these two hydrophilic materials. In view of the paucity of research, the present study was undertaken.

Clinpro™ (CL) was selected as the hydrophobic, unfilled (6% wt) pit and fissure sealant control in the present study. Several in vitro and clinical studies, have reported that CL has exhibited superior performance in comparison with many contemporary pit and fissure sealants.[10, 32, 39-45] It also meets the ISO 6874:2015(E) Dentistry-Polymer-based pit and fissure sealants for a Type II (light-cured) sealant.[10]

Viscosity is the resistance of a liquid to flow, which is controlled by internal frictional forces within the liquid and is usually measured in Centimetre-Gram-Seconds (CGS) units of milli Pascals (mPa.s) or Centipoise (Cp).[30] The viscosity of sealants can be assessed either by using ultrasonic vibratile viscometer such as Brookefield viscometer or by conventional capillary tube method. The ultrasonic vibratile viscometer is more accurate compared to conventional capillary tube method, as it gives a digital read out. The viscometer rotates the spindle in the liquid to overcome the viscous resistance to the induced movement, and thus a reading is obtained. All Brookfield laboratory viscometers are accurate within the range of +/-1%, and have a reproducibility within the range of +/- 0.2. Hence, a Brookfield viscometer (DV-E Viscometer) was used to evaluate the viscosity of the test sealant specimens in the current study.[19, 46, 47]

The volumetric polymerization shrinkage of resin-based materials is a crucial factor and a source of concern to clinicians, as it may lead to failure of restorations. Numerous methods for the determination of shrinkage have been reported, each method has limitations and disadvantages.[12, 13, 48-51] In the current study, the volumetric polymerization shrinkage of the test sealants was measured using the specific density method, American Society for Testing and Materials (ASTM) method D792- "Specific gravity and density of plastics by displacement", based on the Archimedes Principle (buoyancy of a material in fluid). It is relatively simple, convenient, accurate, low-cost method and can capture the entire volumetric change[11, 12, 52] and is also reasonably insensitive to temperature changes.[53] This is the only method for measuring polymerization shrinkage that has published standards for execution in ISO 17304.[7] The method described provides a precise measure of polymerization shrinkage without the use of sophisticated instrumentation. The equipment required is an analytical balance capable of measuring to the nearest 0.1 mg.[7, 11, 12, 52] In the present study, the density of cured samples was measured immediately after 15 minutes post preparation; therefore, any shrinkage associated with postcure was not measured.[12] To obtain stabilized readings, the measurements were done in a temperature-controlled room, protected from air drafts and electrostatic influences.

An important factor to consider in the long term success of sealants is the prevention of microleakage. Kidd in 1976 defined microleakage as the clinically undetectable passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material applied to it.[54] This clinically undetectable passage of fluids and microorganisms has its effect not only on the longevity of the restoration, but also on the vitality of the tooth itself.[55-57] The dye penetration method is the oldest & most common methods of detecting microleakage in vitro and it is simple and fast.[55, 58] The concentration of dye used ranges between 05-10%.[58] The main advantages of dye penetration techniques are: Dyes are detectable in dilute concentrations, they are nontoxic and the results can be evaluated quantitatively. Methylene blue is an adequate indicator of passage of microorganisms and large size endotoxins as well as of toxic agents of low molecular weight.[59, 60] Since Methylene blue has a lower molecular weight (319.9) it penetrates more deeply than other dyes. Matloff et al (1982) found Methylene blue to be the most sensitive indicator of microleakage.[60] Hence, in the present study 1% Methylene blue was used. The time of immersion of specimens in the dye varies between 4 hrs to 72 hrs or more. In the present study the test specimens were immersed in 1% Methylene blue dye for a period of 24 hrs.[58] As higher dye penetration has been observed in specimens that were tested before 24 hrs storage as compared to specimens that were stored in water before thermocycling for at least 24 hrs, a finding attributed to the water sorption potential of composite resin, it has been recommended that microleakage tests on composite restorations be carried out only after 24hrs of specimen storage to permit water sorption of the resin.[55] Hence the specimens in the present study were stored in water for 24 hrs at 37^0C, as recommended, before being immersed in 1% methylene blue dye.

Apart from microleakage, which was scored using a standard grade scale that has been used extensively in research[14,15,32,36,61-64], the present study also simultaneously evaluated penetration depth and marginal adaptation of the sealants using the specimens processed for the Dye Penetration test, employing separate grade scales for penetration depth[15,16] and marginal adaptation[16], by viewing the test specimens under 40X stereomicroscope. Penetration depth is an important parameter that may increase the longevity of sealant and affect the retention and adaptation of the sealant. Marginal adaptation is a major prerequisite for dental restorative materials as microleakage against dental restorations poses a major problem in clinical dentistry.[65] Several other factors also need to be considered during sealant placement, as these may influence the sealing of the pit and fissure system. One of the most important factor is the morphology of pits and fissures. According to Nagano's classification (Nagano T, 1960), there are five major types of occlusal pits and fissures which are described as V, U, I, IK and Inverted Y.[66-68] In the present study, the fissure system of each extracted tooth specimen was visualised under Stereomicroscope at 10 X magnification and only teeth with U and V type fissure system were selected. Various studies have demonstrated that fissure morphology had significant effect on sealant penetration and marginal adaptation with the majority of studies showing better penetration and adaptation for shallow fissure systems.[66, 68-75]

In the present study, the lower mean viscosity shown by the unfilled sealant CL and highest viscosity exhibited by EWS can be attributed to their filler content (CL-6%, EWS-36.6%), which is in agreement with Simonsen RJ (2002)[2] and Reddy VR et al (2015),.[42] Interestingly, UXHS demonstrated the same mean relative viscosity value as CL despite having the highest filler content of 53% filler content by weight, this may be due to its thixotropic nature and the spiral brush action of the Inspiral® Brush tip which causes the shear thinning of the filled, thixotropic UXHS, reducing its viscosity as it is dispensed through the Inspiral brush. Contrary to the results of the present study, Prabhakar J et al (2018)[4] reported higher viscosity values for CL when compared to UXHS.

A material's viscosity is not a single point measurement and it typically depends on a number of factors. This includes how the tested material is handled or processed when used and the rate of shearing which is determined by the speed at which the spindle rotates in a rotational viscometer. The viscometer measures the amount of torque resistance imparted by the test material against the rotating spindle at each speed. The measured parameter (torque) and the control parameter (spindle speed) are combined into an equation that defines dynamic viscosity as the ratio of shear stress to shear rate. Viscosity may not be a single number for a given material. Different rotational speeds (shear rates) can yield different viscosity values. Temperature is yet another parameter that needs to be considered when measuring viscosity. As temperature increases most materials will exhibit a decrease in viscosity.[47, 76]

When a thixotropic material like UXHS, is sheared at a constant rate, the measured velocity will decrease with time, while it is subjected to a constant shear rate. Thus the experimental parameters of viscometer model, spindle and speed all have an effect on the measured viscosity. This measured viscosity is called the "apparent viscosity" of the fluid and is accurate only when explicit experimental parameters are furnished and adhered to.[76]

The significant difference in the volumetric shrinkage between the tested sealants in the present study can be attributed to variation in composition. Other than the amount of filler content or loading, the polymerization shrinkage depends on many factors, including the average molecular weight, size of the filler particles, type of filler material, composition of the resin matrix, amount of monomer, type of monomer, the degree of cure and viscosity of the resin-based materials.[3, 48, 51] In the present study, UXHS demonstrated the least percentage volumetric shrinkage, this could be attributable to the high level of inorganic mineral filler in UXHS. Scanning electron microscopy and energy dispersive X-ray analysis of fracture and polished surfaces carried out by Guclu ZA et al (2016), indicated that UXHS is a ductile material that is highly filled with uniformly distributed micrometre and nanometre sized silicon, aluminium, and barium bearing mineral phases.[28]

The volumetric shrinkage values obtained for CL and EWS are comparable with those reported by Sener Y et al (2014) and Arumugham et al (2018) respectively.

In the present study, the hydrophilic sealant, UXHS, under both dry and moist conditions, demonstrated significantly lower microleakage and better marginal adaptation in comparison with the hydrophilic sealant, EWS and the hydrophobic sealant, CL. The thixotropic nature of UXHS, combined with its hydrophilic chemistry could have potentially contributed to this.

Additionally, this material is hydrophobic after curing unlike other hydrophilic sealants.[9, 28] No significant difference in terms of sealant penetration into the fissure system was observed between the sealants.

Low microleakage values for Ultraseal XT Plus that has a similar composition to UXHS but with filler content of 58% by weight in comparison to 53% filler loading of UXHS, were reported by Zerovu et al (2000) .[61] The results of the present study are also in partial agreement with those reported by Ku J et al (2017)[5] and Khogli et al..[77]

Failure of wet-bonding of the hydrophilic sealant to moist enamel has been put forth as a probable cause for greater microleakage. Acid-etched enamel has high surface energy creating a strong bond to moisture, which if present can plug the microporous surface of the etched enamel, impeding the formation of resin tags and ultimately weakening the resin tooth substrate bond.[5, 78] For effective bonding to moist enamel, the moisture should be displaced or combined with a bonding agent.[5, 79] In addition to hydrophilic monomers, bonding agents contain ethanol or acetone solvents that enhance the displacement of moisture resulting in satisfactory bond strength to moist enamel.[5, 80, 81] Both EWS and UXHS though hydrophilic in composition, lack such solvents.[5, 82] Consequently, the excessive moisture left behind on the bonding interface could interfere with the bonding and polymerization of the resin.[5, 83]

Alternatively, water sorption by hydrophilic monomers, could probably account for impaired bond strength and durability. Increased hydrophilicity of monomers results in increased water sorption.[5, 84 Water] sorption impairs bond durability by accelerating the hydrolytic degradation of the bonding interface[5, 85] and by inducting structural defects in the resin, which could accelerate moisture sorption and generate internal swelling stress.[5, 86] In the present study the teeth were stored in distilled water for 24 hours after sealant placement, thus water sorption by the hydrophilic monomers may have played a role in the increased microleakage encountered in one of the hydrophilic sealants, EWS. However, in contrast to EWS, the other hydrophilic sealant, UXHS, has performed better in the present study, even though it has hydrophilic monomers, which could probably be accounted for the fact that after curing UXHS develops hydrophobicity unlike other hydrophilic sealants.[9]

EWS applied on dry enamel showed more microleakage than CL in the present study. As per EWS manufacturer's instructions, the tooth surface should not be desiccated, as EWS contains acidic monomers which are activated by moisture, which could probably be accountable for this finding.[5]

Gawali PN (2016) reported least microleakage with UXHS under moist surface condition, and also reported that the depth of penetration of the hydrophobic sealant (Fissurit F) was greater than that of the hydrophilic sealant (UXHS) in both dry and moist surface conditions.[64] Toodeshkchooei GD et al (2012) reported no significant difference in the microleakage demonstrated by CL and EWS.[87]

In terms of depth of sealant penetration into fissures, there were no significant differences between the groups in the present study, which is consistent with the findings of Khogli et al and Ku J et al..[5, 77] Iyer RR et al (2013) reported no statistically significant difference in the penetration depth of a contemporary hydrophobic sealant (Seal- Rite) and EWS.[69] On the contrary, some studies have reported that hydrophilic sealant showed less penetration in moist enamel than in dry enamel.[82, 88] Beslot-Neveu et al stated that hydrophilic sealant (EWS) could not penetrate to the bottom of the fissures while displacing water because its surface energy is lower than that of water.[88] Eliades et al also suggested that residual moisture may impede the penetration of the sealant by forming a liquid meniscus due to surface tension at the bottom of the fissure.[82]

The viscosity of the sealant material also seemed to affects the penetration of the sealant into fissures according to various studies.[19, 29, 30, 89] Sealants with low viscosity showed higher penetration than sealants with high viscosity.[19, 30, 90] Therefore, the relatively lower penetration of EWS compared UXHS observed in the present study may be due to its slightly higher viscosity.

Contrary to the results of the present study, Rodriguez et al. (2011) reported significantly lower microleakage scores and better marginal adaptation for CL compared to EWS.[91] Kane et al (2009)[16] compared EWS and CL using the same grade scale as the present study and reported that the marginal adaptation of EWS was statistically superior to CL.

Rangel PEE et al (2018) reported that the hydrophilic sealants used in a dry environment did not adapt to the surface and had a tendency to fail with regard to adhesion. They reported that CL under dry environment showed statistically significant higher values of adhesion to the enamel, in comparison to EWS and UXHS tested under both dry and moist surface conditions.[38]

The results of the present study indicate that as the mean penetration depth score increased, indicating better penetration, there was a highly significant reduction in the mean microleakage scores. With the reduction of marginal adaptation scores, lower mean marginal adaptation score indicating better marginal adaptation, a significant decrease in microleakage scores was observed. When there was significant increase in mean penetration depth score, higher penetration depth score indicating greater penetration of the sealant material into the fissure, significantly better marginal adaptation was observed i.e. lower mean marginal adaptation scores were obtained. Within the limitations and based on the results of the present study, it can inferred that there is highly significant reduction in microleakage when there is greater sealant penetration and superior marginal adaptation. Additionally, superior marginal adaptation is observed with greater sealant penetration.

Though the present study was able to provide valuable insights into the tested physical properties of the test materials viz. viscosity, polymerization shrinkage, microleakage, penetration depth and marginal adaptation, its inherent limitations must be pointed out. The results of the present study are valid for in-vitro conditions. Pit and fissure sealants may perform variably in the oral-environment, influenced by factors such as the type of fissure, fissure preparation, enamel etching, conditioning and application of bonding agent. Hence, further research on the effect of these factors, on sealant microleakage, adaptation and penetration is necessary. One of the limitations of the present study was that thermocycling could not be employed to simulate the temperature changes in the oral cavity on exposure to hot and cold food, while eating and drinking, under clinical conditions. The specimens were not subjected to artificial aging nor subjected to mechanical and brushing simulations or pH cycling, which could be assessed in future research. Long term clinical trials are recommended to confirm the results of the same.

Conclusion

In the present study, Ultraseal XT Hydro^{TM} hydrophilic pit and fissure sealant demonstrated lower volumetric shrinkage, lower microleakage, better penetration depth and better marginal adaptation in comparison with the hydrophilic sealant, Embrace Wetbond^{TM} and the hydrophobic sealant, Clinpro^{TM}. Therefore, within the limitations of the present study and based on the results, UXHS can be considered a suitable alternative to contemporary commercially available pit and fissure sealants, especially for sealant application in young paediatric patients, where moisture control and behaviour management pose a challenge. However, further research is recommended to provide an in-depth understanding of the clinical performance of the tested materials. The results of the present study can help dental clinicians make informed clinical decisions during the selection of commercially available pit and fissure sealants.

Manufacturer name

Clinpro ™ pit and fissure sealant: 3M ™, **Embrace Wetbond ™**, hydrophilic pit and fissure sealant: Pulpdent Corporation, Ultraseal XT Hydro ™, hydrophilic pit and fissure sealant: Ultradent corporation.

Material	Material description	Chemical composition	Manufacturer	Batch no:
Embrace Wetbond™ P & F Sealant [8]	Hydrophilic pit and fissure sealant Sealant	36% by weight- Glass Filler contains Di-Tri multifunctional acrylate monomer in resin acid integrating network. (Silica Amorphous, Uncured acrylic resin and Sodium Fluoride).	Pulpdent™ Corporation	190115 190502
Ultraseal XT® Hydro™ P & F Sealant [9]	Hydrophilic pit and fissure sealant Sealant	Matrix: Triethylene glycol dimethacrylate(TEGDMA), Diurethane dimethacrylate(DUDMA), Methacrylic acid Filler: Mixture of inorganic fillers (53wt %) Aluminium Oxide, Methacrylic acid, Titanium dioxide, Sodium monofluorophosphate.	Ultradent Corporation	BHMY4 BGJJV
Clinpro™ P & F Sealant [10]	Hydrophobic Sealant Pit and fissure sealant	Bis-GMA, Triethylene glycol dimethacrylate (TEGDMA), Ethyl 4-dimethyl aminobenzoate (EDMAB), hydroquinone, silane-treated silica, Tetra Butyl Ammonium Tetra Fluoroborate, Titanium dioxide, Diphenyliodonium Hexafluorophosphate, Triphenyl antimony, Bisphenol A Digilycidyl Ether Dimethacrylate.	3M ESPE	NA82335 N910454 N959657
Etch-Rite™	Acid Etchant	38% Phosphoric acid; Silica gel	Pulpdent™ Corporation	190110
Ultra-Etch®	Acid Etchant	35% Phosphoric acid; Silica gel	Ultradent Corporation	BGYNF

Table 1: Material description, composition and Manufacturer details of the materials used in the study.

GROUPS	N	Mean ± SD
Group I (EWS)	3	2.94±0.0 a
Group II (UXHS)	3	2.79±0.0 a
Group III (CL)	3	2.79±0.0 a

Table 2: Mean values of relative viscosity (cp) of the tested pit and fissure sealants. Same letters imply mean values with no statistically significant differences (p>0.05) using the Kruskal Wallis test.

GROUPS	N	Mean ± SD
Group I (EWS)	6	3.48±0.23 a
Group II (UXHS)	6	1.99±0.06 b
Group III (CL)	6	6.17±0.14 c

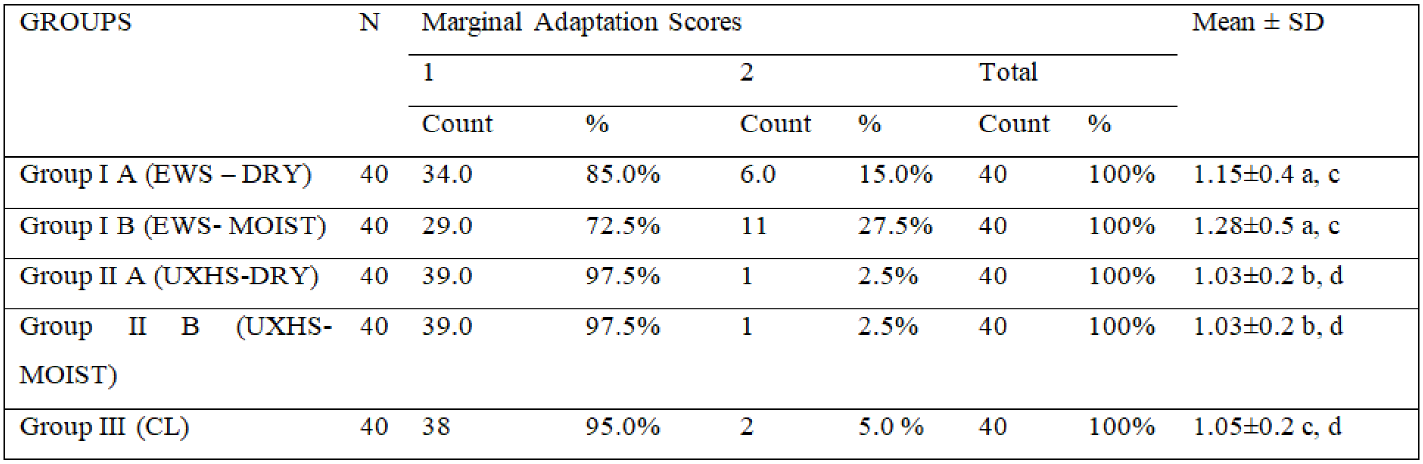
Table 3: Mean values of volumetric shrinkage percentage of the tested pit and fissure sealants. Same letters imply mean values with no statistically significant differences (p>0.05) using the Mann-Whitney U test.

Groups	N	Microleakage Scores										Mean ± SD
		0		1		2		3		Total		
		Count	%	Count	%	Count	%	Count	%	Count	%	
Group I A (EWS – DRY)	40	13	32.5 %	10	25.0 %	12	30.0 %	05	12.5 %	40	100 %	1.23±1.0 a
Group I B (EWS-MOIST)	40	08	20.0 %	13	32.5 %	6	15.0 %	13	32.5 %	40	100 %	1.60±1.2 a
Group II A (UXHS-DRY)	40	38	95.0 %	02	5.0 %	0	0.0 %	0	0.0 %	40	100 %	0.05±0.2 b, c
Group II B (UXHS-MOIST)	40	37	92.5 %	02	5.0 %	0	0.0 %	01	2.5 %	40	100 %	0.13±0.5 b, c
Group III (CL)	40	35	87.5 %	01	2.5 %	0	0.0 %	04	10.0 %	40	100 %	0.33±0.9 c

Table 4: Frequency distribution of microleakage scores and mean values of microleakage scores of the tested pit and fissure sealants. Same letters imply mean values with no statistically significant differences (p>0.05) using the Mann-Whitney U test.

	N	Penetration Depth Scores								Mean ± SD
		1		2		3		Total		
		Count	%	Count	%	Count	%	Count	%	
Group I A (EWS – DRY)	40	0.0	0.0%	07	17.5%	33.0	82.5%	40	100%	2.83±0.4 a
Group I B (EWS-MOIST)	40	0.0	0.0%	10	25.0%	30.0	75.0%	40	100%	2.75±0.4 a
Group II A (UXHS-DRY)	40	0.0	0.0%	04	10.0%	36.0	90.0%	40	100%	2.90±0.3 a
Group II B (UXHS-MOIST)	40	0.0	0.0%	03	7.5%	37	92.5%	40	100%	2.93±0.3 a
Group III (CL)	40	01	2.5%	05	12.5%	34	85.0%	40	100%	2.83±0.4 a

Table 5: Frequency distribution of penetration depth scores and mean values of penetration depth scores of the tested pit and fissure sealants Same letters imply mean values with no statistically significant differences (p>0.05) using the Kruskal Wallis test.



GROUPS	N	Marginal Adaptation Scores						Mean ± SD
		1		2		Total		
		Count	%	Count	%	Count	%	
Group I A (EWS – DRY)	40	34.0	85.0%	6.0	15.0%	40	100%	1.15±0.4 a, c
Group I B (EWS- MOIST)	40	29.0	72.5%	11	27.5%	40	100%	1.28±0.5 a, c
Group II A (UXHS-DRY)	40	39.0	97.5%	1	2.5%	40	100%	1.03±0.2 b, d
Group II B (UXHS-MOIST)	40	39.0	97.5%	1	2.5%	40	100%	1.03±0.2 b, d
Group III (CL)	40	38	95.0%	2	5.0 %	40	100%	1.05±0.2 c, d

Table 6: Frequency distribution of marginal adaptation scores and mean values of marginal adaptation scores of the tested pit and fissure sealants. Same letters imply mean values with no statistically significant differences (p>0.05) using the Mann-Whitney U test.

Correlations				
			Penetration Depth	Adaptation
Spearman's rho	Microleakage	ρ- value (rho)	$-.225^{**}$	$.352^{**}$
		p-value	.001	.0005
		N	200	200
	Penetration depth	ρ- value (rho)		$-.174^{*}$
		p-value		.014
		N		200
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

Table 7: Correlation between mean scores of Microleakage, penetration and marginal adaptation using Spearman's rank correlation coefficient.

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