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An *In vitro* Comparative Evaluation of Microleakage of ACP Containing Pit and Fissure Sealant and Moisture Tolerant Pit and Fissure Sealant

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Authors' contributions

This work was carried out in collaboration between both authors. Author RM conceptualized the study, collected and analyzed the data and wrote the first draft of the manuscript. Author AM performed critical analysis of the study and approved the manuscript for publication of the study. Both authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Background: Pits and fissure sealants are widely used to prevent caries in children. Microleakage is one of the most crucial factors resulting in sealant compromise. Factors pertaining to microleakage like, pre-treatment of occlusal surfaces, moisture control, bonding systems, and flowability of the sealant, will determine the longevity of the treatment. In vitro microleakage studies are useful in predicting the marginal sealing ability of pit and fissure sealants.

Aim: This in vitro study was undertaken to evaluate and compare the microleakage of ACP (Aegis®) containing pit and fissure sealant and moisture tolerant pit and fissure sealant (Embrace[™] Wetbond[™]).

Study Design: In vitro study.

Place and Duration of Study: Department of Pedodontics and Preventive Dentistry, Dr. D.Y. Patil Dental College and Hospital, Pune. between June 2020 and February 2021. **Methodology:** 26 permanent non- carious premolars extracted for orthodontic treatment were used. The sealants used for the analysis of microleakage were Aegis® and EmbraceTM WetbondTM pit and fissure sealants. The premolars were divided into two groups of 13 each. After sealant

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application, they were immersed in methylene blue dye. The teeth were subjected to thermocycling for 24 hours, after which The teeth were sectioned buccolingually and dye penetration was studied under a stereomicroscope (magnification 10X).

Results: Both the groups were studied and statistically evaluated using Mann- Whitney U test. Aegis® showed higher microleakage in (46.2%) than Embrace (38%), though the results were not statistically significant.

Conclusion: Both the materials showed microleakage, more *In-vivo* studies with longer follow- up periods are needed to evaluate and compare the clinical success of these pit & fissure sealants.

Keywords: Aegis®; embraceTM wetbondTM; pit and fissure sealants; microleakage.

1. INTRODUCTION

Despite exhaustive research and implementation of numerous preventive strategies dental caries continues to be one of the most common diseases of mankind. Although the prevalence of dental caries is on a decline in the developed nations but in the developing countries it is still on the rise especially in children. Though fluoride's extensive use through community water fluoridation and topical application has given a significant relief from smooth surface dental caries¹ but caries in occlusal surface still remains a concern. Literature provides enough evidence on the effect of dental caries not just on oral health but also on the overall health of an individual [1,2,3].

Pit & fissure sealants constitute one of the important preventive strategies against the dental caries. Liu W et al. [4] conducted a study in Guangzhou City in 2019 and reported that the risk of caries development was decreased by 44% in the children whose fissure were sealed with sealants as compared to the ones left unsealed in a rural population while it was 35% amongst urban children. Splieth C et al. [5] also reported a significant benefit of fissure sealants in preventing dental caries on occlusal surface in primary as well as permanent dentition in children.

Pit and fissure not just help to seal the susceptible areas but also helps in remineralizing the demineralized surfaces near pits and fissures. The sealants pause the activity of the microbes, halts the caries process further acting as a preventive as well as a remineralizing agents. However, sealants come with few limitations like loss of retention and being technique sensitive. Resin sealants being hydrophobic demonstrate extremely sensitive nature to moisture in the oral cavity [6]. To overcome this moisture control challenge, Pulpdent in 2002 introduced a hydrophilic sealant EmbraceTM WetbondTM. Unlike other sealant

materials that are hydrophobic and demand application of bonding agent for enhancing adaptation, the EmbraceTM WetbondTM material is a hydrophilic resin-based sealant that does not require the application of bonding agent. Aegis® pit & fissure sealant is another newly introduced sealant containing amorphous calcium phosphate that release calcium and phosphate to act as remineralizing agents. It is also known as a smart material [7]. Though newer sealants are marketed and all prove to be efficient in preventing dental caries but they all differ in their survival rate.

The marginal sealing ability of sealant materials is critical for effective treatment. Inadequate sealing leads to marginal leakage, i.e., seepage of bacteria, fluids, molecules and ions through the tooth-material interface, leading to caries progression below the sealant. In vitro microleakage studies can predict the marginal sealing ability of restorative materials Since there is a scarcity of data available on the micro Aegis® data comparing leakage and EmbraceTM WetbondTM pit and fissure sealants, this in-vitro study was undertaken to measure and compare the microleakage of Aegis® and EmbraceTM WetbondTM pit and fissure sealants.

2. MATERIALS AND METHODS

Before starting the study , approval from the institutional scientific and ethics committee was taken. 26 permanent premolars that were extracted for orthodontic treatment were included for the study. The sealants used for the analysis of microleakage were Aegis® pit and fissure sealant manufactured by Bosworth Companies, Keystone Industries and EmbraceTM WetbondTM pit and fissure sealant manufactured by Pulpdent. The samples were stored in aseptic medium in an accordance with CDC guidelines. Premolars with caries or developmental defects or fractures were excluded from this study.

Score	Penetration		
0	No dye penetration		
1	Dye penetration down the mesial or distal wall		
2	Dye penetration down the mesial and distal wall		
3	Dye penetration underneath the sealant and down		
	the mesial and distal wall		
4	Dye penetration all around the sealant		

 Table 1. Scoring criteria for penetration proposed by Theodoridou

Manufacturer's instructions were followed for the application of sealants. Teeth were cleaned with pumice and water to remove debris from fissures and dried .Etching was done with 37% phosphoric acid using applicator tip, rinsing the tooth was done after 20 seconds to remove etchant material and teeth were dried to appreciate chalky white appearance to confirm adequate etching. Application of Aegis® pit and fissure sealant and EmbraceTM WetbondTM pit and fissure sealant on 13 extracted teeth of each of the two groups. The sealants were cured using a light cure gun for 40 seconds. All surfaces of the teeth were coated with a double layer of nail varnish except the occlusal surfaces, following with the teeth were then immersed in 1% methylene blue dye for 24 hours. Then the samples were removed from the dye and the excess was brushed off. Thermocycling (5°-55°C) was carried out for 500 cycles, with a dwell time of 30 seconds. The teeth were sectioned longitudinally in the mesio-distal section with the help of a diamond wheel measuring 0.2mm thickness. The root portions were removed after sectioning. The samples were studies under Stereomicroscope with 10X magnification for microleakage analysis.

2.1 Evaluation of Microleakage

For evaluating microleakage the sections were cleaned and examined under Stereomicroscope

for penetration of dye independently by two examiners. Scoring criteria for penetration proposed by Theodoridou P et al. [8] was followed (Table 1).

The data on microleakage were compared statistically between Aegis® and $Embrace^{TM}$ WetbondTM pit and fissure sealants using the Man-Whitney test which was run in Statistical Package of Social Science (SPSS) software version 21. The significance was set at p<0.05.

3. RESULTS

This study was undertaken to compare the microleakage of Aegis® and EmbraceTM WetbondTM pit and fissure sealants through an in-vitro study.

The difference in microleakage scores between Aegis® and EmbraceTM WetbondTM sealant material for all the samples is represented in Table 2. After assessing the difference between the two groups it was found that the teeth sealed with Aegis® sealant displayed higher microleakage over Embrace[™] Wetbond[™] sealant (Graph 1) but the difference between them was not statistically significant with Man-Whitney test value of 54.00 and p=0.09 The microleakage with both the pit and fissure sealant materials was thus statistically comparable.

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Scores	Aegis® (n)	Embrace (n)	Man- Whitney U	Significance (p)
Score 0- No dye penetration	0	1		
Score 1- Dye penetration down the mesial or distal wall	4	6		
Score 2- Dye penetration down the mesial and distal walls	6	6	54.00	0.089
Score 3- Dye penetration underneath the sealant and down the mesial and distal wall	2	0		
Score 4- Dye penetration all around the sealant	1	0		
Total	13	13		
	Significance at	p<0.05		

Table 2. Comparison between Aegis® and Embrace "Wetbond" for microleakage score	Table	2. Com	parison	between	Aegis® and	Embrace	'‴Wetbond''	for microleaka	ge score
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Graph 1. Microleakage scores between Aegis® and embrace



Fig. 1. Aegis®: (a)Dye penetration down mesial wall (b) Dye penetration all around the sealant





Fig. 2. Embrace: (a)No dye penetration (b) Dye penetration down the mesial and distal wall

4. DISCUSSION

The success of pit and fissure sealants is governed by various factors. Few of them include bonding of sealant with the tooth surface, clinician's expertise, carious lesion in the pits and fissures, microleakage and penetration of sealant deep in the fissures. Microleakage is one of the biggest limitation of sealants, as it may lead to bacterial invasion and secondary caries. The present study used scoring criteria proposed by Theodoridou P et al. [8] which is widely accepted for the evaluation of microleakage.

As children are most benefited from sealants, preventing moisture contamination becomes challenging because of poor cooperation during the procedure leading to partial or complete sealant loss from the tooth surface and reporting a failure rate between 5 to 10% in a year [9]. Embrace[™] Wetbond[™] was introduced by Pulpdent as the first moisture friendly material for sealing deep pits and fissures. The chemical composition of Wetbond incorporating di. tri and multifunctional acrylate monomers activates it when in contact with moisture thus, when sealant comes in contact with oral cavity moisture it evenly spreads over tooth surface which is not seen in hydrophobic sealants. In the present study only one sample showed no penetration of the dye when assessed for microleakage in the Embrace[™] 46.1% group. of samples demonstrated dye penetration down the mesial or distal wall. Similar percentage was noted for penetration of the dye underneath the mesial and distal walls. Remarkably, no samples were reported with dye penetration underneath the sealant and down or all around the sealant. A similar results were reported by Marks D et al. [7], Shafiei L et al. [10] and Ku J et al. [11]. On the contrary Tavangar M et al. [12] and Khatri SC et al. [13] reported Embrace[™] Wetbond[™] to have higher microleakage scores.

Aegis® is a material containing amorphous calcium phosphate. The best property of this material is that the sealant releases calcium and phosphate ions and the ions will be released only when the pH drops down to 5.9 from 7.4 making it a smart material. The released calcium and phosphate ions help in remineralizing the demineralized areas of the fissures.

Aegis® was used on thirteen premolar samples and scanned under stereo-microscope for dye penetration to assess the microleakage associated with the material. It was found that none of the teeth showed complete adaptation and thus all presented with some amount of microleakage. Our results are comparable to studies conducted by Marks D et al. [7]. The results of the present study were in contrast with another group of Selecman JB et al. [14]. In their study 90% of the molar samples presented no dye penetration while only one sample displayed dye penetration upto one-half of the sealants length. The variation in the results may be attributed to the pre-treatment of the enamel surface before sealant placement with air abrasion that was followed in the study by Selecman JB et al. [14] but not in the present study.

When the two sealants were compared for microleakage in the present study it was found that though higher scores of microleakage were observed in Aegis® as compared to EmbraceTM WetbondTM sealant material but the difference between them was not statistically significant. Both the materials were comparable in terms of microleakage. Though the literature does not mention any studies on the comparison of Aegis® with $\mathsf{Embrace}^\mathsf{TM}$ WetbondTM for microleakage but they do report about the retention rates which can be associated with the microleakage. The success of the sealant depends on the adaptation of material to the tooth structure which will ultimately improve the retention rate thereby indicating less microleakage.

Therefore indirect comparisons can be made from studies evaluating retention rate of Aegis® and Embrace[™] Wetbond[™]. Study conducted by Acharya et al. [15] concluded that there existed no significant difference in the retention rate between Aegis® and Embrace[™] The Aegis® presented with 72% completely retained sealants while Embrace reported with 65.6% at 12 months [15]. On the contrary, the study reported by Khatri SC et al. [13] stated that 78.91% of sealants were intact when sealed by Embrace[™] Wetbond[™] while it was 81% for Aegis® sealant material at 3 months. The retention of the sealant further decreased to 65.6% for $\mathsf{Embrace}^{\mathsf{TM}}$ and 71.9% for Aegis® at 12months period. They reported a significant difference between Aegis® and Embrace[™] Wetbond[™] which was not found in the present study for microleakage. Likewise, the difference of Embrace[™] Wetbond[™] with other flowable pit and fissure sealants too was reported to be significant for nanoleakage [16].

Pit and fissure sealants are becoming a part of the protocol for managing high risk carious groups. Several countries have developed their national programs of pit and fissure sealants for school children. India too has initiated a pilot program which will be conducted in all the districts of Maharashtra in government aided schools. The private as well as government dental Institutes in the state are a part of this program [17]. Therefore the clinical success of pit and fissure sealants is an integral step in maintaining the caries free state of the oral cavity.

5. CONCLUSION

It can be concluded that both the pit and fissure sealant materials; Aegis® and Embrace[™] Wetbond[™] show microleakage when observed under Stereomicroscope. Among the two the higher scores of microleakage were observed with Aegis® pit and fissure sealant material compared to EmbraceTM WetbondTM but the difference between them was not statistically significant. The most common type of microleakage observed in Aegis® sealant was dye penetration down the mesial as well as the distal walls while that with $Embrace^{TM}$ Wetbond was dye penetration down the mesial or the distal walls as well as dve penetration down the mesial as well as the distal wall. Further in-vivo studies with a longer follow up periods are required to evaluate and compare the clinical success of these pit & fissures sealants.

CONSENT

All authors declare that waiver of consent was obtained from the scientific committee of D.Y Patil Vidyapeeth (Ref: DPU/R&R(D)/32(24)/19) for publication of this study and accompanying images. A copy of the written consent is available for review by the Editorial office/ Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL

All authors declare that ethical approval was obtained from the Review and Research committee of D.Y Patil Vidyapeeth (Ref: DPU/R&R(D)/32(24)/19).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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