COMPARATIVE EVALUATION OF MARGINAL SEALING ABILITY OF TWO MATERIALS USED AS PIT AND FISSURE SEALANTS USING DYE PENETRATION METHOD-AN INVITRO STUDY

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In partial fulfilment for the Degree of

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BRANCH VIII

PEDODONTICS AND PREVENTIVE DENTISTRY

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CHENNAI

DECLARATION BY THE CANDIDATE

I hereby declare that the dissertation titled **"COMPARATIVE EVALUATION OF MARGINAL SEALING ABILITY OF TWO MATERIALS USED AS PIT AND FISSURE SEALANTS USING DYE PENETRATION METHOD-AN INVITRO STUDY "**is a bonafide and genuine research work carried out by me under the guidance of **Dr. C. Joe Louis MDS** Professor and Head, Department of Pedodontics and Preventive Dentistry Chettinad Dental College & Research Institute, Kelambakkam, Chennai.

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CERTIFICATE

This is to certify that the dissertation titled **"COMPARATIVE EVALUATION OF MARGINAL SEALING ABILITY OF TWO MATERIALS USED AS PIT AND FISSURE SEALANTS USING DYE PENETRATION METHOD-AN INVITRO STUDY"** is a bonafide and genuine research work done by **Dr. V. DHANALAKSHMI** under my guidance during her postgraduate study period between 2017-2020.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY** in partial fulfilment for the degree of **Master of Dental Surgery in the Branch VIII - Pedodontics and Preventive Dentistry**. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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ABSTRACT

INTRODUCTION

Dental caries is still highly prevalent all over the world. The occlusal surface of the teeth has pit and fissures which provide good environment for microbes causing it. Sealing these areas reduces the risk of occlusal caries which is done by pit and fissure sealant. The success of pit and fissure sealant depends on the marginal sealing ability of the material. There is limited literature evidence on Conseal F and Embrace Wet Bond in terms of marginal sealing ability. With this background the present invitro study was carried out to evaluate and compare the marginal sealing ability of Conseal F and Embrace Wet Bond.

AIM

The present study was carried out to evaluate and compare the marginal sealing ability of two commercially available pit and fissure sealants using dye penetration method.

MATERIALS AND METHODS

A total of 50 human premolar teeth were used in this invitro study and were divided into two groups. Group I included 25 teeth for application of Conseal F sealant and Group II included 25 teeth for application of Embrace Wet Bond sealant. Samples were stored in artificial saliva for 72 hours before thermocycling. The samples were immersed in 1% methylene blue solution for 24 hours, rinsed to remove excess dye and sectioned.

The sectioned samples were examined under an Optical Stereomicroscope and compared in terms of the extent of microleakage based on the amount of dye penetration between the sealant and tooth substance interface. The dye penetration scores in both the groups was statistically analyzed using Chi square test and Mann Whitney Test. A P-value < 0.05 was considered to be statistically significant.

RESULTS

It was seen that in Group I, 9 samples (36%) had no dye penetration (Grade 0) while in Group II, 6 samples (24%) demonstrated Grade 0 penetration. Group II had the most extensive dye penetration (Grade 3) in 8 of 25 samples (32%) ($P<0.001$).

CONCLUSION

On comparison of the microleakage scores of the groups, a statistically significant difference was found between the two groups; indicating a better performance of Conseal F as compared to Embrace wet bond.

KEY WORDS

OCCLUSAL CARIES, MICROLEAKAGE, SEALANT, PITS AND FISSURES.

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LIST OF ABBREVIATIONS

BIS-GMA	-	Bisphenol A glycidyl dimethacrylate
ICDAS	-	International Caries Detection and Assessment System
Df	-	Degree of freedom
GIC	-	Glass ionomer cement
HEMA	-	Hydroxyethyl methacrylate
TEGDMA	-	Triethylene glycol dimethacrylate

LIST OF ANNEXURES

INTRODUCTION

Prevention is always better than cure, inspite of all preventive methods, the most commonly occurring dental disease is dental caries, which still remains the most prevalent childhood disease.[1] On the other hand there is definite decline in the prevalence of dental caries particularly in developed countries due to various preventive protocols such as Fluoride therapy, Plaque control and Dietary sugar control. But all preventive approaches are highly effective only on the smooth surface caries and not on the occlusal surface caries.

According to Shafer (1993) "Dental caries is an irreversible microbial disease of the calcified tissues of the teeth characterized by demineralization of the inorganic portion and destruction of organic substance of the tooth which leads to cavitation."[2] National health and nutrition examination survey (2011-2012) done in USA reveals that nearly 1/4th of the children and ½ of the adolescents experienced dental caries lesions in their permanent teeth.

In the study done by Ripa (1970) on the distribution of dental caries of the school children in North America, which reveals that, in the total tooth surface, occlusal surface accounts for only 12.5% but in the caries distribution it accounts for about more than 50%.[3]

Epidemiological data and researches suggest that particularly in the children pit and fissures contributes to (80-90%) of carious lesions (Beltran-Águilas et al 2005) of which 90% of carious lesions in permanent teeth and 44% of primary teeth are found to be of pit and fissure type.[4]

Occlusal pits and fissures are classified by Nagano into five major types as V, U, I, IK and Inverted Y. According to him there exists relationship between the fissure morphology and the susceptibility of the tooth to caries and concluded that V- and U-type fissures are self-cleansing and have less risk of developing caries than other types (I, IK, and Y). The prevalence of U-type (14%), V-type (34%), I-type (19%), IK-type (26%), and Inverted Y-type was 7%.[5]

The morphological pattern of the pit and fissures which favors the food retention thereby providing shelter and favorable environment for the microbes. Studies have been documented that the occlusal surfaces of posterior permanent and primary teeth are known to be the most susceptible areas for the development of dental caries. International Paediatric Dentistry Guidelines, recommend sealing the primary and permanent molars in children and adolescents to prevent the onset of cavities and minimize the progression of non cavitated occlusal carious lesions.

As molars are the pillars of occlusion both in primary and permanent dentition, preserving this is of utmost importance in the preventive dentistry. Many researches and development were done and techniques and ideology were created to preserve the natural enamel and dentin which are till now "**NATURAL EXCELLENT BARRIER**" to dental caries.[6] As Pedodontist we solely bear the responsibility to provide adequate preventive measures to the pediatric population.

According to Simonsen placement of dental material over the pit and fissures of the enamel of caries susceptible teeth which forms a micromechanical bonded protective layer in such a way it forms the barrier between tooth surface and oral environment, thereby preventing the accumulation of food which in turn eliminates the microbial colonization and prevents dental caries formation.[7] They not only prevent occlusal caries, but also arrest the progression of incipient lesions. This concept leads to the invention of pit and fissure sealants which are the major cornerstone of modern preventive dentistry.

The journey of development of pit and fissure sealant started with idea of obliteration of the fissures which was given by Arthur in 1987 undergoes various modifications in the materials and techniques such as blocking off the susceptible fissures with zinc phosphate cement, mechanical fissure eradication, prophylactic odontotomy, and chemical treatment with silver nitrate.[8]

Dr. Buonocore was the first person to develop pit and fissure sealants of resin based which is used commonly nowadays. The first commercial dental pit and fissure dental sealant was introduced in 1971; it was Nuva-Seal (LD Gaulk, Milford, Del).[9]

The advantages of sealing the occlusal surfaces are the decrease in caries prevalence when compared with non-sealed teeth, and t lower cost compared with the cost of placing a restoration.[10] Currently two types of pit and fissure sealants are in use, which are termed as Resin based pit and fissure sealants and Glass ionomer-based pit and fissure sealants.

Resin-based sealants are categorized into generations based on their mechanism of polymerization or their content such as light activated, self-cured, chemically cured, and fluoride-releasing sealant,[11] Filled and Unfilled respectively.

Glass ionomer cement was introduced in 1974 by McLean and Wilson, as an alternative sealant material, especially in situations where moisture control is difficult due to its hydrophilic properties.[12] Glass ionomer also has good adhesion, biocompatibility, and fluoride release.

Resin-based materials are the commonly preferable material but in the case of difficult isolation, the use of glass ionomer is recommended.[12] By intense investigations it was concluded that no single pit and fissure sealant was reported to be ideal.

For a variety of reasons, pit and fissure sealants have not been widely accepted by the dental profession despite overwhelming evidence in support of their caries preventive potential. The primary problems associated with sealants are lack of retention of the sealant or microleakage leading to the deterioration of the material itself which increases potential for the development of caries.

The retention of pit and fissure sealants is one of the most important features of success in preventing dental caries, which depends on various factors such as microleakage at the sealant/tooth interface, wear, and abrasion resistance, the expertise of the clinician and most important is the cooperation of the patient. [13]Among the above factors the marginal sealing ability of a sealing material is extremely important for success of treatment.

In Pediatric dentistry, maintaining isolation is the most difficult task to perform during the process of sealant therapy as due to lack of cooperation of the pediatric patient. Thus, inadequate isolation further increases the risk of microleakage and subsequent treatment failure.[14] Therefore, microleakage is an unwanted outcome and the sealant capacity to prevent microleakage in fissures is an important factor to evaluating the clinical success of such materials since microleakage can also affect the process of decay under the sealant.[15] The majority of commercially available resin-based pit and fissure sealants are hydrophobic materials whereas recently introduced hydrophilic sealants, which bond effectively to moist enamel surfaces, present a distinct advantage in Pediatric dentistry where patient-compliance, isolation, and moisture-control can be particularly challenging.

EMBRACE WETBOND is a moisture-tolerant, self-adhesive, light-cured, acrylate-based, hydrophilic pit and fissure sealant which has been developed by PULPDENT.[16] Before any general recommendation can be made regarding the use of any products, it is necessary to evaluate the performance of the products both in invitro and clinical studies.[17]

To the best of my knowledge, the fluoride releasing sealant (CONSEAL F) conventional resin sealant commonly used by the Dental practitioners and moist bonding sealant (EMBRACE WET BOND) had never been compared in the studies before.

Hence the purpose of the study is to compare the marginal sealing ability of two commercially available pit and fissure sealants namely **CONSEAL F** and **EMBRACE WET BOND** using Dye penetration method.

AIM AND OBJECTIVES

The main aim of the study is to compare the marginal sealing ability of two commercially available pit and fissure sealants namely

- ❖ CONSEAL F
- ❖ EMBRACE WET BOND

Using Dye penetration method.

Objective:

To evaluate the microleakage of two commercially available pit and fissure sealants namely

- ❖ CONSEAL F
- ❖ EMBRACE WET BOND

REVIEW OF LITERATURE

S. Hatibovic-Kofman et al (1998)[18] Conducted an in-vitro study to compare the microleakage of unfilled and filled sealants after conventional (pumice with rubber cup), bur, and air-abrasion tooth preparation. The study results showed there was no statistically significant difference in retention of sealant with regard to technique of tooth preparation before sealant placement.

Blackwood J A et al (2002)[19] conducted an in vitro study to evaluate microleakage of pit and fissure sealants after using three different pit and fissure preparation techniques: (1) traditional pumice prophylaxis and acid etching, (2) fissure enameloplasty and acid etching and (3) air abrasion and acid etching. Sixty extracted third molars with no clinical evidence of caries were randomly divided into 3 groups of 20 each. Teeth were prepared using 1 of 3 occlusal surface treatments prior to placement of Delton opaque light-cured sealant. The teeth were thermocycled between 5±2°C and 55±2°C for 500 cycles with a dwell time of 30 seconds and then stored in 0.9% normal saline. All teeth were sealed apically and coated within 1.5 mm of the sealant margin with two layers of nail varnish. The teeth were immersed in a 1% solution of methylene blue for 24 hours to allow dye penetration into possible gaps between enamel and sealant. Three buccolingual cuts parallel to the long axis of the tooth were made yielding 4 sections and 6 surfaces per tooth for analysis. The surfaces were scored 0 to 3 for extent of microleakage using a binocular microscope at 25X magnification.

Neither air abrasion nor enameloplasty followed by acid etching produced significantly less microleakage than the traditional pumice prophylaxis with acid etching technique.

Ansari et al (2004)[20] evaluated the effect of pumice prophylaxis on the level of microleakage around and between the sealant and enamel. A total of 32 freshly extracted sound upper first premolars, assigned as suitable for sealant application, were chosen and divided randomly into two groups: (1) a test group, without prophylaxis; and (2) a control group, with prophylaxis. Sealant was applied to all teeth using the same conventional technique, with prophylaxis being omitted in the test group. The sealed teeth were thermocycled and then immersed in 2% Basic Fuchsin solution for 72 h. Each tooth was sectioned and examined for dye penetration under a stereomicroscope. Using a chi-square test for trend, the frequency of microleakage was significantly higher in the test group compared to the controls ($P < 0{\cdot}016$) which proves prophylaxis has a role in improving sealant retention. Removing this step may cause an increase in microleakage.

Vanessa et al (2006)[21] evaluated in his invitro study the marginal microleakage of different materials used as pit-and-fissure sealants (Delton, Filtek Flow, Dyract Flow and Vitremer). Fifty-six extracted sound human third molars were randomly assigned to 4 groups (n=14). After sealant placement, the teeth were thermocycled isolated, immersed in 2% buffered methylene blue dye for 4 h, included in acrylic resin and sectioned longitudinally in a buccolingual direction. The sections were analysed for leakage using a stereomicroscope. A 4-criteria ranked scale was used to score dye penetration. All materials exhibited dye penetration to some extension and no statistically significant difference was observed among the groups (p >0.05). In conclusion, the findings of this study showed that a flowable composite resin, a flowable compomer and resin-modified glass ionomer placed on occlusal pits and fissures had similar marginal sealing as the unfilled self-cured resin-based sealant.

Ganesh et al (2007)[15] done an in vitro study using forty premolars extracted for orthodontic reasons. The teeth were divided into two groups and sealants were applied. One was an experimental group using Fuji VII ® as a pit and fissure sealant and the other a control group using Concise®. The teeth were kept in gentian violet dye for 24 hours, the sectioned samples were observed for the extent of dye penetration, and scores were based on established scoring criteria. The comparison of the performance of the two groups showed a statistically significant difference and Concluded the Concise® resin-based sealant performed better in terms of sealing ability than did the Fuji VII ® glass ionomer sealant.

Nahid et al (2008)[22] Evaluated the effect of a bonding agent on the microleakage of a sealant material following contamination with saliva. In this experimental research, 48 sound premolars were divided into two groups. The first group received sealant without bonding and the other group was given sealant with bonding. After prophylaxis, the occlusal surfaces were etched with 37% phosphoric acid gel and the teeth were then placed in fresh human saliva for l0 s. Following this, in the first group fissure sealant (Kerr) was applied directly and cured; for the second group sealant was placed and cured after bonding (Single Bond; 3M). All samples were thermocycled (500 cycles; between 5°C and 55°C; dwell time of 30 s). Silver nitrate was used as the leakage tracer. The teeth were sectioned. Microleakage evaluation was made by stereomicroscope at 40x magnification and the results were evaluated with the Mann-Whitney U test. In the group that received sealant without bonding extensive microleakage was seen; placement of sealant with bonding significantly reduced microleakage. In the presence of contamination with saliva, use of bonding under the fissure sealant can reduce microleakage

Marks et al (2009)[23] evaluated the effect of adhesive agents and fissure morphology on the microleakage and penetrability of pit and fissure sealants. Sealants used in this study included Aegis (Bosworth), Conseal f (Southern Dental Industries), and Admira Seal (Voco). Adhesive agents included Optibond Solo Plus (sds/Kerr) and Clearfil S3Bond (Kuraray). Ninety extracted permanent molars were randomly assigned to 9 groups combining sealant and adhesive agent materials. A sealant and control group (phosphoric acid etch only) was also included. Dye penetration (microleakage), penetrability, and fissure morphology assessment was performed for the treatment groups through microscopic evaluation. No correlation was found between the extent of microleakage and penetrability and Concluded application of sealants using phosphoric acid as a conditioning agent revealed superior results, while the use of adhesives was found to be unnecessary.

Parco et al (2010)[24] Compared the microleokage of a self-etching sealant with a traditional phosphoric acid-etched sealant under noncontaminated and saliva-contaminated conditions. Fifty-two sound extracted human molars were randomly divided into 4 groups (N=I3). Teeth in Groups I and II were cleaned with pumice, etched with phosphoric add, rinsed, coated with a drying agent, placed with sealants (UltraSeal XT Plus), and light cured. Teeth in Groups 3 and 4 were cleaned with a proprietary pour pumice arid rinsed prior to being sealed with a self-etching sealant (Enamel Loc). Teeth in Groups 2 and 4 were contaminated with saliva and thoroughly air-dried prior to the sealant placement. All teeth were subjected to a thermocycling process, stained with silver nitrate, and sectioned, and images of the sealant on the occlusal surface were recorded. Saliva contamination did not significantly affect the microleakage distance ($P<0.17$). Under the conditions used in this in vitro study the self-etching sealant, regardless of contamination condition, had extensive microleakage distances vs. little microleakage in the traditional phosphoric acid-etched sealant

Zahra Bahrololoomi (2011)[25] evaluated the microleakage of flowable composite resins and conventional fissure sealants with or without dentin bonding agent. The occlusal surface of 60 intact extracted human premolars, divided into four groups, were cleaned with pumice/slurry, etched with 37% phosphoric acid for 15 seconds, rinsed and dried. Groups were treated differently: Excite bonding agent followed by Helioseal F fissure sealant in group1; Helioseal F alone in group 2; Excite bonding agent followed by Tetric Flow in group 3; and Tetric Flow alone in group 4. Light-curing was done after each application. After thermocycling, the whole surface of each specimen was coated with nail varnish except for one millimeter around the fissure sealant. The teeth were immersed in 2% basic fuchsin for 24 hours and then sectioned buccolingually. The sections were analyzed for leakage under a stereomicroscope. Data was analyzed by Kruskal-Wallis and Mann-Whitney tests at a significance level of $P < 0.05$. There were no statistically significant differences between the study groups in terms of the mean microleakage scores ($P > 0.05$), except for groups 2 and 4 ($P = 0.002$) and groups 3 and 4 ($P = 0.033$),and he concluded use of a flowable composite with bonding agent is a good alternative for sealing pits and fissures.

Prabhakar et al (2011)[26] study aims to correlate the relationship between the viscosity of the sealant, resin tag length and microleakage.30 third molars were selected for study. The teeth were randomly divided into 3 groups. Group E: Embrace wetbond, H: Helioseal, G: Guardian seal. Teeth were cleaned with pumice prophylaxis and pretreated with acid etching and bonding agent. The respective pit and fissure sealants were applied. Teeth were placed in 1% methylene blue dye and sectioned mesio-distally into two halves. These were used to assess the microleakage using stereomicroscope and resin tag length using SEM. Viscosity was assessed using Brooke's field viscomet. Viscosity was lowest for Embrace wetbond and highest for Guardian seal. Microleakage scores were highest with Guardian seal and lowest with Embrace wetbond. Resin tag lengths were longer with Embrace wetbond as compared to other groups. There is a definite negative correlation between viscosity, resin tag length and microleakage.

Khogle et al (2012)[16] compared the microleakage and penetration depth of a hydrophilic sealant and a conventional resin-based sealant using one of the following preparation techniques: acid etching (AE) only, a diamond bur + AE, and Er: YAG laser combined with AE, and (ii) to evaluate the microleakage and penetration depth of the hydrophilic pit and fissure sealant on different surface conditions. Eighty recently extracted 3rd molars were randomly assigned to eight groups of ten teeth according to the material, preparation technique, and surface condition. For saliva contamination, 0.1 mL of fresh whole human saliva was used. All samples were submitted to 1000 thermal cycles and immersed in 2% methylene blue dye for 4 h. Sections were examined by a light microscope and analysed using image analysis software . The combination of Er:YAG + AE + conventional sealant showed the least microleakage. The sealing ability of the hydrophilic sealant was influenced by the surface condition. Er:YAG ablation significantly decreased the microleakage at the tooth–sealant interface compared to the non-invasive technique. The hydrophilic sealant applied on different surface conditions showed comparable result to the conventional resin-based sealant.

Bhatia et al (2012)[27] evaluated the retention of two resin based fissure sealants (Embrace and Delton FS+) for a period of twelve months. Sixty eight first permanent molars were sealed for seventeen healthy children in the age group of six‑eight years. According to random selection, the first permanent molars on the right side of both arches were sealed with Embrace (Group A) and on the left side of both arches were sealed with Delton FS+ (Group B). The total retention of Embrace was 23.50% and for Delton FS+ was 17.60% at one year. At one year the total retention of Embrace was 23.50% and for Delton FS+ was 17.60%, however the results were statistically insignificant.

Singla et al (2012)[28] compared the microleakage of traditional composite (Charisma/GlumaComfortBond) and self-priming resin (Embrace Wetbond). Standardized Class V cavities partly in enamel and cementum were prepared in 20 extracted human premolars. Teeth were divided into two groups. Group 1 was restored with Charisma/Gluma Comfort Bond and Group 2 with Embrace Wetbond. The specimens were stored in distilled water at room temperature for 24 h and then subjected to 200 thermocycles at 5°C and 55°C with a 1 min dwell time. After thermocycling teeth were immersed in a 0.2% solution of methylene blue dye for 24 h. Teeth were sectioned vertically approximately midway through the facial and lingual surfaces using a diamond saw blade. Microleakage was evaluated at enamel and cementum surfaces using 10 X stereomicroscope. The statistical analysis was performed using Wilcoxon signed-rank test. Wetbond showed less microleakage at occlusal and gingival margins as compared with Charisma/Gluma Comfort Bond and the results were statistically significant ($P < 0.05$). Class V cavities restored with Embrace Wetbond with fewer steps and fewer materials offers greater protection against microleakage at the tooth restorative interface.

Anna Eliades et al (2013)[29] evaluated the properties of self-adhesive restorative materials used as sealants in comparison with sealants with hydrophobic or hydrophilic monomer. The self-adhesive materials tested were Fusio (FS) and Vertise-Flow (VF) and the sealants Embrace Wetbond (EM/hydrophilic) and Helioseal-F (HS/hydrophobic). The properties tested were: (a) degree of cure (%DC, *n*: 5, ATR-FTIR), (b) extent of oxygen inhibition (*n*: 5, transmission optical microscopy), (c) flow (*n*: 5, ASTM D-4242 method), (d) hardness(VH0.2kp/10s dry/1 w in water), (e) adaptation, microleakage and fissure penetration (*n*: 10, 1% fuschin dye, reflection optical microscopy and ESEM) VF showed the highest % DC(76.1) followed by HS(68.7) and EM(61.3), FS(59.2). HS demonstrated the highest extent of oxygen inhibition (23 _m vs. 13–10 _m of the rest). EM and HS exhibited the greatest flow, followed by FS and VM. The VHN(0.2kp/10s) ranking before and after 1week water exposure was FS≥VF > EM≥HS. Water storage increased VF and reduced HS values. The lowest adaptation and microleakage scores were found in HS.FS and VF after alumina sandblasting showed the worst adaptation and leakage scores, that were improved after acid-etching. Improved fissure penetration was found in HS, EM and FS, VF after acid-etching. Although the self-adhesive materials presented improved setting characteristics, their low flow affected fissure penetration capacity. When combined with enamel acid-etching, adaptation and microleakage scoring were substantially improved in comparison with enamel sandblasting. The sealant with the hydrophobic monomers demonstrated the best sealing characteristics.

Kamal El- Din et al (2013)[7] assessed the nanoleakage and resin tag length of three different Pit and Fissure Sealants using scanning electron microscope. The occlusal surfaces of 15 intact extracted human maxillary first premolars (divided into three equal groups), were cleaned with pumice, etched with 37% phosphoric acid for 15 sec, rinsed and dried. Premolars were then sealed with; Group A: Fisseal Flowable composite, Group B: Vertise Flow composite and Group C: Embrace WetBond. Teeth were stained with modified silver staining technique. With the aid of SEM, nanoleakage was measured using the Dye absorbance method and length of resin tags were determined. This study indicated that, there is a negative correlation between resin tag length and nanoleakage; the longer the resin tags, the lesser the nanoleakage, and the better the cariostatic action of Pit and Fissure Sealants and the use of Vertise Flow composite as good alternative for sealing pits and fissures is recommended.

Prasanna kumar et al (2013)[30] had evaluated and compared the retention and development of caries when sealed with moisture-tolerant resin-based sealant, conventional resin-based sealant with and without a bonding agent, and Glass Ionomer Cement Sealant in young permanent teeth. A total of 80 healthy cooperative children aged 6-9 years who were at high caries risk with all four newly erupted permanent first molars were included in the study. Teeth were divided into 4 groups using a full-factorial design, and each of the molars was sealed with the four different sealant material. Evaluation of sealant retention and development of caries was performed at 6 and 12 months using Modified Simonsen's criteria. The data obtained were tabulated and subjected to statistical analysis using Kruskal-Wallis Test and Mann-Whitney Test. The result from the present study indicated that moisture-tolerant resin-based sealant could be successfully used as a pit and fissure sealant because its hydrophilic chemistry makes it less technique sensitive and simplifies the sealant application procedure.

Joshi et al (2013)[31] in his invitro study 120 therapeutically extracted premolars devoid of any caries, anomalies or morphogenic diversity were collected and distributed equally in three groups (40 in each). Group – I: Composite based Pit and fissure sealant, Group

-II: Compomer- restorative material and GROUP-III: Glass ionomer cement based pit and fissure sealant. Samples were cleaned with slurry of pumice and etched with phosphoric acid etchant. After thorough washing and drying, teeth were treated and cured with three sealants having different composition followed by thermocycling and immersion in methylene blue dye for 24 hours. Teeth were then observed and score was given for microleakage. The sections were photographed to show score of "0", "1", or "2" microleakage and the data was statistically analyzed with the non-parametric test (Kruskal Walis test). Composite material was found better for sealant material as it was showing significantly least microleakage as compare to Glass Ionomer Cement and promising result with compomer. Besides many inventions, researches and nano-technology implementation in dental materials, composite material is comparatively better than Glass Ionomer Cement and compomer as sealant materials.

Priya Subramaniam et al (2015)[32] evaluated and compared the retention and caries incidence with use of the two newly introduced moisture tolerant pit and fissure sealants. One hundred and eight children formed the study group. The glass carbomer sealant and Embrace WetBond sealant were two moisture tolerant sealants used. The sealant was applied on the occlusal surface of the teeth following the manufacturer's instructions. Children were recalled for assessment of sealant retention and the teeth were examined for dental caries on the occlusal surface using mouth mirror and blunt probe following 1, 3,6,12,18 and 24 months. Sealants were assessed according to a modified version of the CCC sealants evaluation system described by Deery et al. At 18 and 24 months, both GC and EBW showed similar pattern of sealant retention At 24 months, enamel caries was observed in 3 teeth sealed with EBW as compared to only 1 tooth sealed with GC. There was no significant difference between the retention of glass carbomer sealant and Embrace WetBond sealant, at the end of 2 years. There was no significant difference in the caries incidence between both these sealants.

Imam et al (2015)[14] had compared the marginal microleakage of fissure sealants and self-adhering flowable composites in permanent teeth. This in vitro, experimental study was conducted on 60 extracted human premolar teeth. The teeth were divided randomly into two groups of 30. In the first group, fissure sealant (Clinpro, 3M ESPE, USA) was placed on the teeth. In the second group, self-adhering flowable composite (Vertise Flow, Kerr, USA) was applied as the sealant. Then, both groups were immersed in 0.5% fuchsin dye solution for 24 hours. Sectioned samples were observed with a stereomicroscope for the extent of dye penetration. Data were analyzed using SPSS 21 and the Mann-Whitney test ($P<0.05$).Microleakage in the fissure sealant group was significantly higher than that in the self-adhering flowable composite group ($P<0.001$). Microleakage was less using self-adhering flowable composite compared to conventional fissure sealant; therefore, self-adhering flowable composite can be used as a suitable fissure sealant in permanent teeth

Akurathi Ratnaditya (2015)[33] in her in vivo study compared and evaluated the retention of the traditional hydrophobic pit and fissure sealant with a hydrophilic resin-based sealant on first permanent molars and to compare the sealant retention in maxillary and mandibular first permanent molars. In this randomized clinical trial 212 permanent first molars from 76 subjects are considered in this study. Out of which 106 right side upper & lower first molars were sealed with Delton FS (Group I) and the remaining 106 left side upper & lower first molars were sealed with Embrace Wet Bond sealant (Group II). Clinical evaluation by both visual and tactile examination was carried out -immediately after sealant application, at 1, 6, 12, 18 and 24 months using Simonsen's criteria. At the end of two years 67.9% of permanent molars of group II showed completely retained sealants compared to 45.3% of permanent molars of group I. Conspicuously 56.6% upper teeth in group II retained sealant, whereas only 17% of upper teeth retained sealant in group I and is statistically significant ($p=0.01$). It can be concluded that hydrophilic sealant may be used as effective pit and fissure sealants especially in children with high risk of caries, excessive salivation, mentally and physically challenged, very young children, uncooperative child and partially erupted molars and community care programs.

Panigraha et al (2015)[34] According to him contamination of etched enamel with saliva has been shown to result in sealant failure. Recently, a hydrophilic sealant has been introduced. In absence of documented literature, this in vitro study was undertaken to ascertain the efficacy of Embrace Wet Bond without reduction of microtensile bond strength in the different moisture contamination. A 5mm block of sealant were built over prepared occlusal surface of 40 non-carious therapeutically extracted third molars which were sectioned into 1mm thick stick and tested using Zwick micro tensile tester. Obtained data were subjected to descriptive analysis, one-way ANOVA and Scheffe's post-hoc tests. Mean microtensile bond strength of Embrace sealant was not significantly lowered in different moisture contamination groups except Group 3 (air drying), which showed very highly significant ($p<0.001$) decrease in μTBS as compared to Group 1 (non-contaminated).Mean μTBS of Embrace sealant remains largely unchanged even in presence of moisture. Owing to its hydrophilic property, this sealant can be a great help in cases where maintaining isolation is difficult.

Sridhar et al (2016)[35] had evaluated and compared the marginal sealing ability of two commercially available pit and fissure sealants. A total of 50 human premolar teeth were used in this invitro study. Group 1 included 25 teeth for application of Clinpro sealant and Group 2 included twenty 25 for application of Helioseal F sealant. Samples were stored in artificial saliva for 72 hours before thermocycling. The samples were immersed in 2% methylene blue solution for 24 hours. The sectioned samples were examined under an Optical Stereomicroscope and compared in terms of the extent of microleakage based on the amount of dye penetration between the sealant and tooth substance interface. The dye penetration scores in both the groups was statistically analysed using Chi square test and Mann Whitney Test. A p-value of less than 0.05 was considered to be statistically significant. On comparison of the microleakage scores of the groups, a statistically significant difference was found between the two groups; indicating a much better performance of Clinpro as compared to Helioseal F.

Guclu Z A et al (2016)[36] conducted an invitro study to characterise the new hydrophilic fissure sealant, UltraSeal XT® hydro™ (Ultradent Products, USA), and to investigate its *in vitro* resistance to microleakage after placement on conventionally acid etched and sequentially lased and acid etched molars. The sealant was characterised by Fourier transform infra-red spectroscopy (FTIR), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), and Vickers indentation test. Occlusal surfaces of extracted human molars were either conventionally acid etched (n=10), or sequentially acid etched and laser irradiated (n=10). UltraSeal XT® hydro™ was applied to both groups of teeth which were then subjected to 2,500 thermocycles between 5 and 55°C prior to microleakage assessment by fuchsin dye penetration. UltraSeal XT® hydro™ is an acrylate-based sealant that achieved a degree of conversion of 50.6±2.2% and a Vickers microhardness of 24.2±1.5

under standard light curing (1,000 $mWcm^{-2}$ for 20 s). Fluoride ion release is negligible within a 14-day period. SEM and EDX analyses indicated that the sealant comprises irregular submicron and nano-sized silicon-, barium-, and aluminium-bearing filler phases embedded in a ductile matrix. Laser preconditioning was found to significantly reduce microleakage (Mann-Whitney U test, $p<0.001$). The lased teeth presented enhanced surface roughness on a 50 to 100 µm scale that caused the segregation and concentration of the filler particles at the enamel-sealant interface. Laser preconditioning significantly decreased microleakage and increased enamel surface roughness, which caused zoning of the filler particles at the enamel-sealant interface.

Deshpande A et al (2016)[37] In his invivo study evaluated the retention rate, marginal integrity and marginal discoloration of two different sealant using rubber dam isolation on molars of 7 to 10 year old children. 20 children of 7 to 10 year with intact deep and retentive fissures, who were reporting to the Department of Pedodontics and Preventive Dentistry were selected. The two sealants that were studied included (a) Conseal F (SDI) and (b) Constic (DMG, Germany). The four permanent first molars of each child were randomly assigned for placement of each of the two materials. The data was collected in an evaluation form provided for every patient. Pit and fissure sealant was applied by clinician and children were examined by blinded investigator. To evaluate occlusal fissure sealants, visual and tactile examination was performed with a mouth mirror and an explorer in follow-up appointments and clinical examination. All the cases were clinically evaluated after 1, 3 and 6 months follow up. The data obtained were tabulated and compared statistically using the Chi-square test of significance for marginal integrity between group I and II at 1 month, 3month and 6month interval. Highly significant difference were found between the groups ($p<0.01$) except for maxillary molars at 1 month follow up which showed significant value (0.029). Results shows significant difference between groups except for 1 month follow up of mandibular teeth. The conseal-F sealant was better than flowable composite as sealant with respect to marginal integrity and anatomical form. Both the materials showed similar results with respect to marginal discoloration.

Hoobi et al (2016)[38] in his study estimated and compared the microleakage of pit and fissure sealant after various methods of occlusal surface preparation. Thirty non-carious premolars extracted for orthodontic reasons were equally divided into three groups. In group one, occlusal fissures were opened with round carbide bur, in group two, occlusal surfaces of the teeth were cleaned with a dry pointed bristle brush and samples of group three were cleaned with a slurry of fine flour of pumice in water using rubber cup. Then fissures of all teeth were etched using 35% phosphoric acid gel prior to placement of Conseal F (SDI) light cured sealant, the teeth were thermocycled, then they were immersed in 1%methylene blue for 24hours. Each tooth was sectioned bucco-lingually to detect the microleakage. Different levels of microleakage were observed among various groups, highest level was recorded for brushing group followed by pumice group, while round bur samples showed the least microleakage when compared with other groups. Statistically the difference was not significant between brushing and pumice groups, while it was significant between round bur and other groups and Concluded that preparation of occlusal surface with round bur was very effective in reduction of microleakage in comparison with the traditional pumice slurry and bristle brush.

Babuji et al (2016)[39] had done a study to evaluate microleakage and shear bond strength of various fissure sealants. Thirty‑ six extracted molars were randomly allocated equally (n = 12) into three groups with three different sealants to evaluate shear bond strength and microleakage at sealant space. Tetric flow (16.8 MPa) recorded the highest shear bond strength and the difference was statistically significant with enamel loc (12.8 MPa). There was no statistically significant difference in relation to microleakage ($P > 0.05$) in the tested groups. and Tetric flow recorded the highest shear bond strength and the difference was statistically significant with enamel loc. However, there was no statistically significant difference among the groups regarding microleakage.

Harsha PP et al (2017)[40] evaluated the marginal microleakage of conventional fissure sealants and self-adhering flowable composites as fissure sealant in permanent teeth. An experimental study was conducted on 40 extracted human premolar teeth. The teeth were divided randomly into two groups of 20. In the first group, fissure sealant (Fissurit F, Voco) was placed on the teeth. In the second group, self-adhering flowable composite (Dyad Flow, Kerr, USA) was applied as the sealant. Then, both groups were immersed in 2% fuchsin dye solution for 24 h. Sectioned samples were observed with a stereomicroscope for the extent of dye penetration. Data were analyzed using Mann–Whitney test ($P < 0.05$). Microleakage in the fissure sealant group was significantly higher than that in the self-adhering flowable composite group. Microleakage was less using self-adhering flowable composite compared to conventional fissure sealant; therefore, self-adhering flowable composite can be used as a suitable fissure sealant in permanent teeth.

Maryam et al (2017)[41] conducted the study to compare the microleakage of class V cavities restored with the newly introduced Embrace Wet Bond class V (EWC) composite resin and conventional Opallis composite resin. In this in vitro study, class V cavities were prepared on 30 extracted bovine incisors, with the gingival floor and the coronal margin of the cavities 1 mm apical and coronal to the cementoenamel junction (CEJ) respectively. In group I, the cavities were restored with Opallis composite resin in association with ExciTE adhesive system (total-etch); in group II, the EWC composite resin was used for restorations. After 500 thermocycling procedures, the teeth were immersed in 0.5% fuchsin solution for 24 hours. Then, the samples were placed within a polyester model and sectioned in the buccolingual direction. The samples were evaluated under a stereomicroscope at ×30 for the penetration of dye. There was signifcantly more microleakage in group II at both the enamel and dentin margins (coronal margin: p = 0.04; gingival margin: p = 0.21). In both groups, microleakage at gingival margins was signifcantly higher than that at coronal margins (group I: p = 0.008; group II: p = 0.26**).** Despite the high speed and the short process of restoration with Embrace WetBond, it is not a reliable restorative material for class V cavities due to its inadequate marginal seal.

Alqarni et al (2017)[42] had carried out an in vitro study to evaluate the microleakage and sealing ability of Helioseal F, Seal-it and GCP Glass seal and Compared sealing ability of GCP Glass seal with resin fissure sealants. 80 extracted premolars free of caries, cracks or morphogenic diversity were collected and distributed equally in four groups (20 in each). Group-I (Helioseal F), Group-II (Seal-it), Group-III (GCP Glass seal) and Group-IV (GCP Glass seal without etching). Samples were cleaned with pumice slurry and etched with phosphoric acid etchant except Group-IV without etching. Air and water sprays were used for washing and drying, the teeth were treated with sealants followed by thermocycling and immersion in methylene blue dye for 48 hours. The samples were sectioned and examined under a stereomicroscope at x15 magnification then the score was given for microleakage. Seal-it material was the best sealant material as it was showing significantly least microleakage as compare to Helioseal F and GCP Glass seal and the microleakage was found to be the highest for the GCP Glass seal with etching..

Zayat et al (2017)[43] had evaluated microleakage and antimicrobial efficiency of two pit and fissure sealants, SeLECT Defense® and Ultra Seal XT Plus. Intact human premolars were selected for the microleakage test and assigned randomly to two groups (n=20) according to the sealant used. After sealants application, the specimens were thermocycled then subjected to silver nitrate staining. Thin sections were obtained from each specimen, photo-developed then observed with polarizing light microscope. Microleakage was assessed according to a (0-4) scoring method. Data obtained from microleakage and bacterial viability testes were analyzed using one-way ANOVA, Chi-square tests and Yuen-Welch tests ($\alpha=0.05$). Microleakage scores were significantly lower with SeLECT Defense compared to Ultra Seal XT Plus ($p<0.01$).

Nahid et al (2017)[44] had compared the one-year clinical success of a hydrophilic and a hydrophobic fissure sealant in permanent first molars. This split-mouth clinical trial was conducted on 23 six to nine year olds who had four fully erupted sound first molars. Helioseal-F and Embrace sealants were randomly applied on the first molars, and follow-ups were scheduled at three, six and 12 months to examine the teeth according to USPHS criteria (retention, marginal adaptation, color match, surface smoothness and caries recurrence). The Wilcoxon signed rank test, the Friedman test and the Mann Whitney test were applied for statistical analyses (P0.05). Friedman test revealed no significant difference in any of the five parameters at different time points in any sealant group ($P>0.05$).

Embrace hydrophilic and Helioseal-F hydrophobic sealants have the same one-year clinical success rate.

Pitchika V et al (2018)[45] evaluated the shear bond strength (SBS) and microleakage of a new self-etch adhesive-based fissure sealant (EG) on a prismatic enamel in comparison to conventional fissure sealing with 30 s acid etching (CG). The fissures were sealed according to the manufacturer's instructions. Each group was divided into 3 subgroups: 1-day water storage, 3-month water storage, and 5,000× thermocycling. After measuring SBS using the Ultradent method, failure mode was analyzed. In additional 16 teeth, microleakage was tested using dye penetration method. Pairwise comparisons were analyzed using Mann-Whitney U-Test. Multiple linear regression was performed to assess the factors influencing on SBS. EG had significantly lower mean SBS (4.1 MPa±2.1) than the CG (17.6 MPa±6.4). CG (1.1%) performed significantly better than the EG (12.8%) in microleakage analysis. The tested EG yielded significantly inferior results and its clinical use should be decided after weighing its pros and cons.

Amey manohar panse (2018)[46] evaluated the various parameters and compare the efficacy of the new material to the conventional sealant. Seventy-six non carious primary molars were randomly assigned into two groups. Fissurit F (Group A) and Constic (Group B). Each group was further subdivided into four groups:G1–Microleakage (n=18), G2–Fracture strength (n=18), G3–Tensile strength (n=20) ,G4–Shear strength h(n=20)The parameters were evaluated and compared to check the efficacy of the two groups. Nonparametric tests Kruskal–Wallis and Mann–Whitney tests were applied to the values obtained to compare microleakage and fracture strength and comparison of shear and tensile bond strengths is done by independent t-test. Microleakage and fracture strength of Constic were found to be better, Use of a flowable composite without bonding agent is a good alternative for sealing pits and fissures.

Nahvi A et al (2018)[47] conducted an invitro study to compare microleakage in self-etching fissure sealants and conventional fissure sealants with total-etch or self-etch adhesive systems. This experimental in vitro study was conducted on 60 healthy third molars extracted from humans. The first group received Acid etch + Clinpro sealant, the second group received Acid etch + Single bond 2 + Clinpro sealant, the third group received Single bond universal (self-etching bonding) + Clinpro sealant, and the fourth group received prevent seal self-etching sealant. An incision was made on the teeth after they were immersed in methylene blue 5%. The samples were then examined under a stereomicroscope and the dye penetration rate was measured based on the Williams and Winter criteria. The Kruskal–Wallis and Mann–Whitney tests were used for data analysis in SPSS-18 ($P < 0.05$). The results showed that the use of bonding results in a significant reduction in the microleakage of fissure sealants. The microleakage caused when using self-etch fissure sealant was not different from that caused by the use of the conventional method.

Sandra Marıa et al (2018)[48] evaluated the microleakage of a conventional light-cured, resin-based fissure sealant (LCRBS), GrandiO Seal, and a resin-modified glass ionomer sealant (RMGIS), Vitremer, after application of a fluoride varnish, Bifluorid 12, on demineralized enamel.80 human third molars were divided into eight groups. The groups combined the three study factors (1) type of enamel (intact or demineralized); (2) enamel non-varnished or varnished with Biflourid12; and (3) type of sealant (GrandiO Seal or Vitremer. The percentage of microleakage after thermocycling was measured using imaging analysis software. The Kruskal-Wallis plus Dunn tests were used to compare differences in microleakage in the different groups. The lowest microleakage was in the unvarnished groups, and was the same for GrandiO Seal and Vitremer. When varnish was applied, microleakage was greater in demineralized enamel than in intact enamel for both LCRBS and RMGIS.The application of fluoride varnish on demineralized enamel increases the microleakage of both GrandiO Seal and Vitremer.

Alsabek et al (2019)[49] conducted a study which was a single-blind, split-mouth, randomized controlled clinical trial. Forty patients between age 6–9 were selected. First permanent molars' occlusal surfaces with scores of 1, or 2 according to the International Caries Detection and Assessment System II (ICDAS II) and with scores between 14–30 by using DIAGNOdent device (Kavo®, Biberach, Germany) were selected and readings recorded. One side of the mouth was randomly chosen to have either the moisture tolerant resin sealant or the glass ionomer sealant placed, and then the second material was placed on the other side. The retention of these materials was analyzed at 3 and 6 months. The sealants were then removed and DIAGNOdent readings were subsequently taken. After three months, full retention was found in 38/40 (95%) teeth in Group A (Embrace™ WetBond™) and 35/40 (87.5%) teeth in Group B (Fuji TRIAGE®). Additionally, no sealant suffered a total loss in group A, whereas, three sealants were totally lost (7.5%) in group B. The difference in sealant retention in two groups in this period was not found to be statistically significant ($P > 0.05$). At six months, full retention was found in Group A 34/40 (85%) and 25/40 (62.5%) in Group B. Also, the partial loss in Group A was 2/40 (5%) whereas in Group B 7/40 (17.5%). Also, the total loss was 4/40 (10%) and 8/40 (20%) in Group A and B, respectively. The difference in sealant retention in two groups after six months follow-up was found statistically significant ($P < 0.05$).Embrace™ WetBond™ showed superiority over the glass ionomer sealant tested in retention after six months follow up.

Arora et al (2019)[50] evaluated and compared the depth of penetration and effect of resin infiltration system (Icon) and other conventional pit and fissure sealants (Clinpro and Embrace) on enamel surface properties of WSLs. Freshly extracted premolars were obtained. Enamel surfaces were treated with resin infiltrant and fissure sealant. Depth of penetration and surface roughness of specimens were measured with an optical profilometer.

Microhardness was determined by a Vickers' hardness tester. Normality of the data was checked by Shapiro–Wilk test. Icon resin infiltrant showed the highest depth of penetration and microhardness followed in descending order by Embrace and Clinpro whereas Clinpro was found to cause maximum surface roughness followed in descending order by Embrace and Icon. The resin infiltration technique for treating WSLs seems adjusted to the philosophy of minimally invasive dentistry in a single appointment, making it beneficial for the patients, especially children.

Khatri et al (2019)[51] evaluated and compared the retention rates and development of caries in permanent molars of children sealed with amorphous calcium phosphate-containing (Aegis™) and moisture-tolerant fluoride-releasing (Embrace WetBond™) sealant over a period of 1 year. This was a double-blind, split-mouth, randomized controlled trial among children aged 6–9 years. Sixty-eight permanent mandibular first molars in 34 children were randomly assigned to be sealed with Aegis™ or Embrace Wetbond™ sealant. The follow-up examinations were conducted at 3, 6, and 12 months for evaluating the retention and development of caries. . Within-group comparison of retention and development of caries at 3, 6, and 12 months was evaluated using the Friedman's test. At 12 months, 23 of 32 (72%) sealants were completely retained in Aegis™, whereas 21 of 32 (65.6%) were retained in Embrace Wetbond™ group. There was no significant difference in the retention rates of Aegis™ and Embrace Wetbond™ sealants at 12 months (*P* > 0.05). Aegis™ was superior to Embrace Wetbond™ sealant as Aegis™ exhibited higher retention and lower caries scores.

Sara Arastoo et al (2019)[52] compared the microleakage of a flowable nanocomposite and materials conventionally used as pit and fissure sealants. A total of 185 extracted mandibular third molar teeth were selected and randomly divided into 5 groups (n=36): flowable nanocomposite, flowable composite, filled sealants, nano-filled sealants, and unfilled sealants. Five teeth were reserved for examination under a scanning electron microscope (SEM). The samples were thermocycled (5-55°C, 1-minute dwell time) for 1000 cycles and immersed in 0.2% fuchsine solution for 24 hours. Teeth were sectioned buccolingually. Microleakage was assessed qualitatively and quantitatively by means of dye penetration and SEM. Data were analyzed using chi-square, Kruskal-Wallis, and Bonferroni-corrected Mann-Whitney U tests. Qualitative microleakage assessment showed that flowable composite and nanofilled flowable composite had almost no microleakage.

Rishi et al (2019)[53] had evaluated the microleakage and depth of penetration of four recently available pit & fissure sealants. Forty sound extracted human molars were randomly divided into 4 groups(N=I0). After sealant placement, the teeth were stored in simulated saliva for 7 days and thermocycled (500 cycles; 5°C, 37°C and 55°C), isolated, immersed in 5% methylene blue dye for 24 hrs, subsequently embedded in acrylic resin and sectioned longitudinally in a buccolingual direction The sections were analyzed for leakage using an stereomicroscope. Microleakage was assessed using a dye penetration scoring system (score=0-3) and also measured in millimetres. All materials exhibited a similar pattern of penetration into the pits and fissures, and no statistically significant difference between the study groups was found. ($p>0.05$). Microleakage was found to be highest for the flowable giomer, and least for the Gic Fuji VII. Under the conditions used in this in vitro study the flowable giomer, showed higher microleakage depth while micro leakage of Gic Fuji VII was comparatively better.

ARMAMENTARIUM USED IN THE STUDY

- Gloves
- Mouth mask
- Periodontal Curettes
- Explorer
- Hydrogen per oxide
- Artificial saliva
- Slow speed contra angle hand piece
- Rubber cup
- Pumice slurry
- Applicator brush
- EAZETCH gel (37%Phosphoric acid)
- Conseal F (SDI)
- Embrace Wet bond (PULPDENT)
- Light curing unit (Blue phase NMC LED, Wood pecker Mini S)
- Self cure glass ionomer cement (Golden yellow, Japan)
- Mixing pad and Spatula
- Nail varnish (Pink, Purple)
- 1% methylene blue dye solution
- Diamond disc (0.02mm)
- Self cure acrylic resin (Clear, Pink)
- Hard tissue microtome (Accutome 100 saw microtome)
- Stereomicroscope. (Lawrence and mayo-meiji techno)

METHODOLOGY:

This invitro study was approved by the Institutional Human Ethical Committee.

SAMPLE SIZE CALCULATION:

Sample size was calculated using G Power 3.0.10 software

Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	One
	Parent distribution	=	Normal
	Effect size d	=	0.74
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Non centrality parameter δ	=	2.5566567
	Critical t	=	1.6788516
	Df	=	45.7464829
	Sample size for Group I	**=**	**25**
	Sample size for Group II	**=**	**25**
	Total sample size	=	50
	Actual power	=	0.808

A total of 50 human premolar teeth extracted for orthodontic purpose were included in the study. The teeth included were non hypoplastic and non carious and free of cracks and restoration with an ICDAS code 0-1.

Initially the root portion of the teeth were cleaned with periodontal curettes to remove the remnants of soft tissues and debris, disinfected with hydrogen peroxide and stored in artificial saliva at room temperature. Artificial saliva was prepared following the composition recommended by Sieck et al.[54]

Cleaning of occlusal surfaces:

The occlusal surface of the teeth were cleaned with pumice slurry and rubber cup using low speed contra-angle handpiece and no invasive technique was used. Then rinsed and dried with air water spray.

Fifty human premolar teeth were randomly divided into two groups.

Group I – 25 teeth for Conseal F application

Group II – 25 teeth for Embrace wet bond application.

CONSEAL F (SDI)

Composition:

- Urethane dimethacrylate
- Titanium di oxide
- Silica
- Fluoride releasing system
- Photo initiator

Features:(According to manufacturer)

- ❖ Continuous fluoride release for extra protection
- ❖ Self priming

- Self adhesive
- Colour - Natural tooth shade

EMBRACE WET BOND: (PULPDENT)

Composition:

- Uncured acrylate ester monomers -55-60%
- Silica, amorphous -5%
- Glass Filled Resin 36.6 % Di, tri- and multifunctional acrylate monomers

Features:(According to manufacturer)

- Continuous fluoride release for extra protection
- Self priming
- Self adhesive
- Hydrophilic and hydro balanced
- Water miscible
- Colour - Natural tooth shade

Pit and fissure sealant Placement:

GROUP -I

The enamel on the occlusal surface (which includes pit and fissures) was etched with EAZETCH gel (37% phosphoric acid gel) for 20 seconds then rinsed with water for 20 seconds and dried with air syringe for 10 seconds and inspected visually for uniform frosty appearance.

After etching and drying CONSEAL -F was applied with disposable cannula supplied by the manufacturer and sharp explorer is used to remove the air bubble and to check the uniform flow for 15 seconds.

GROUP -II

Occlusal surfaces were etched and rinsed as similar, as above. According to the manufacturer with Embrace Wet Bond, the typical dull, frosted appearance of the etched surface is not needed. The surface can be lightly dried with a cotton pellet to remove the excess moisture. The sealant Embrace Wet Bond was applied as per manufacturer's instructions with disposable cannula supplied by the manufacturer and sharp explorer is used to remove the air bubble and to check the uniform flow for 15 seconds.

Then the pit and fissure sealants were cured with light curing unit for 20 seconds and stored in artificial saliva for 72 hours before thermocycling.

Thermocycling:

Thermocycling was done and the purpose of thermocycling is to simulate the oral environment. In this study manual method of thermocycling was carried out. The teeth were thermocycled for 500 cycles between 5°C and 55°C with 30 second dwelling time.

Samples sectioning:

After thermocycling the samples were carefully coated with two layers of nail varnish (Purple- Conseal F, Pink-Embrace Wet Bond) excluding 1mm around the sealant and the root apices of the teeth were sealed with GIC(Golden yellow,Japan) to prevent seepage of dye. Then the samples were immersed in 1% methylene blue dye for 24 hours, washed in plain water to remove excess dye. Then the crown and root portion were sectioned using diamond disc and high speed lathe.

Then the crown portion of the teeth were embedded in self-cure acrylic resin (Pink - Conseal F, Clear-Embrace Wet Bond) and sectioned buccolingually using hard tissue microtome (Accutome 100 saw microtome) under water coolant to avoid thermal damage resulting in section sample of 1mm thickness.

Microleakage Evaluation:

The sectioned specimen were examined under an optical stereomicroscope (Lawrence and mayo-meiji techno) of 40 X magnification, for dye penetration and the images of the sectioned teeth samples were recorded.

The extent of microleakage was evaluated based on amount of dye penetration between the sealant and the tooth substance interface using Williams B and Winter GB criteria[55]

Criteria for grading microleakage:

Grade 0: No dye penetration

Grade 1: Dye penetration extending 1/3rd the total length of interface between sealant and tooth structure.

Grade 2: Dye penetration extending between 1/3rd and 2/3rd the total interface length

Grade 3: Dye penetration extending beyond 2/3rd of the total interface length.

Statistical analysis:

Data was collected, tabulated and statistically analysed using IBM SPSS Version 20.0 (IBM Corp. Released 2011.IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.) The chi-square test was used to analyse frequency distribution of microleakage scores. Inter group comparisons of microleakage scores was done using Mann-Whitney test. The level of significance was considered at P value <0.05.

PHOTOGRAPHS/FIGURES

Figure -1 Armamentarium used in the study

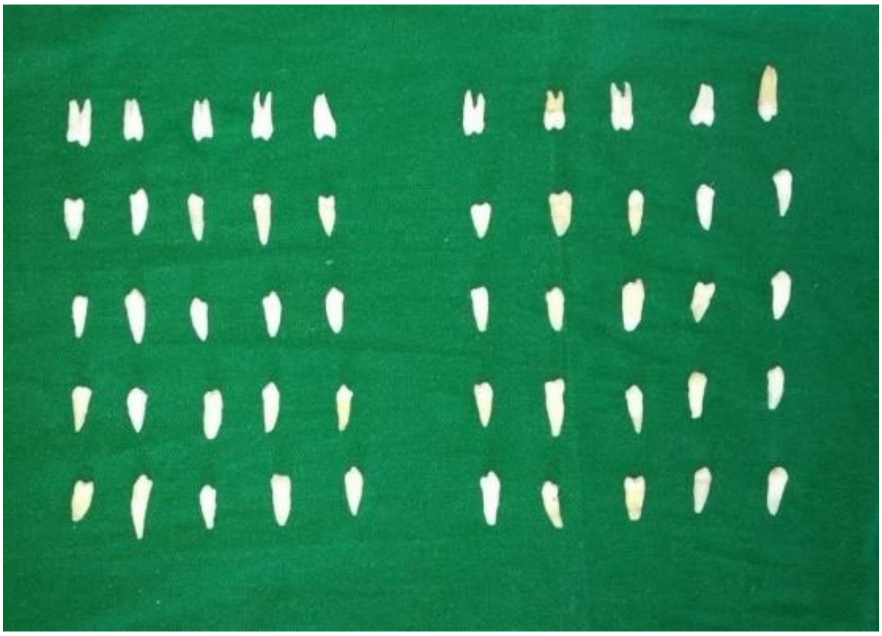


Figure -2 Premolars collected for the study

Figure-3 Instruments used for Tooth prophylaxis

Figure -4 Pumice prophylaxis

Figure- 5 Application of Etchant

Figure - 6 Application of Conseal F

Figure - 7 Application of Embrace Wet Bond

Figure - 8 Process of curing

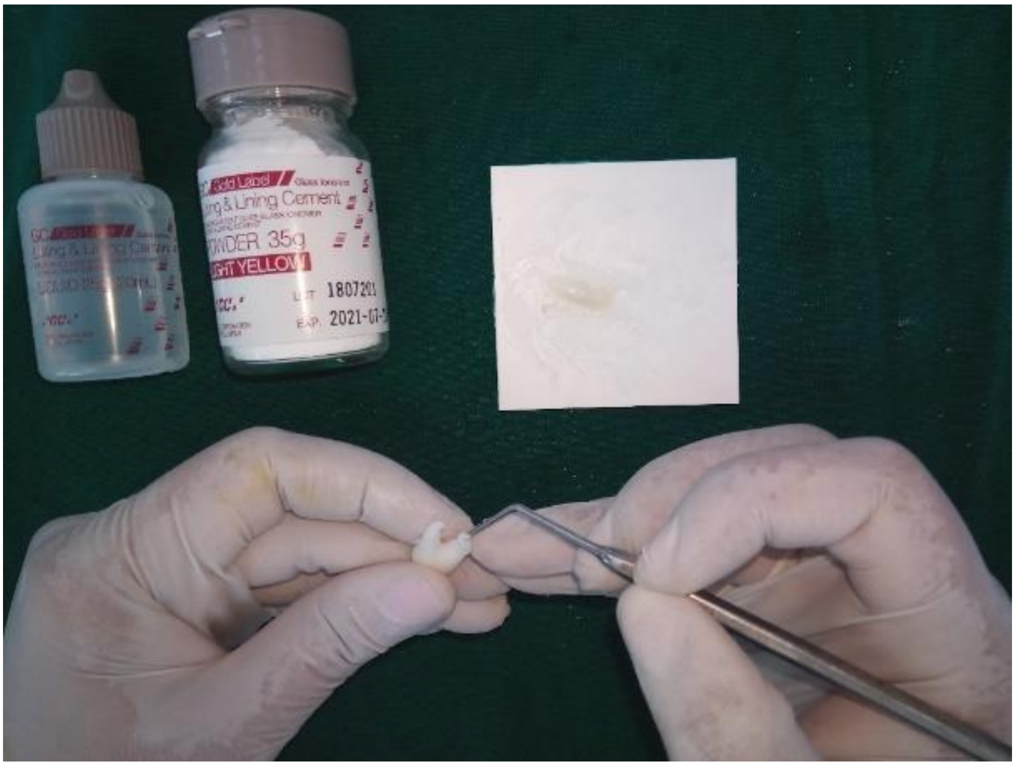


Figure - 9 Sealing of tooth apex

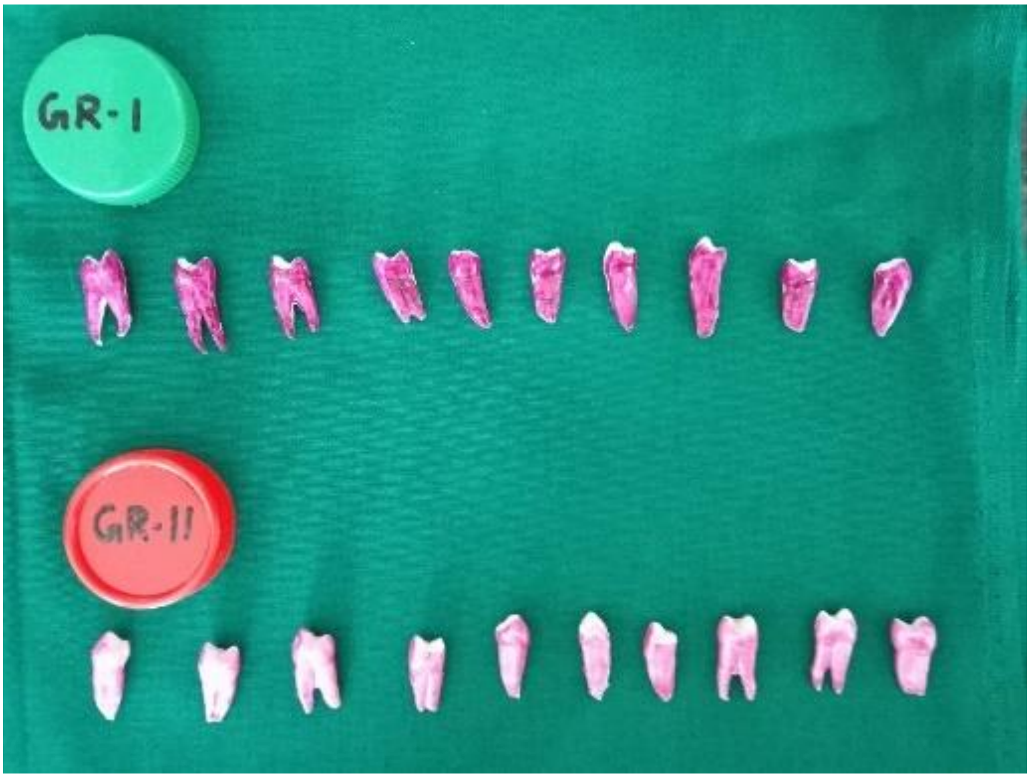


Figure - 10 Application of nail varnish

Figure - 11 Application of nail varnish leaving 2 mm around the sealant

Figure - 12 Methylene blue

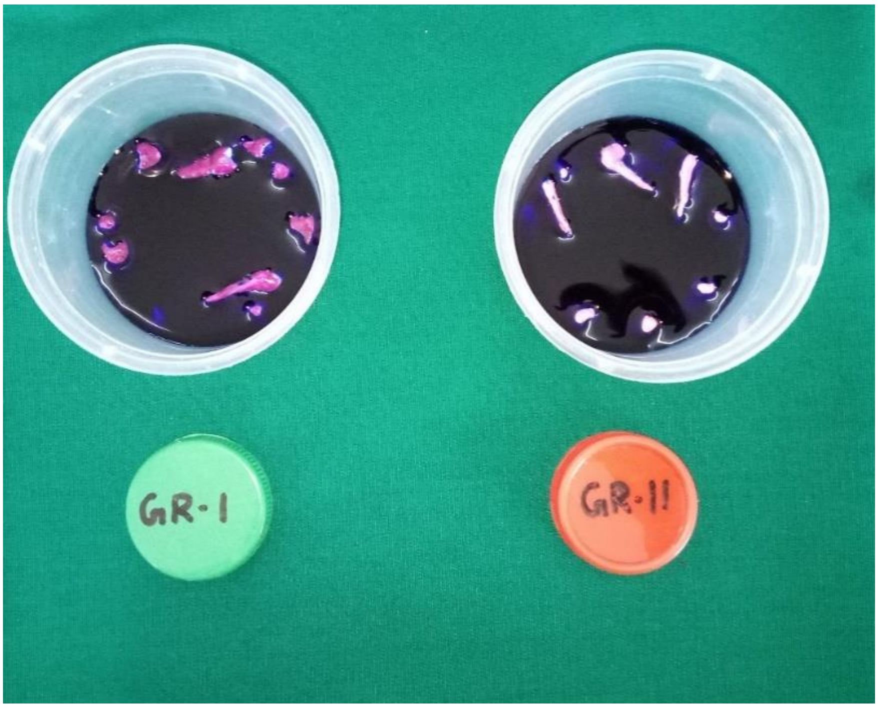


Figure - 13 Immersion of samples in methylene blue

Figure - 14 Crown portion of tooth embedded in clear acrylic resin

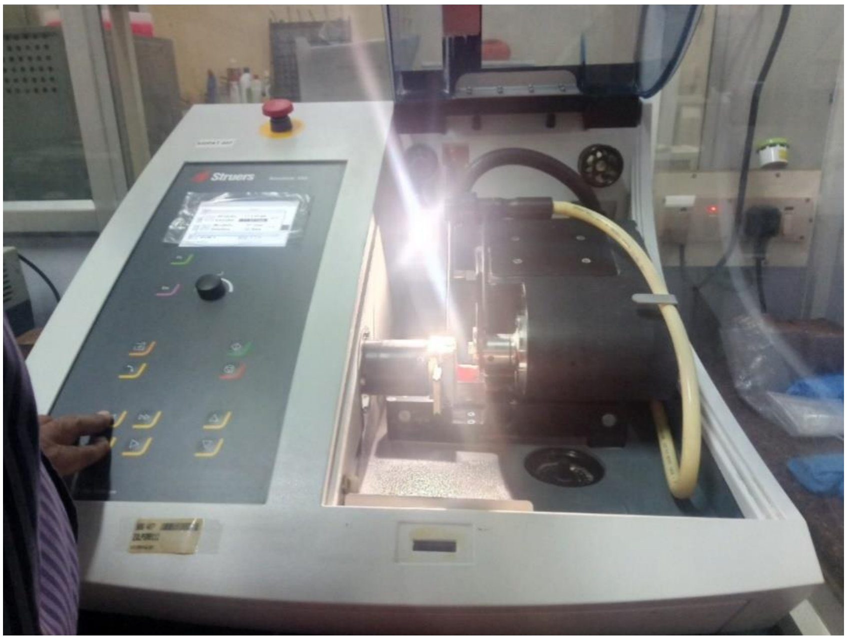


Figure - 15 Sectioning of Tooth Samples in Hard tissue Microtome

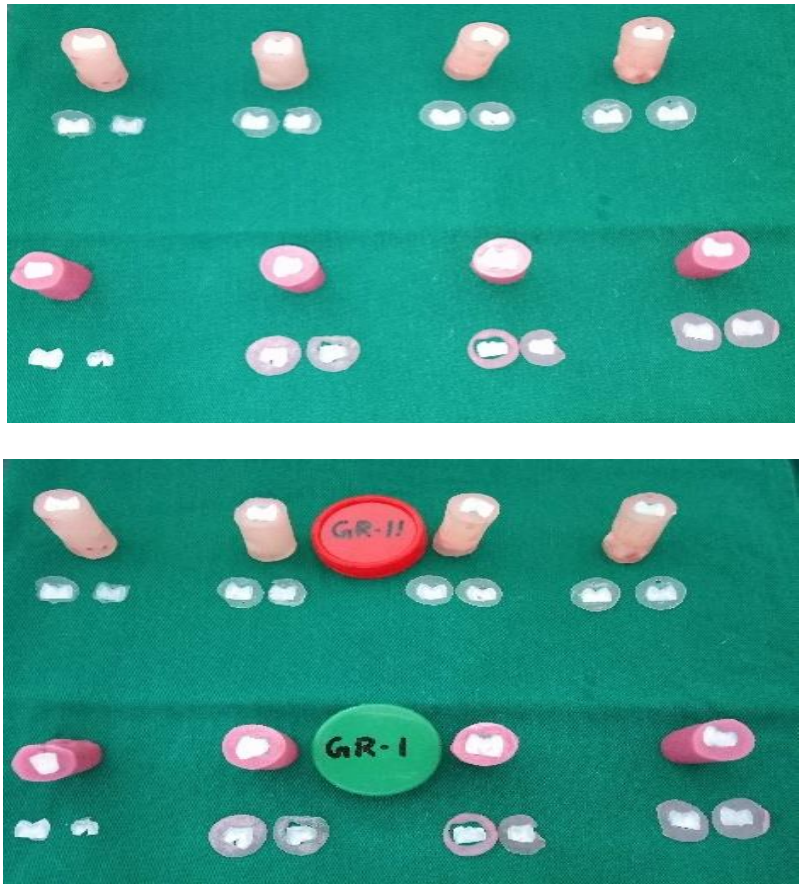


Figure - 16 Sectioned samples

Figure - 17 Stereomicroscope

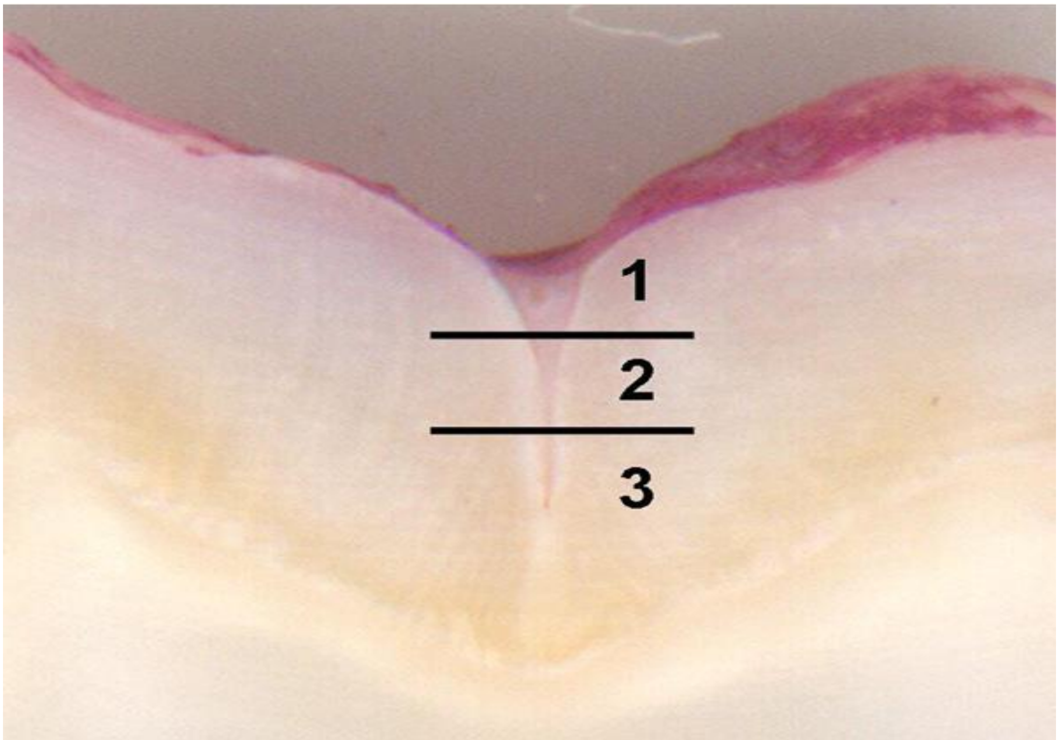
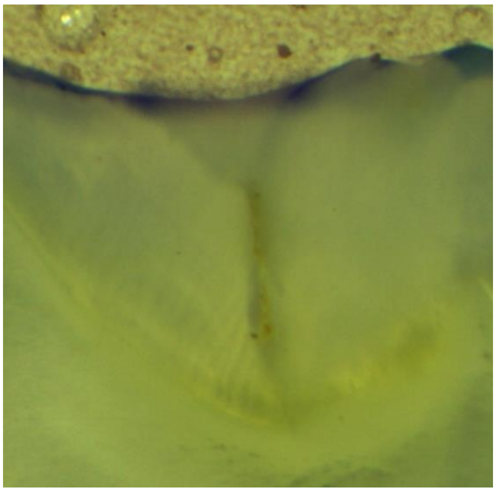
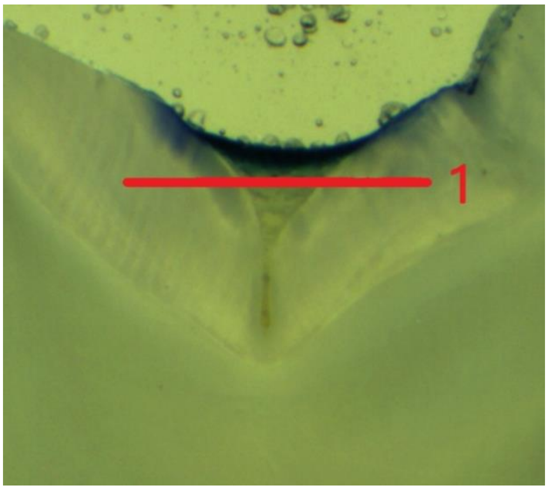


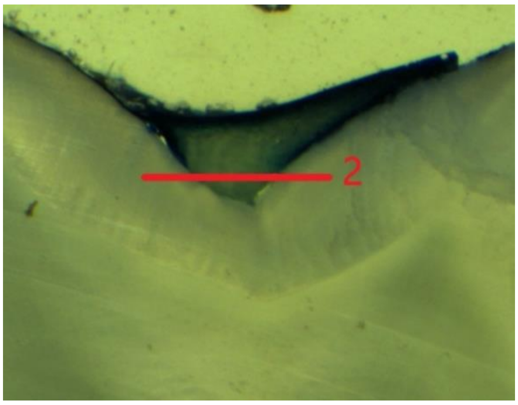
Figure - 18 Scoring criteria



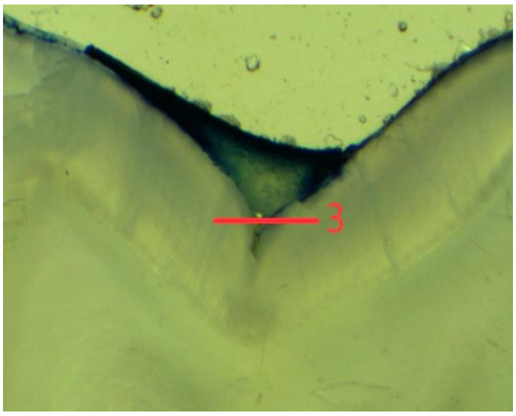
Grade 0



Grade 1



Grade 2



Grade 3

Figure - 19 Microleakage photographs

RESULTS

The present invitro study was carried out to evaluate and compare the marginal sealing ability of Conseal F and Embrace Wet Bond.50 extracted premolar teeth which were non hypoplastic and non-carious removed for orthodontic purpose were divided into 2 groups of 25 each. 25 teeth for application of Conseal F classified as Group I and 25 teeth for application of embrace wet bond as Group II. Teeth were cleaned with pumice prophylaxis and pre-treated with acid etching and the respective pit and fissure sealants were applied, cured and the samples stored in artificial saliva for 72 hours before thermocycling.

Thermocycling was done and the samples were carefully coated with two layers of nail varnish (Purple-Conseal F, Pink-Embrace Wet Bond) excluding 1mm around the sealant and the root apices of the teeth were sealed with GIC(Golden yellow, Japan) to prevent seepage of dye .Then the samples were immersed in 1% methylene blue dye for 24 hours, washed in plain water to remove excess dye. The crown and root portion were sectioned using diamond disc and high-speed lathe.

Then the crown portion of the teeth were embedded in self-cure acrylic resin and sectioned buccolingually using hard tissue microtome to a thickness of 0.1mm. The sectioned specimen was examined under an optical stereomicroscope of 40 X magnification for dye penetration and the images of the sectioned teeth samples were recorded.

The extent of microleakage was evaluated based on amount of dye penetration between the sealant and the tooth substance interface using Williams B and Winter GB criteria. Data was collected, tabulated and statistically analysed using IBM SPSS Version 20.0 The chi-square test was used to analyse frequency distribution of microleakage scores. Inter group comparisons of microleakage scores was done using Mann-Whitney test.

Table 1 shows the Frequency distribution of microleakage grades for the study variables In Group I (Conseal F) the number of samples with microleakage grades 0,1,2,3 was 9,13,3,0 respectively with the % of 36,52,12. In Group II (Embrace Wet Bond) the number of samples with microleakage grades 0,1,2,3 was 1,5,9,10 respectively with the % of 4,20,36,40 respectively.

Table 2 explains Chi square cross tabulation for the distribution of cases in various microleakage grades among the groups with a P value <0.001 which reveals that there is a significant difference in the distribution of microleakage grades among the two groups, owing to the greater distribution of cases among the higher grades (Grades 2 and 3) in Group II.

Table 3 shows descriptive statistics for the study variables. Since the outcome variable (Microleakage) is measured in ordinal scale (as grades), Mean and Standard deviation cannot be calculated for the data, hence Mean ranks were used to classify the data. The mean rank for Group I is 16.26 whereas Group II is 34.74

Table 4 Shows the comparison of microleakage grade between the two groups Since the outcome is measured in an ordinal scale, the non-parametric equivalent of Student's 't' test, Mann Whitney U test is used here reveals that there is a highly significant difference (P <0.001) in the microleakage grade between the two groups owing to the increased microleakage in group II (Mean rank of Group II > Mean rank of group I).

When the two groups (Group I- Conseal F and Group II- Embrace Wet Bond) were compared in terms of the extent of microleakage based on the amount of dye penetration between the sealant and tooth substance interface, it was seen that in Group 1, 9 samples (36%) had no dye penetration (Grade 0) while in Group 2, 1 samples (4%) demonstrated Grade 0. Similarly, Group 2 had the most extensive dye penetration (Grade 3) in 10 of 25 samples (40%). On the other hand, Group 1 had 0 of 25 samples (%) in the Grade '3' category. This difference in frequency of distribution between the groups were found to be significant at P <0.001, denoting that greater distribution of cases among the higher grades (Grades 2 and 3) in Group II.

On comparison of the microleakage scores of the groups, a statistically significant difference was found between the two groups with a P-value <0.001 indicating a much better performance of Conseal F as compared to Embrace Wet Bond.

Representative tooth sections for Grade 0, Grade1, Grade 2 and Grade 3 have been shown.

TABLES AND GRAPHS

Table 1. Frequency distribution of microleakage grades for the study variables

Group	Microleakage score	N	%
Conseal F	0	9	36.0
	1	13	52.0
	2	3	12.0
	Total	*25*	*100.0*
Embrace Wetbond	0	1	4.0
	1	5	20.0
	2	9	36.0
	3	10	40.0
	Total	*25*	*100.0*

(N = No of samples in each of the microleakage grades)

Table 2. Chi square cross tabulation for the distribution of cases in various microleakage grades among the groups

Groups	**Microleakage Grade**				**Chi square**	**Df**	**P value**
	0	**1**	**2**	**3**			
Conseal F	9	13	3	0	22.95	3	<0.001
Embrace Wetbond	1	5	9	10			

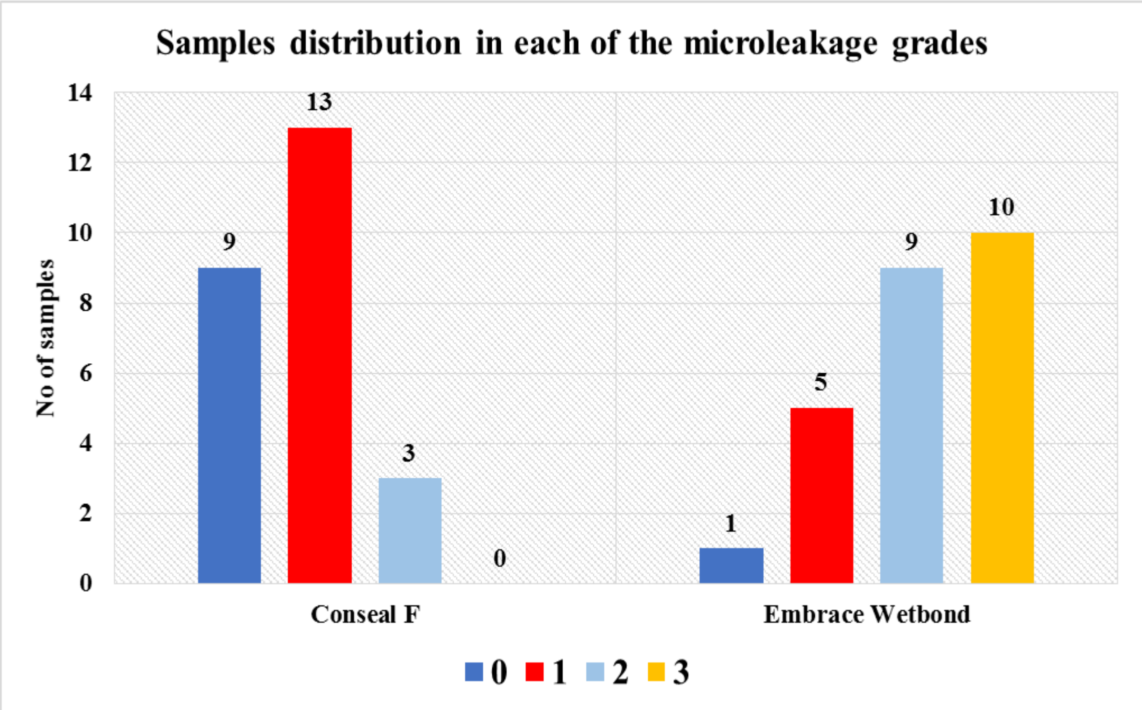
Table 3. Descriptive statistics for the study variables

	Group	N	Mean Rank	Sum of Ranks
Microleakage Grade	*Conseal F*	25	16.26	406.50
	Embrace Wetbond	25	34.74	868.50

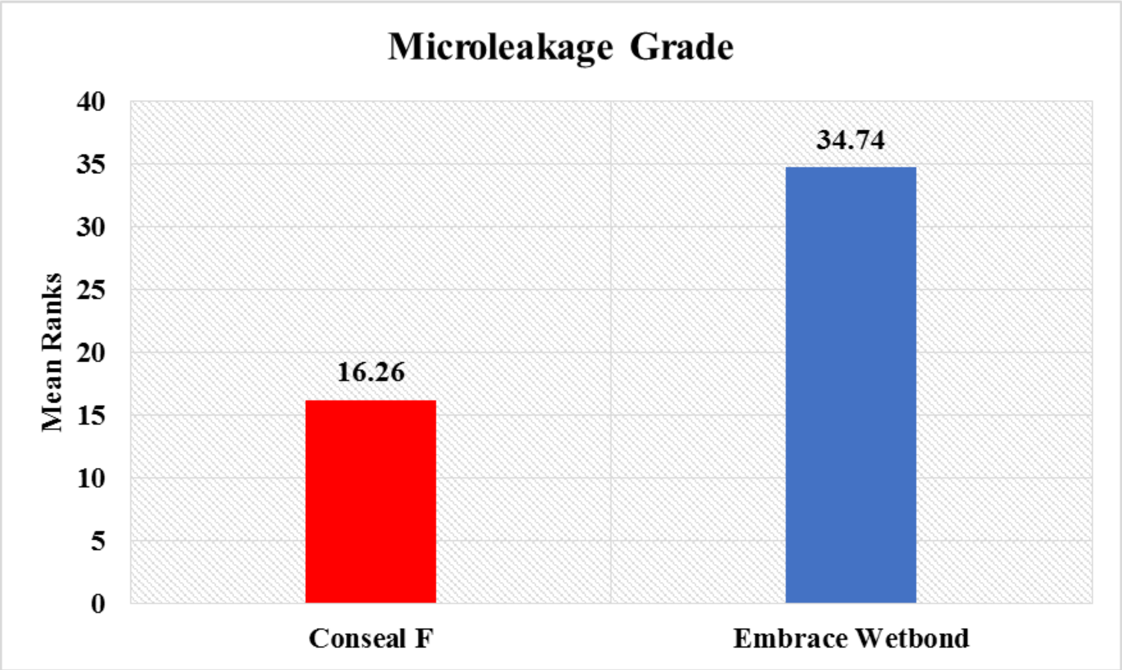
Table 4 Shows the comparison of Microleakage grade between the groups

	Group	Mean Rank	Mann Whitney U	Z statistic	P value
Microleakage Grade	*Conseal F*	16.26	81.5	4.663	<0.001
	Embrace Wetbond	34.74			

Graph 1 shows the frequency of distribution of microleakage scores of two test groups



Graph 2 shows Mean ranks for the two Pit and Fissure sealant groups



DISCUSSION

Dental caries is a microbial disease which is caused by an ecological shift in the bacterial biofilm composition and activity, when exposed over time to fermentable carbohydrates, which leads to a break in the balance between demineralization and remineralization.[56]

Major focus of modern dentistry in the present era is to preserve and stimulate preventive measures as often as possible instead of invasive procedures.[57] Current preventive strategies are fluoride therapy either systemic or local application, Dietary regulation, Plaque control, Pit and Fissure sealant therapy.

Although fluoride therapy is considered to be the gold standard, it is effective only on reducing smooth and interproximal caries not on occlusal caries.[15] Evidential studies done by Brown et al. [1996] and Kastle et al. [1996] showed that more than 90% of dental caries in fluoridated communities is exclusively pit and fissure caries.

According to Manton and Messer (1995) Pits and fissures which are present on the occlusal surface of the posterior teeth are more susceptible to caries formation.[58]

Occlusal pits and fissures are classified by Nagano into five major types as V, U, I, IK and Inverted Y. according to him there exists relationship between the fissure morphology and the susceptibility of the tooth to caries and concluded that V- and U-type fissures are self-cleansing and have less risk of developing caries than other types (I, IK, and Y). U-type (14%), V-type (34%), I-type (19%), IK-type (26%), and Inverted Y-type was 7%.[5]

Because of the morphology of the pit and fissures which favors the food retention thereby providing shelter and favorable environment for the microbes studies have been documented that the occlusal surfaces of posterior permanent and primary teeth are known to be the most susceptible areas for the development of dental caries lesions.

Due to the narrowness and tortuosity of the pit and fissures (~0.1mm wide) they are not only considered to be ideal site for food accumulation but also inaccessible areas for the mechanical debridement with toothbrush bristles which is (0.2mm wide) too large to penetrate most of the fissures.[26]

The other factor which is considered to be natural self-cleanser of food debris is saliva, fails in the pit and fissures due to their morphology.[59]

Several studies had proved sealing these caries susceptible sites with a dental material which acts as mechanical barrier between enamel and biofilm thus prevents food accumulation thereby preventing caries formation.

Hence the concept of pit and fissure sealants evolved. The journey of development of pit and fissure sealant started with idea of obliteration of the fissures which was given by Arthur in 1987 undergoes various modifications in the materials and techniques such as blocking off the susceptible fissures with zinc phosphate cement, mechanical fissure eradication, prophylactic odontotomy, and chemical treatment with silver nitrate.[8]

For more than thirty years, pit and fissure sealants have been recommended for caries prevention by researchers and practitioners. In 1950s Dr.Buonocore was the first person to introduce acid etching technique of enamel with 85% phosphoric acid for 60 sec. With this ideology by 1960s first pit and fissure sealant methyl cyanoacrylate was developed which utilizes acid etching technique. Cyanoacrylates were not suitable as they undergone bacterial degradation in the oral cavity.[60]

In 1965 Bowen reported that BISGMA, a resin material which bond well with acid etched enamel as well as resistant to bacterial degradation. Hence Buonocore and Cueto tried this resin to seal the pit and fissures in 1967. The first commercial dental pit and fissure sealant was introduced in 1971; it was Nuva-Seal (LD Gaulk, Milford, Del).[61]

Commonly two types of pit and fissure sealants in practice

1.Resin based

2.Glass ionomer based

1. Resin based pit and fissure sealants

BISGMA is the viscous resin formed by reacting bisphenol A and glycidyl methacrylate which is similar to the composition of composites used for dental restoration but differs only in viscosity. To obtain low viscosity 3 parts of BISGMA mixed with 1 part of methyl methacrylate or tri ethylene glycol or diurethane dimethyl acrylate.[11]

According to their mechanism of polymerization resin-based sealants are categorized into four generations.

1. First generation sealants that were activated using an ultraviolet light are no longer in the market.[62]

   Ex: Cyanoacrylate,
2. Second generation sealants are BIS-GMA di-methacrylate, can be self-cured and chemically cured. The major drawback in this is the etched enamel surfaces is contaminated with water and oral fluids, and this leads to incomplete penetration of acrylic resin and improper bonding which leads to marginal failure[63] and
3. Third generation- sealants is of photo activated resin which uses visible light,
4. Fourth generation - fluoride-releasing sealant.[64]

Another classification is filled and unfilled resin systems based on the presence or absence of filler particles on the system.

Different types of sealants are also available such as clear, tinted, and opaque dental sealants based on their translucency.[65]

Several studies demonstrated the resin-based sealants had improved the marginal integrity mainly through a micromechanical retention. In the late 1970s and early 1980s, pit and fissure sealants played an important role in the reduction of caries prevalence.

2. Glass Ionomer based pit and fissure sealant:

The first poly-carboxylate was introduced by smith in 1968 which bonds chemically to the enamel.

In 1974 Glass ionomer material was introduced by Mclean and Wilson as a restorative material due to its hydrophilic properties and rapid setting, considered to be best alternative for the patients in which moisture control is difficult.[66] Prolonged fluoride release is another advantage of glass ionomer cement over the conventional resin based sealants and studies had proved that it increases the resistance of the fissure to demineralization and may prevent occlusal caries even after the sealant has been removed.[67]

Ahovuo-saloranto et al (2008)[10] in his randomized controlled clinical trials, concluded that pit and fissure sealants application reduces the risk of dental caries development by 78% and 60% in 2 and 4 yrs. application respectively compared to unsealed occlusal surfaces in permanent molars. Furthur he confirmed in his review article in 2013 it reduces caries up to 48 months compared to unsealed occlusal surfaces.

Despite their documented efficacy sealants are still underused by the dental professionals. Hence it is essential to update evidence-based recommendations and help health care providers in clinical decision making.

The success or failure of the pit and fissure sealants depends on many parameters of which retention and marginal sealing ability plays the key role because failure of it leads to penetration of microorganisms beneath the sealants which will initiates carious lesions, which is further confirmed by Ganesh and Shobha[15] 2007 study which reveals that the primary factor affecting the performance and durability of a sealant is its marginal adaptation to the enamel, which provides a good seal and minimizes microleakage.

JUSTIFICATIONS FOR EXPERIMENTAL PARAMETERS

With this background an invitro study was conducted to evaluate the marginal sealing ability of Conseal F and Embrace wet bond using dye penetration method.

Sample selection and storage:

In this study, maxillary and mandibular premolars extracted for orthodontic purpose, which were free of caries, developmental defects, enamel microfractures and discoloration were included, artificial saliva was used as a storage solution for extracted teeth, as it has no effect on the protein structure and neither does it alter the enamel structure.[26] Other storage media used in various studies are distilled water,saline,0.2% thymol, chloramine.

Pumice prophylaxis:

Controversial ideas exist in whether the occlusal tooth surfaces should be cleaned before etching and Pit and fissure placement. According to Raadal et al (2001) removal of plaque and pellicle from the occlusal surfaces either by pumice or air polishing will enhance the acid etch pattern of the enamel and leads to increased retention whereas Harris and Garcia-Godoy (1999) suggests that acid etching alone is sufficient for surface cleaning. Blackwood et al (2002)[19] in his comparative study between enameloplasty, air abrasion and pumice prophylaxis, proved the least microleakage was seen with the conventional pumice prophylaxis, which was similar to Hatibovic-Kofman et al (1998) results.

Ansari et al (2004)[20] reported that prophylaxis has a role in improving sealant retention and reduces microleakage.

Based on above studies, pumice prophylaxis was used for cleaning the occlusal surfaces of premolars prior to etching, no invasive procedures like enameloplasty was used which may affect the nature of the sealants as given in the in vitro study by Prabhakar AR et al.(2011).[26]

Material selection:

Conseal F (SDI) - as Group I which was resin based, fluoride releasing and light curable sealant and BIS-GMA free. Commonly used by the dental professionals.

Embrace Wet Bond (Pulp dent) - as Group II

The majority of commercially available resin-based pit and fissure sealants are hydrophobic materials whereas recently introduced hydrophilic sealants, which bond effectively to moist enamel surfaces, present a distinct advantage in Paediatric dentistry where patient-compliance, isolation, and moisture-control can be particularly challenging. Embrace Wet Bond incorporates di-, tri- and multifunctional acrylate monomers into an advanced acid-integrating chemistry that is activated by moisture. Because of its unique chemistry, Embrace Wet Bond is miscible with water and flows into moisture-containing etched enamel and combines with it.[68]

Thermocycling:

All dental materials that are present in the oral cavity are constantly exposed to heat and pH changes during eating, drinking, and breathing.[69] Due to the difference in the coefficients of thermal expansion of resin materials (25-60 ppm/°C) are greater than that of enamel (11.4 ppm/°C) and dentin (8 ppm/°C) leading to formation of marginal gaps by thermal stress and ends up in microleakage[70] Therefore, to assess the microleakage in invitro studies, thermocycling is one of the commonly used methods to simulate the oral environment and long-term stresses to which the restorations are exposed. Studies documented a various range of cycling from 100 to 500 cycles, while the temperature extremes range from 4°C to 15°C for the cold bath, and up to 45°C to 60°C for the hot bath and dwell time from 15 to 60 sec. Moreover, ISO specification (ISO TR 11450)[71] recommends marginal microleakage test should be preceded by the thermocycling in water bath temperatures at 5^0c and 55^0c to simulate the oral environment

In this study, the teeth samples were subjected to 500 thermal cycles between 5°C and 55°C, with a dwell time of 30 seconds which were similar to studies done by Penugonda et al[72]., and Sytner D et al.[73], to be most clinically relevant .

Microleakage assessment:

Microleakage of dental restorations can be described as leakage of minute amounts of oral fluid, bacteria and their toxins between sealant margins which may result in adverse effects like enamel demineralization and dental caries.[74]

Various invitro methods to assess microleakage:[75]

- Chemical markers,
- Radioactive isotopes,
- Penetration of bacteria, neutron activation analysis,
- Scanning electron microscopy,
- Creating artificial caries,

- Electrical conductivity and
- Dye penetration methods.

Dye penetration is a commonly used technique as it is inexpensive and non-toxic and detects even small amounts of leakage. When compared with bacterial penetration, dye penetration method is more accurate as the dye particle diameters are less than those of bacteria and they are the same size as the bacterial endotoxins. Hence dye penetration method was used in this study to evaluate microleakage.

Commonly used dyes are[76]

0.6-2% rhodomine B,

1-5% methylene blue,

1-5% basic fuschin and

50% silver nitrate.

In this study 1% Methylene blue dye was used because it is readily detectable under visible light, soluble in water and diffuses freely which is similar to the studies of Topaloglu et al (2010), Joshi k et al (2014)[31], Lele G S et al (2016), Panse et al (2018).[46]

Sectioning and evaluation of microleakage:

In this present study the samples were sectioned buccolingually using hard tissue microtome (Accutome 100 saw microtome) under water coolant to avoid thermal damage resulting in section sample of 1mm thickness and viewed under stereomicroscope as in the study done by Priya Sridhar et al (2016)

Stereomicroscope is the gold standard in microleakage studies and hence used in this study.

The sectioned specimens were then analyzed at 40x magnification scored using score rank scale.

Different methodologies have been used to measure microleakage in-vitro by assessing the dye penetration along the tooth structure-dental restoration interface.[77]

- Some studies assess the dye penetration by measuring the percentage of dye penetration along the enamel-sealant interface.

  Criteria given
- By Colley et al.
- By Grande et al
- By Hosoya et al
- By Williams B and Winter GB

In this study for the microleakage score, a ranked scale method described by Williams B and Winter GB was used.

Result:

The results of the present study showed 9 of 25 samples (36%) had no dye penetration (Grade 0) in Group I (Conseal F sealant), while only 1 of 25 samples (4%) were Grade 0 in Group II (embrace wet bond sealant). Group II had the most extensive dye penetration (Grade 3) in 10 of 25 samples (40%). On the other hand, Group 1 had 0 of 25 samples (%) in the Grade '3' category On comparison of the microleakage scores of the groups, a statistically significant difference was found between the two groups with a p-value<0.001 indicating a better performance of Conseal F as compared to embrace wet bond. This was in accordance with the results obtained in Anna Eliades et al (2013)[29] which states that sealant with the hydrophobic monomers demonstrated the best sealing characteristics than the hydrophilic sealants.

The above result was further supported by the studies done by Maryam et al (2017)[41],Priya Subramaniam et al (2015)[32],Kamal el din et al (2013)[7] which believed that self-adhesive sealants cannot form resin micro-tags and an acceptable hybrid layer in the enamel, which result in microleakage in the long run.[31]

On the other hand the obtained results of the present study were in contrary to the results obtained in the studies of Prabhakar et al (2011)[26], Prasanna Kumar et al (2013)[30],Nahid et al (2017)[44],Ratnaditya et al(2015)[33] shows better retention and sealability of Embrace Wet Bond.

SUMMARY

Dental caries is still highly prevalent all over the world. The occlusal surface of the teeth has pit and fissures which provide good environment for microbes causing it. Filling of fissure areas reduces the risk of occlusal caries which is done using pit and fissure sealant. The success of pit and fissure sealant depends on the marginal sealing ability of the material. There is limited literature evidence on Conseal F and Embrace Wet Bond in terms of marginal sealing ability. With this background the present invitro study was carried out to evaluate and compare the marginal sealing ability of Conseal f and Embrace Wet Bond. The aim of the study is to compare the marginal sealing ability of two pit and fissure sealants using dye penetration method. Hence an invitro study was conducted in the Department of Pedodontics and Preventive Dentistry. 50 extracted premolar teeth which were non hypoplastic and non-carious removed for orthodontic purpose were divided into 2 groups of 25 each. 25 teeth for application of Conseal F classified as group 1 and 25 teeth for application of embrace wet bond as group II. After extraction all the teeth were cleaned to remove debris, disinfected with hydrogen peroxide and stored in artificial saliva at room temperature. All fissures in the teeth were cleaned for 15 seconds with aqueous slurry of pumice using rubber cup in slow speed contra angle hand piece. Then the teeth were rinsed and dried with air water spray. In Group I the enamel on the occlusal surface (which includes pit and fissures) was etched with

EAZETCH gel (37% phosphoric acid gel) for 20 seconds then rinsed with water for 20 seconds and dried with air syringe for 10 seconds and inspected visually for uniform frosty appearance. After etching and drying Conseal F was applied with disposable cannula supplied by the manufacturer and sharp explorer is used to remove the air bubble and to check the uniform flow for 15 seconds.

In Group -II Occlusal surfaces were etched and rinsed as similar, as above. According to the manufacturer with Embrace Wet Bond, the typical dull, frosted appearance of the etched surface is not essential as it is moisture bonded. Hence a cotton pellet was used to remove the excess moisture, and not total drying done as in case of Group I. The sealant Embrace Wet Bond was applied as per manufacturer's instructions with disposable cannula supplied by the manufacturer and sharp explorer is used to remove the air bubble and to check the uniform flow for 15 seconds.

Then the pit and fissure sealants were cured with light curing unit for 20 seconds and stored in artificial saliva for 72 hours before thermocycling.

Thermocycling was done and the purpose of thermocycling is to simulate the oral environment as all the dental materials that are present in the oral cavity are constantly exposed to heat and pH changes during eating, drinking, and breathing.[25] Due to the difference in the coefficients of thermal expansion of resin materials (25-60 ppm/°C) are greater than that of enamel (11.4 ppm/°C) and dentin (8 ppm/°C) leading to formation of marginal gaps by thermal stress and ends up in microleakage. After thermocycling the samples were carefully coated with two layers of nail varnish (Purple- Conseal F,Pink-Embrace Wet Bond) excluding 1mm around the sealant and the root apices of the teeth were sealed with GIC(Golden yellow, Japan) to prevent seepage of dye .Then the samples were immersed in 1% methylene blue dye for 24 hours, washed in plain water to remove excess dye. Then the crown and root portion were sectioned using diamond disc and high-speed lathe.

Then the crown portion of the teeth were embedded in self-curing acrylic resin (Group I -Pink, Group II-Clear) and sectioned buccolingually using hard tissue microtome under water coolant to avoid thermal damage resulting in section sample of 1mm thickness.

The sectioned specimen were examined under an optical stereomicroscope (Lawrence and mayo-meiji techno) of 40 X magnification, for dye penetration and the images of the sectioned teeth samples were recorded.

The extent of microleakage was evaluated based on amount of dye penetration between the sealant and the tooth substance interface using Williams B and Winter GB criteria. Data was collected, tabulated and statistically analysed using IBM SPSS Version 20.0 The chi-square test was used to analyse frequency distribution of microleakage scores. Inter group comparisons of microleakage scores was done using Mann-Whitney test. On comparison of the microleakage scores of the groups, a statistically significant difference was found between the two groups with a P-value <0.001 indicating a better performance of Conseal F as compared to Embrace Wet Bond. The least microleakage was seen with Conseal F sealant and can be a better material of choice to be used in day to day clinical practice. Clinical performance of the sealants differs compared with in vitro results due to change in oral conditions and patient performance. Hence long term clinical studies with larger sample is required.

CONCLUSION

Dental caries is considered as a major public health issue especially for children. We should give sufficient importance, to preventive measures rather than restorative procedures. Pit and fissure sealants have been found not only to prevent occlusal caries, but also to arrest the progression of incipient lesions. The advantages of sealing the occlusal surfaces are associated with the decrease in caries prevalence when compared with non-sealed teeth. Moreover, sealing is associated with lower cost when compared with the cost of placing restorations. In developing country like India, the preventive measures toward oral health are imperious. The cost of preventive measures like sealants seems to be higher than the cost of restorative materials, but in the long term, it would be more cost-effective as the tooth would be maintained in the state of health.

The retention of sealant and the ability to seal are important factors in the success of sealants in preventing dental caries. Indeed, the longer the material remains bonded to the occlusal surface, the greater the protective benefit it provides to the teeth. In addition, the success of preventing leakage between the sealant and the enamel tooth surface is considered to be an important feature of the success of fissure sealants. Sealants are still underused despite their documented efficacy and the availability of clinical practice guidelines. New sealant materials and techniques continue to emerge for managing pit-and-fissure caries, further complicating the clinician's decision making. Accordingly, continuous critical review of the available evidence is necessary to update evidence-based recommendations and assist health care providers in clinical decision making.

With this background the present invitro study was carried out to evaluate and compare the marginal sealing ability of Conseal F and Embrace wet Bond using dye penetration method.

Within the parameters of in-vitro study the following conclusion can be drawn:

1. Both the sealants used in this study showed some degree of microleakage.
2. Chi square test shows that there is a significant difference in the distribution of microleakage grades among the two groups, owing to the higher distribution of cases among the higher grades (Grades 2 and 3) in Group II
3. The test reveals that there is a highly significant difference ($P < 0.001$) in the microleakage grade between the two groups owing to the increased microleakage in group II
   (Mean rank of Group II > Mean rank of group I).
4. On comparison of the microleakage scores of the groups, a statistically significant difference was found between the two

groups with a P-value <0.001 indicating a better performance of Conseal F as compared to embrace wet bond.

5. The least microleakage was seen with Conseal F sealant and can be a better material of choice to be used in day to day clinical practice.
6. It should be noted that the results of the present study are valid for in vitro conditions. Clinical performance of the sealants differs compared with in vitro results due to change in oral conditions and patient performance. Hence long-term clinical studies with larger sample is required.
7. The study concludes that for long term retention of pit and fissure sealants good isolation and dry environment is essential.

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ANNEXURE I

PARTICIPANT INFORMATION SHEET (ENGLISH)

NAME OF INVESTIGATOR: Dr. V. DHANALAKSHMI

PHONE NO : 9940663763

TOPIC : COMPARATIVE EVALUATION OF MARGINAL SEALING ABILITY OF TWO MATERIALS USED AS PIT AND FISSURE SEALANTS USING DYE PENETRATION METHOD-AN INVITRO STUDY

The purpose of the study is to find the marginal sealing ability of two pit and fissure sealants. The study will be done by collecting teeth from the patients who are extracting for orthodontic reasons only. No risk will be involved to the patients. Your records will be maintained confidential and you have freedom to participate or withdraw from research at any point of time.

The details of the research has been explained to me in a language which I under stand . I hereby give permission for using my records, extracted teeth for professional research and education purpose only.

SIGNATURE OF PATIENT

ANNEXURE II

PARTICIPANT INFORMATION SHEET (TAMIL)

முதன்மை ஆய்வாளரின் பெயர்- டாக்டர். வெ. தனலட்சுமி

கைப்பேசி என் - 9940663763

தலைப்பு: இரண்டு பிட் மற்றும் பிஷ்ஷர் அடைப்பான்களின் ஒட்டுத்திறனை சாய ஊடுருவல் முறை மூலம் ஒப்பிடுதல் - ஆய்வுக்கூட சோதனை முறைப்படி.

இந்த ஆராய்ச்சியின் நோக்கம் இரண்டு பிட் மற்றும் பிஷ்ஷர் அடைப்பான்களின் ஒட்டுத்திறனை சாய ஊடுருவல் முறை மூலம் ஒப்பிடுதல். இந்த ஆராய்ச்சியில் பல் சீரமைப்பு பொருட்டு நீக்கப்படும் பற்கள் உபயோகப்படுத்தப் படுவதால் எந்த வித பாதிப்பும் ஏற்படாது என்று உறுதியளிக்கிறேன

தங்களது பதிவுகள் ரகசியமாக பாதுகாக்கப்படும் என்றும் தாங்கள் விருப்பமின்றி தங்களுக்கு பயன்படுத்தபடாது என்றும் உறுதியளிக்கிறேன்.

இந்த ஆராய்ச்சியின் குறிப்புகள் எனக்கு தெளிவாக விளக்கப்பட்டன, அவற்றை நான் முழுமையாக புரிந்து கொன்டேன்

இந்த ஆராய்ச்சிக்காக எனது பிடுங்கிய பற்களை உபயோகிக்கலாம் என முழுமனதுடன் சம்மதிக்கிறேன்.

நோயாளியின் கையொப்பம்

ANNEXURE III

PARTICIPANT INFORMED CONSENT FORM (PICF)-ENGLISH

IHEC Proposal S.No: 384 Date:_______________

Title of the project:

COMPARATIVE EVALUATION OF MARGINAL SEALING ABILITY OF TWO MATERIALS USED AS PIT AND FISSURE SEALANTS USING DYE PENETRATION METHOD-AN INVITRO STUDY

Name of the Principal Investigator: Dr. V. DHANALAKSHMI

Mobile No: 9940663763

The contents of the information sheet dated _____________ that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from CARE. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

Date:

____

Place:

(Signatures /Left Thumb Impression)

Name of the Participant:___________________________________

Son/Daughter/Spouse/of___________________________________

Complete Postal Address:

____

____

This is to certify that the above consent has been obtained in my presence.

Date:

_______________________________________ Place:

Signature of the principal Investigator

Witness – 1 Witness – 2

_________________________________ _________________________________

Note 1: Three copies should be made, for (a) Participant, (b) Researcher, (c) Institution 2: Submit the modified participants informend consent as per the study proposal 3: Investigators are advised to prepare the translation in simple understandable

Tamil on their own

ANNEXURE IV

PARTICIPANT INFORMED CONSENT FORM (PICF)-TAMIL

முறையான அனுமதி படிவம்

நாள்.

தலைப்பு. இரண்டு பிட் மற்றும் பிஷ்ஷர் அடைப்பான்களின் ஒட்டுத்திறனை சாய ஊடுருவல் முறை மூலம் ஒப்பிடுதல் - ஆய்வுக்கூட சோதனை முறைப்படி.

முதன்மை ஆய்வாளரின் பெயர். டாக்டர். வெ. தனலட்சுமி

கைப்பேசி எண். 9940663763

இத்தகவல்களின் உள்ளடங்களை நான் கவனமாக படித்தேன். அது என்னுடைய மொழியில் எனக்கு விளக்கப்பட்டது. நான் அவற்றை முழுமையாக புரிந்து கொண்டேன். எனக்கு ஏற்பட்ட சந்தேகங்களை தீர்த்துக் கொள்ள வாய்ப்பு அளிக்கப்பட்டது என்பதை உறுதி படுத்துகிறேன்.

இந்த ஆராய்ச்சியின் முடிவில் என்னுடைய பெயர் மற்றும் என்னைப் பற்றிய தகவல்கள் வெளியே வராது என்பதனையும் நான் விரும்பும் பட்சத்தில் இந்த ஆராய்ச்சியில் இருந்து எப்போது வேண்டுமானாலும் விலகிக் கொள்ளலாம் என்றும் அவ்வாறு விலகும் பட்சத்தில் எனக்கு அளிக்கப்படும் சிகிச்சையை அது ஒருபோதும் பாதிக்காது என்பதனையும் அறிந்துக்கொண்டேன்.

இந்த ஆராய்ச்சியில் பங்கேற்க முழுமனதுடன் சம்மதிக்கிறேன்.

நோயாளியின் கையொப்பம் / இடது பெருவிரல் ரேகை

ஆராய்சிசியாளரின் கையொப்பம்.

பங்கேற்பவரின் பெயர். ______________________________

பங்கேற்பவரின் முகவரி. ____

சாட்சி (1)	சாட்சி(2)
பெயர்.	பெயர்.
முகவரி.	முகவரி.
கையொப்பம்	கையொப்பம்

ANNEXURE V

CERTIFICATE FOR THERMOCYCLING PROCEDURE

Chettinad Hospital & Research Institute Rajiv Gandhi Salai, Kelambakkam, Kancheepuram Dist. Tamil Nadu 603 103 India
T + 91 (0) 44 4741 1000, 3300 F + 91 (0) 44 4741 1011 mail@chettinadhealthcity.com www.chettinadhealthcity.com

Chettinad
Hospital & Research Institute

26.09.2019

TO WHOMSOEVER ITMAY CONCERN

This is to certify that Dr. V. Dhanalakshmi , III MDS, Post Graduate Student, Department of Pedodontics and Preventive Dentistry has done the Thermocycling Procedure utilizing the apparatus in Research Laboratory, Faculty of Allied Health Sciences , Chettinad Hospital and Research Institute, Kelambakkam.

Prof. Sripathi Kandula
Principal -Faculty of Allied Health Sciences

Principal
Faculty of Allied Health Sciences
Chettinad Hospital and Research & Institute
Chettinad Health City, Rajiv Gandhi Salai,
(OMR Chennai), Kelambakkam - 603 103. India.

CHETTINAD HOSPITAL & RESEARCH INSTITUTE
Kelambakkam
603 103.

ANNEXURE VI

CERTIFICATE FOR HARD TISSUE MICROTOME SECTIONING

Tele : 0471-2340801

Grams : CHITRAMET
Fax : 0471-2341814

श्री चित्रा तिरुनाल आयुर्विज्ञान तथा प्रौद्योगिकी संस्थान
बायो मेडिकल टैक्नोलॉजी विंग
पूजापुरा, तिरुवनन्तपुरम-695 012, इन्डिया

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
BIO MEDICAL TECHNOLOGY WING
POOJAPPURA, THIRUVANANTHAPURAM-695 012, INDIA
(An Institute of National Importance under Govt. of India)

Ref:

Date: 29.08.19

This is to certify that **Dr V.Dhanalakshmi**, Post Graduate student from Chettinad Dental College and Research Institute, Chennai who had used the Accutome 100 saw microtome to cut her teeth samples as a part of her dissertation work. The work was carried out at Histopathology laboratory, Biomedical Technology Wing, SCTIMST.

Dr.SABAREESWARAN. A, M.V.Sc (Path)
Scientist-in-Charge,
Histopathology Laboratory
Biomedical Technology Wing, Sree Chitra Tirunal
Institute for Medical Sciences & Technology,
Thiruvananthapuram 695 012

ANNEXURE VII

CERTIFICATE FOR STEREOMICROSCOPE IMAGE RECORDING

MHRD G.O. No. F.9-1/2002-U.3 dt. 21.01.03

Dr. M.G.R.
EDUCATIONAL AND RESEARCH INSTITUTE
(Deemed to be University)
(An ISO Certified Institution)
Maduravoyal, Chennai - 600 095, Tamilnadu, India.

UGC No. F. 6-6 / 2002 (CPP-I) dt. 13.03.2003

THAI MOOGAMBIGAI DENTAL COLLEGE AND HOSPITAL
(A Constituent Unit of Dr.M.G.R.Educational and Research Institute)

Dr. Rathika Rai,MDS.,
Principal

10.09.2019

THAI MOOGAMBIGAI DENTAL COLLEGE & HOSPITAL
DATE

CERTIFICATE

This is to certify that Dr. V. Dhanalakshmi, Post Graduate Student in the department of Pedodontics and Preventive Dentistry, Chettinad Dental College, Chennai had used the Stereomicroscope which was available in our college to record the images of sectioned tooth samples needed for her main dissertation work.

10/9/19

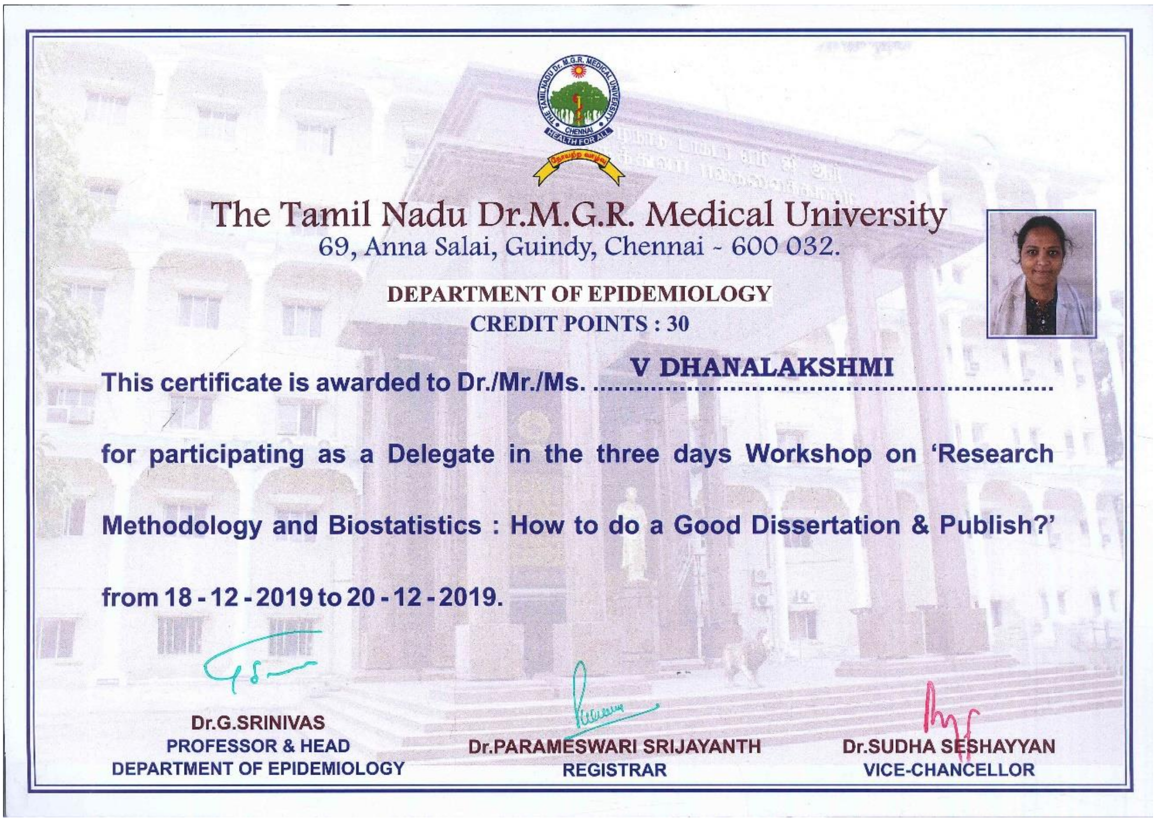
Principal

PRINCIPAL
Thai Moogambigai Dental College & Hospital
Golden George Nagar, Mugappair
Chennai-600 107

Golden George Nagar, Mugappair, Chennai - 600 107. Ph : 2653 6400 / 2653 3178 Fax : 2653 3884 E-mail: tmdcaho@gmail.com | www.tmdch.ac.in

ANNEXURE VIII

RESEARCH METHODOLOGY CERTIFICATE



The Tamil Nadu Dr.M.G.R. Medical University
69, Anna Salai, Guindy, Chennai - 600 032.

DEPARTMENT OF EPIDEMIOLOGY
CREDIT POINTS : 30

This certificate is awarded to Dr./Mr./Ms. V DHANALAKSHMI

for participating as a Delegate in the three days Workshop on 'Research Methodology and Biostatistics : How to do a Good Dissertation & Publish?' from 18 - 12 - 2019 to 20 - 12 - 2019.

Dr.G.SRINIVAS
PROFESSOR & HEAD
DEPARTMENT OF EPIDEMIOLOGY

Dr.PARAMESWARI SRIJAYANTH
REGISTRAR

Dr.SUDHA SESHAYYAN
VICE-CHANCELLOR