YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PEDIATRIC DENTISTRY

IN VITRO EVALUATION OF EFFECTS OF DENTAL OPERATION MICROSCOPE AND ADHESIVE SYSTEMS ON SHEAR BOND STRENGTH AND MICROLEAKAGE IN FISSURE SEALANT PROCEDURE

DOCTORAL THESIS

DDS

DİLARA UYSAL

İstanbul-2019

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 14.06.2019 and numbered 2019/10-03

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DECLARATION

I hereby declare that this thesis is my own work, I had no unethical behavior at any stage from its planning to writing, I obtained all the information in the thesis in accordance with the academic and ethical rules, I provided references for all the information and interpretations that are not obtained by the present study, I included these sources in the references part, and I had no violation of patent rights or copyrights.

14.06.2019

Dt. Dilara UYSAL

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ABBREVIATIONS

4-MET	:	4-methacryloxyethyl Trimellitic Acid
ACP	:	Amorphous Calcium Phosphate
ADA	:	American Dental Association
AI	:	Amelogenesis Imperfecta
Bis-GMA	:	Bisphenol-A Glycidylmethacrylate
C^{+4}	:	Carbon
Ca^{+2}	:	Calcium
$Ca_{10}(PO_4)_6OH)_2$	:	Hydroxyapatite Crystals
CaF_2	:	Calcium Fluoride
GIC	:	Glass Ionomer Cements
CPP	:	Casein Phosphopeptide
CPP-ACP	:	Casein phosphopeptide-amorphous calcium phosphate
EDTA	:	Ethylene Diamine Tetra Acetic Acid
FDI	:	World Dental Federation (Fédération Dentaire Internationale)
Phenyl-P	:	2-methacryloxyethyl Phenyl Hydrogen Phosphate
$H_3[PO]_4$	:	Phosphoric acid
HAp	:	Hydroxyapatite
HEMA	:	Hydroxyethylmethacrylate
I^-	:	Iodine
IEPS	:	Insoluble extracellular polysaccharides
LED	:	Light Emitting Diode
mm	:	Millimeter
MMA	:	Methyl Methacrylate
MMPs	:	Matrix metalloproteinases
Mn^{+4}	:	Manganese
MPa	:	Mega Pascal
Na^{+2}	:	Sodium
NCSS	:	Number Cruncher Statistical System
nm	:	Nanometer
NPG-GMA	:	N-phenylglycine Glycidyl Methacrylate
NTG-GMA	:	N-tolglycine Glycidyl Methacrylate
^oC	:	Celsius degree

OH^-	:	Hydroxyl Ion Group
P^{+5}	:	Phosphorus
PENTA	:	Dipentaerythritol Pentaacrylate monophosphate
pH	:	Power of hydrogen
PMDM	:	Pyromellitic dianhydrate dimethacrylate
PO_4^+	:	Phosphate
QTH	:	Quartz Tungsten
Rb^+	:	Rubidium
RMGIs	:	Resin-modified glass ionomers
S. mutans	:	*Streptococcus mutans*
S^{+2}	:	Sulfur
SDF	:	Silver Diamine Fluoride
SEM	:	Stereo Electron Microscope
sec	:	Second
TEGDMA	:	Triethylene glycol dimethacrylate
TiF_4	:	Titanium Tetrafluoride
USPHS	:	United States Public Health Service
UV	:	Ultraviolet
WHO	:	World Health Organization
µm	:	Micrometer
µTBS	:	Tensile Bond Strength

ABSTRACT

Uysal D. 2019. In vitro evaluation of effects of dental operation microscope and adhesive systems on shear bond strength and microleakage in fissure sealant procedure. Yeditepe University, Institute of Health Sciences, Ph.D. Thesis, Istanbul.

The aim of this study is to evaluate the shear bond strength and microleakage in fissure sealant procedure using dental operating microscopy and adhesive systems. The third human molar tooth used in the study and four groups of 15 samples and two groups of 15 samples were formed for the microleakage experiment and for the shear bond strength test, respectively. Fissure procedure was performed following the acid etching in two of the experimental groups subject to microleakage test, while fissure sealant was applied by using a dental operating microscopy in one group. In the other two groups, fissure sealant was applied after the application of the bonding and one of these groups was performed by using dental operating microscopy. A stereomicroscope was used for the evaluation of microleakage. Two samples from each group were visualized in a scanning electron microscope (SEM). Fissure sealant was applied to one of the groups formed to test the shear bond strength only after acid etching, while it was applied to the other group after the bonding agent application following the acid etching procedure. Shapiro-Wilk normality test was used for testing the normal distribution one way variance analyse test was used for multiple intergroup comparison, Tukey multiple comparison test was used for subgroup comparisons, Kruskal Wallis Test was used to compare the variables with no normal distribution, Dunn's multiple comparison test was used for subgroup comparisons, and Chi-Square test was used to evaluate the qualitative data. A p value of <0.05 was considered statistically significant.

In conclusion, no statistically significant difference was found between the groups for the microleakage test. However, when the groups were evaluated according to the shear bond strength results, the bond strength of the samples subject to fissure sealant procedure after the bonding agent application following the acid etching was found to be significantly higher ($p<0.05$).

Keywords: Fissure sealant, dental operating microscope, adhesive system

ÖZET

Uysal D. 2019. Dental operasyon mikroskobu ve adeziv sistemlerin kullanılmasının fissür örtücülerde makaslama bağlanma dayanımı ve mikrosızıntı üzerine etkilerinin ın-vıtro olarak değerlendirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, İstanbul.

Bu çalışmanın amacı, dental operasyon mikroskobu ve adeziv sistemler kullanılarak yapılan fissür örtücü uygulamasının makaslama bağlanma dayanımı ve mikrosızıntısının değerlendirilmesidir. Çalışmada yapılan mikrosızıntı deneyi için, çekilmiş insan 3. büyük azı dişi kullanıldı ve 15'er örnekten oluşan 4 grup, makaslama bağlanma dayanımı deneyi için de 15'er örnekten oluşan 2 grup oluşturuldu. Mikrosızıntı testi yapılan deney gruplarından ikisinde asit pürüzlendirmesi sonrası fissür örtücü uygulaması yapılırken bu gruplardan bir tanesinde de fissür örtücü dental operasyon mikroskobu ile uygulandı. Diğer iki gruba asit sonrasında bonding ajanı uygulanıp ardından fissür örtücü yapıldı ve bu gruplardan bir tanesinin bonding ajanı ve fissür örtücü uygulaması dental operasyon mikroskobu ile yapıldı. Mikrosızıntı değerlendirmesi için stereomikroskop ile görüntü alındı. Her bir gruptan 2'şer adet örnek taramalı elektron mikroskobunda (SEM) görüntülendi. Makaslama bağlanma dayanımı deneyi için oluşturulan gruplardan birine sadece asit pürüzlendirmesi sonrası fissür örtücü yapılırken diğer gruba asit sonrası bonding ajanı uygulanıp ardından fissür örtücü yapıldı. Bu çalışmadaki istatistiksel analizler, Shapiro – Wilk normallik testi ile değişkenlerin dağılımına bakılıp, çoklu gruplar arası karşılaştırmalarında tek yönlü varyans analizi, alt grup karşılaştırmalarında Tukey çoklu karşılaştırma testi, normal dağılım göstermeyen değişkenlerin çoklu gruplar arası karşılaştırmalarında Kruskal Wallis Testi, alt grup karşılaştırmalarında Dunn's çoklu karşılaştırma testi, nitel verilerin değerlendirilmesi ki-kare testi ile yapıldı. Sonuçlar, anlamlılık $p<0,05$ düzeyinde değerlendirildi.

Sonuç olarak, mikrosızıntı deney grupları arasında istatistiksel olarak anlamlı bir fark bulunmazken, makaslama bağlanma dayanımı sonuçlarına göre asit pürüzlendirmesi sonrası bonding ajanı uygulanıp ardından fissür örtücü yapılan örneklerin bağ dayanımı istatistiksel olarak anlamlı derecede yüksek bulunmuştur ($p<0,05$).

Anahtar Kelimeler: Fissür örtücü, dental operasyon mikroskop, adeziv sistem

1. INTRODUCTION AND AIM

Oral and dental health is reported to be one of the cornerstones of human health. Besides its importance in terms of nutrition, it has been reported to be of great importance for the individual to speak, smile and have self-confidence in the social environment (1).

Dental caries is reported to be a chronic and preventable infectious disease that is caused by many factors and that causes physical and chemical destruction of hard tissues. In developed countries, dental caries affects 60-90% of school-age children, while it is one of the leading health problems in the vast majority of adults. Preventive dental practices for the prevention of dental caries and training courses on oral and dental health are reported to become increasingly widespread throughout the world (2).

Significant progress has been reported to be made in recent years in terms of the prevention of dental caries in which many factors play a role, reducing the risk of dental caries, and development of non-invasive or minimally invasive treatment techniques protecting oral and dental health as much as possible. As a result of such a preventive clinical approach, It has been reported that the prevalence of caries decreases and the rate of progression decreases, however, the decrease in the prevalence of caries has been reported not to be equal in all tooth surfaces (3). Pits and fissures on the occlusal surface, which are the most prone to caries due to their complex morphologies, have been reported to constitute 85% of decayed surfaces (4–7).

Enamel acid etching is an inevitable step during the application of pits and fissure sealants. With the procedure, it is stated that microporosities are formed in the enamel and the bond strength of the fissure sealant material increases with the increase in wettability of the enamel. The efficacy of fissure sealants has been reported to be measured by the retention of the material to the tooth and the level of leakage between the material and tooth surface (8,9). It has been suggested that the amount of retention vary depending on appropriate isolation of the operation site, preparation of enamel surfaces, viscosity of fissure sealant material, and use of adhesive systems (10–12). A surface layer, which cannot be removed by washing and impairs the retention of the fissure sealant material, is reported to be formed due to the contamination of the acid-etched enamel surface with saliva (13,14). It has been reported that the use of adhesive agents under the fissure sealant to eliminate this clinical problem, reduce microleakage and increase the clinical success rate will improve success (12,15).

In the literature, there are several studies on the use of bonding agents in addition to acid etching for improving the retention rate of fissure sealants (11,12,16). There are studies indicating that the use of bonding agent prior to fissure sealant increases the retention rate, whereas there are also studies reporting that this application has no contribution to retention (10,11,17).

One of the recent applications used in dentistry is reported to be the use of dental operating microscope in treatments. It has been reported to support the physician's angle of view and the ergonomic positioning in a positive way. It has been further reported to ensure that treatments are made more detailed and the angle of view is clearer. It helps the establishment of the connections between the material and the tooth during restorations as well as making the condensation of the material in the best way. Thus, the possibility of secondary caries and pulp infections due to microleakage is suggested to reduce (18,19).

The aim of this study is to evaluate the shear bond strength and microleakage in fissure sealant procedure using dental operating microscopy and adhesive systems. The rate of retention and microleakage effect of the adhesive systems used during the fissure sealant procedure were evaluated. It has been concluded that the dental operating microscope provides a clearer view and significantly reduces the margin of error as well as increasing treatment success compared to conventional treatment methods. Although dental operating microscope is frequently used during root canal treatment, it is aimed to expand its use in restorative procedures. It is aimed to increase the use of dental operating microscope during treatments, to reduce the errors in the treatments, and to increase the use of new developing technology in dentistry.

2. GENERAL INFORMATION

2.1. Dental Caries and Its Etiology

Organic acids released due to the fermentation of carbohydrates by microorganisms in the plaque are known to initiate dental caries. Therefore, dental caries is reported to be a multifactorial, infectious and contagious disease, which is developed due to the demineralization of hard tissues by organic acids resulting from the deterioration of the balance between dental tissue and plaque fluid. Being prevented by changing nutrition habits, dental caries has been reported to be controllable as the number of cryogenic microorganisms in the dental plaque can be increased or decreased depending on the carbohydrate uptake of the host (7,20).

Dental caries is caused by a combination of many factors; the presence of microorganisms alone is not sufficient for the development of caries in enamel and dentin. Therefore, teeth (host), *Streptococcus mutans* (*S. mutans*), and carbohydrates fermentable with cryogenic microflora such as lactobacilli must coexist for a certain period of time for the formation of caries (21). As in other diseases, dental caries occurs when the disease-causing factors come together under suitable conditions in a sensitive host. It has been emphasized that no decay formation is observed unless all of these factors coexist. *S.mutans*, which is considered one of the primary causative agents of dental caries since the 1980s, causes dental caries due to its virulence properties. Acid-forming capacity and adhering to enamel surface and dental plaque are reported to be among these properties. It produces lactic acid by fermenting sucrose, resulting in the dissolution of the enamel matrix. The production of insoluble extracellular polysaccharides (EPS) has been suggested to facilitate the adhesion of bacteria to the tooth surface (22).

2.1.1. Types of Caries

2.1.1.1. Enamel Caries

Deterioration of the balance between the normal flora and the host in the dental plaque and the acids produced by bacteria create the initial level of demineralization in the tooth enamel. When adequate oral and dental care is not made and the other factors that cause caries are not eliminated, these demineralization areas become deeper and cause a loss of enamel in the amount requiring a probe to be inserted during the clinical examination **(Picture 1)** (7,23).

Enamel caries is found to be investigated as four layers from the center to periphery.

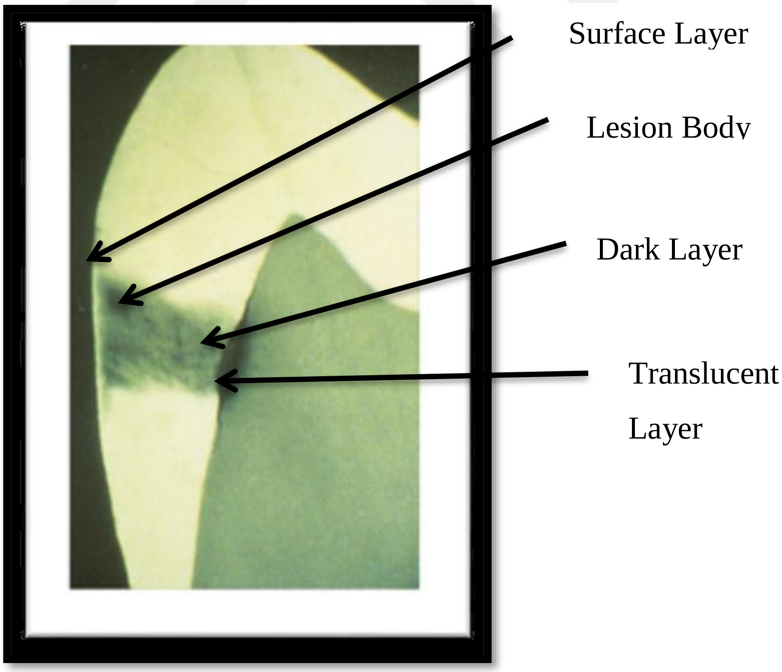
Translucent layer: It is located in the part of the lesion moving towards the dentin tissue in the region adjacent to the healthy enamel; is the first layer deviating from the healthy enamel **(Picture 2)**; is found in 50% of permanent teeth with enamel carious lesions and in 25% of deciduous teeth; and about 1% mineral loss occurs in this layer (7,23).

Dark Layer: It is reported to be located above the translucent layer **(Picture 2)**. It is observed in 85-90% of the enamel teeth in permanent teeth and 85% of the deciduous teeth. The width of this layer depends on the speed and intensity of caries and the structural properties of the enamel. It has been reported that the caries is very fast in the lesions not having this layer. The presence of gaps of different sizes in this layer and the fact that the lesions, which do not contain dark layer before, are seen in this layer following the remineralization are reported to suggest that remineralization may be effective in the formation of the dark layer (7,23).

Lesion Body: This region between the superficial layer and the dark layer constitutes the largest part of the lesion **(Picture 2)**. It has been reported that the maximum loss of substance is observed in this layer (7,23).

Surface Layer: This layer just below the enamel surface is reported to be a superficial layer not affected by the attack of the cryogenic factor covering the demineralized layer **(Picture 2)**. It has been reported to be observed as a mineralized layer, the integrity of which is not deteriorated by the precipitation of calcium and phosphate ions released by dissolution of the enamel layer under the surface or arisen from saturated solution in dental plaque to this part of the enamel. Together with the increase in demineralization, the enamel caries developing under the bacterial plaque cause the dissolution of crystals on the tooth surface and the formation of micro-craters on the surface. Reduction of plaque power of hydrogen (pH) of microorganisms with proteolytic fermented products causes the lesion in the enamel to progress to deeper regions. The demineralization of the organic structure continues during this progression and as a result, the caries lesion spreads towards the dentin (7,23).

Picture 1. Enamel Caries

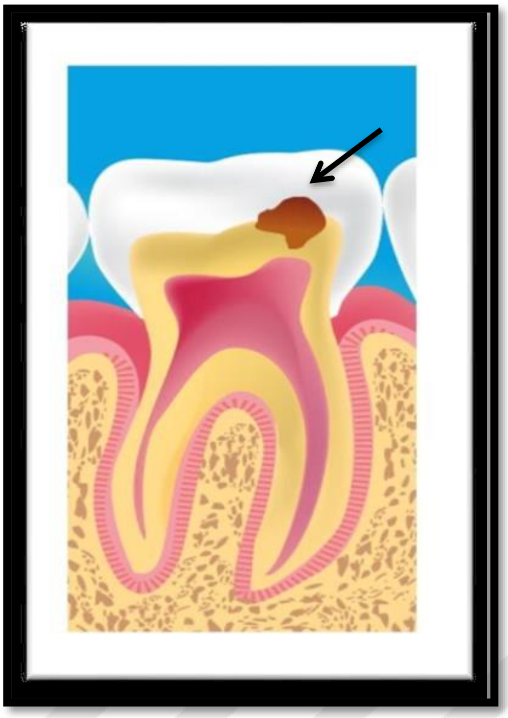


Picture 2. Enamel Caries Layers

2.1.1.2. Dentine Caries

It has been reported that a cavitation focus occurred at the enamel-dentin border after the enamel caries progressed and the entire enamel disappeared **(Image 3)**. This cavitation, in which most inorganic and organic molecules are dissolved in water, has been reported to be called “destruction region”. It is known that there are no calcified hard tooth tissues in this region and decalcified organic tissues are denatured, depolymerized, and decayed as their calcium-binding active ends are remained uncovered. Since inorganic parts in dentistry are under the protection of high organic parts, decay is progress slowly but cavitation develops rapidly. The decalcified dentin in the zone of softening can be easily distinguished during the clinical examination and easily treated with the help of the excavator. Microscopic examination revealed that microorganisms proliferate in dentin channels. in a layer known as invasion zone under the zone of softening, microorganisms filling dentin channels are unlikely to multiply because of the insufficient oxygen, moisture, and nutrients required for them to reproduce and produce metabolic residues. However, it is known that it is possible for some toxic or irritant substances to reach this region from the upper zone of softening. Hard zones of the tissue do not undergo any biochemical change (7,23).

Tertiary dentin is a layer formed in the pulp-dentin line due to trauma and irritation such as caries and restorative procedures, which is also known as irritation dentin, irregular secondary dentin, reaction dentin, and repair dentin. It is a dentin layer which is repaired faster, less mineralized, irregular, has less tubular structure and has higher organic content than primary dentin. In some cases, the pulp horns fill the hypermineralized peritubular dentin tubules in areas close to the dentin-enamel junction, and this is seen in the peripheral tubules adjacent to the root and cement. The thickness of this layer, which is also called sclerotic dentin or transparent dentin, increases with age to protect the pulp, just as in the reparative dentin. (7,23)



Picture 3. Dentin Caries

2.1.1.3. Cement Caries

The cement tissue is known to be a layer covering the anatomical root surface of the tooth. For the development of caries in the cement tissue, cement should be in direct contact with the oral environment. The cervical part of the cement tissue becomes apparent due to the regression of epithelial attachment in the apical direction and when bacterial plaque is accumulated in this region, cavities with unclear borders may occur in the cement tissue **(Picture 4)**. Cement caries is also known as old-age caries as the cement becomes apparent at an advanced age. It has been reported to be named as dentin caries if it progresses (7,23).

Because is thought that the decay can be prevented by eliminating the factors affecting the development of caries. For instance, the starting of caries can be prevented by correctly arranging a diet with frequent carbohydrate consumption, strengthening of tooth structure with fluoride applications, and removing dental plaque by using mechanical and chemical agents. However, these methods are reported to be not effective in the removal of caries after the formation of caries cavity. In such cases, caries layer should be removed by mechanical or chemical methods . (7,23)

Picture 4. Cement Caries

2.1.1.4. Pit and Fissure Caries (Occlusal Caries)

The morphological structure in the form of hollows and grooves in the occlusal surface of the enamel layer is called fissure. Besides the genetic factors, the morphology of the fissures is determined depending on the environmental factors in the development period and this recessed morphological structure facilitates plaque retention (24).

The caries starts easily on the fissure floor where the thickness of the enamel gradually decreases **(Picture 5)**. In the past, it was thought that caries in the pits and fissures started from the fissure floor, and the fissure walls and tubercle slopes were affected later. Nowadays, it is thought that these caries progress in the walls of the fissure in the direction of enamel prisms, merge into two separate independent lesions at the fissure floor and become a single lesion. The lesion has become cone-shaped by reaching the enamel-dentin border at later stages (22). Since organic deposits in the fissures act as a plug and create a barrier against acid attacks, the lesions start to occur from the tubercle slopes. It has been reported that most of the caries lesions in the pit and fissures occur within the first four years following the tooth eruption (25).

It has been further reported that the occlusal dark fissure appearance or insertion of the probe into the fissure is not sufficient for the diagnosis of fissure caries unless a soft tissue is detected with a probe in the clinical examination. Caries reaching to dentin border by passing the occlusal surface enamel layer expands parallel to the dentin border and it may sometimes trigger pulpitis without being realized by the patient. The regions of the permanent teeth that are the most susceptible to caries are first and second permanent molar pit and fissures and buccal and palatal pits of first molars (26).

Although pit and fissures account for only 10% of the total dental enamel surface, most of dental caries starts and progresses here. Even in the Netherlands, where preventive therapies are applied most intensively, caries in these regions is reported to be 1.5 times more than the approximal surfaces(27).

Picture 5. Pit and Fissure Caries (Occlusal Caries)

Pit and Fissure Morphology and Susceptibility to Caries

It is known that the susceptibility of posterior teeth to caries is directly related to the shape and depth of the pit and fissures on the occlusal surfaces. On occlusal surfaces, there are uneven spiral grooves of different morphology and about 0.1 mm wide, where food residues and bacteria can hold mechanically (28). The molars have primary, secondary and complementary fissures and may have up to 10 pits in addition to those fissures (29).

It is known that morphology of pits and fissures generally consisting of narrow and numerous uneven grooves may vary from person to person and from tooth to tooth and this feature of pit and fissures is emphasized to be among the most important reasons increasing their susceptibility to caries. It is known that the susceptibility of fissures to caries is related to the steepness of the tubercle slopes and thus to the depth of the fissures. The angle of the fissure between tubercle slopes is 70-90° and in cases where the angle is less than 70°, the susceptibility to caries increases (30). Fissure type and progression rate of caries lesion has been reported to be associated (30,31). In deep fissures with uneven grooves, mechanical retention of nutrient residues and bacteria is more common. The deepest regions of the fissures are known to contain inanimate bacteria and tartar. These regions cannot be cleaned in a sufficient way by mechanically and benefit less from the cleaning effect of saliva. Therefore, it is known that the caries susceptibility is higher here (31).

Fissures are classified into five types on the basis of fissure morphology **(Picture 6):**

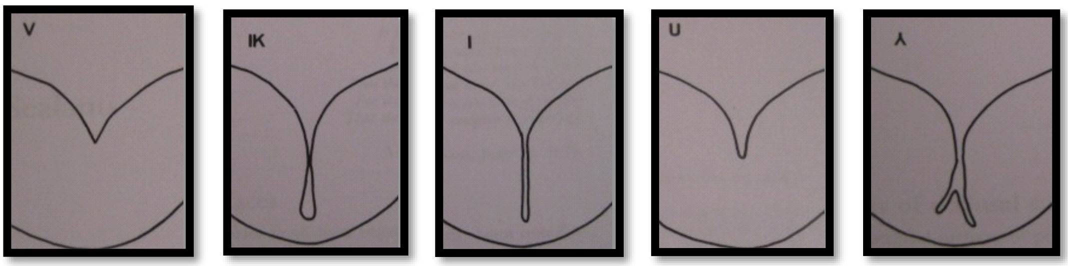
1. V-Type: 34% (wide at the top and gradually narrowing to the bottom)

2. IK-Type: 26% (hourglass-shaped)

3. I-Type: 19% (in the form of a narrow slit)

4. U-Type: 14% (same width at top and bottom)

5. Y-Type 7% (It starts in the form of a narrow slit and shows branching towards the dentin) (32).



Picture 6. Schematic representation of fissure morphologies

In another classification, fissures are divided into three different types according to fissure cleft depths: superficial, medium and deep.

Superficial fissure: When examined clinically, the tubercle slopes intersect at a wide angle and it is possible to see the fissure base between the tubercles by using the traditional light source. The enamel thickness is reported to be about 1.5-2 mm.

Medium fissure: The fissure slopes intersect at a narrower angle than shallow fissures and the fissure base can be generally seen via transillumination.

Deep fissure: Tubercle slopes intersect at a narrow angle, the fissure base cannot be seen by transillumination, and the enamel thickness is 0.2 mm or less. In such fissures, initial caries rapidly progresses to dentin (10).

Although there are different classifications, some reasons are reported for the high risk of caries in pits and fissures. The morphology of the occlusal surfaces of the teeth has been reported to be the most important cause for the formation of pit and fissure caries. The facts that the occlusal pits and fissures generally have many narrow and uneven grooves and the washing effect of salvia is less on these structures facilitates the retention of food and bacterial plaque. In addition to these adverse effects, it is known that the inability to perform mechanical cleaning sufficiently causes caries development (33). In many studies on fissure sealants, fissure sealants have been reported to stop the initial caries lesions by eliminating the nutritional source required for *S.mutans* (34,35).

The enamel thickness in pits and fissures has also been reported to be another factor that affects caries formation. It has been suggested that the enamel thickness is less in pits and fissures than smooth enamel surfaces. Therefore, many years are required for caries lesions starting on smooth enamel surfaces to reach the dentin, whereas the caries lesions starting on pit and fissures has been reported to progress rapidly to dentin. Furthermore, the enamel thickness is known to vary depending on the type of fissure. In deep and narrow fissures with an enamel thickness of less than 0.2 mm, caries can progress quickly to the enamel and reach dentin in a short time. However, in wide fissures with an enamel thickness of 1.5-2 mm, it takes a longer time for caries to reach the dentin (36,37).

Another reason that increases the accumulation of bacterial plaque and hence the risk of caries in pits and fissures are uneven morphological structures occurred due to tissue anomalies in teeth. It has been reported that developmental anomalies in enamel and dentin increase the susceptibility of pit and fissures to caries by causing the tissues to decrease in these regions and inadequate mineralization For instance, the risk of pit and fissure caries is reported to be quite high in cases of Amelogenesis Imperfecta (AI) and Molar Incisor Hypomineralization (MIH) (38,39).

Fluoride with greater efficacy on smooth surfaces has less effect on pit and fissure surfaces; which is one of the factors causing a high rate of caries on these surfaces. Even after the pit and fissures have been brushed and etched with a proper acid, debris and pellicle can be found on the fissures. Therefore, fluoride cannot reach these areas easily as on smooth surfaces, which are easy to clean (40,41). Fluoride shows its remineralization effect and caries-preventive effect in plaques with high pH values like 6.7-7.3. Nutrients and microorganisms accumulated in pits and fissures, which are very difficult to be removed, are reported to reduce the pH levels of these regions. As a result of this situation, it has been reported that fluoride could not provide its remineralization effect. Therefore, pits and fissures with low pH levels become caries susceptible regions (41).

2.2. Pit and Fissure Caries Prevention Methods

Although progress has been made in the prevention of pit and fissure caries, epidemiological findings show that the problem has not been clearly identified yet (42,43). Therefore, it has been reported that the search for methods for the prevention of pit and fissure caries is still continuing (44,45).

2.2.1. Oral and Dental Health Training Programs

In order to prevent pit and fissure caries, special training programs have been developed to understand the importance of mechanical cleaning and oral health. It is stated that the program is a preventive protection method with a proven benefit and includes training for the family as well as for the patient. Studies have shown that this method decreases both the plaque and caries on occlusal surfaces of molar teeth in the process of eruption when applied in cooperation with the family (46–49). Besides these training programs, it is of great importance to evaluate dietary habits, make dietary arrangements and to control sugar consumption (50–52).

Mechanical cleaning should be supported by such several methods as topical fluoride, fissure sealant applications, and antimicrobial agents (50–52).

It has been stated that the risk of caries of the child should be taken into consideration when choosing the protective method to be applied (53).

2.2.2. Fluoride Applications

Fluoride, which has been reported to be one of the protective applications commonly used to prevent dental caries, is reported to show its prevention effect in different ways. Fluorine application prevents enamel demineralization occurred due to acid attacks and provides remineralization on the teeth. Furthermore, it prevents the production of acid by inhibiting bacterial metabolism, and thus increases plaque pH (54,55).

In the mechanism of fluoride application, fluoride ions are replaced by the hydroxyl (OH^-) ion group in the apatite crystals and form the fluorapatite crystals more resistant to caries, the solubility of which is lower (54).

In topical fluoride applications, calcium fluoride (CaF_2) that is a loosely bound fluoride compound is produced on the enamel surface. In studies, CaF_2 accumulating in tissues such as plaque and saliva has been reported to support remineralization by providing fluoride ion to the medium when the enamel is demineralized (56–58).

One of the topical fluoride application methods, fluoride varnishes are reported to be one of the significant protective applications frequently used to protect the teeth from caries. Fluoride varnishes are easily applicable on teeth for which moisture isolation is difficult to provide, ensures the release of fluoride for a long time and can spread easily to fissures. Therefore, it is reported to be one of the preferred protective dental applications (59).

In studies comparing the antioxidant activities of fluoride varnish and fissure sealants applied to primary molars, fissure sealants have been reported to be more effective (60,61).

2.2.3. Using Antimicrobial Agents

In individuals with a high risk of caries, supporting the oral and dental care with antimicrobial agents, besides the mechanical cleaning, is reported to increase the effectiveness of brushing (50,52).

Chlorhexidine, which is used in the prevention of caries and chemical control of dental plaque, is reported as an antibacterial agent. It is reported to be available in the forms of gel, mouthwash and varnish. Chlorhexidine can be used as an adjuvant agent in preventing caries in patients with active caries because it stops the development of *S. mutans* (62).

2.2.4. Ozone Treatments

The use of ozone in dentistry was first reported in 1932 (63). It has been reported that the liquid form of ozone is primarily used for the treatment of infected wound sites and chronic periodontal infections (64).

Ozone is suggested to be a non-invasive agent with antibacterial, antiviral and antifungal properties, which is used to reduce the number of bacteria causing caries (65–67). It has been reported to realize the disinfection function by neutralizing pathogenic microorganisms or by inhibiting the growth of microorganisms and by destroying cell walls (65–70). Ozone molecules provide the blockage of enzymatic control systems of microorganisms and enter into the cells by increasing the permeability of the membrane, resulting in the destruction of microorganisms (63,68).

Ozone inhibits microorganisms by eliminating the protein layer protecting the caries lesion and biomolecules necessary for the survival of the bacteria thanks to its oxidizing effect. Thus, it is stated that the metabolic balance is returned to the remineralization side. The oxidation properties of the ozone have shown that the pyruvic acid formed by the bacteria is converted to acetate with a more alkaline structure and carbon dioxide. In this way, it has been emphasized that a suitable environment for the accumulation of minerals into the caries lesion is created (71).

When ozone is used for the disinfection of cavities, it is stated that there is no decrease in the bond strength of resin-based materials to the tooth tissues on the contrary to other antimicrobial agents (sodium hypochlorite and chlorhexidine). Therefore, ozone can be used for cavity disinfection purposes prior to the composite and fissure sealant applications (72). Furthermore, in the literature, the antibacterial effect of ozone is reported to be effective only for the initial enamel lesions and although there is no cavitation, antibacterial effect is not observed in the caries lesions associated with the dentin (73).

2.2.5. Laser Applications

In dentistry, the laser is reported to be used for various purposes such as diagnosis and treatment of enamel and dentin caries, amputation treatment of deciduous teeth, bone contouring, and preparation of the surface before the fissure sealant application (74). In studies conducted with carbon dioxide laser, it has been reported that the progression of caries lesions in the enamel can be stopped up to 85% via in vitro laser application. The laser beams are absorbed from the surface of the tooth and heat up the surface layer for a short period of time (75). After the heating period, crystals that are more resistant to acid attacks in the process of caries occur following the carbonate loss from carbonated apatites (75,76). It has been reported that the resulting resistance is the most important factor to prevent the progression of sub-surface caries and is a treatment method that can be used for the treatment of suspicious areas in the teeth (75). Furthermore, lasers have been reported to be used in caries prophylaxis because they have antibacterial effects on the surfaces when applied at different wavelengths and reduce the number of cryogenic microorganisms (77).

2.2.6. Remineralization Treatments

In dentistry, non-invasive treatment and remineralization are of great importance for non-cavitated caries lesions. The fact that the remineralization capacity of the fluoride applications is limited leads to the search for new remineralization agents (78,79).

Amorphous calcium phosphate (ACP) has been reported as one of the agents used to provide remineralization. Casein phosphopeptide (CPP) found in the milk protein transforms into casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex in order to stabilize the calcium phosphate (80,81). Besides having remineralization properties, CPP-ACP has been reported to also have anti-caries properties (82). It has been reported that CPP-ACP binds to bacteria on the tooth surface and plaque to form a dense calcium reservoir on the tooth surface and adjacent areas (83). In acidic conditions that may lead to demineralization, it releases calcium and phosphate ions into the medium, increasing the calcium and phosphate concentration of the plaque, and provides remineralization by preventing demineralization (81).

The use of remineralization agents containing ACP in dentistry has been reported to be started with toothpaste and chewing gum containing CPP-ACP. In recent years, it has been reported to be used in the contents of restorative and protective materials used in dentistry (83). In literature, remineralization efficacy of CPP-ACP is reported to be superior to sodium fluoride mouthwash (84) while it has a similar remineralization efficiency with resin-based fissure sealants (84,85).

Silver diamine fluoride (SDF) is another remineralization agent which is reported to be safe to use and effective to prevent caries by the World Health Organization (WHO). Silver affects many vital activities of bacteria, such as cell wall synthesis, hydrogen bonding and cell division, and renders bacteria ineffective. In this way, it stops caries by preventing the formation of biofilm (86,87). In the in-vitro studies, it has been reported that SDF penetrates the enamel more than the flora and therefore its effect is higher than sodium fluoride (86). In clinical studies, SDF has been reported to have anti-caries effect superior (88) or similar to (89) sodium fluoride. Some researchers have stated that its effects are almost similar to fissure sealant and glass ionomer cement applications (90,91).

Another remineralization agent used in dentistry is reported to be titanium tetrafluoride (TiF_4). Titanium in the content of TiF_4 is known to increase the activity of fluoride and provide faster ion uptake to the tooth surface. TiF_4 forms fluorapatite on the enamel surface and decreases the solubility of the enamel. Moreover, it provides better protection and remineralization against the development of caries and erosion compared to topical fluoride agents (92,93). Topical application of TiF_4 has been reported to change the surface morphology of the enamel as well as increasing the fluoride content, unlike other remineralization agents. Following the topical application, a protective layer covering the surface, which does not go away for 24 hours and is resistant to acid application, is formed on the enamel. Because of these properties, TiF_4 differs from fluoride applications (94,95).

In a study by ***Büyükyılmaz et al.(1994)*** examining the application of TiF_4 as a sealant, the preventive layer formed as a result of 4% TiF_4 application on occlusal surfaces was reported to remain attached on the pits and fissures for 12 months despite masticatory and abrasive forces. Furthermore, it has been reported that the topical TiF_4 method can be an effective method for sealing pits and fissures since its technical sensitivity is less than that of the modified glass ionomer cement and resin-based sealants and can be applied in a shorter time. High acidity leading to suspicion at first was reported to disappear within a few days, but caused the color change in teeth and required polishing (93,96). It was concluded that more clinical studies are needed for TiF_4 (97).

2.3. Pit and Fissure Sealants

Pit and fissure sealants have been reported to be resin composite materials applied to pits and fissures on occlusal surfaces which are susceptible to caries. These materials have been reported to be layers that bind to the tooth, micromechanically; prevent the access of bacteria and bacterial products, which lead to the formation of caries, to teeth; reduce the food accumulation; and thus prevent the formation of caries (98).

2.3.1. History of Pit and Fissure Sealants

The history of occlusal caries dates back many years when Black was reported that more than 40% of permanent teeth caries develop in occlusal pits and fissures (99). ***Paynter (1962)*** stated that the presence of pits and fissures on occlusal surfaces containing food and microorganism was the most important factor in the formation of caries (100).

Several methods have been reported to be tried to reduce and prevent pit and fissure caries. ***Wilson (1895)*** proposed the sealing of occlusal pits and fissures with zinc phosphate cement. ***Hyatt (1924)*** suggested that prophylactic restorations, in which conservative class I cavities were prepared by including the pits and fissures suspected for development of caries and restored with the use of amalgam, would be effective in terms of protection (101,102). In a study by ***Bodecker (1929)***, pit and fissure caries were reported to be prevented if fissures were sealed with a thin layer of zinc oxyphosphate cement. They further reported that they developed prophylactic odontotomy technique as an alternative method aiming at more comfortable cleaning of deep and retentive areas by mechanically expanding fissures (103). ***Kline (1942)*** suggested the protective effect of the application of silver nitrate with ammonia composition to prevent the expansion of occlusive caries lesions. However, it was later understood that this method was insufficient to stop the caries (104). Until the widespread use of fissure sealants, prophylactic restorations and prophylactic odontotomy methods were used to prevent the development and progression of caries on occlusal surfaces (103).

The development of fissure sealants has been reported to be based on the process of acid etching of tooth enamel with phosphoric acid, which was found to increase the retention of resin restorative materials and strengthen marginal integrity. The first studies evaluating the effects of acid etching on retention were performed by ***Buonocore (1955)***(8). Cyanoacrylates, the first fissure sealant material, where acid etching technique was first used, was reported to appear in the mid-1960s. It has been reported that the cyanoacrylates are not suitable for use as a fissure sealant material because of their low binding strength and wear resistance and also due to being exposed to bacterial degradation in the oral cavity in time. Among other materials used as fissure sealants, there are polyurethane derivatives, use of which was discontinued since they were toxic and retention capability was not sufficient and polycarboxylate cement, use of which was discontinued because they were not fluid enough to spread to the deepest parts of the fissures and their wear resistance was low (105,106). At the end of the 1960s, it was found that a viscous resin formed by the reaction of bisphenol-A and glycidyl methacrylate could establish a strong bond with the etched tooth enamel. Thus, Bisphenol-A Glycidyl Methacrylate (Bis-GMA), which is a type of dimethacrylate resin, derivative fissure sealants came to the market (107).

2.3.2. Indications of Pit and Fissure Sealants

Although fissure sealants have been used for many years, it is stated that there is still no consensus on their indications. Guidelines have been reported to be developed for providing guidance on pit and fissure sealant applications (108).

When deciding to apply fissure sealant to a tooth; clinical observation, medical and social risk factors, dental history of the patient and susceptibility to caries should be taken into consideration and supporting the diagnosis with radiographic examination when required is emphasized to be of great importance. Furthermore, the individual's systemic or topical fluoride intake, tooth brushing and dietary habits have been reported to be taken into consideration for the indication of fissure sealant (41,44).

It has been emphasized that evidence-based clinical evaluations provide recommendations for the pit and fissure indications. In the guideline published by American Dental Association (ADA) in 2008, it was stated that patient selection, selection of teeth and application time (eruption level and eruption time) of the sealant were required to be taken into consideration while deciding the application of pit and fissure sealant (108). It was reported that the patient’s age, caries activity and caries risk, as well as the general health status of the patient, should be taken into consideration when choosing the patient at the indication of the fissure sealant (41,108).

Pit and fissure morphology are suggested to be important factors that should be considered while determining the fissure sealant application (109,110). While the effects of caries lesions on the occlusal surface on fissure sealant indication are evaluated, enamel caries cannot be clearly identified. Therefore, researches are reported to suggest the principle of “If you remain in dilemma, apply fissure sealant” in cases of caries suspicion (108). It has been stated that the health status of the approximal area should also be evaluated in the indications of fissure sealant. In the application of fissure sealants, it is reported that tooth eruption time is one of the factors to be taken into account (109).

2.3.3. Properties Required to be Present in an Ideal Pit and Fissure Sealant Material

- Biocompatible,
- Resistance to abrasion,
- Adequate bond strength and being able to remain in the mouth for a long time,
- Easy to apply,
- Being able to penetrate the fissures, having low viscosity and high fluidity,
- Low solubility in oral fluids,
- Resistant to functional and masticatory forces
- Having the effect of preventing caries formation,
- Having thermal and mechanical properties similar to enamel, and
- It should not undergo dimensional changes during polymerization (111).

2.3.4. Classification of Pit and Fissure Sealants

2.3.4.1. Resin-Based Pit and Fissure Sealants

It is stated that the scope of use of these materials in dentistry is increasing with the acidification method developed to ensure better bonding of resin-based materials to the enamel. In the literature, there are numerous studies on resin material and it has been found that resin has a strong bonding ability to the acidified enamel and is resistant to bacterial degradation (36).

Bis-GMA is one of the first methacrylates used in dentistry and is currently the basis for the resin-based materials currently used (105).

Today, the most preferred material as a fissure sealant has been reported to be Bis-GMA based polymers. Bis-GMA resins, which is a combined form of methyl methacrylates' ability to rapidly polymerize and epoxy resins' ability of minimal polymerization shrinkage, strongly bond to the enamel following the acidification process and can penetrate the fissures very well. Bis-GMA is reported to be capable of forming cross-linking during polymerization, compared to previously developed and used monomers. Furthermore, it has been emphasized that it has less polymerization shrinkage (112).

Despite these advantages, it is difficult to add a filler to the monomer due to its high viscosity; which is reported to be the disadvantage of that material (112). Therefore, monomers called triethylene glycol dimethacrylate (TEGDMA) and hydroxyethylmethacrylate (HEMA) are added to the structure of the fissure sealant to increase the viscosity and penetration ability of the polymer matrix (113,114).

TEGDMA can form a large number of bonds with monomers and is added to the resin matrix to reduce the viscosity of the Bis-GMA monomer but increases the polymerization shrinkage, which is a disadvantage (113,114).

HEMA, which is a monomer similar to TEGDMA, has both hydrophilic and hydrophobic properties. HEMA has been reported to be added to resin systems to reduce the viscosity of the polymer matrix and to increase the bonding of the resin material to the tissue, which is slightly moist (113,114).

Restorative resin materials (e.g. composite) are most commonly composed of Bis-GMA resins. It is indicated that methyl methacrylate (MMA) monomer is added to the resin in the ratio of 1/3 to increase the flowability of Bis-GMA and to ensure that Bis-GMA is better able to bond to pits and fissures and acidified enamel. This ensures better retention between the material and the dental tissue. However, despite these advantages, there are also disadvantages of resin-based fissure sealants; polymerization shrinkage leading to bacterial penetration and failure of restoration is the greatest disadvantage (113,114).

Based on the structural properties, resin-based fissure sealants are reported to be examined under three classes according to their polymerization method, color, and filler types (98,115).

According to Polymerization Methods

Bis-GMA-containing resin-based fissure sealants, polymerization of which occurs in various ways, are classified as first, second and third generation (36,98):

First generation fissure sealants: They are single component systems that do not require mixing and they are reported to be polymerized with ultraviolet (UV) light. It has been reported that UV light requires a wavelength of 365 nm (nanometer) to initiate polymerization. The use of first generation fissure sealants was discontinued because the irregularities in the wavelength of UV light had a negative effect on the retention of the material and had detrimental effects on the retina (36,98).

Second generation fissure sealants: Second generation fissure sealants consisted of two components are autopolymerizing materials. The first component contains Bis-GMA and benzoyl peroxide as initiator, whereas the second component contains Bis-GMA and the 5% organic amine accelerator. After the two components are mixed homogeneously, the mixture is applied to tooth. Despite the exothermic reaction following the mixing process, the heat generated is harmless due to the limited amount used. Following the preparation stage, the most important issue to be considered during the mixing process is to prevent air bubbles from leaking to the material since the application time is limited. Otherwise, air bubbles leaking into the material can present in the form of roughness and pits after application of fissure sealant (36,98).

Third generation fissure sealants: It is reported to be a type of fissure sealant which is polymerized with visible light. The contents of these materials are aromatic ketones and diketones activated by light at a wavelength of 170 nm. Camphorquinone which initiates the polymerization reaction and aliphatic amines having the ability to accelerate the reaction is added to the resin content. Visible blue light sources (halogen light sources) and light emitting diodes (Light Emitting Diode [LED], modified type visible blue light sources) are mostly used in the polymerization of third generation fissure sealants. Other light sources have been reported as Quartz Tungsten Halogen (QTH) light sources, plasma arc light sources, and laser light sources. Argon laser light sources have been reported to reduce the polymerization time by 75% by increasing the physical properties and polymerization degree of resin materials (36,98).

Second generation fissure sealants and visible light-polymerized fissure sealants have been shown to have similar clinical success rates. Nevertheless, the use of third generation fissure sealants, which are more easily applied, do not require mixing, have a more homogeneous structure and polymerize with visible light, is gradually increasing and use of second-generation fissure sealants has been suggested to have lost its popularity (36,98).

According to Filler Type

Several substances such as quartz, glass, and porcelain are reported to be added to the resin-based fissure sealants in order to increase the abrasion resistance and reduce the polymerization shrinkage. It has become suspicious that the fluidity of the material will decrease due to the addition of fillers into the fissure sealants and as a result, the material will not penetrate well into the details of the fissure. Nevertheless, no significant difference has been reported between the filler-containing fissure sealants and other fissure sealants in terms of fissure penetration. However, it has been reported that the fillers in the fissure sealants should be 30–65% by weight to better penetrate to the fissure details and for the fracture resistance to be acceptable (98).

In order to benefit from the ability to make the teeth more resistant to acid attacks, fluoride is considered to be added to resin-based fissure sealants. With this addition, it is intended to ensure that secondary caries is prevented and dental tissues adjacent to the restoration benefit from this effect of fluoride (116).

There are two methods in the process of fluoride addition to fissure sealants (117,118):

In the first method, soluble fluoride salt is added to the non-polymerized resin and it is thought after the application that the fluoride salt will be dissolved from the polymerized resin and released into the mouth medium. However, the protective effect of the fissure sealant has been reported to decrease with time because its structure is weakened due to the dissolution of the salt (117,118).

The second method has been reported to be the placement of mobile fluoride with covalent bonds that can be replaced by ions from saliva. In this way, it is thought that fluoride can be released to the medium with only ion exchange mechanism without any structural deterioration. Although there is a very low amount of fluoride in the structure in this application, it has been reported that there is no significant decrease in the protective effect of the fissure sealant because the fluoride lost is replaced over time (117,118).

It has been reported that to replace conventional fissure sealants, fluoride-containing fissure sealants should provide better retention or at least as much retention as the conventional fissure sealants, should maintain fluoride release for a long time and be able to form a fluoride reservoir in the mouth to form fluorapatite crystals to make the enamel more resistant to acid attacks. In this regard, fluoride release levels of fluoride-containing fissure sealants have been investigated in many studies (119,120).

In a study by ***Garcia-Godoy et al. (?) (1997)***, fluoride release properties of five resin-based fissure sealants containing fluoride (FluoroShield, Helioseal F, Ultraseal XT, Baritone L3, and Teethmate F-1) and a fluoride-free (Delton) fissure sealant were examined and all fluoride-releasing materials were observed to release a significant amount of fluoride for 30 days. They stated that among the materials, resin-based fissure sealant containing fluoride (Teethmate F-1) released a higher amount of fluoride than fissure sealants containing fluoride salt from the second day (121).

Locker et al. (2003) reported that two materials used as resin-based fissure sealant (Fissurit F and Helioseal F) released fluoride for 112 days (109).

Some researchers believe that fluoride-containing fissure sealants will degrade the integrity of the material after fluoride release, resulting in a weakening of the physical properties of the material (118). However, there are also researchers who reported that there were no significant differences between the fluoride-containing resin fissure sealants and the fluoride-free conventional fissure sealant materials in terms of retention values (122).

In the mid-1990s, concerns about possible estrogenic effects of Bisphenol-A and Bisphenol-A Dimethacrylate in fissure sealants were reported. However, short-term estrogenic effects of Bisphenol-A based resins were reported to be nonsignificant by

Soderholm and Mariotti (1999) and ***Fung et al. (2000)*** reported that Bisphenol-A released from the fissure sealants into the oral environment was not fully absorbed and was too low to be detected in the systemic circulation (123,124).

The most important factor affecting retention and clinical success of resin-based fissure sealants, which are considered as the most successful materials in terms of high retention rates and protective effects, are reported to be their contact with moisture and saliva (125,126). It is very difficult to isolate the teeth that cannot complete the eruption process completely from saliva because of gingiva on the distal surfaces. Therefore, various approaches have been developed to reduce moisture sensitivity while applying resin-based fissure sealants. It is very difficult to isolate the teeth that cannot complete the eruption process completely from saliva because of gingiva on the distal surfaces. Therefore, various approaches have been developed to reduce moisture sensitivity while applying resin-based fissure sealants (127,128). There is no consensus among researchers on the effectiveness of these approaches. Some researchers believe that these methods are successful, while some researchers report that such methods increase the number of steps and costs of the application, do not contribute to the retention ability of fissure sealants and are not practical (129–132). For this reason, it is reported that in teeth that are not completely erupted, glass ionomer-based fissure sealants with less moisture sensitivity should be used temporarily until the teeth are completely occluded (133,134).

According to Colors

Opaque, transparent and colored versions of resin-based fissure sealants have been produced to ensure that clinical practices and follow-ups are done easier. Although researches show that fissure sealants of different colors show clinically similar results, colored and opaque fissure sealants are more preferred by dentists because their clinical application and follow-up are easy and can be recognized by families. Although opaque fissure sealants are less preferred than others, they allow the detection and control of any possible caries that can be developed under the fissure sealant; which is reported to be the greatest advantage of such sealants (115).

2.3.4.2. Glass Ionomer Based Pit and Fissure Sealants

Glass ionomer cements (GIC) used in dentistry were reported to be formulated in 1969 by Wilson and Kent and developed by Mclean and Wilson in the 1970s (108).

It has been suggested that this material is produced with the idea of combining color match of silicate cement with the teeth, fluoride release, and the tooth-bonding ability of polycarboxylate cement. The resistance of the GIC to the chewing forces is similar to that of the silicate cement, however, its resistance to acids is higher than that of the silicate cement (108,135).

GIC, which is a material that contains fluoride in its structure and can physically bond to the teeth, is used in many areas of dentistry such as base material, adhesive cement, and restoration material and is reported to be able to release a high level of fluoride (135).

They are acid-resistant and biocompatible materials with low viscosity, film thickness of which is less and thermal expansion coefficients of which is close to the dental tissues. They are also known to be used as fissure sealants as they can chemically bond to enamel and dentin and have anti-caries properties as a result of fluoride release for a long time. However, in the initial stages of hardening reactions, extreme sensitivity to moisture and low mechanical resistance are reported as their disadvantages (135).

In many studies on retention rates of GIC-based fissure sealants, the rate of positive outcomes is very low; only a few studies have reported positive results. There is a consensus among the researchers that its clinical success is lower than that of resin-based fissure sealants and that it is often necessary to be renewed and therefore is not an ideal fissure sealant. There are studies showing that retention rate can be increased with the advancements made in resin-modified GICs (RMGIC), however, there are also studies that show the exact opposite (135,136).

GIC-based fissure sealants containing water in their structures are reported to be not affected by the moisture and saliva as much as resin-based fissure sealants. Therefore, their clinical success on the enamel surfaces contaminated with saliva has been reported to be higher than that of resin-based materials. In spite of this clinical advantage, as their retention rate is less, it is recommended that GIC-based fissure sealants should be applied as a temporary fissure sealant for the erupting molars only during the eruption period and they should be replaced with resin-based fissure sealants, which are more long-lasting, when the tooth erupts and moisture insulation becomes available (137,138).

In numerous clinical studies comparing resins-based fissure sealants and GIC-based fissure sealants, it has been generally observed that the retention rate of GIC-based fissure sealants was low compared to resin-based materials. It has been reported that GIC is chemically bound to the dental tissue, release fluoride and show anticariogenic effect. However, it has been reported to have several disadvantages such as uneven surface, sensitivity to early water contact, low abrasion resistance, and different retention rates when applied as fissure sealant (139,140).

2.3.4.3. Resin-Modified Glass Ionomer Cement-Based Pit and Fissure Sealants

The hardening mechanism of cement has been changed by adding a small amount of resin to the cement to eliminate the negative properties of conventional GIC (e.g. being affected from the moisture and low abrasion resistance) and to obtain a physically stronger material and as a result, RMGIC bonding to dental tissues both chemically and micromechanically has been obtained (141).

Although the resin caused a slowing in the acid-base reaction as it leaks into the cross-links, an increase has been reported in its physical properties compared to GICs. Nevertheless, it is stated that the resin in the material absorbs some amount of water from the oral environment and decreases the abrasion resistance (141).

As in conventional GICs, RMCIS is capable of releasing fluoride and has been reported to be a biocompatible material (141). Thanks to its improved physical properties and ability to release fluoride, RMCIS has been thought to be used as a fissure sealant and become the subject of many studies in the literature (142).

2.3.4.4. Polyacid Modified Composite Resins (Compomers)

Compomers (polyacid-modified composite resin) that started to be developed in the early 1990s is produced with the idea of combining aesthetic properties of composites and chemical bonding and fluoride release properties of GICs in a single material (143).

It has been reported that the fluoride source of the compomers is the ion-releasing bioactive glass filler and start releasing fluoride when coming into contact with water. Compomers have been reported to be more similar to composite materials in terms of structure rather than GICs and RMGIC (143,144). In a study by ***Saito et al. (?) (1999)***, tooth bonding strength of the compomers was reported to be higher than the GICs, however, their abrasion resistance was reported to be lower than that of conventional GICs (144).

In a study where resin- and compomer-based fissure sealants were applied to the first permanent molars, the eruption of which completed, of children at 6-7 years of age, authors reported that retention was lower in compomer-based fissure sealants after two years of clinical follow-up (145).

2.3.4.5. Ormocers

Ormocers have been started to be used in the field of dentistry as a biocompatible material that significantly reduces polymerization shrinkage. Having minimum amount of residual monomer after polymerization, ormocers are highly resistant to abrasion, have easy condensation and have extremely low marginal leakage rates (146).

It has been emphasized that the number of clinical studies investigating the long-term effects of ormocers is insufficient. They are produced to be used in two types: restorative materials and fissure sealants (146).

2.3.5. Factors Affecting the Clinical Success of Pit and Fissure Sealants

It has been reported that the complex morphologies of pits and fissures are one of the most important factors affecting the success of fissure sealants. Regardless of the material used, fissure sealants hardly penetrate into deep and narrow fissures, while they can easily reach large fissure bases (147).

Non-disinfection of the tooth surface from plaque and debris during the fissure sealant application is a factor that negatively affects the retention and penetration of the sealant. Therefore, the tooth surface must be cleaned by various methods such as air abrasion and bristle brush before applying the fissure sealant (147).

Contamination of acid-etched enamel surface with saliva and moisture is reported to be another factor that negatively affects the bonding of fissure sealants to enamel (147).

Another factor that causes pit and fissure sealants to fail is the material used; fissure sealants with high viscosity are more difficult to enter into pits and fissures, which adversely affects the retention of the material. Therefore, it has been reported that sealants with low viscosity should be considered during the selection process of pit and fissure sealant (147).

Other material-based factors affecting the clinical success of fissure sealants also include low abrasion resistance of the selected pit and fissure sealant against occlusal forces, surface tension, polymerization shrinkage, and thermal expansion coefficient (147).

One of the most important factors affecting the success of fissure sealants is the contamination of the tooth, to which fissure sealant will be applied, with saliva and moisture. Particularly in young children, it is difficult to prevent the contamination of tooth with saliva during fissure sealant applications; which has been reported to affect clinical success as well as retention of fissure sealants. Thus, resin-based fissure sealant materials that are currently considered the most successful fissure sealant with their high level of retention and protective effects should be used with different approaches to minimize contamination. Because resin-based fissure sealants are highly susceptible to moisture and saliva contamination, they can only show high retention as long as they are applied under ideal conditions (108,109,115,125,126,148).

Although it is recommended to use a rubber dam to provide moisture control in children, rubber is difficult to use particularly in pediatric patients and increases the cost, which reduces its rate of use (110). It has been reported to be hard because isolation is provided via dental cotton rolls, it causes swallowing problems in children, cotton rolls can be moved with tongue, and there is a risk of contamination during the placement of cotton rolls. It is stated that the four-hand technique is required during the cotton roll isolation. During the application of fissure sealants, adaptation problem, particularly in young children, and the presence of operculum in the teeth that are still in eruption process have been reported to be factors having negative effects on saliva and moisture isolation (149).

In pediatric patients, for whom moisture and saliva isolation is so important and difficult, different approaches have been developed to increase the clinical success of fissure sealants. Hydrophobic fissure sealants are recommended to be used with dehydration agents to prevent moisture. However, the increase in procedure time and increased cost in pediatric patients with adaptation problems have been reported as the disadvantages of this method (150).

Application of bonding agents containing hydrophilic monomers in their structure to the enamel surface prior to fissure sealants has been reported to be another recommended method. This method is also reported to be disadvantageous because of the increase in the number of procedure steps and cost of application as in the other method (151).

In recent years, studies have been carried out to develop hydrophilic fissure sealants showing better retention against the risk of contamination of moisture and saliva (152,153).

It has been proposed to use GIC-based fissure sealants temporarily instead of resin-based fissure sealants until the risk of moisture contamination is eliminated (115).

2.4. Use of Bonding Agents in Pit and Fissure Sealants

2.4.1. Enamel and Adhesion

Dental enamel is the most rigid structure of the human body, which is formed by ameloblasts, covering the anatomical crown of the tooth. The enamel which is the only structure of tooth with ectodermal origin protects dentin and pulp by surrounding the tooth and is reported to be very flexible according to its hardness and be also fragile. Enamel hardness is shown to decrease gradually towards the enamel-dentin combination (154).

Enamel formation begins with the baby's development in the mother's womb and forms following the synthesizing of dentin by odontoblasts. Ameloblasts first secrete the enamel-dentin membrane onto the dentin layer and move on towards the periphery by secreting a 4 μm enamel matrix on this membrane with no prism every 24 hours (155).

During the formation of the enamel, it has been found to replace the carbonate forming the core structure. The caries progress from the center of the crystals to the periphery due to the apatite part of this carbonate, which is not resistant to acid attacks in hydroxyapatite (HAP) crystals (155). In the enamel layer extending from the enamel-dentin boundary to the outer surface of the crown, there are reported to be nested prisms of 4-6 μm in thickness. This passage in keyhole-shaped prisms and settlement of HAP crystals the regions between the prisms are determined during the formation of enamel matrix (156). As HAP crystals are about 10 times larger than dentin and bone crystals, it can be observed more easily via electron microscopy (155).

It has been reported that the enamel is composed of 94-98% inorganic material, 1-4% water and 1-2% organic material in weight; and 86% inorganic material, 12% water and 2% organic material in volume (157).

A large part of the inorganic part of the enamel has been reported to be composed of HAP crystals. HAP crystals [$Ca_{10}(PO_4)_6OH)_2$] are formed by the lining up of calcium (Ca^{+2}), phosphate ($PO4^{+}$) and hydroxyl (OH^{-}) group crystallites and composed of small amounts of carbonate, fluoride, chlorine, zinc, strontium, magnesium and aluminum elements. Among these elements involved in the dental structure, fluoride, boron, barium, lithium, magnesium, molybdenum, strontium and vanadium have protective properties against caries (155).

There is an inter-prismatic matrix between the enamel-dentin joint and the HAP crystals located on the outer surface of the tooth (154). The location of the prisms has been reported to affect the resistance of the tooth to acid attacks (158). In case of encountering fluoride during amelogenesis or applying fluoride to immature enamel, fluoride and hydroxyl ions create fluorapatite crystals by changing their places. In this way, the crystal becomes bigger and thicker and the dental structure has become more resistant to acid attacks (156).

It has been reported that there are water and organic materials in the inter-prismatic region between the HAP crystals in the tooth enamel. These micropores have been shown to ensure that the enamel is in contact with the dentin and pulp. It is emphasized that the enamel that loses its moisture in the micropores due to air-drying process has an opaque and chalky appearance and regains its moisture thanks to saliva in the oral environment and returns to its former appearance. It has been reported that this is due to the semi-permeable feature of the enamel. Substances with a low molecular weight can diffuse into the enamel, whereas substances with high molecular weight cannot be diffused from micropores (159).

The organic part of the enamel is composed of large protein complexes, sheathelin, free amino acids and lipid (155). During the development of the enamel, the organic structure (30%), which was initially higher, then hydrolyzes and gives its place to minerals. After that, its ratio has been observed to decrease to 1%. During this mineralization stage at the time of embryological development, protein catabolism occurs and as enamel proteins (amelogenin, ameloblastin, amelotin, enamelin) collapse, their residues remain in the periphery of enamel prisms. As a result, the locking process of HAP crystals in the mature enamel weakens and is exposed to acid attack, resulting in the occurrence of more affected areas (155).

Regardless of localization or depth, the enamel is homogeneous except for the aprismatic external layer and its surface energy is high as its inorganic material content is higher (160).

"Adhesion" is a term that defines the gravitational force between different molecules in contact with each other. “Cohesion” describes the gravitational force between the same molecules. Substance that is used for adhesion process is called "adhesive" and the surface/substance to which adhesive is applied is called "adherent". It has been reported that for a robust adhesion, adherent must be in tight contact with the adhesive. In dentistry, adhesives are reported to correspond to bonding agents, and adherent tooth corresponds to dental surface (161).

It has been reported that adhesion types can be examined as chemical, diffusion, electrostatic and mechanical:

<u>Chemical (Adsorption) Adhesion:</u> It is the type of bonding occurred between the adherent and adhesive due to primary and secondary forces. Primary forces occur due to the ionic bonds between positive and negative atoms, covalent bonds formed by shared electrons, and metallic bonds formed by ions delocalized in the electron cloud. Hydrogen bonds, Van der Walls forces, Keesom dipole interaction forces, London dispersion forces, and Debye dipole induction forces have been reported as secondary forces. It is emphasized to be a weak bonding type form (162).

<u>Diffusion Adhesion:</u> It is a weak bonding type which is formed between two moving molecules by the diffusion of two polymers and polymer chain ends in the interface (135).

<u>Electrostatic Adhesion:</u> It is a weak bonding type which is formed by electrostatic interaction between different surfaces (160).

<u>Mechanical Adhesion:</u> It is formed by the bonding of the adhesive to the grooves on the surface of adherent due to geometric and rheological factors. Geometric factors ensure the mechanical locking on microporosities on uneven surfaces, while rheological

factors ensure that adhesive flows into the grooves and holds to adherent by shrinking due to the fluidity of liquids and deformation of solids (39). Polymerization shrinkage in resin-based dental materials is suggested to be also due to rheological factors (162).

Micromechanical bonding is the basis of adhesion technologies used in dentistry. The adhesive material flows into microporosities occurred following the acid etching procedure and provides micromechanical locking (163).

Adhesion provided between the dental surface and restorative agents is considered as the restoration success in dentistry. Adhesive dentistry is a branch of dentistry developed after the concept of acid etching defined by ***Buonocore (1955)*** (8). It has been shown that acrylic resin bond to microporosities formed due to the acid etching of enamel and thus, Black's principle of "expand to protect" has been reported to lose its validity. As a result, it has been paved the way for minimally invasive approaches using the adhesive system (163).

During the development of adhesive systems, ***Bowen (1965)*** reported that the bonding of resin-based dental materials to the tooth surface was facilitated by surface-active monomers (164). ***Nakabayashi (1998)*** defined the formation of the hybrid layer and important steps were taken in the development of the adhesive system (165). Advances in the adhesive system have prevented the expansion of the cavity design to damage the healthy tissue to provide retention. Restoration repair became possible, tooth tissue weakened after preparation was supported, micromechanical attachment, microleakage, postoperative tenderness, and secondary caries were prevented, the functional stresses were ensured to be better transmitted to the tooth throughout the connection interface, and aesthetically successful results have been achieved (166).

With the development of adhesive systems, many classifications have been reported to be created. In general, adhesive systems are classified into three main groups: generations, smear layer treatment strategy, and clinical application steps (166).

2.4.1.1. Classification of Adhesive Systems by Generations

There are seven subgroups in this classification which is frequently used in the classification of adhesive systems.

<u>First Generation:</u> The first generation adhesive systems have been reported to be based on the principle of Buonocore, who demonstrated the chemical bonding of N-

phenylglycine Glycidyl Methacrylate (NPG-GMA) with dentin (164). The first generation of adhesive agents is based on NPG-GMA, a surface active comonomer. A water-resistant bond between calcium and resin on the tooth surface was aimed to be provided, however, binding strength of this hydrophobic agent was limited to 2-3 MPa (Mega pascal) (167). Although it was thought that there was ionic bonding to HAP crystals on the enamel surface and covalent bonding to collagen, Carbon-13 NMR analyzes revealed no bonding between NPG-GMA and HAP (168).

Second generation: In this generation developed in the early 1980s, it was reported that bonding mechanism involved formation of ionic bond between the negatively charged phosphate groups in adhesive resins and positively charged calcium ions in the smear layer (169). Adhesives with bond strengths of 1-10 MPa have been reported to be unable to resist the polymerization shrinkage of composite resins and form micropores leading to microleakage (167).

It has been reported that the smear layer, which has very low cohesive durability and is not firmly bonded to the dentin, cannot be removed in the second generation adhesive agents. Adhesion has been tried to be provided by this layer. However, these adhesives with no hydrophilic character have not been able to penetrate the smear layer, so resin tags cannot be formed. Thus, it has been reported that adequate adhesion cannot be achieved (170). In in vitro tests, restorations made with second-generation adhesives have been reported to provide acceptable adhesion for only six months (171).

Third Generation: In the late 1980s, it was understood that the smear layer had to be modified or removed to ensure adequate adhesion and thus, multistep applications have been reported to come to the fore. Modifying or removing the smear layer has been reported to allow the penetration of acidic monomers such as Phenyl-P or Dipentaerythritol Pentaacrylate monophosphate (PENTA) and to allow the passage of the resin to the dentin tubules. However, it has been reported that the desired penetration and bonding strength cannot be achieved due to the hydrophobic character of the adhesive agent (135).

During the development of the third generation adhesives, it has been reported that many etching agents like 6.8% ferric oxalate and Ethylene Diamine Tetra Acetic Acid (EDTA), 2.5% nitric acid and NPG, maleic acid and HEMA solution in water, PENTA in HEMA ethanol solution, 10% citric acid and 20% calcium chloride, NPG-

GMA or N-tolglycine Glycidyl Methacrylate (NTG-GMA) have been mixed with Pyromellitic Acid Dimethylmethacrylate (PMDM) solution in acetone and many primers and resins have been used like the combination of PMDM, HEMA and glutaraldehyde; and Bis-GMA and HEMA combination (163,172). Among these agents, ferric oxalate has been reported to be used with the aim of creating a precipitate layer comprising of calcium oxalate and ferric sulfate on the surface and thus, the protection of the pulp. However, its use has been discontinued due to the occurrence of coloration in follow-ups (172).

Although the bonding strength in this generation has increased to 10-14 MPa, it has been reported that the use of first, second and third generation adhesives has been discontinued since they failed to provide adequate binding resistance (173).

Fourth Generation: There are three steps in this generation of adhesive applications. In the first step, the smear layer is removed with the washed acid following the application, the dentin tubules are opened, dentin permeability is increased, intertubular and peritubular dentin is decalcified and a collagen network is formed (174).

In the second step, the primary solution of the hydrophilic monomers which are reactive in acetone and/or water is applied and the surface energy as well as wettability of the dentin surface increase. As the hydrophilic part of the primer shows an affinity to dentin whereas hydrophobic part has an affinity to the resin, penetration into dentin collagen is easier (175).

In the third step, adhesive resin with or without filler is applied in which the resin is polymerized by entering into the collagen network and provides a hybrid layer (174). Thus, an adhesive group having a bonding strength of 20 MPa or more has been reported to be formed (176).

Fifth Generation: In this generation, it is aimed to make the third-step system of the fourth generation, which is time-consuming and requires a careful application, to be more practical. For this purpose, one bottle system has been provided by decreasing the number of steps by combining primary and bonding agents. However, it has been reported that it is wrong to call this application "one bottle" or "single step" because the acid application is performed prior to the procedure and primer and adhesive resin require two or more applications (175).

Although the bonding strength of the fifth generation adhesives has been found to be 30 MPa in many studies, there were no different results in some studies (177). This situation has been reported to be explained by technical factors such as the failure of acetone or ethanol-containing adhesives to penetrate into the air-dried dentin surfaces (178). In air-dried dentin, the spaces between the collagen network disappear, resulting in collagen collapse, and thus, adhesive applied on the dentin cannot penetrate to the dentine (179).

Sixth Generation: This generation was reported to be created in the early 2000s to prevent the failures occurred during the acid application, washing and drying processes in the fourth and fifth generations and to shorten the procedure duration as much as possible. In order to achieve this, acid has been added to the primer and "self-etch adhesives" have been obtained (180).

The sixth generation is based on the formation of the hybrid layer by modifying the smear layer. In the following periods, one bottle applications combining acid, primer and adhesive resins have been reported to reduce the number of application steps and to shorten the procedure duration (181). Although the sixth generation adhesives create a sufficient bond strength in dentin, they were found to be ineffective in enamel. The reason for this ineffectiveness has been attributed to the fact that demineralization obtained from the enamel etched with phosphoric acid cannot be obtained in the self-etch system and adhesion is affected in a negative way (182).

Seventh Generation: Acid, primary and adhesive resins have been reported to be combined in one single bottle in this generation. In most of the studies on bonding strength, the bonding strength of the sixth and seventh generation adhesives have been reported to be similar, however, this adhesive generation could not achieve the expected demand due to the phase separation observed in the first versions (183).

2.4.1.2. Classification of Adhesive Systems by Their Smear Layer Interactions

Adhesive systems have been reported to be classified according to the criteria whether they do or do not remove/modify the smear layer. It has been observed that researchers have different views in this classification. In the early development periods of adhesive systems, some researchers have reported that the smear layer acts as a barrier and reduces the permeability of dentin by 86% and that they protect the pulp from microorganisms and its products (135). This principle applied in the first and second generation adhesives has been reported to be insufficient in the studies carried out in a later period. Adhesive systems, which are still used today and have sufficient bonding strength, have been reported to remove or modify the smear layer (135,160,163).

It is emphasized that the systems that completely remove the smear layer (etch and rinse system) form a hybrid layer by removing the smear layer completely in the acid etching stage. However, it has been reported that this situation may cause toxic effects on pulp by causing the penetration of acid, microorganisms, and toxic products into the pulp through the dentine tubules opened and although there is no penetration, post-operative sensitivity may be encountered due to hydrodynamic mobility (160).

In smear layer-modifying systems (self-etch system), the smear layer is removed with a weak acid + primer application and joins to the hybrid layer (163).

2.4.1.3. Classification of Adhesive Systems by Clinical Applications

When developing adhesive systems, many acid types and application methods have been used to increase the bonding strength. Thus, adhesive systems have been divided into three groups according to their clinical applications: etch and rinse, self-etch and multi-mode combining two systems (184).

Etch and Rinse Adhesive Systems

The etch and rinse system is based on obtaining a hybrid layer by allowing resin monomers to flow instead of minerals and smear layer removed from the dentin through acid etching and creating a micromechanical bonding to microporosities (184).

Etch and rinse system has been reported to be a three-step (fourth generation) adhesive system that involves acid-etching, priming, and applying adhesive (185). It has been reported that primer should be applied on the acid-etched tooth surface, whose wettability, penetration, and retention are improved through the formation of microporosities and surface energy of which is reduced. Wettability has been reported to be increased through surface activation by hydrophilic primer application. Thus, the tooth surface is prepared for adhesive resin application (186). However, this three-step fourth generation application takes time and the risk of contamination increases in each step. For this reason, the primary was added to the bonding agent and in this way, two-stage fifth generation adhesive systems were created (187). Thus, one bottle agents containing hydrophilic and hydrophobic resin and solvent (ethanol, acetone, water) have been started to be used (163). Following the application of adhesive resin, macrotags surrounding the enamel prisms and microtags penetrating into the enamel prisms have been reported to be formed. It has been suggested that interprismatic microtages are much more effective in providing retention on the enamel (181).

Following the acid etching process, the majority of the HAP crystals on the dentin surface have been reported to disappear and the microporous collagen network is revealed. Resin has been reported to diffuse into this collagen network and the hybrid layer is formed. Thus, it is said that adhesion occurs as a result of micromechanical locking. However, since functional groups of resin monomers have a poor affinity to the collagen network lacking the HAP, chemical adhesion cannot occur (188).

In the etch and rinse adhesive system, it has been reported that the solvents in the bonding agent have an important role in adhesion. In the adhesives where the acetone is used as a solvent, wet bonding occurs while dry bonding occurs where the solvent is water/ethanol (189).

As etch and rinse adhesive systems applied in three stages have high bonding strength to enamel and dentin and they are resistant to hydrolytic destruction due to low hydrophilicity of polymerized resin, they are recognized as the most effective system in the present day (190).

<u>Acid Etching and Adhesion to Enamel:</u> The main principle in the bonding of restorative materials to the tooth is the micromechanical locking of the restorative material to microporosities caused by the removal of inorganic material from hard dental tissues (191). The high inorganic structure of the enamel and its almost uniform distribution throughout its thickness have been reported to be seen as an advantage for this micromechanical locking (192).

The aim of the acid etching of enamel surface is to clean the enamel and to increase the wettability, penetration, and retention of microporosities on the enamel surface

(193). It has been reported that with the removal of prismatic and interprismatic crystals on the enamel surface, the surface energy of the enamel increases and the number of microorganisms on the enamel surface decreases by 75-95% (194). Following the acid etching, microporosities with a depth of 25-75 µm are formed in the enamel tissue and thus, the area of 10 µm on the upper surface is removed and the bonding surface has been reported to be increased by about 2000-fold in this way (195).

Acid-etched enamel surface has been reported to be classified under three main categories according to the concentration of the acid applied, application duration, invasive or non-invasive procedure, amount of fluoride in the enamel, and angle of HAP crystals (196):

Type I: a honeycomb appearance is produced as a result of prism core materials being dissolved.

Type II: a cobblestone appearance is produced as a result of peripheral regions of the enamel prisms being dissolved.

Type III: a colorless appearance is produced.

Although it is known that it can vary according to many parameters and that what kind of etching pattern will occur on the surface of the same tooth clinically, the most common etching pattern has been reported to be as in type I classification (163). It has been suggested that there is no relationship between etching type and bond strength to the enamel although the etching types are important to understand how the adhesion occurs (197).

In the enamel etching process, various agents such as 16% EDTA, 10% maleic acid, 10% citric acid, 1.6-3.5% oxalic acid, 2.5% nitric acid, 20-25% polyacrylic acid, and 10% pyruvic acid can be used but the most commonly used agent has been reported to be 37% phosphoric acid ($H_3[PO]_4$) (195). The use of phosphoric acid in the form of solutions makes the control of the enamel surface difficult and therefore, gel forms have been developed and the application has been made easier by adding colorants to its content. In the application of phosphoric acid, the concentration of acid has been found to be between 30-40% although there is a relationship between the concentration of acid and the depth of microporosities. If the concentration is above the recommended concentration, which is 40%, less calcium is dissolved from the enamel and it has been reported that the adhesion cannot be successful in this way (198).

In relevant studies published, it has been determined that the restorative material applied to the tooth enamel should provide a bonding strength of at least 17-20 MPa. The use of phosphoric acid at concentrations ranging from 35-37% allows the restorative material to bond with the desired bond strength and prevents deeper penetration compared to other acids. Therefore, other alternative acid agents have lost their effectiveness before they become widespread (163).

After the acid etching process, it should be ensured that there is no contamination in the enamel. It has been reported that acid etching procedure should be repeated in cases where there is suspected contamination because the microporosities on the enamel surface are affected negatively from the calcium and phosphorus in the saliva (160).

In the etch and rinse system, primer and bonding agent are applied to form microtag and macrotag structures in the enamel following the acid etching procedure. Microtags are known as resins on the inner surface of the enamel prisms and macrotags are known as resin tags surrounding the enamel prisms. As the bonding surface of the microtags is very large and their number is high, they have been reported to contribute more to adhesion (163). However, it has been reported that there is no relationship between the length of resin tags, i.e. depth of resin penetration and resin-enamel bond strength (199).

Self-Etch Adhesive Systems

Self-etch adhesive system was developed to remove the washing requirement, decrease the number of application steps, and to shorten the application duration (161). It has been reported to have mainly two stages. At the first stage, the enamel and dentin are simultaneously rinsed and the primary agent (self-etch primer) is applied and in the second stage, adhesive resin (self-etch adhesive) is applied. With the continuous development of adhesive systems, it has been observed that self-etch adhesives have been formed which have been able to reduce these two stages to one stage. In the single-stage self-etch system, primary and adhesive resin agents are combined in a single bottle (200).

There is no washing and drying step, which etch and rinse system has, in self-etch adhesive systems. Thus, it has been observed that both the number of application stage is reduced and the technical sensitivity is decreased (181,201). This situation has been reported to be advantageous by reducing the risk of saliva contamination in young children, particularly in children with whom cooperating is difficult (201).

It has been reported that the acidic monomer structure in the self-etch adhesive system changes the smear layer while demineralizing the enamel and dentin. This modified smear layer is also reported to be involved in the hybrid layer that occurs simultaneously (202). The smear layer prevents dental fluid movement, protects the pulp, and is incorporated into the hybrid layer by being modified. Thus, both these properties of smear layer are benefited and adequate demineralization occurs in the dentin (167). Furthermore, there are also studies reporting that resin tags provide good sealing and significantly reduce postoperative sensitivity as the smear layer is not removed (203).

With self-etch primer application, minerals are dissolved in the smear layer and it is reported that the etched surface at the depth of 0.5-1.5 μm occurs in the underlying solid dentin. Although the depth of the resulting hybrid layer is 1-2 μm, sufficient bonding strength has been achieved and microleakage remained at low levels (188). However, as the primer is effective throughout the smear layer in the self-etch adhesive system, it is stated that the thickness of the smear layer has a significant effect on the adhesion. In the literature, it has been reported that there is a low bonding when the primer fails to reach the dentin underneath the smear layer. Inversely proportional effects of etching effect of self-etch primer and the buffering effect of adherent result in low bond strength in the enamel (202).

Self-etching adhesives have been observed to be classified according to their pH acidity as strong, intermediate, and mild, however, it has been further observed that some researchers classify these adhesives only as strong and mild (181). Besides this classification, some researchers have reported that self-etch adhesives with a pH of above 2.5 are ultra-mild (204).

Since strong self-etch adhesives have a pH value of ≤1, it is stated that they completely dissolve the smear layer as in the etch and rinse adhesive system and create similar etching in enamel and dentin (203). However, calcium phosphate which is caused by the dissolution of the smear layer is not removed through washing as in etch and rinse system and leads to microleakage by preventing the chemical bonding with collagen due to its low hydrolytic effect (205).

As the mild self-etch adhesives have a pH value of ≥2, it has been reported to cause a demineralisation on the dentin surface only at a depth of 1 μm. Therefore, HAP crystals have been observed to be formed in the hybrid layer (190,206). These crystals have been reported to form an adherent surface for chemical binding (181). calcium carboxylate and calcium phosphate bonds formed with the chemical bonding of carboxylic acid based monomers such as 4-methacryloxyethyl Trimellitic Acid (4-MET) in the self-etch adhesive system, phosphate-based monomers such as phenyl-P (2-methacryloxyethyl Phenyl Hydrogen Phosphate), and 10-methacryloxysyl dihydrogen phosphate to calcium in the HAP crystal have been reported to remain stable for a longer period in the hydrophilic environment (181,206).

The thickness of the hybrid layer in the self-etch adhesive system is not as thick as in the etch and rinse adhesive system and does not provide sufficient bond strength (181,203). It has been reported to increase the restoration bonding thanks to both micromechanical and chemical adhesion. Micromechanical adhesion has been shown to increase the resistance to detachment stresses. Chemical adhesion has been reported to increase resistance to hydrolytic degradation (190). As collagen is surrounded by HAP crystals, it prevents the degradation of adhesion and resists collagen collapse (207).

The pH values intermediate self-etch adhesives have been found to vary between 1-2. In this intermediate self-etch adhesives, a hybrid layer is formed, the upper layer of which is completely demineralized and lower layer of which is partially demineralized with the primer application. It has been shown that HAP crystals under the hybrid layer provide chemical adhesion. The effect of acidic primer can reach 1-2 μm depth in dentin. In both etch and rinse adhesive system and strong self-etch adhesive system, there is a sudden transition from the unaffected dentine to the collagen network, whereas there is a gradual transition in the intermediate self-etch adhesive system. They have been reported to provide better micromechanical adhesion to enamel and dentin because they contain lower pH value than mild self-etch adhesives (181).

Multimode Adhesive Systems

With the adhesive dentistry gradually developing day by day, the number of application steps is decreased, application duration is shortened, and the need for technical precision is minimized (181).

If the selected adhesive system is etch and rinse system, it is ensured that the adhesive resin penetrates deeper with the removal of smear layer through acid etching step, the thickness of the hybrid layer increases and a strong micromechanical locking is provided (184). Compared to the self-etch system, long-term stability of the hybrid layer and endogenous matrix metalloproteinase (particularly MMP-2 and MMP-9) activity have been found to be higher in etch and rinse system. However, the acid application takes more time as it is an additional step and increases the risk of contamination. This, in turn, affects the adhesion success negatively (191). During the washing and drying phase, the need for moist bonding has also been reported to result in the need for an extra technical sensitivity (208,209). Moreover, many researchers have reported that post-operative sensitivity in etch and rinse adhesive system is due to the failure of adhesive resin to completely penetrate to the collagen network (184,210). In many studies, although the binding strength of the three-step etch and rinse system is accepted as the gold standard, it has been emphasized that the disadvantages cause an increase in the views towards the development of the adhesive system (201,209).

Self-etch adhesive systems have reduced the application steps by eliminating acid etching-washing-drying steps, allowed the application to be completed quickly and ensured the reduction of postoperative sensitivity thanks to the more superficial demineralization and integration of the smear layer with collagen and minerals (161). The greatest advantage of the self-etch adhesive system is that the phosphate or carboxyl group of the functional resin monomer in the chemical structure bonds chemically with the residual HAP crystals in the collagen network (206). However, the hybrid layer formed by the penetration of the hydrophilic primer by removing or modifying the smear layer is not as thick as in the etch and rinse system and long-term bonding stability provided by the three-step etch and rinse systems cannot be achieved (201,209).

In the self-etch system, adequate adhesion and demineralization with an adequate depth cannot be achieved as in the step of etching with phosphoric acid in the etch and rinse system; which have been reported to be other disadvantages of this system (208). In order to eliminate this disadvantage, it has been thought that the enamel should be selectively etched (184,211). In order to perform the clinically selective acidification process correct, acid gels with high viscosity should be used and the physician should not dry the surface until a chalky image is obtained to ensure acid etching; otherwise, collagen network degradation leads to a reduction in bond strength and microleakage in the long-term (109). It has been reported that the bonding strength of self-etch adhesives applied to the enamel which is not acid etched is lower than those applied to the acid-etched enamel (177).

With the application developed with the advancements in the adhesive technology in recent years, multimode (universal) adhesives that can be both used in self-etch and etch and rinse system have been produced (182). This new system has been reported to be applied following the selective acid etching procedure applied to the enamel as well as being able to be used for both clinical applications. Thus, the restoration can be performed by applying the most appropriate adhesive system with the ultraconservative approach after cavity preparation. Furthermore, it has been stated that using the multimode adhesive system would be a rational solution to exclude the complications encountered in these techniques for the purpose of decreasing the number of application steps (179,212).

Since multimode adhesives are a new class in adhesive system, in vitro and clinical studies are known to be limited. In in vitro studies, zymographic examination, dentine penetration level, bonding strength test (μTBS) and microleakage levels have been reported to be considered (182,213,214). In clinical trials, clinical criteria such as retention, postoperative sensitivity, marginal adaptation, and marginal coloration have been reported to be taken as basis (215).

In an in vitro study of the zymographic examination by ***Marchesi (2014)***, the increase in the pro and active forms of MMP-2 and MMP-9 following the application of the adhesive system were examined and MMP-2 and MMP-9 were found to be increased, regardless of which method of application (whether self-etch or etch and rinse) was chosen (182).

In the in-vitro studies that examined the level of dentine penetration, it was reported that the hybrid layer was thicker and the resin labels were longer when the etch and rinse mode of the multimode adhesive system was preferred (213).

In many studies in which the bonding strength of multimode adhesives was measured, it was observed that there was no significant difference between the self-etch or etch and rinse mode of universal adhesives and the control groups in the self-etch or etch and rinse system (182,213,214).

In the literature, there is limited number of studies investigating the retention, postoperative sensitivity, marginal adaptation, and marginal coloration of the different application strategies of universal adhesives. However, in the studies, it has been emphasized that there is no difference between the application modes at the end of six- and 18-month follow-ups (215).

2.5. Retention of Pit and Fissure Sealants

Although the retention of resin-based fissure sealants is reported to be higher than most of the other materials used as fissure sealants, the choice of the material to be used should be decided on the basis of moisture control. It has been reported that in newly erupted teeth or teeth still in the eruption process, the use of glass polyalkenoate sealants would be more accurate as the resin-based sealants will reduce retention (41).

Effective isolation should be established, resin-based sealants should be used and applied following the complete eruption of teeth and there should be a good practitioner technique and protocols to increase the retention of fissure sealants (216). Other methods used to increase retention include the use of bonding agents, the use of viscose resin, surface preparation with an adhesive and air abrasion following the fissure preparation with phosphoric acid gels (11,127,142,217).

Researchers have also reported that, besides the above-mentioned techniques, interventional preparation before fissure sealant application also increases retention and provides benefits in terms of caries that cannot be detected (9). It has also been reported that the retention of the fissure seal increased and the microleakage was reduced with the use of the interventional technique (218). However, there are also recent studies suggesting that removal of intact enamel tissue to extend the fissures does not provide any additional benefit (219). Interventional preparation technique has been reported to cause teeth to enter a recurrent restoration cycle. According to the current approach in this regard, the removal of the organic debris in the fissures is sufficient to ensure proper bonding and there is no need to remove the intact enamel tissue (41). In the evaluation of the retention of restorations, it has been reported that bond strength tests against tensile and shear bond strength can be performed (220).

2.5.1. Bond Strength Tests

The eruption level of the tooth, the technique used in application, and performing the application carefully have been shown to be important factors in terms of the retention, which is accepted as the primary factor in the clinical success of pit and fissure sealants (4).

Bond strength tests have been reported to be in vitro tests to measure and compare the bond strength of restorative materials to dental tissues such as enamel or dentin and to evaluate their bonding ability (221,222).

In the literature, it has been reported that the ideal bond strength test should be easily applicable. In general, the advantages of laboratory tests include followings: it is easy to obtain data for a particular feature; a certain parameter can be measured by keeping all other variables constant; the performance of new material or technique can be directly compared to the current gold standard; many experimental groups can be tested simultaneously with certain limitations in a study; and simple and inexpensive testing protocols can be often used (223).

Another objective of the laboratory test is to obtain data that can predict the possible clinical outcome (223). It has been reported that there are different methods used for the measurement of bonding strength to enamel and dentin (220). The bond strength can be measured by using a macro or micro-test assembly, depending on the size of the binding site. The macro-bond strength can be measured with the shear, tensile, or push-out protocol (223).

Besides the bond strength, it has been reported that the type of bonding failure can be also determined and this should be considered as a more important parameter. Failure types:

Adhesive failure: between restoration and tooth

Cohesive failure: In restoration or teeth,

Mixed failure: defined as partly adhesive and partly cohesive failure. The bond strength value has been reported to non-significant unless the type of failure was specified (224).

2.5.1.1. Macroshear

Shear has been observed to be the most common technique used among the bond strength tests and to be used in most of the scientific articles related to bond strength (220). A strong relationship has been found between mean bond strength and failure type; the higher the bond strength, the higher the rate of adhesive failure (223).

In the studies on the best performances of materials, the bond strength test against shear bond strength has been reported to be one of the tests in determining the retention degree of the materials to pits and fissures (15,140). Low shear bond strength has been reported to be related to improper bonding and gaps between tooth and restoration. These cavities have been shown to cause bacterial infiltration, postoperative pulpal inflammation and microleakage (225).

2.5.1.2. Macrotensile/Push-out

It has been described as a less popular method except for measuring the bond strength of cement with hard materials such as ceramic and metal alloys (226,227). It has never been accepted as a universal bond strength test method, the reason of which has been attributed to the fact that it is time consuming and sample preparation process is tiring. However, this method has been reported to be a very useful method for testing the retention of the posts applied to the root canals (228,229).

2.5.1.3. Microtensile

micro-binding tests have been started to be generally and typically performed in tensile type with the development of micro-tensile bond strength test by ***Sano et al. (1994)*** (230). The tested bonding area has been found to be much smaller than the macro tests. It has been reported that the samples should be prepared following the bonding procedure and the original preparations of the micro-specimens are required. This has been interpreted as a situation which is more laborious and requires technical sensitivity. Nevertheless, more economical use of teeth (e.g. more than one samples can be obtained from a tooth), easier control of regional differences, and better stress distribution to the right surface have been reported to be listed as its advantages (230,231).

2.5.1.4. Microshear

Microshear bond strength test was developed in 2002 to obtain a large number of samples from a single tooth (232). This test has been shown to facilitate manipulation by testing a large number of samples per tooth. There might be cases of bending or variable and irregular force loading because it has a very thin material structure (cylinder) with a typical diameter of 0.7 mm and a relatively thicker adhesive layer. This irregular stress distribution is often mentioned when it is compared with the macro-shear test. Due to these negative aspects, only 7% of the studies on bond strength has been observed to use this protocol (201).

2.5.2. Microleakage and Evoluation Methods

Microleakage, which is defined as the transition of the microorganism, liquid, molecule and ions between the dental and restorative material, which cannot be determined clinically, has been reported as one of the factors that directly affect the success of any restorative material applied to the teeth. The occurrence of microleakage has been reported to cause discoloration between the tooth and restorative material, post-restorative sensitivity, secondary caries, loss of restoration, and even destruction of pulp (233).

Many factors have been reported to cause microleakage including different expansion levels between the restorative material used and dental tissue, failure of the adhesion of restorative material to dental tissues, insufficient penetration of restorative material, shrinkage of the restorative material during polymerization, abrasion of the restoration surface over time, deformation of restoration under occlusal forces, failure of performing restoration under ideal conditions, and carelessness of the dentist (234). Another factor causing microleakage has been also reported as the properties of restorative material; it should completely inhibit the microleakage, provide thermal expansion between the restorative material and the dental tissue, not be any difference in its coefficient, not be shrunk during polymerization, and not absorb water (235). Resin-based fissure sealants have been reported to arise from the type of monomers in the polymer chain and to have a volumetric shrinkage which can vary between 1.5-3%. It has been reported that because of their high thermal expansion coefficients, they may be affected by the heat changes in the mouth. Therefore, it has been emphasized that the clinical success of fissure sealants as in all restorations is related to the resistance of the fissure seal and its resistance to microleakage. The fissure sealant forms a complete sealing surface on the tooth enamel, creating a barrier between the oral fluids, microorganisms and the tooth. Therefore, the microleakage resistance of the fissure seal has been reported to be directly related to the time that the tooth remained healthy (4).

There are several methods used for the assessment of microleakage. Although clinical evaluation is considered to be more important in examining a material, it causes difficulties in the assessment of rapidly developing restorative materials and adhesive systems as it takes too much time and standardization is difficult to be provided. In vitro tests, therefore, have been reported to be one of the methods used for the assessment of materials, microleakage studies, detection of gaps in the tooth-restoration interface, and early assessment of restorative materials (236).

Visual and penetration methods have been reported to be used in the determination of microleakage. In visual examinations, the consistency in tooth-restoration combination is evaluated via electron microscopy. In the penetration method, stereo microscope has been reported to be used to examine the amount of leakage caused by stainer, chemical markers, radioisotopes, bacteria or compressed air in tooth-restoration combination (237).

2.5.2.1. Clinical Diagnostic Methods

The United States Public Health Service (USPHS) criteria were developed by Dr. Gunnar Ryge to evaluate the clinical performance and aesthetics of a restoration. These criteria have been reported to include color matching, marginal discoloration, anatomical form, marginal adaptation, and parameters for the evaluation of secondary caries (238). Among these parameters, marginal discoloration, marginal adaptation, and secondary caries occurrence are indicated as indirect clinical markers of microleakage (239).

World Dental Federation (FDI) criteria developed by ***Hickel et al. (2007)*** have been collected under three headings: aesthetic, functional and biological properties. Surface and marginal discoloration among the aesthetic properties and marginal adaptation among the functional properties have been reported to be the clinical criteria used in the indirect evaluation of microleakage (240).

Neutron Activation Analysis

This method has been reported to allow for assessment in both in-vivo and in-vitro conditions for the determination of microleakage. When applied under clinical conditions, the tooth is extracted after the manganese (Mn) is placed on the margin of the restoration and the microorganism can be determined through Mn25 bombardment in the nuclear reactor (241). As tooth contain manganese in its structure, the results may vary and it might not be understood that which area of the tooth has an Mn absorption. Neutron activation analysis has been reported to microleakage assessment method that is not suitable for clinical use because of its disadvantages such as being expensive, requiring sensitive operation, radiation hazard, and being unable to determine the localization of microleakage (241,242).

2.5.2.2. In vitro Diagnostic Methods

Dye Penetration Test

Dye penetration, which is one of the oldest methods used to determine microleakage, is still the most widely used method because of its practical application. In the past, organic dyes were used for the determination of microleakage, however, in the present, the use of fluorescent dyes has been increased with the techniques developed. The dyes used in this technique are said to be available in the form of suspension or ready-to-use solutions containing different amounts of particles (243). The most preferred dyes for microleakage detection have been reported as methylene blue (0.2-2%), basic fuchsin (0.5-2%), fluorescent (2-20%), crystal violet (0.05%), aniline blue (2%), silver nitrate (50%), toluidine blue (0.25%), erythrocyte (2%), rhodamine B (0.2%), phosphoric acid (37%) and acridine orange (0.1%). It has been emphasized that while selecting the dyestuff to be used for the determination of microleakage, its properties should be taken into consideration. For example, it is stated that methylene blue is acidic when it is not buffered with phosphate and may lead to misleading results because it can dissolve calcium present in the tooth (244).

In the dye penetration method, root ends have been reported to be sealed with resin material following the restoration of the extracted and cleaned tooth. All surfaces, except the tooth surfaces where the microleakage is to be evaluated, are covered with a sealant (such as nail polish); then the samples should be immersed in the prepared solution. Duration of stay of the samples in the solution is determined depending on the selected dyestuff and solution density. Samples kept at the specified time and room temperature should be washed under the running water after taken from the solution to remove the sealing material (181,190,242,245). The following methods have been reported to be used to assess the samples:

Sectioning: Samples are prepared or etched and the area to be investigated is examined under a binocular microscope. The measurement process is completed by determining the penetration level of the dye (245). Taking three sections from the samples provides a more reliable evaluation for the detection of microleakage (246).

Clearing: The teeth whose restorations are completed are first left into nitric acid for 48 hours to be decalcified. Then, they are waited in 80% ethyl alcohol for 24 hours to be dehydrated. Following these steps, they are left into 90% ethyl alcohol for two hours and lastly, 100% ethyl alcohol for three hours. After that, samples should be kept in methyl salicylate for 24 hours to ensure demineralization and clearing. For the evaluation of the microleakage, the teeth that are fully cleared and dye penetration of which become visible should be photographed and recorded (244).

Volumetric Measurement: The teeth that are kept in the dye solution for a required period of time are left in the nitric acid solution for thawing. A spectrophotometer is used to calculate the dye concentration in nitric acid; thus, the amount of microleakage can be determined. However, this method does not provide any information about the localization of microleakage (247).

Advantages of dye penetration method (244):

• Low cost,

• Easy to apply,

• Reliable,

• No toxic effect,

• Ability to determine under visible light,

• Ensures fast, direct and error-free measurements,

• Soluble in water,

• Not reacting with hard tissues,

• It is held on the surface by the dentin matrix or apatite crystals.

The disadvantages of dye penetration method include followings: as the samples are damaged after the sectioning, those samples cannot be re-used for another examination; the three-dimensional leakage can only be seen in two dimensions; and differentiation of leakage density according to localization can not be evaluated. However, it has been observed that this problem is greatly eliminated by taking a few sections from the same sample (242,244).

In studies where the dye penetration method was used, the air trapped in the root canal has been reported to not allow the dye to progress to the leakage regions. Therefore, the researchers have reported that it would be more accurate to apply the vacuum method instead of leaving the teeth in the dye in the studies, in which the dyeing method will be used, to minimize the impact of capillary pressure by pushing the paint from the area of penetration (248).

Use of Radioisotopes

This technique, which was developed in 1951, has been reported to be the most commonly used method after dye penetration technique. It has been stated that the tooth, microleakage of which will be examined, is left into the solution prepared with Ca^{45} (calcium), I^{131} (iodine), S^{35} (sulfur), Na^{22} (sodium), Rb^{86} (rubidium), C^{14} (carbon) ve P^{32}(phosphorus) isotopes for several hours (245). Afterwards, the section is taken from the region to be examined and transferred to the photographic film (242). In radiographs taken, the samples to which radioisotope method is applied have been observed to pass through the restoration and tooth tissue. Isotope selection, the distance between light source and emulsion, duration of irradiation time, duration of exposure of the film, rinsing and washing after irradiation have been reported to be of great importance to obtain accurate results from these radiographs (249).

Isotopes, which are much smaller (40 µm) than the dyestuff particles of 120 µm, allow the detection of the smallest microleakages. Furthermore, the radioactive isotope method is considered to be more advantageous as radiographs taken from samples are permanent and can be stored. It has also several disadvantages and one of them is that it is a very sensitive and expensive method as radioactive material is used (245). Another one has been reported to be the subjective evaluation of the results. Furthermore, the tendency of tooth and restorative materials to draw isotopes to their structures has been reported to cause misevaluation of isotope distributions in leakage (249).

Using Chemical Agents

It was first mentioned by ***Komfield (1953)*** that the non-radioactive chemical agents could be used as a method for the detection of microleakage. In his study, he left the samples, for which acrylic restoration was done, into the barium sulfate (250). As a result, marginal discoloration and microleakage regions have been shown. It has been reported that two colorless penetration agents react to form an opaque appearance and that both of the penetration agents should have small molecules and must allow the penetration. Microleakage is revealed by photographing technique and silver salts containing silver ions that are extremely small when compared to the size of microorganisms and is more penetrative are used. For this purpose, 50% silver nitrate salt has been reported to be used (245). Leinfelder et al. used soluble calcium hydroxide in their study and determined the microleakage by changing the pH value (251).

One of the most important advantages of detecting microleakage with chemical agents is that there is no radiation hazard. It has been reported that obtaining the results quantitatively has been reported to be one of the advantages. However, both chemical agents should be penetrative and the results are evaluated subjectively. These factors have been reported as negative aspects of the method. It has been asserted that the use of silver nitrate in glass ionomer cement and amalgam restorations does not provide successful results in microleakage detection. Silver ion has been reported to be included in the structure of glass ionomer cement restoration. However, in amalgam restorations, silver has been found to prevent the determination of microleakage by reacting with other (252).

Bacterial Methods

In this method, bacterial toxins leaking from the restoration margins and other bacterial products are examined based on the relationship between bacterial penetration and microleakage (244,245). The samples prepared are left into gram positive and gram negative media and microleakage is detected by observing the color change in the marker solution in the course of incubation period (244,253). In this method, qualitative results are obtained and microleakage in areas of tooth-restoration combination narrower than the width of the bacteria (0.5-1 µm) cannot be evaluated; which have been reported to be the disadvantages of this method (245,253).

Electrochemical Methods

It has been suggested that this analysis is based on the measurement of the electric current occurring between two separate electrodes connected to the external power source immersed in the electrolyte (254). This method involves the following steps: the sample prepared is isolated from the dental root in a way to be in contact with the restoration, an electrode is placed, the assembly prepared is immersed in the physiological solution to provide the electrolytic environment, and an electric current is applied. Microleakage can be evaluated by measuring the alternating current generated by the electric current in the electrochemical environment when passing through the sample. As the samples are not damaged, quantitative measurements that can be repeated at certain intervals is possible to be carried out. However, this method has been reported not to be used in the examination of microleakage of metal restorations. Although it is frequently used in the detection of root canal leaks, it has been reported that incorrect data can be obtained because it does not contact with apical leakage (211).

Microscopic Examination Methods (SEM Analysis)

Examination of the sections taken from the samples following the dye penetration method under the stereo electron microscope (SEM) is another method for detecting microleakage levels and localizations. SEM analysis has been reported to allow the detailed examination of the surface properties with the ability to enlarge the image. Thus, the surface microstructure can be visualized to evaluate the particle size and different crystalline phases (245). It can be also used to verify other microleakage methods, however, it has several disadvantages including gap formation while obtaining sections from the samples, damage to the samples in a way not to allow to take a section from the same sample, and expensive. It has been emphasized that researchers have developed the method of obtaining replicates by the measurements taken from the teeth to solve these problems (242,245). In these methods, polyvinylsiloxane materials are not preferred as a measurement material due to their high dimensional stability and epoxy resins are not preferred to obtain a replication (242).

Analysis by Microtomography

Adaptation of composite restorations to the tooth surface and micro-spaces in the interface have been reported to be evaluated in high-resolution computer systems using three-dimensional imaging using X-ray (255,256). Moreover, the polymerization contraction in the composite restorations is subject to angular, spatial and volumetric examinations and cavity shape, configuration factor, and the effect of the restorative materials can be compared (255). In microtomography, micro-gaps arising from unsaturated or partially saturated adhesives with minimal X-ray absorption may not be recognized (256). This method has been suggested to be used only in cases where there are insufficient radiographic contrast between the tooth surface and restoration and where restoration is performed with materials with high X-ray absorption, water absorption levels similar to dentin or with non-absorbing materials (255).

Liquid Filtration Technique

In this technique first described by ***Derkson (1986)***, it has been reported that the movement of air bubbles in the capillary tube is used to measure the occlusion capacity of the investigated material (257). By means of the tubes connected to the coronal section and apex, it is stated that the water in the tubes is filtered through the duct into the air spaces towards the apex as a result of the pressure applied to the coronal section. Besides its advantage that liquid measurement per minute (mL/min) does not damage samples compared to dye penetration, it has been reported that the margin of error can be minimized thanks to the repeatable measurements (258).

2.6. Dental Operating Microscope

In recent years, thanks to rapidly developing technology, it has been reported that there are advancements in the science and technology benefited in dentistry. The use of any device that increases the vision of the dentist in sensitive dentistry applications has been reported to be very useful (259). Tools providing magnification and improved lighting are known to be used together mostly to obtain a more detailed view in dental health clinics (259).

2.6.1. History of Dental Operating Microscope

Dental operating microscope is known to be introduced by ***Apotheker*** in 1981 (260). It has been emphasized that the use of this microscope is not widespread due to the facts that it is inadequately configured, is ergonomically difficult to use, has only one magnification (8x) capacity, is placed on a platform, has a weak balance, and has only flat binoculars and a very long focal length (250 mm) (261).

Carr (2010) has reported that he developed an ergonomic operating microscope that provides easy operation in almost all endodontic procedures (259). This microscope has five different magnifications from 3.5x to 30x, can be mounted on the wall or ceiling, and has angled binoculars that allow the practitioner to perform the operation while sitting. In the literature on endodontics, ***Selden (1989)*** is observed to be the first author who has a study on non-surgical applications of the operating microscope in endodontics (262).

In recent years, the operation microscopes used in dentistry clinics have been reported to be highly advanced devices with different magnification levels and many settings of which including focusing can be done electronically. Operating microscopes, which are used in many fields in dentistry, have been reported to have positive effects on the quality and success of treatment, particularly in areas where there is a need for a detailed view and good lighting (261).

2.6.2. Scope of Use of Dental Operating Microscope

Restorative dentists, periodontists, pedodontists, and endodontists have been reported to perform procedures requiring a resolution of more than 0.2 mm, which is the resolution limit of the human eye. Some of them have been reported to be challenging procedures such as alignment of crown margins, scaling, incisions, determination of root canals, removal of caries, furcation and perforation repairs, placement and removal of posts, and bone and soft tissue grafting. The use of advanced magnification and lighting techniques during these procedures has been reported to contribute to the success of the treatment. Dental operating microscope has been reported as one of the tools used for magnification and lighting (259).

2.6.3. Advantages and Disadvantages of Dental Operating Microscopy

The most important benefits of dental operation microscopy are reported as lighting and magnification (261).

It has been known that dentists have to work in a small area that receives little light in the oral environment. For some areas in dentistry, particularly in endodontic procedures, it is stated that lighting is much more important. It is known that the pulp room and root canals in the teeth, which receive less light in the oral environment, are involved in the procedures. Because the ambient light is less for dentists, moving light sources (reflector) have been added to the dental treatment units (261).

According to the inverse square law in which light intensity is determined, the amount of light received from a source is reported to be inversely proportional to the square of the distance. This situation can be explained with such an example that the amount of light in the object is reduced by four times when the distance between the light source and the object is increased by two times (259). It has been reported that the light needed in working inside the mouth, particularly in the root canals, is insufficient due to the possible distance of the light source. When the amount of light coming to the object is increased, the visual efficiency can be increased. Being one of the options that can be used to lighten the operation area, these lamps, which are also called surgical headlight lamp mounted on magnification loupes to transmit light, can be divided into two: lamps providing light transmission through fiber optic cable from a light source and LED dental lights. It has been reported that devices that use fiber optic cable can restrict the movements of the physician since they have a cable connection to a light source which is close to the physician. LED light source provides more freedom of movement to physician and headlamps can increase light levels to four times that of conventional dental lights. Furthermore, the light is moving in accordance with the movement of the physician's head. These have been reported as the advantages of such light sources (259).

Besides the strong lighting power of the operating microscope, it also provides comfort to the physician with different light filters. It is stated that different color filters in the microscope can eliminate the potential negativity in the operation area due to strong lighting. Green filters are used to prevent the complexity caused by the reflection of the red color of blood in surgical procedures, whereas yellow or orange filters are used to prevent premature polymerization of resin materials (261).

Magnification, which is another advantage of a dental operating microscope, has been reported to have three main benefits: compensation of presbyopia, ergonomic benefits, and optical benefits (261).

Compensation of presbyopia: Presbyopia is defined as the impaired visual acuity in which the close objects cannot be clearly seen due to the loss of flexibility of the eye lens with age. In such cases, the closest point where the eyes can focus clearly is farther away than the ideal working distance (263). In a study by ***Gilbert (1980)*** investigating the age-related problems, it has been reported that a regular eye examination should be performed every two years until the age of 50 and every year after the age of 50 (264). ***Burton ve Bridgeman (1990)*** stated in their research that the working distance increased significantly with age. They reported that physician can use magnification loupes instead of increasing the working distance to see the tooth clearly to ensure a standing upright and comfortable posture at a fixed working distance (263).

Ergonomic benefits: The correct working position has been reported to be a factor with a great importance for the dentist who uses magnification. Most of the dentists have been observed to experience recurrent chronic neck and back pain. The opinion that the use of suitable magnification loupes reduces chronic neck and back pain, and even in some cases, it completely eliminates the pain (265–268). These studies have also reported that the appropriate selection, adjustment and use of the magnification systems facilitated the adoption of a straight and upright posture. However, they have further emphasized that learning and adaptation are always necessary when using any new method (261).

Optical benefits: In parallel with the development of technology, the techniques used by physicians in clinics have also advanced and it has been reported that magnification has become a more sought criterion. Some studies have focused on the benefits of magnification as a diagnostic tool (269–271). ***Thomas (1989)*** reported that the number, length, and direction of the fracture lines in the teeth can be easily determined using magnification and lighting (272). ***Whitehead and Wilson (1992)*** showed that the clinical performance of a group of experienced dentists increased when they used magnification (273). In another study, it has been shown that magnification is beneficial in fixed prosthodontic procedures in the laboratory (274). On the contrary, there are some studies that show that the use of a magnification loupe does not provide an optical benefit during dental practice. ***Donaldson et al. (1998)*** have shown that the use of a magnification loupe in clinical, pediatric and operative dentistry applications by university students did not provide a significant benefit (275). Moreover, in a study by ***Lussi et al. (2003)*** using a magnification loupe in the preparation of cavities, it has been reported that the magnification loupes did not significantly reduce the iatrogenic damage to adjacent tooth surfaces (276).

Potential hazards of long-term use of magnification

There is no evidence regarding that the long-term use of magnification for the procedures carried out at close range has a risk for the dentists. Ophthalmologists have reported that the use of magnification loupes do not damage or weaken the eyes (277). However, after using the magnification loupe for a while, the user gets used to seeing more in detail than with the naked eye and he/she may feel that something is overlooked psychologically when he/she works with the naked eye. If the use of magnification is not possible, it has been emphasized that this will start to be an uncomfortable feeling. The eyes will need a couple of hours to re-adapt to the normal vision. When using magnification, the eye muscles get used to contracting to a specified level and need to rest to regain their normal functions. It is recommended that those who use a magnification loupes should use them for certain operations, not every day to reduce this situation and they should prefer normal vision for other operations. However, individuals with convergence insufficiency have been reported to be under a potential risk when using magnification loupes. Convergence insufficiency is reported as a medical condition in which there is a weak relationship between the extrinsic eye muscles responsible for the medial movement of eye and muscles responsible for divergence (simultaneous outward movement of both eyes away from each other). In this case, which is also called lazy eye (amblyopia), eye of the individual fails to achieve normal visual acuity and visual fatigue can occur. It has been observed to be characterized by headaches, eye pain, blurred vision, or fatigue when working for a long time. It has been reported to be frequently observed in young people and young adults, sometimes in middle-aged individuals. If the individual can compensate for this anomaly, it has been reported to remain undetected. The individual is at potential risk if he/she is continuously exposed to artificial visual conditions, such as a limited field of view, i.e. using magnification loupes. Anomalies have been reported to become even more advanced due to the focusing errors of magnification loupes. This has been reported to cause blurred vision, treatment of which might be difficult. It has been reported that this risk can be eliminated by undergoing a special eye test before purchasing the magnification loupe and by stopping its use if symptoms persist (278).

3. MATERIAL AND METHOD

In this study, sample preparation, microleakage, and shear bond strength tests were performed in Yeditepe University Faculty of Dentistry Hard Tissue Laboratory and Yeditepe University Laboratory of the Department of Genetics and Bioengineering.

Ethics committee approval was obtained from the Ethics Committee of Istanbul University Faculty of Dentistry (Meeting no. 81 of 27.02.2019). In the study, 90 human third molars with no caries, which were extracted for treatment purposes in the last month, were used.

Written informed consent was obtained from the patients whose teeth were extracted for the evaluation of extracted teeth used in the study in laboratory conditions.

3.1. Materials Used in the Study

Fissurit® FX (VOCO GmbH, Germany) was used as a resin-based and fluoride-containing fissure sealant **(Picture 7)**, 37% **Jumbo Etch Royale Kit™** (PULPDENT, U.S.A.) was used for the pit and fissure etching in accordance with the manufacturer's instructions **(Picture 8)**, and **Adper™ Single Bond 2 Adhesive** (3M ESPE, U.S.A.) was used as a bonding agent **(Picture 9)**. The brand names, manufacturers, contents and application methods of the materials are shown in **Table 1**.

Table 1. The contents of the materials used and their application methods

Material	Manufacturer	Ingredients	Method of Application
Fissurit® FX	VOCO GmbH, Germany	Bis-GMA, diurethane dimethacrylate, BHT, Benzotriazolderivat, sodium fluoride	Applied to the fissures from the edges, and is waited for 15 sec to spread. The air bubbles are removed with a thin probe. 20 sec light is applied.
Jumbo Etch Royale Kit ™	PULPDENT, USA	37% Phosphoric acid	It is applied to cover the enamel surface. After 30 sec, the compressed air is washed with water spray for 30 sec. Dry for 10 sec.
Adper™ Single Bond 2 Adhesive	3M ESPE, USA	BISPHENOL DIGLYCIDYL ETHER DIMETHACRYLATE HEMA 2-HYDROXY-1, 3-DIMETHOXYPROPANE 2-Propenoic acid, 2-methyl-, 7,7,9 (or 7,9,9) - trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl ester	It is applied to the dental surface for 15 sec, dried for 5 sec and light is applied for 10 sec.

Picture 7. Fissurit® FX

Picture 8. Jumbo Etch Royale Kit ™

Picture 9. Adper™ Single Bond 2 Adhesive

3.2. Preparation of Teeth

The upper and lower continuous third molars of the patients applied to Yeditepe University Faculty of Dentistry, Oral and Maxillofacial Surgery Clinic, for which tooth extraction was performed, were collected. Power Analysis test was used to determine the number of study groups. As a result of the statistical analysis, it was determined that there should be at least 15 teeth in each group to obtain significant results. The enamel surfaces of the collected teeth were examined via eye and mirror. A total of 90 third molars without caries, which were consistent with the ICDAS score 0 and 1 and had no hypomineralization and gravity-related defects on the enamel surface, were included in the study **(Picture 10)**. Decayed teeth and teeth with hypomineralization and gravity-related defects on the enamel surface were excluded from the study.

Plaque and soft tissue residues on the teeth were thoroughly brushed and cleaned under running water. In order to provide disinfection until the experiment time, the teeth were left into 100 mL distilled water containing 0.2% thymol crystal at room temperature and used within three months. The collected teeth were randomly divided into groups: 60 for microleakage test and 30 for shear bond strength test. Among the 60 teeth to be used for microleakage, SEM image was obtained for eight randomly selected teeth (two teeth from each group).

Picture 10. Example of third molars used in the study

3.3. Study Groups

Group 1: Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA) + Fissure sealant - **Fissurit® FX** (VOCO GmbH, Germany)

Fissure sealant was applied to the acid-etched tooth surfaces. Microleakage test was performed.

Group 2: Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA) + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure sealant**Fissurit® FX** (VOCO GmbH, Germany)

A bonding agent was applied to the acid-etched tooth surfaces and then fissure sealant was applied. Microleakage test was performed.

Group 3: Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA) + Fissure sealant - **Fissurit® FX** (VOCO GmbH, Germany) (with dental operating microscope Carl Zeiss f170)

Fissure sealant was applied to the acid-etched tooth surfaces under dental operating microscope. Microleakage test was performed.

Group 4: Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA)+ Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure sealant - **Fissurit® FX** (VOCO GmbH, Germany) (with dental operating microscope Carl Zeiss f170)

A bonding agent was applied to the acid-etched tooth surfaces under dental operating microscope and then fissure sealant was applied. Microleakage test was performed.

Group 5: Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA) + Fissure sealant - **Fissurit® FX** (VOCO GmbH, Germany)

Following the acid etching process, fissure sealant was applied on the surfaces of teeth, whose surface was flattened at the enamel level and embedded in acrylic blocks, with the help of cylinder blocks. Shear bond strength test was performed.

Group 6: Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA) + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure sealant - **Fissurit® FX** (VOCO GmbH, Germany)

Following the acid etching process, bonding agent and fissure sealant were applied respectively on the surfaces of teeth, whose surface was flattened at the enamel level and embedded in acrylic blocks, with the help of cylinder blocks. Shear bond strength test was performed.

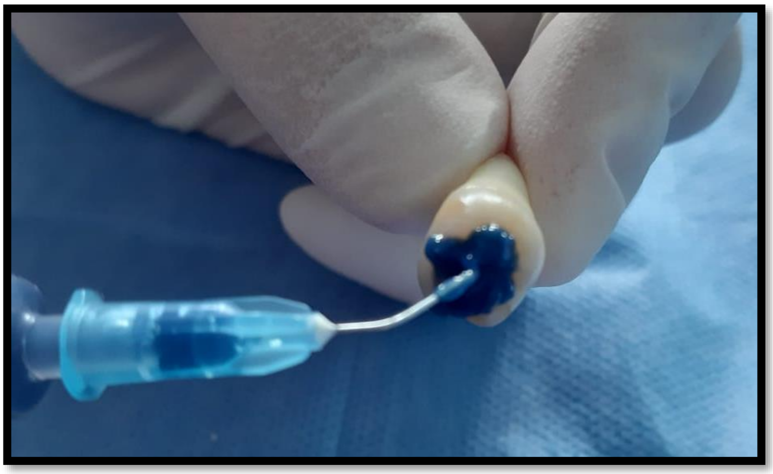
3.4. Microleakage Test

Microleakage tests were performed in Yeditepe University Faculty of Dentistry Hard Tissue Laboratory.

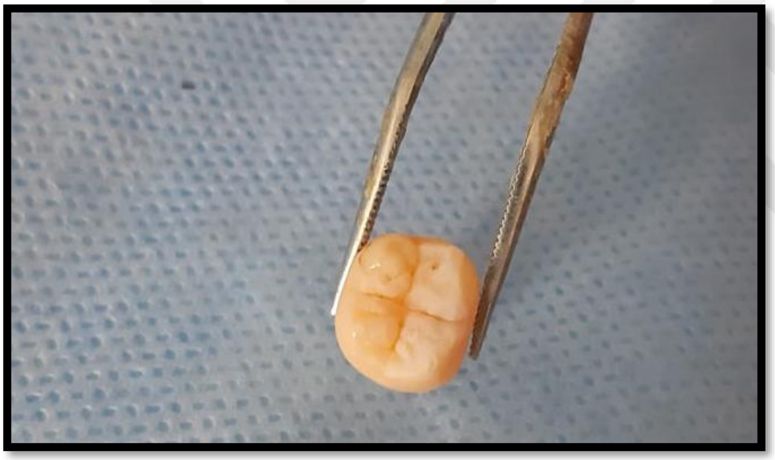
3.4.1. Preparation of Samples for Microleakage Test

Acid Etching Pit and Fissures

The teeth in all groups were washed for 30 sec and dried for 30 sec before the experiments. In accordance with the manufacturer's instructions, the teeth of the patients in Group 1 (Acid- **Jumbo Etch Royale Kit ™** [PULPDENT, U.S.A.] Fissure sealant- **Fissurit® FX** [VOCO GmbH, Germany]), Group 2 (Acid- **Jumbo Etch Royale Kit ™** [PULPDENT, U.S.A.] + + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure sealant - **Fissurit® FX** [VOCO GmbH, Germany]), Group 3 (Acid- **Jumbo Etch Royale Kit ™** [PULPDENT, U.S.A.] + Fissure sealant - **Fissurit® FX** [VOCO GmbH, Germany] [dental operating microscope - Carl Zeiss f170]), and Group 4 (Acid- **Jumbo Etch Royale Kit ™** [PULPDENT, U.S.A.] + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure sealant-**Fissurit® FX** [VOCO GmbH, Germany] [dental operating microscope - Carl Zeiss f170]), were etched for 30 sec using 37% phosphoric acid **Jumbo Etch Royale Kit ™** **(Picture 11)**. The acid layer on the acid-etched enamel surface was removed by washing with air-water spray for 30 sec and the tooth surface was dried for 10 sec. After the washing and drying process, the enamel surface in pits and fissures was ensured to become a homogeneous, chalky and opaque structure **(Picture 12)**.



Picture 11. Acid-etched enamel surface



Picture 12. Enamel surface following the washing and drying of acid

Bonding Agent Application to Pit and Fissures

Group 2: [Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, USA) + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure Sealant - **Fissurit® FX** (VOCO GmbH, Germany)], the bonding agent [**Adper™ Single Bond 2 Adhesive** (3M ESPE, U.S.A.)] was applied **(Picture 13)**. The manufacturer's instructions were followed during the application of bonding agent. Bonding agent was applied to the tooth surfaces with a fine brush for 15 seconds and then dried for 5 seconds and diluted. Then, light was applied for 10 seconds by using halogen light device Optilux 501 (Kerr, Sybron Dental Facilities, Japan).

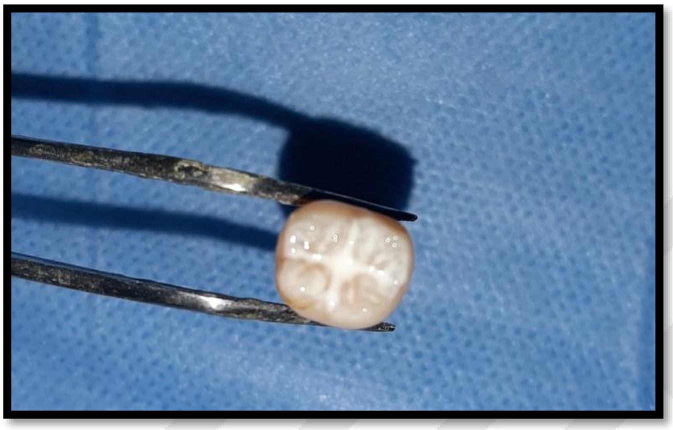
Group 4 [Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, U.S.A.) + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) + Fissure sealant- **Fissurit® FX** [VOCO GmbH, Germany] [dental operating microscope - Carl Zeiss f170 **(Picture 14)**, the bonding agent [**Adper™ Single Bond 2 Adhesive** (3M ESPE, U.S.A.)] was applied following the acid etching process under dental operating microscope. The manufacturer's instructions were followed during the application of bonding agent. Bonding agent was applied with a fine brush for 15 seconds and then dried for 5 seconds and diluted. Then, light was applied for 10 seconds by using halojen light device Optilux 501 (Kerr, Sybron Dental Facilities, Japan) **(Picture 16)** .

Picture 13. Application of bonding agent to acid-etched and dried teeth

Picture 14. Dental operating microscope used in the study - Carl Zeiss f17

Fissure Sealant Application to Pit and Fissures

The surfaces of the teeth in the groups were prepared for material application. Fissure sealants were applied in accordance with the manufacturer's instructions **(Picture 15)** and after every application, the sealants were waited for 15 sec in order to better penetrate the fissures. For the polymerization of fissure sealants, the halogen light device Optilux 501 (Kerr, Sybron Dental Facilities, Japan) was used for 20 seconds and the tip of the device was touched to the cusp peaks in order to obtain standardization.



Picture 15. Fissure sealant applied tooth surface

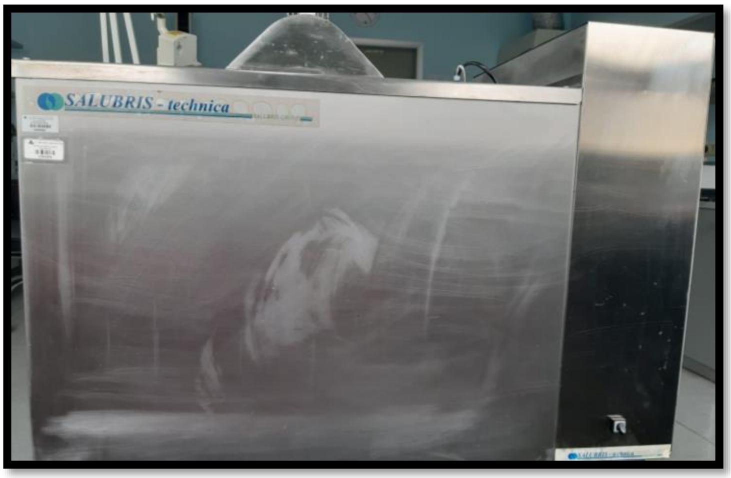
Picture 16. The halogen light device Optilux 501

Preparation of Samples for Microleakage Analysis

Fissure sealant application was performed on a total of 60 teeth in 15 teeth each group, and it was kept in sterile distilled water at Yeditepe University Faculty of Dentistry Hard Tissue Laboratory in an incubator with a temperature of 37°C (Memmert, Germany) for 24 hours.

Aging was performed by using thermal cycler for the teeth waited in an incubator for 24 hours to mimic the oral environment **(Picture 17)**. All teeth were subjected to 5000 cycles of thermal cycling at 5-55°C for 30 sec each **(Picture 18)**. After the aging process, the materials in the occlusal faces of the teeth that were left to dry for a certain period of time were checked. No material loss was observed in any group after aging.

Picture 17. Incubator of 37°C where samples were kept during the experiment period



Picture 18. Thermal cycling unit

Then the root ends of the teeth were closed with a composite material and the whole tooth surface was stained with two layers of nail polish so that the edges of the fissure sealants were open 1 mm **(Picture 19)**. After the polish was dried, all samples were kept in an incubator containing 0.5% basic fuchsin solution at 37°C for 24 hours. The teeth were washed under running water to remove the residual dye and dried at room temperature.

Picture 19. Teeth, roots of which were covered with composite material and to which nail polish was applied

The teeth were separated from their roots with the sectioning device (Izomet Buehler, Ltd, Lake Bluff, II, USA) to detect microleakage. It was then cut in the buccolingual direction **(Picture 20)**.

Picture 20. Sectioning device

Picture 21. Sample groups after microleakage experiment

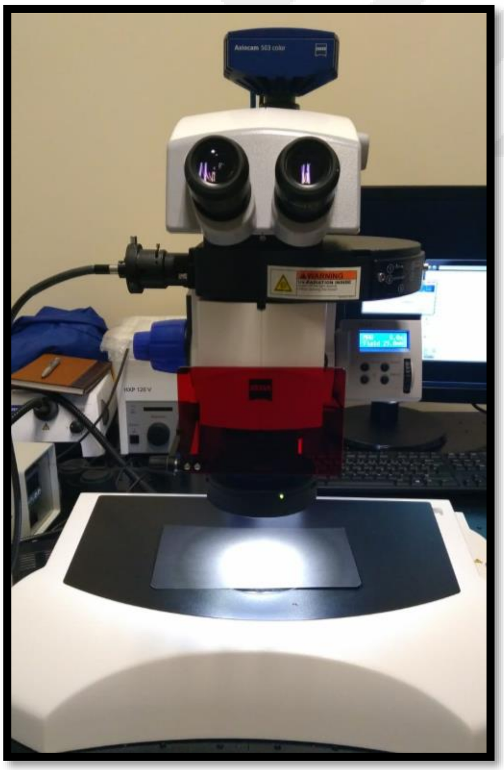
3.4.2. Evaluation of the Microleakage in Samples via Stereo Microscope

After the teeth were cut in bukko-lingual direction with sectioning device, the degree of dye penetration was examined with stereomicroscope (Zeiss, Stereo Discovery.V12, Switzerland) in the Department of Histology of Yeditepe University Faculty of Medicine **(Picture 22)**. The criteria developed by ***Pardi et al. (2006)*** (3)

were used to evaluate the microleakage score of tooth sections. Scores and evaluation are shown in **Table 2**. Microleakage scores were evaluated at 30x magnification.

Table 2. Criteria used for evaluating microleakage scores

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



Picture 22. Stereo Microscope used in the study

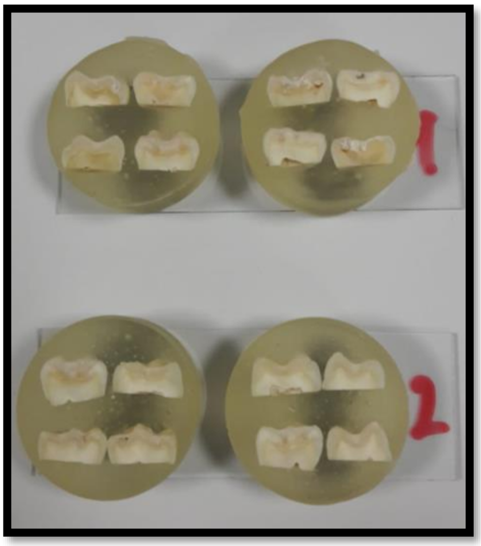
3.4.3. Imaging of Samples with Scanning Electron Microscopy (SEM)

The imaging of the adaptation of fissure sealants to the enamel was performed by SEM (EVO 40, Carl ZEISS, Germany) **(Picture 23)** in Yeditepe University Department of Genetics and Bioengineering.

A total of eight teeth, two teeth from each, were prepared for SEM analysis. The samples were glued to the sample holder with double-sided carbon paper. The surfaces of the samples were coated with gold film using a sputter coater device to ensure conductivity **(Picture 25)**. SEM examinations were performed at 1500x magnification to assess the penetration level of fissure sealants.

In this imaging, the adaptation of fissure sealants to the enamel walls and the continuity of the connection between the enamel wall and the fissure sealant were examined.

Picture 23. SEM device used in the study



Picture 24. Samples to be covered with gold film

Picture 25. Samples covered with gold film

3.5. Shear Bond Strength Test

Samples of this in vitro test were prepared and shear bond strength tests were performed at Yeditepe University Faculty of Dentistry Hard Tissue Laboratory.

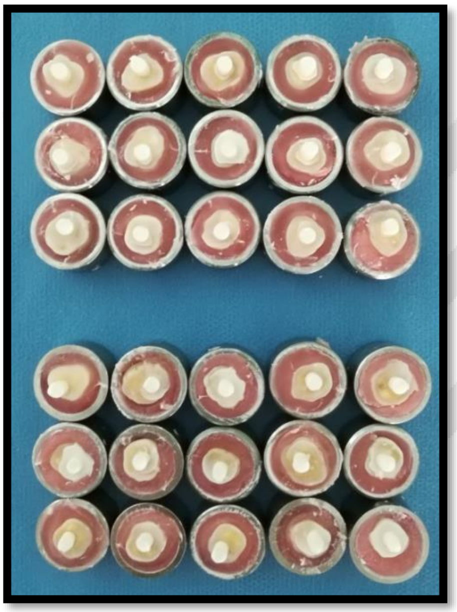
3.5.1. Preparation of Samples for Shear Bond Strength Test

Group 5 [Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, U.S.A.) Fissure sealant- **Fissurit® FX** [VOCO GmbH, Germany]) and Group 6 [Acid- **Jumbo Etch Royale Kit ™** (PULPDENT, U.S.A.) + Bonding agent - **Adper ™ Single Bond 2 Adhesive** (3M ESPE, USA) Fissure sealant - **Fissurit® FX** (VOCO GmbH, Germany)], were removed from the roots of the teeth so that the roots of the teeth were 1 mm below the enamel-cement boundary **(Picture 30)**. Low-speed precision sectioning device (Isomet, Buehler Ltd., Lake Bluff, Illinois, USA) was used. Chemically hardening autopolymerization in copper molds of 3 cm x 2 cm was embedded to the denture repair acrylic (Pen Acryl, Armada Dental, Istanbul, Turkey) in a way that either buccal or lingual/palantinal surfaces would be facing up .The remaining part of the acrylic on the tooth was cut with a diamond disc to obtain the flat surfaces to be studied. Enamel surfaces were abraded using 600 grit silicon carbide sanding sheets. The surface where the samples were adhered and the surface where the shear bond strengths would be applied were provided to be parallel to each other and perpendicular to the ground plane **(Picture 31)**.

Picture 26. Separation of samples from the roots

Picture 27. Flat enamel surface prepared for shear bond strength test

The teeth in group 5 and group 6 to be used for the shear bond strength test were etched with acid prior to surface preparation. After acid etching, the bonding agent was applied to the teeth in group 6. Following the completion of the surface preparation of the teeth, fissure sealant was applied to the surfaces with the help of cylinder molds of 4 mm in diameter and 6 mm in height. It was, then, polymerized for 30 seconds with a halogen light source and the cylinder molds were removed.The samples were then left into 37°C distilled water for 24 hours.



Picture 28. Samples prepared for shear bond strength test

3.5.2. Shear Bond Strength Assessment

For the shear bond strength test, samples were placed on the universal test machine (LF Plus, Lloyd Instruments, Ametek Inc., UK) **(Picture 33)**. Shear bond strength was applied at a head speed of 1 mm/min. The connection breaking point was determined as the highest bonding value. The data obtained were calculated as Megapascals (MPa).

Picture 29. Samples placed on the universal test machine

3.6. Statistical Analysis

Statistical analysis was performed using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA).

Descriptive data were expressed as mean, standard deviation, median, and interquartile range. Shapiro – Wilk normality test was used to check the normality of variables.One-way analysis of variance (ANOVA) was used for the intergroup comparison of normally distributed variables and Tukey multiple comparison test was used for subgroup comparisons, whereas intergroup comparisons of variables Kruskal Wallis Test was used for intergroup comparison of non-normally distributed variables, and Dunn's multiple comparison test was used for subgroup comparisons. A p value of <0.05 was considered statistically significant.

4. RESULTS

4.1. Fissure Sealant Microleakage Assessment Results

No statistically significant difference was observed between group 1 (acid + fissure sealant), group 2 (acid + bonding agent + fissure sealant), group 3 (acid + fissure sealant - with dental operating microscope) and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) in terms of occlusal microleakage score distributions ($p=0.437$). SEM images also showed fissure sealants with a good penetration to fissures in each group. The penetration level in each group was evaluated as “present”.

No statistically significant difference was observed between occlusal microleakage score distributions of group 1 (acid + fissure sealant) and group 2 (acid + bonding agent + fissure sealant), group 3 (acid + fissure sealant - with dental operating microscope) and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) ($p>0.05$).

No statistically significant difference was observed between occlusal microleakage score distributions of group 2 (acid + bonding agent + fissure sealant), group 3 (acid + fissure sealant - with dental operating microscope) and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) ($p>0.05$).

No statistically significant difference was observed between group 3 (acid + fissure sealant - with dental operating microscope) and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) in terms of occlusal microleakage score distributions ($p=0.843$).

Table 3. Percentage distribution of microleakage scores by groups

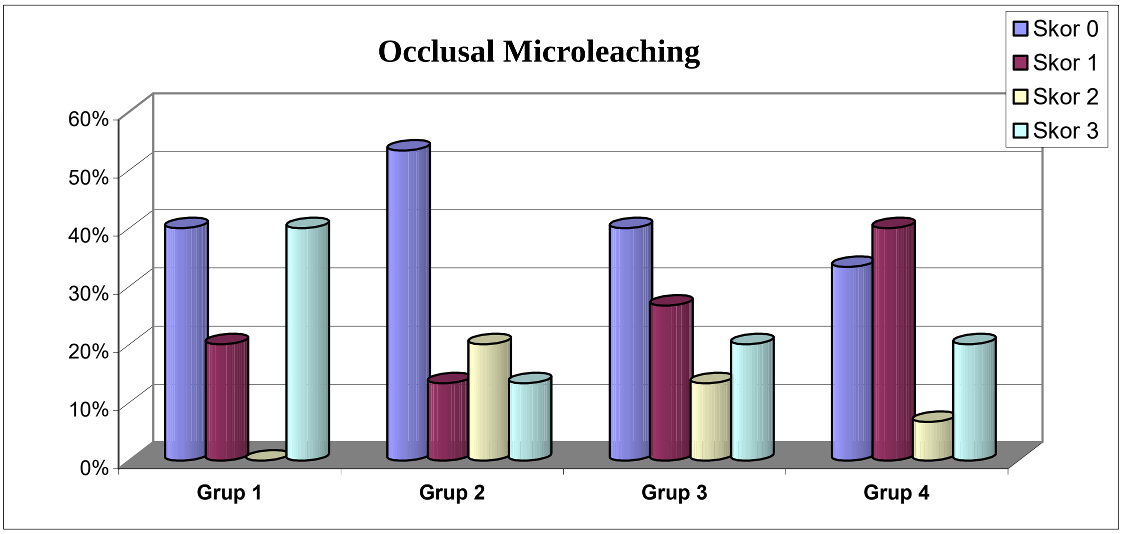
		Group 1 (Acid + fissure sealant) n:15		Group 2 (Acid + bonding agent + fissure sealant) n:15		Group 3 (Acid + fissure sealant - dental operating microscope) n:15		Group 4 (Acid + bonding agent + fissure sealant - dental operating microscope) n:15		p
Occlusal microleakage	Score 0	6	40%	8	53.3%	6	40%	5	33.3%	0.437
	Score 1	3	20%	2	13.3%	4	26.67%	6	40%	
	Score 2	0	0%	3	20%	2	13.3%	1	6.67%	
	Score 3	6	40%	2	13.3%	3	20%	3	20%	

$^*p<0.05$

Table 4. Comparison of microleakage values in binary groups

	p
Group 1 (Acid + fissure sealant) / Group 2 (Acid + bonding agent + fissure sealant)	0.139
Group 1 (Acid + fissure sealant) / Group 3 (acid + fissure sealant - with dental operating microscope)	0.370
Group 1 (Acid + fissure sealant) / Group 4 (with acid + bonding agent + fissure sealant - with dental operating microscope)	0.377
Group 2 (Acid + bonding agent + fissure sealant) / Group 3 (acid + fissure sealant - with dental operating microscope)	0.717
Group 2 (Acid + bonding agent + fissure sealant) / Group 4 (with acid + bonding agent + fissure sealant - with dental operating microscope)	0.273
Group 3 (acid + fissure sealant - with dental operating microscope) / Group 4 (with acid + bonding agent + fissure sealant - with dental operating microscope)	0.843

$*p<0.05$



Graph 1. Graphical representation of the percentage distributions of the microleakage scores by groups

(score 0: no dye penetration, score 1: penetration limited to the outer half of the sealant, score 2: leakage up to the inner half of the sealant, score 3: penetration extending to the underlying fissure)

4.2. Evaluation of Stereo Microscope Images of Fissure Sealant Microleakage

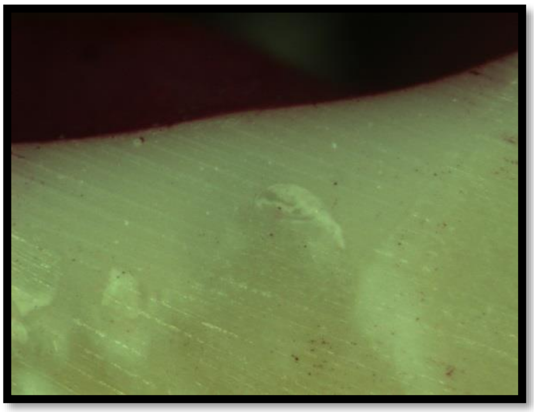
Samples obtained from group 1 (acid + fissure sealant), group 2 (acid + bonding agent + fissure sealant), group 3 (acid + fissure sealant - with dental operating microscope) and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) were examined under 30x magnification by using stereo microscope.

Score distributions of 15 samples from Group 1 after stereo microscope examination were found as follows: score 0: six samples **(Picture 30)**, score 1: three samples **(Picture 31)**, score 2: zero sample, and score 3: six samples **(Picture 32)**.

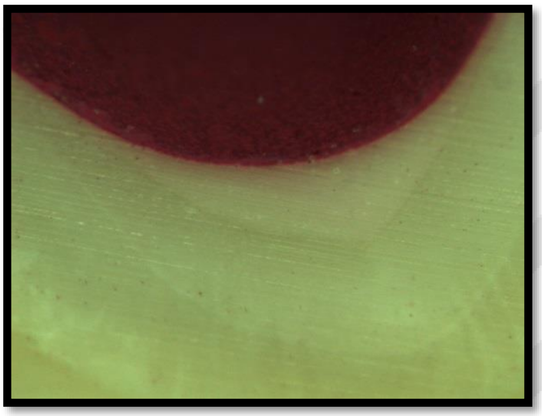
Score distributions of 15 samples from Group 2 after stereo microscope examination were found as follows: score 0: eight samples **(Picture 33)**, score 1: two samples **(Picture 34)**, score 2: three samples **(Picture 35)**, and score 3: two samples **(Picture 36)**.

Score distributions of 15 samples from Group 3 after stereo microscope examination were found as follows: score 0: six samples **(Picture 37)**, score 1: four samples **(Picture 38)**, score 2: two samples **(Picture 39)**, and score 3: three samples **(Picture 40)**.

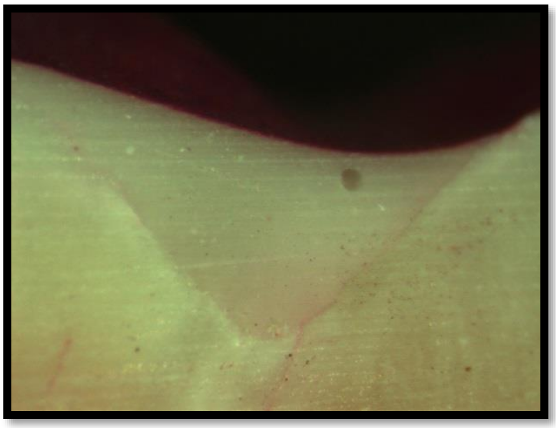
Score distributions of 15 samples from Group 4 after stereo microscope examination were found as follows: score 0: five samples **(Picture 41)**, score 1: six samples **(Picture 42)**, score 2: one sample **(Picture 43)**, and score 3: three samples **(Picture 44)**.



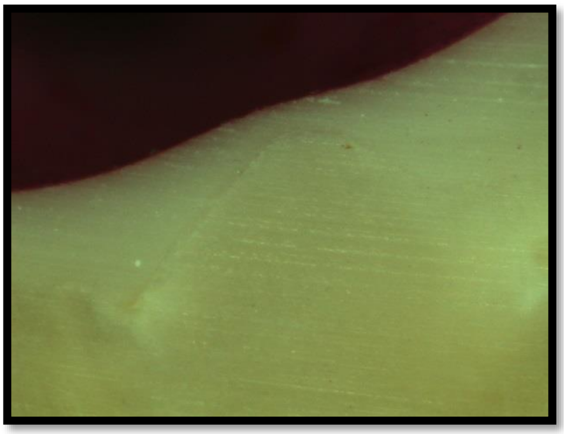
Picture 30. Stereo microscope image of a sample with a score of 0 at 30x magnification in acid + fissure sealant group



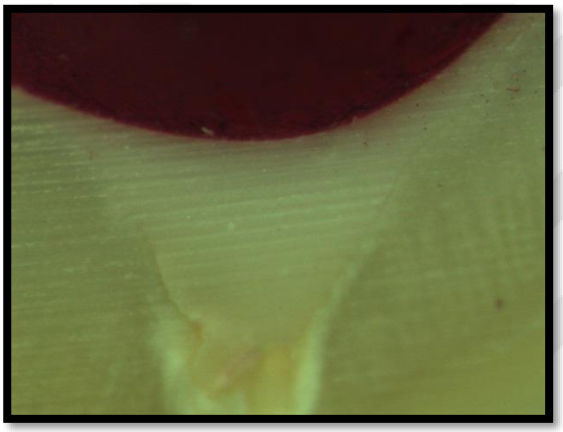
Picture 31. Stereo microscope image of a sample with a score of 1 at 30x magnification in acid + fissure sealant group



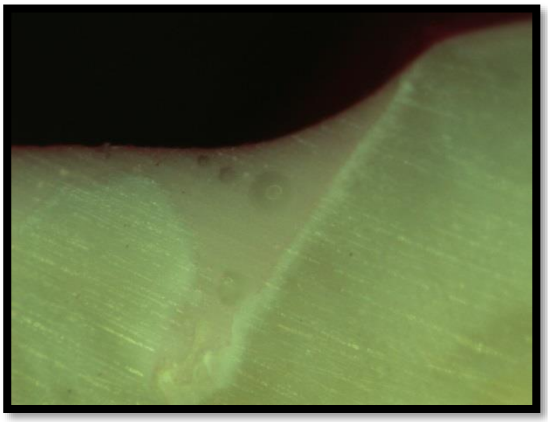
Picture 32. Stereo microscope image of a sample with a score of 3 at 30x magnification in acid + fissure sealant group



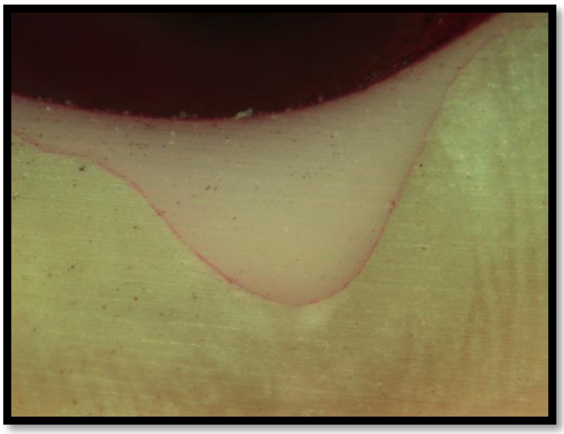
Picture 33. Stereo microscope image of a sample with a score of 0 at 30x magnification in acid + bonding agent + fissure sealant group



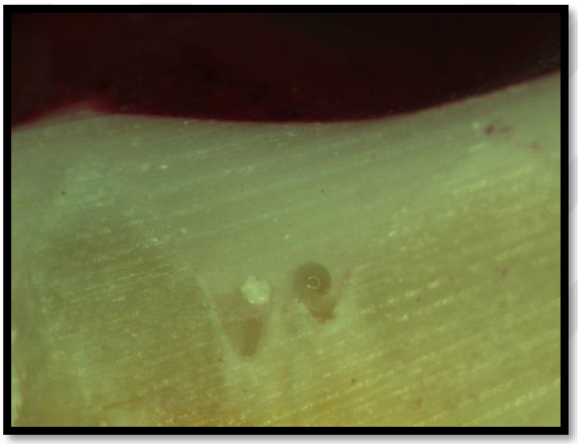
Picture 34. Stereo microscope image of a sample with a score of 1 at 30x magnification in acid + bonding agent + fissure sealant group



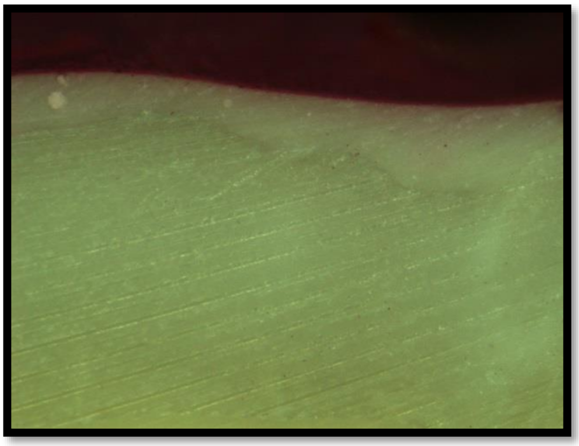
Picture 35. Stereo microscope image of a sample with a score of 2 at 30x magnification in acid + bonding agent + fissure sealant group



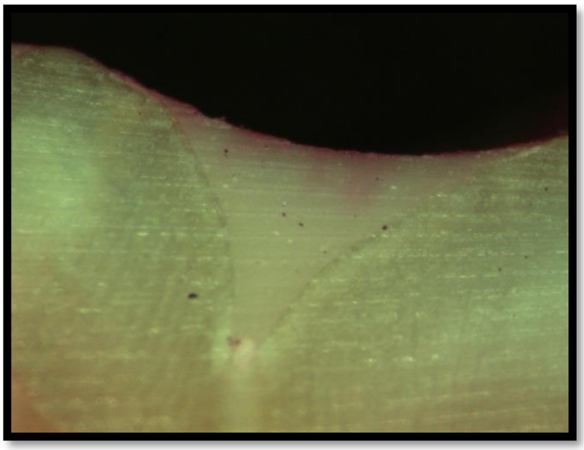
Picture 36. Stereo microscope image of a sample with a score of 3 at 30x magnification in acid + bonding agent + fissure sealant group



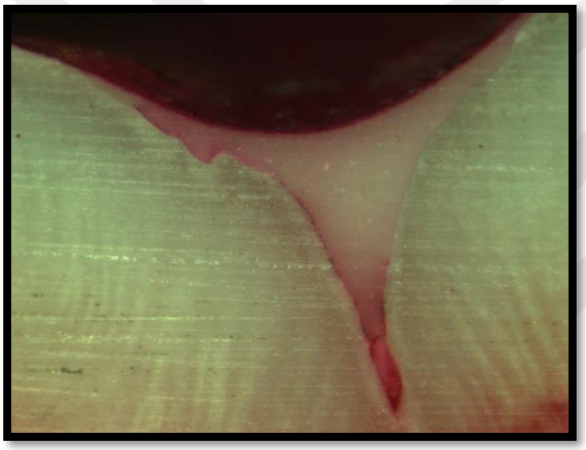
Picture 37. Stereo microscope image of a sample with a score of 0 at 30x magnification in acid + fissure sealant (with dental operating microscope) group



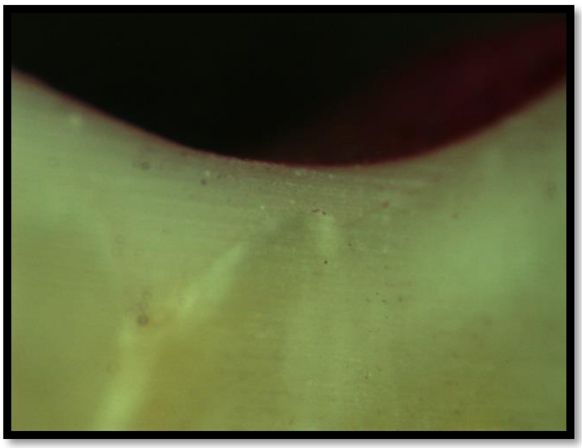
Picture 38. Stereo microscope image of a sample with a score of 1 at 30x magnification in acid + fissure sealant (with dental operating microscope) group



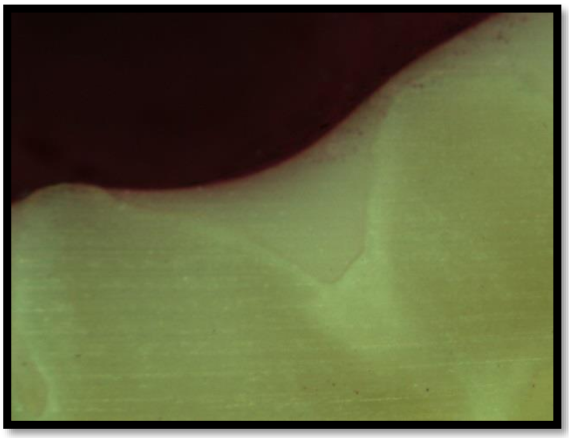
Picture 39. Stereo microscope image of a sample with a score of 2 at 30x magnification in acid + fissure sealant (with dental operating microscope) group



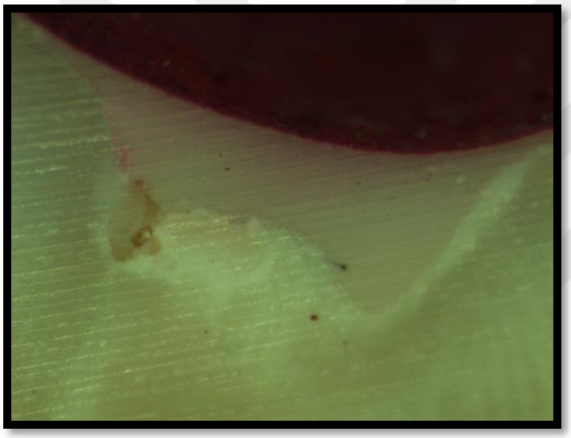
Picture 40. Stereo microscope image of a sample with a score of 3 at 30x magnification in acid + fissure sealant (with dental operating microscope) group



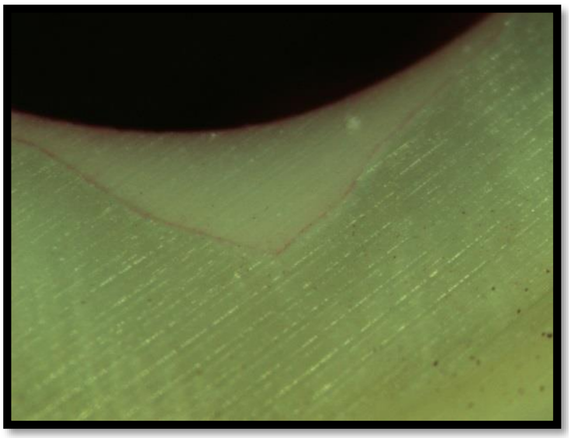
Picture 41. Stereo microscope image of a sample with a score of 0 at 30x magnification in acid + bonding agent + fissure sealant (with dental operating microscope) group



Picture 42. Stereo microscope image of a sample with a score of 1 at 30x magnification in acid + bonding agent + fissure sealant (with dental operating microscope) group



Picture 43. Stereo microscope image of a sample with a score of 2 at 30x magnification in acid + bonding agent + fissure sealant (with dental operating microscope) group

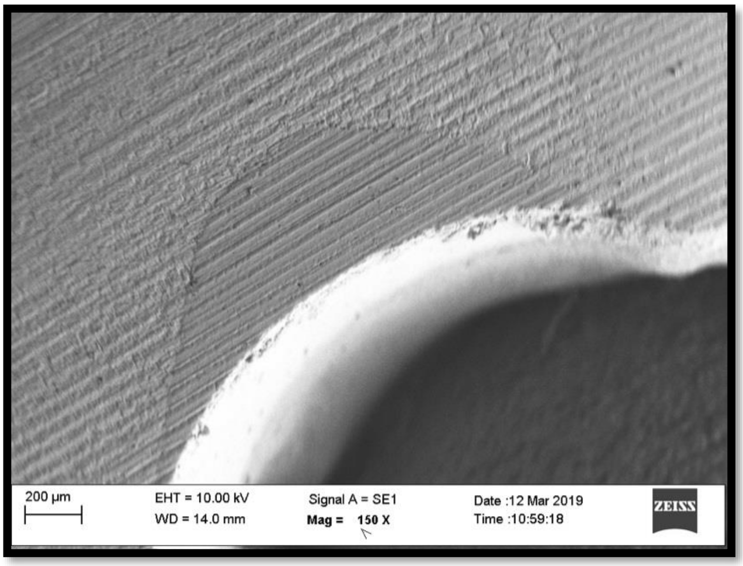


Picture 44. Stereo microscope image of a sample with a score of 3 at 30x magnification in acid + bonding agent + fissure sealant (with dental operating microscope) group

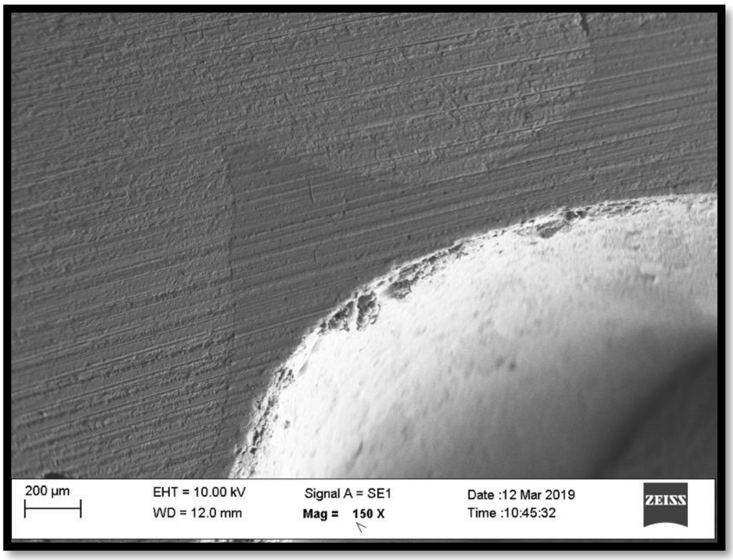
4.3. Evaluation of SEM Images of Fissure Sealant Microleakage

Samples obtained from group 1 (acid + fissure sealant), group 2 (acid + bonding agent + fissure sealant), group 3 (acid + fissure sealant - with dental operating microscope) and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) were examined under 150x magnification via SEM. In SEM imaging, penetration of fissure sealants, the adaptation of fissure sealants to the enamel walls and the continuity of the connection between the enamel wall and the fissure sealant were examined.

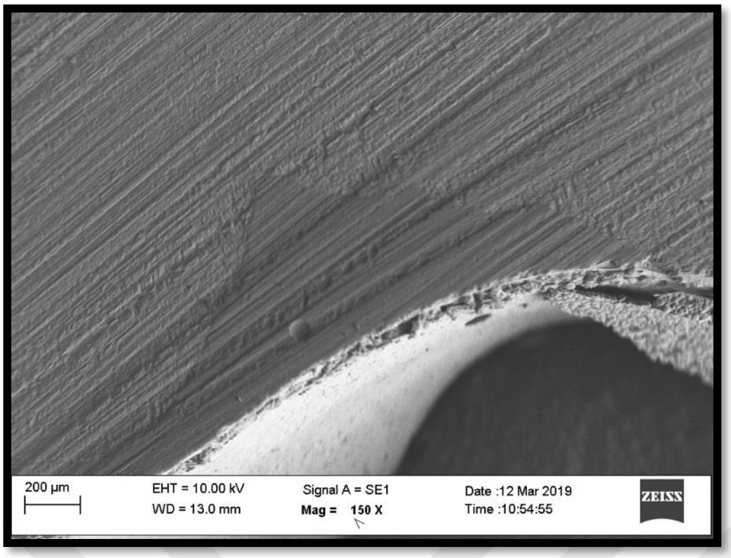
In the SEM image at 150x magnification of group 1 (Acid + fissure sealant) **(Picture 45)**, group 2 (acid + bonding agent + fissure sealant) **(Picture 46)**, group 3 (acid + fissure sealant - with dental operating microscope) **(Picture 47)** and group 4 (acid + bonding agent + fissure sealant - with dental operating microscope) **(Picture 48)**, fissure penetrations of fissure sealant materials were evaluated as “present”. In all groups, adaptations of fissure sealants to enamel walls were observed to be continuous. There was a continuous adhesion between the fissure sealants and enamel walls.



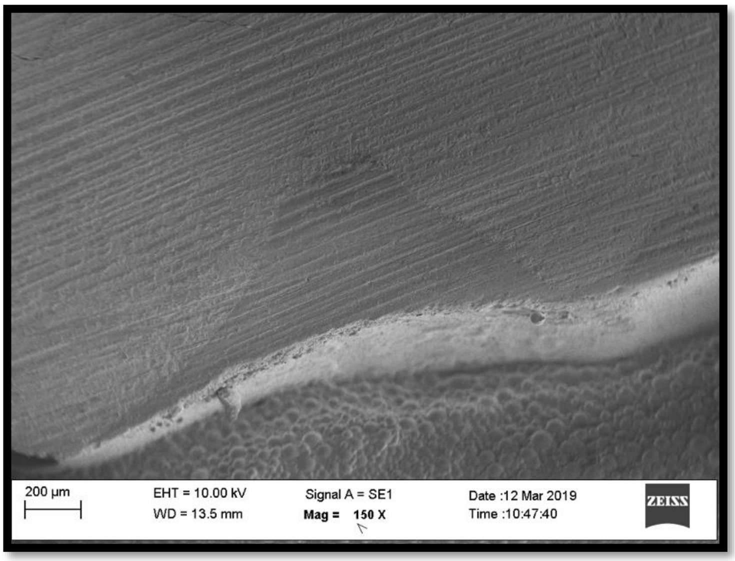
Picture 45. SEM image at 150x magnification of acid + fissure sealant group



Picture 46. SEM image at 150x magnification of acid + bonding agent + fissure sealant group



Picture 47. SEM image at 150x magnification of acid + fissure sealant - dental operating microscope group



Picture 48. SEM image at 150x magnification of acid + bonding agent + fissure sealant - dental operating microscope group

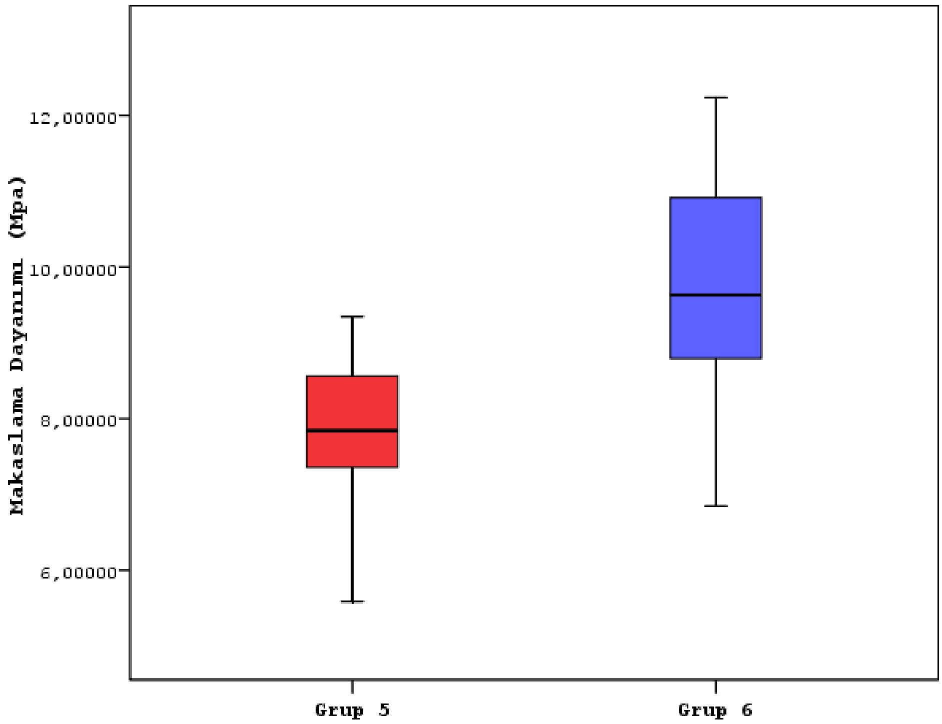
4.4. Assessment Results of Shear Bond Strength of Fissure Sealants

The mean of shear bond strength (Mpa) of Group 6 (Acid + bonding agent + fissure sealant) was found to be significantly higher than Group 5 (Acid + fissure sealant) (p = 0.001).

Table 5. Mean shear bond strength scores and p value

	Group 5 (Acid + fissure sealant) n:15	Group 6 (Acid + bonding agent + fissure sealant) n:15	p
Shear Strength (Mpa)	7.82 ± 0.92	9.67 ± 1.65	*0.001

***p<0.05**



Graph 2. Mpa values of shear bond strength groups

5. DISCUSSION

Although dental caries, which is an infectious and contagious disease that may occur due to various causes, can be prevented and controlled, it has been reported to become the most important public health problem in many countries throughout the world (79,279). Particularly in developing countries, the cost of dental treatment has been emphasized to exceed the total cost of all public health problems (280). Therefore, as in all branches of medicine, the prevention of caries formation and protection of oral health has been suggested to be one of the most important factors in dentistry. The main objective of today's dentistry has been reported to protect the oral and dental health by extending the use of protective practices (281).

The use of protective dentistry applications started in the 1930s have been reported to gradually increase in recent years. In particular, it has been stated that protective practices involving 6-11 age group children come into prominence. Identifying the problems correctly and carrying out studies for these problems are determined to be prerequisites by WHO to ensure that protective applications related to oral and dental health are effective. Therefore, besides the epidemiological studies in which the level of caries is determined, studies on extending protective applications particularly for the prevention of occlusal surface caries are also carried out nowadays (281).

Although dental caries is prevented through oral and dental health protection methods, epidemiological studies have reported that there is a decrease in the interface and flat surface caries, but the rate of pit and fissure caries is still high. Therefore, the importance of new applications and studies for the protection of occlusal surfaces has been emphasized to increase day by day (282).

Many methods including oral and dental health training and motivation, systemic and topical fluoride applications, plaque control, diet arrangement, use of remineralization agents, ozone, laser and fissure sealant applications have been reported to be used to prevent occlusal surface caries developing in pits and fissures (46,108,115,283–285). Fissure sealants are defined as materials that are applied to decayed pits and fissures, are micromechanically attached to the tooth surface, prevent the access of the microorganisms and nutrient residues that cause caries to teeth. Fissure sealant materials have been reported to be applied to the occlusal, buccal and palatinal surfaces of permanent teeth with suspicion of caries to prevent the formation of caries or stop the progression of the non-cavitated caries lesion (41). Therefore, it is of great importance that fissure sealant material should be clinically long lasting and durable (286).

It has been reported in many studies that resin-based fissure sealant application is the most successful protective method in the occlusal region with the highest caries rate (108,208,287). In many studies, bonding systems providing the adhesion of fissure sealants to tooth have been emphasized to be the most critical determinants contributing to sealing and mechanical retention and the clinical success of pit and fissure sealants has been reported to be directly related to sealing and mechanical retention (4). Regardless of the contents of the fissure sealants, the most important reason for their failure after the application is the weak bond due to the lack of penetration and the microleakage caused by the deterioration of this weak bond between the tooth surface and the material (4,111,132,288–291).

Therefore the aim of this study was to evaluate the shear bond strength and microleakage in fissure sealant procedure using dental operating microscopy and adhesive systems.

The bond strength between tooth and restoration has been reported to be one of the most important factors affecting microleakage. Despite the developments in dental materials in recent years, full sealing has not been achieved yet, but it is emphasized that the aim is to minimize microleakage (242). In the literature, it has been reported that there are many methods for the microleakage assessment of dental materials. Although in vitro microleakage tests cannot fully mimic the oral environment, it is possible to control many variables in the laboratory environment such as humidity, heat, and tooth type change, which are difficult to control in the clinical environment, in this type tests. Therefore, they are reported to be a preferred method for the microleakage assessment (236,248). It is emphasized that the clinical assessment model is considered as the most acceptable method for the assessment of material. As clinical assessments take too much time and there are standardization difficulties, it is difficult to carry out a timely assessment for the restorative materials, adhesive systems and their mode of application. Clinical trials have been reported to be less preferred due to the facts that microleakage is determined as marginal discoloration or degradation and that it is difficult to distinguish the cause of restoration loss in the long term in such trials. In vitro tests, therefore, have been reported to be frequently used for the assessment of materials, microleakage studies, detection of gaps between tooth and restoration, and early assessment of restorative materials (292).

Pashley et al. (1988) reported in their study that there were no significant differences between the in vivo and in vitro test results (293).

In a study by ***Barnes et al. (1993)***, it has been stated that in vitro tests provide clearer results in the determination of microleakage compared to in vivo tests (294).

In the present study, assessments were carried out under in vitro conditions, as in the studies by ***Pashley et al. (1988)*** and ***Barnes et al. (1993)***, due to the facts that clinical trials are time-consuming, there are standardization difficulties and it is difficult to assess the microleakage of materials on time.

Resin-based fissure sealants applied to acid-etched tooth surfaces have been reported to have high retention and protective effects (109,126,140,208,295). However, the contamination of the enamel surface with moisture and saliva has been reported to be the most important criterion that negatively affects both the retention and clinical success of resin-based fissure sealants (153,289). Due to this negative aspect of resin-based fissure sealants, it has been recommended that glass ionomer-based fissure sealants with less moisture sensitivity should be applied temporarily to the pit and fissures of first molars that are not completely erupted until the teeth are completely occluded (288,296). Glass ionomer based fissure sealants with less moisture sensitivity have been reported to fail to meet the expectations as its clinical success is lower in terms of retention and should be repeated frequently (137,297). In a guideline published by ADA in 2008 on fissure sealants, it has been emphasized that resin-based fissure sealants should be preferred primarily in the teeth where moisture isolation can be provided rather than glass ionomer based fissure sealants (108).

In an evidence-based systematic review by ***Ahovuo-Saloranta et al. (2008)***, permanent first molar teeth to which resin-based fissure sealant is applied have been reported to have 78% and 60% less caries rate at the end of two and four years, respectively, compared to teeth to which no fissure sealant is applied (43).

Similar to the study by ***Ahovuo-Saloranta et al. (2008)***, Fissurit® FX was used as a resin-based fissure sealant , which was reported to be more resistant in terms of retention and considered to be the most successful fissure sealant material, in the present study because there was no problem like moisture and saliva isolation under in vitro conditions.

Most of the studies conducted in recent years have been reported to aim to increase the retention rate of fissure sealants (117,298). The use of adhesive agents between the fissure sealant and the tooth surface has been reported to be a method of increasing the bond strength and clinical success (299–303). In studies evaluating the success of the resin-based fissure sealant applied on tooth surfaces with or without saliva and moisture contamination, the use of adhesive agent has been reported to contribute to clinical success by reducing microleakage and increasing bond strength and retention (16,30,40,304). However, it has also been reported that there are also studies reporting that this intermediate step increases the duration of treatment and costs, its efficacy is reduced when primers interact with moisture and it does not contribute to the retention (305–307). It has been emphasized that there is no consensus on the benefits of using fissure sealants with adhesive agents. ADA recommends that further laboratory and clinical trials evaluating the contribution of adhesive systems to the retention of fissure sealants should be carried out (108).

In this study, the effect of the adhesive agent on microleakage and shear bond strength of fissure sealants was evaluated. Similar to the study by ***Witzel ve ark. (2000)***, the adhesive agent was thought to contribute to the retention rate of the fissure sealant. Although this study is an in vitro study performed under laboratory conditions, it was taken into consideration that the fissure sealant application was a procedure performed for individuals at 6-11 years of age in the clinical practice. Since the study duration was thought to be limited, Adper™ Single Bond 2 Adhesive, which was applied through etch-rinse technique, was used as the adhesive agent in the subgroups of this study experiment because of being a single-step application, providing ease of application and saving time.

In studies using extracted human teeth, it has been reported that the standardization of the samples increases the reliability of the experimental results (148,308). In the standardization of the teeth, the age of the patient, whether the tooth samples used are decayed and the conditions under which the teeth are kept during the experiment are of great importance (148,308).

In this study, the extracted human third molars were used to obtain results close to the clinical applications. Similar to the study by ***Jacobs et al. (1986)***, third molar teeth of the patients at the age of 25-35, which were extracted, completed their root development, and had no infection in surrounding tissues as well as no caries and tissue loss, were used in the present study.

It has been suggested that there are controversial results about the effects of structural changes at the chemical and micro level emerging during the maturation stage of enamel following the eruption process on the retention of fissure sealants (145).

In studies by Jacobs et al. (1986) , ***Türfekçi et al. (2007)*** and ***Grewal et al. (2009)***, it has been reported that there is no difference between the bond strength of the restorative materials to the enamel which has completed or not completed its maturation stage, the microporous structure of the enamel of the teeth that have just erupted has no effect on the penetration of the fissure sealant, the maturation stage of the enamel following the eruption process has no role in the clinical success of the fissure sealants(145,308–310).

No clear information could be obtained about the time elapsed from the completion of the eruption process of extracted human third molars used in the present study. Furthermore, this duration was not evaluated because ***Jacobs et al. (1986)***, ***Türfekçi et al. (2007)*** and ***Grewal et al. (2009)*** suggested that the maturation stage of the enamel following the eruption did not have a significant effect on the bonding ability of fissure sealants. In the present study, only the third molars with no caries, no hypoplasia in the enamel and no deformation due to extraction were included.

In in vitro studies by ***Civelek et al. (2003)*** and ***Attam et al. (2009)***, the teeth were removed from the blood and tissue debris after the extraction and then, were kept in the distilled water and formalin containing tap water, demineralized water, sterile saline, and thymol until the test (311,312).

In a study by ***Srinivasan et al. (2005)***, it was reported that the teeth should be stored in thymol-containing distilled water to prevent bacterial contamination (313).

Similar to the study by ***Srinivasan et al. (2005)***, human third molars were left in distilled water containing 0.2% thymol to prevent the teeth from bacterial contamination during the waiting period until the test in the present study.

Nejad et al. (2012) reported that tooth surfaces can be cleaned before the fissure sealant application through such several methods as brushing with toothpaste and water, hydrogen peroxide, prophylaxis, air abrasion, air polishing, ultrasonic devices, and laser (314).

Simonsen et al. (2011) reported in their study that the pads to be used for cleaning enamel surfaces should not be oil based so as not to affect acid etching procedure (208).

On the other hand, ***Avinash (2010)*** reported that materials should not contain fluorine so that they do not affect retention (283).

In a study by ***Garcia-Godoy et al. (1997)***, penetration of fissure sealants was reported to be affected if the teeth were cleaned with pumice and pumice residues could not be completely removed from the fissures and therefore, they emphasized that occlusal surfaces should not be cleaned with pumice (121).

Muller-Bola et al. (2006) and ***Deery (2013)*** reported that there is no difference between the fissure sealants applied to tooth surfaces only brushed with water and fissure sealants applied to tooth surfaces brushed with pumice in terms of retention and penetration (315,316). However, SEM studies have shown that pumice remains in the depths of pits and fissures of the teeth washed under running water and prevents the penetration of acid and fissure sealants and thus, affects retention negatively (10,22).

In the present study, similar to the study by ***Garcia-Godoy et al. (1997)***, the extracted human third molars to be used for the experimental groups were cleaned under running tap water not to affect the retention and penetration of fissure sealants considering the fact that SEM imaging would be taken.

Literature review has revealed that there are many studies on the preparation of the enamel surface before the application of fissure sealants. It has been reported that various methods have been applied such as acid etching, air abrasion, laser applications, drilling applications, and their combination (36,284). Most of the studies have emphasized that acid etching is the most reliable method among those. As a result of acid application, the microporosities on the enamel surface, which are 5-50 µm wide, have been reported to increase the wettability of the surface and the retention of the material (22,36).

Herle et al. (2004) suggested that acids used in the preparation of the surfaces of the teeth prior to the fissure sealant application could not reach the deep regions of the fissures where there were bacteria and organic wastes (317).

In a study by ***Hatibovic-Koffman et al. (2008)***, it has been recommended that acid should be applied to the surfaces of the teeth after abrasion of the fissures through drilling to eliminate the aforementioned disadvantage of acid etching (318).

Brown and Barkmeier (1996) reported that acid etching combined with air abrasion would be more successful, whereas ***Nejad et al. (2012)*** reported that combined use of acid etching and laser application could provide more successful clinical results (314,319).

Marotti et al. (2010) suggested that the use of laser and air abrasion together with acid etching increased the number of application steps and such applications did not have any contributions to the success of material used and thus, the use of acid etching alone was sufficient (320).

Similar to the study by ***Marotti et al. (2010)*** , the fact that the fissure sealant application was a common dental application in patients at the age of 6-11 was taken into consideration in the present study. Acting with the idea of keeping the application steps to a minimum and avoiding applications, the necessity of which has not been proven yet, the tooth surfaces were prepared using only the acid etching method.

It has been observed that many studies are aimed at finding the acid type that will provide the strongest bonding by creating minimal surface loss without damaging the living tissues. Majority of these studies have reported that applying phosphoric acid at 35-40% concentrations for about 15-60 sec provides the strongest bonding in primary and permanent teeth (321). In some researches, besides the phosphoric acid, acids such as maleic acid, oxalic acid, citric acid, and polyacrylic acid were also included in in vitro studies to obtain better results, however, the widespread use of these acids has been prevented because of the negative results obtained (322,323). Besides determining the type of acid to be used in the surface preparation of teeth, how many seconds this acid should be applied to the tooth surface has been observed to be discussed in many studies.

The in vivo and in vitro studies by ***Bilgin (1993)*** and ***Eidelman et al. (1988)*** have emphasized that the similar results were obtained from the acid etching application for 15, 30, and 60 sec, the application duration did not affect the clinical success of fissure sealants, and acid etching application for 30 sec was sufficient to prevent moisture and saliva contamination (324,325).

In the present study, acid etching was applied to the occlusal surfaces of the extracted human third molars, to which the fissure sealants were applied, for 30 sec considering the findings reported by ***Bilgin (1993)*** and ***Eidelman et al. (1988)***. In accordance with the manufacturer’s instructions, Jumbo Etch Royale Kit ™ 37% phosphoric acid gel was applied for 30 sec. After the washing and drying processes, the enamel surface was ensured to be homogeneous, chalky and opaque.

In studies conducted by ***Waggoner et al. (1996)*** and ***Özel et al. (2003)***, it has been reported that visible blue light sources and light emitting diodes are used to provide polymerization of resin-based fissure sealants. The success of the polymerization has been reported to be affected by the power, intensity and light wavelength of the light source. It has been further reported that the distance between the light source and fissure sealant affects the quality of the polymerization. The light device must have a light intensity of at least 300 mW/cm and it should be kept as close as possible to the fissure sealant to achieve the desired polymerization. Furthermore, the device should be checked frequently and used in accordance with the manufacturer’s instructions (149,326).

Similar to the study by ***Özel et al. (2003)***, in this study, Optilux 501 (400-505 nm wavelength), a halogen light, was used to ensure optimal polymerization in a way that the light flashed on tubercle peak of teeth following the fissure sealant application and polymerization was carried out for 20 seconds in accordance with the manufacturer's instructions.

It has been reported that there is a need for the use of dental magnification devices in dentistry to provide ergonomic working conditions, to increase the angle of view, and thus, to increase the success rate of dental treatments. Particularly, during the application of endodontic treatments, the use of dental operating microscopes, which is one of the magnification devices used in dentistry, has been observed to be quite widespread. It has been reported that dental operating microscopes are used not only during endodontic treatments but also during restorative procedures (180,259–261,271).

The literature review has revealed that there is no study using dental operating microscope during fissure sealant application.

In this study, the findings obtained by ***Carr et al. (2010)*** were taken into consideration and dental operating microscope was used to provide ergonomic working conditions during fissure sealant application, to increase the angle of view, and thus, to increase the success rate of dental treatments. It was aimed to investigate the effect of dental operating microscope on penetration and bonding ability of fissure sealants. There was no statistically significant difference in the microleakage assessment results of the fissure sealants applied to the teeth to which fissure sealant was applied with the use of dental operating microscope. However, the SEM images showed that the fissure sealants were successful in terms of their penetration to the fissures of the teeth. As reported by ***Ferrari et al. (1997)***, dental operating microscope with the advantage of providing a more detailed image increased the treatment success. However, due to the small number of samples in the experimental groups in this study, statistical data did not reflect the expected result. The reason for the small number of samples included in the experimental groups was attributed to the fact that it was difficult to find samples that meet the standardization criteria (human third molars with no caries, no extraction deformation, and no hypoplasia on enamel belonging to individuals at the age of 25-35) to be used for the experiment and therefore, it was thought that more studies should be carried out with more samples.

It has been reported that thermal stresses can lead to the formation of gaps in the interface of dental tissue and restorative material and then to microleakage and thermal and mechanical load cycle can be applied to the samples prepared to mimic the oral environment before the microleakage assessment of the materials (327). The temperature values applied in the thermal cycle vary between 0-68ºC to reflect the effects of the coldest and hottest temperatures in the mouth on the materials used in dentistry and temperature changes between 5-55°C are usually used in the studies (328–330).

In a study by ***Türkün and Ergücü (2004)*** comparing the materials and methods used in different microleakage tests, thermal cycling has been reported to be often preferred. They reported that thermal cycling was performed between 5-55°C (98.7%) and using tap water (96.6%) (292).

Mandras et al. (1991) reported that thermal cycling, which was repeated for 250 and 1000 times, had no effect on the microleakage of resin-based restorative materials. In other studies, the thermal cycling process has been suggested to increase microleakage (331,332).

In the present study, 5-55°C temperature changes were used to mimic the oral environment as in ***Türkün and Ergücü (2004)*** considering the fact that more thermal cycling process should be repeated based on the findings obtained by ***Mandras et al. (1991)***. Similar to the study by ***Schuldt et al. (2015)***, each of these mimicked environments was subject to 5000 thermocycles for 30 sec. In the present study, it was further aimed to assess the long-term microleakage and shear bond strength of fissure sealants with the thought that the aim of the aging process was to mimic the oral environment. For this reason, it was preferred to apply the thermal cycling process for a long time.

In the studies on determining the microleakage of restorative materials, several methods have been reported to be used including dye penetration, radioactive isotope, bacteria, compressed air, chemical agents, electrochemical technique, neutron activation analysis, SEM, confocal electron microscopy, and multi-photon laser methods (151,242,244). Dye penetration has been suggested to be the most commonly used method in the determination of microleakage (333). Dye penetration technique has been reported to have many advantages: it is easy-to-apply, reliable, nontoxic, and cost-effective, ensures the determination of microleakage under visible light and provides error-free measurements, it is water soluble and not reacting with hard tissues, and (iv) it is retained on the surface by dentin matrix or apatite crystals (151,242,244,334). In the dye penetration method, methylene blue (0.2-10%), basic fuchsine (0.5-2%), fluorescence (2-20%), crystal violet (0.05%), aniline blue (2%), silver nitrate (50%), toluidine blue (0.25%), erythrocyte (2%) and rhodamine B (0.2%) are used (181,242,244,334). One of the important factors in the selection of dyes has been reported to be the particle size of the dye. Since the bacteria are 0-5 μm in diameter, they can easily enter into the restoration and dental interface, so the dye should be able to enter into every area that the bacteria can enter (335,336).

In a study by ***Türkün et al. (2004)***, dye penetration has been reported to be used by 86.8% among the in vitro tests used for the microleakage assessment and basic fuchsine has been reported to preferred by 40.8% for the dye penetration tests (236).

In the present study, microleakage was detected through dye penetration technique using 0.5% basic fuchsine as it has many advantages including (i) it is easy-to-apply, reliable, nontoxic, and cost-effective, (ii) ensures the determination of microleakage under visible light and provides error-free measurements, (iii) it is water soluble and not reacting with hard tissues, and (iv) it is retained on the surface by dentin matrix or apatite crystals .It was observed that ***Türkün et al. (2004)*** also preferred to use dye penetration technique to determine the microleakage. Basic fuchsine of 0.5% was preferred in the present study because of its low toxicity and cost-effectiveness features and its ability to pass through the spaces of 0-5 μm.

In the studies on microleakage performed under in vitro conditions with the dye penetration method, it was reported that the tooth surfaces outside the region where the leak would be examined were covered with an insulating agent such as nail polish (236,248). It is then reported that the samples are kept in the solution selected for leakage for a certain period of time, which varies between one week to six months, and it is preferred to wait for 24 hours (236,248).

Similar to the study by ***Türkün et al. (2004)***, in this study, the tooth surfaces outside the area where the leak was to be examined were covered with nail polish because microleakage assessment was performed through dye penetration method and under in vitro conditions. Samples were left into the incubator at 37°C for 24 hours. The waiting duration in the incubator was thought to have no effect on the results. For this reason, it was considered appropriate to left them in the incubator which was kept constant at 37°C.

In a study by ***Raskin et al. (2001)*** assessing the microleakage via dye penetration method, the number of sections taken from each sample was reported to be important for the reliability of the study and they, therefore, reviewed 144 articles by compiling microleakage studies, revealing that single section, two sections, and three sections were taken in 47.1%, 20.2%, and 12.7% of the studies, respectively. They also reported that there was no significant difference between the highest value obtained from five sections and the highest value obtained from three or four sections (248).

Türkün et al. (2004) evaluated 84 microleakage studies and reported that single section was taken in 50.9% of the studies, whereas three sections were taken in 8% of studies (236).

In the present study, the results obtained by ***Raskin et al. (2001)*** in their review were taken into consideration. In most of the studies, it has been observed that microleakage assessment is performed by taking a single section and the number of sections has no effect on the microleakage assessment results. Therefore, a single section was taken from the samples to be subject to microleakage assessment based on the results obtained by ***Türkün et al. (2004)***. These values were visualized via stereo microscope and scored by the same person in accordance with the criteria developed by ***Pardi et al. (2006)***.

In some of the studies using SEM for imaging, it has been reported that the quality of the bond between tooth and restoration surfaces and structural properties of enamel and dentin can be examined more extensively through SEM (337). In studies evaluating the marginal adaptation of fissure sealants and the quality of resin extensions via SEM, the dimensions of the spaces formed between the fissure sealant and the enamel surface were measured in µm or evaluated as present or absent (338).

Similar to the study by ***Kantovitz et al. (2008)***, the images were evaluated via SEM at 150x magnification in the present study because the surface can be examined in more detail and the quality of the bond between the tooth and restoration surfaces can be seen more comprehensively via SEM. In studies by ***Botsalı et al. (2015)*** and ***Kanemura et al. (1999)***, SEM images were examined at 1000x magnification because they evaluated the shear bond strength and fracture pattern of restorations. In the present study, only the penetration of fissure sealants into the fissures was evaluated. Therefore, 150x magnification was used in SEM analysis. In the assessment of the penetration of fissures, 150x magnification was sufficient to interpret the SEM images .Since it was thought that the margin of error might be high in SEM imaging, the gap between the fissure sealant and the enamel surface was examined and the penetration was interpreted as present or absent .

As with many restorative materials, the integrity of the bond between the tooth surface and the material is one of the most important factors in preventing the leakage of bacteria and oral fluids for the fissure sealant materials (288). It has been emphasized that the caries prevention properties and retention ability of fissure sealants are directly associated with the bond strength and quality between the material and the enamel surface and important in evaluating clinical successes and improving physical properties (339,340).

In the literature, shear and tensile bond strength tests have been reported to be used the most to assess the bonding forces of the materials (223,289,301,328,341–351). Shear and tensile bond tests have been reported to be divided into two according to the area of the surface bonding to the dental tissues: micro and macro tests. In the macro bond strength test, the bonding area of the material has been reported to be ≥3 mm² and in most of the studies comparing macroshear and macrotensile methods, the results have been emphasized to be in parallel with each other (181,352).

As cohesive fracture occurs more frequently in samples where the shear bond strength value is above 20 MPa, it has been reported that tensile tests should be used for the samples that are expected to have high bond strength (181). Microtensile and microshear bond strength tests have been developed due to the facts that fractures seen in macro bond strength tests are mostly cohesive-type and the adhesive bond strength of the restorative material cannot be determined exactly. The aim of these tests is to measure the physical properties of the bonding agent systems as close to real as possible. In this way, researchers have been reported to provide more sensitive test methods (181,353). It has been reported that micro tests can assess 1 mm² and smaller surface areas (223).

In a study by ***Salz and Bock (2010)***, it has been shown that similar results are obtained from macroshear and microshear tests, but they have suggested that microtensile values were four times of the macrotensile values (354).

Microtensile tests have been reported to have more advantages compared to macrotensile tests: regional bond strengths can be measured, small samples with a binding surface area of up to 0.25-1 mm² can be tested, multiple samples can be prepared from a single tooth and mean and variance values can be calculated for a single tooth, and higher bond strength values can be obtained in samples with smaller surface area (181,223,349,353–355). However, there are also disadvantages of microtensile tests: the laboratory processes require technical precision, samples can be easily dehydrated because of being too small, the bond strength below 5 MPa is hardly measured, and the weakened adhesion due to the microfractures that may occur during the preparation of the samples may lead to lower values than the actual values (181,223,353–355).

Microtensile bond strength tests have been suggested to be considered as standard in the evaluation of the bond strength of adhesives to dentin, whereas shear bond strength tests have been considered to be one of the most reliable and widely used techniques in the evaluation of enamel bond strength (354).

Similar to studies by ***Meerbeek et al. (2003)*** and ***Salz et al. (2010)***, the present study benefited from the shear bond strength test to assess the bond strength of fissure sealants. However, considering the disadvantages of microtensile tests (requires technical precision, samples can be easily dehydrated, the bond strength below 5 MPa is hardly measured, and the weakened adhesion due to the microfractures that may occur during the preparation of the samples may lead to lower values than the actual values), shear bond strength test was decided to be performed to assess the bond strength of fissure sealant application because it was easily applicable and the most reliable and widely used method to assess the bond strength of enamel.

Barkmeier et al. (1994) reported that forces varying in the range of 0.5-5 mm/min were applied to measure the restoration-tooth adhesion in the bond strength tests (307).

In the present study, the bond strength values of the fissure sealants were determined by applying a force of 1 mm/min to the samples, compatible with the study of ***Sano et al. (1994)***.

In shear bond tests, the surface area to which the samples are to be bound must be wide and flat. Moreover, sanding the surfaces prior to the test is not an accurate approach as the sanded surface loses its natural structure. In the literature, there are studies reporting that non-abraded enamel increases the resistance of the layer with no prism on its surface to the acid, affects the shear bond strength of resin-based fissure sealants, and provides lower bond strength values compared to the abraded surfaces (223,356).

Kanemura et al. (1999), reported no difference between shear bond strengths of resin-based restorative materials applied to the abraded and non-abraded enamel surfaces (343). In most of the studies in the literature, silicon carbides of different grits have been observed to be used to smoothen the enamel surface in the shear bond strength tests (289,297,346,357–359).

Suzuki et al. (2013) used silicon carbide of 180, 320 and 600 grits, ***Gomes et al. (2008)*** used silicon carbide of 600 and 1200 grits, and ***Mine et al. (2008)*** used only 600-grit silicon carbide for 60 sec (348,358,360).

In the present study, a wide and flat surface was obtained for the reliability of the shear bond strength test. As it was thought that non-abraded enamel would increase the resistance of the layer with no prism on its surface to the acid, would affect the shear bond strength of resin-based fissure sealants, and would provide lower bond strength values than it should be, the enamel surfaces were abraded with 600-grit silicon carbide under water cooling in this study, similar to the study by ***Mine et al. (2008)*** .

In studies assessing the shear bond strength, it has been reported that the samples are kept in water for a certain period of time to mimic the oral environment prior to the test and the waiting period of the fissure sealants in water did not change their shear bond strengths (223,349,361,362).

In the present study, in accordance with the results of the studies by ***Meerbeek et al. (2010)***, ***Peutzfeld et al. (2004)*** and***Powers et al. (2005)***, samples were left into water at

37°C for 24 hours before the shear bond strength test considering that the time spent in the water had no effect on the result.

There have been many studies that support and do not support the use of bonding agents to reduce microleakage of pits and fissure sealants (16,127,301,305). In both in vivo and in vitro studies, it has been shown that effective and long-term adhesion can be obtained following the phosphoric acid etching of enamel in both etch-rinse and self-etch adhesive systems (363). In cases where self-etch adhesives are used, the enamel should be firstly etched with phosphoric acid selectively, the self-etching primer should be applied to dentin, and then, the bonding agent should be applied to the enamel and dentin (184,364). Even in single-step self-etch adhesives, it has been reported that the acid-etching of the enamel as the first step increases the bonding values. Phosphoric acid forms a micro-retentive surface on the enamel and prepares a path for resin infiltration (175,365).

In the present study, teeth in all groups were subject to acid etching considering the study findings reported by ***Pashley et al. (2011)***. Direct fissure sealant was applied to the teeth in Group 1 and Group 3 after acid etching, whereas Adper™ Single Bond 2 was applied to the teeth in Group 2 and Group 4 after acid etching. Fissure sealants were applied to the teeth in group 3 and group 4 with the guidance of a dental operating microscope. Statistical analysis revealed no significant difference between the groups. These results were also supported by SEM images which showed fissure sealant penetration in all groups and there was no relative difference between groups. It was observed that fissure sealants penetrated all fissures without any differences.

In a clinical study by ***Sakkas et al. (2013)***, retention and anti-caries property of fissure sealants applied using adhesive system were evaluated. The study included a total of 87 patients aged from 6.5 to 11.5 years who were divided into two groups. Group 1 consisted of a total of 164 permanent first molars from 41 patients, four teeth from each patient, and group 2 consisted of 182 permanent premolars from 46 patients, four teeth from each patient. Fissure sealant was applied by using a different technique for each of the four teeth included in the study. The resin-based fissure sealant (Fissurit FX, VOCO) was applied to one of the teeth after acid application. Optibond FL (Kerr), Optibond Solo Plus (Kerr), and Prompt-L-Pop (3M ESPE) adhesive systems were applied respectively to other teeth and then, resin-based fissure sealant was applied. At the end of six, 12, 18, 24, and 36 months follow-up, retention, and new caries formation were evaluated visually. No statistically significant difference was found between the samples in terms of caries prevention properties, however, a statistically significant difference was found between the retention of fissure sealants applied following the acid etching through the self-etch adhesive system (Prompt-L-Pop) and retention of fissure sealants applied using Optibond FL and Optibond Solo plus adhesive systems. Researchers have concluded that Optibond FL and Optibond Solo plus adhesive system can be used to increase the clinical success of fissure sealant in terms of retention (366).

In an in vitro study by ***Botsalı et al. (2015)*** including 210 human teeth, 105 teeth were reserved to be used for shear bond strength test and the remaining half of the teeth were reserved to be used for the microleakage assessment. They divided into 14 groups of 15 teeth in each. Fissure FX was applied to the control group directly after the acid etching process. In the other groups, Clearfil SE Bond, G Bond, Clearfil DC Bond, Prelude, Adper Single Bond and Optibond S adhesive agents were applied following the acid etching process. According to the results of the study, the clinical success of microleakage and shear bond strength test results of the Adper Single Bond-applied groups were found to be statistically significantly higher than the other groups. Adper Single Bond adhesive systems have been reported to be used to increase the retention of fissure sealants and to prevent microleakage (367).

In the present study, similar to the study by ***Botsalı et al. (2015)***, Adper™ Single Bond adhesive system was applied via the etch-and-rinse technique and then, fissure sealant was applied. No statistically significant difference was found between the results of the microleakage tests. However, SEM examination revealed that there was a continuous and smooth penetration of fissure sealants into the fissures of the teeth. In the shear bond strength test, Adper™ Single Bond-applied group was found to have a higher shear bond strength. The use of a bonding agent was thought to increase bonding. The reason for the non-significant results in the microleakage test was attributed to the small number of samples in the experimental groups. It was concluded that the bonding agent applied via etch-rinse technique increased shear bond strength.

In a meta-analysis study by ***Botton et al. (2016)*** , authors evaluated the effect of the use of self-etch adhesive systems before the application of pit and fissure sealant on the retention of the pit and fissure sealants. When self-etch adhesive systems were used, an additional acid etching step was not applied during the process. It has been reported that technical sensitivity was required less and the patient spent less time on the seat. It was concluded that the retention of fissure sealant applied through self-etch systems was less. This was thought to be due to the fact that the primer in self-etch systems could not etch the enamel surface sufficiently (368).

In a clinical study by ***Unverdi et al. (2017)***, the authors compared the effects of different adhesive systems applied before the pit and fissure sealant applications on the retention. Their study included 51 patients and 204 teeth, which were divided into four groups: In group 1, which was called control group, the resin-based fissure sealant was applied after acid etching process. In Group 2, XP bond was applied following the acid etching process, Clearfill SE bond was applied alone in Group 3, and Clearfill SE bond was applied following the acid etching process in Group 4 and then, resin-based fissure sealants were applied. Fissure sealants were evaluated after first, third, sixth, 12th, 18th and 24th month follow-ups. According to the evaluation made at the end of the 24th month, the rate of retention in Group 2 and Group 4 was found to be similar to each other but higher than the other groups. No significant difference was found between other groups (369).

In the present study, acid etching procedure was applied to all of the teeth in the groups to be subject to microleakage test at first and then, fissure sealant application was performed directly after the acid etching in Group 1 and Group 3, whereas in Group 2 and Group 4, the bonding agent was applied first and then, fissure sealant was applied following the irradiation. Although there was no statistically significant difference between the groups in terms of test results, the SEM images showed similar penetrations in each group and fissure sealants were observed to be placed on fissures properly and continuously. This present study was found to be similar to the study findings of ***Botton et al. (2016)*** and ***Unverdi et al. (2017)*** and the acid etching step was found to be of great importance in terms of the penetration of fissure sealants.

In an in vitro study by ***Nirwan et al. (2017)***, the effect of sixth, seventh and eighth generation adhesive systems on the retention of fissure sealants was investigated. They included 148 teeth in the study, which were examined in four groups. Group 1 was determined to be control group. Adper bond, a sixth generation adhesive system, Optibond, a seventh generation adhesive system, and Futurabond, an eighth generation adhesive system, were applied to teeth in Group 2, Group 3, and Group 4, respectively. No significant difference was found between the groups following the assessments made in the third and sixth month controls. However, the retention of the fissure sealants was reported to vary depending on the isolation of the tooth during the application of pit and fissure sealant, the level of cooperation of the child, and the application technique (370).

In a study by ***McCafferty et al. (2016)*** using 100 permanent first molars of 20 children aged between 5 to 15 years, effects of adhesive systems and cooperation of child on retention of fissure sealants were evaluated through 12-month follow-ups. The results showed that the retention of fissure sealants in children with better cooperation was observed to be significantly higher, however, the use of adhesive system was reported to have no significant effect on the retention of fissure sealants applied to the occlusal surfaces of the teeth (371).

In an in vitro study by ***Pınar et al. (2015)***, the authors divided 48 premolars into two groups and only fissure sealant (Kerr) was applied following the acid etching procedure in one group, whereas bonding agent (Single Bond; 3M) was applied after acid etching procedure and then fissure was applied in the other group. Then, thermal cycling was carried for aging and microleakage was evaluated via stereo microscope. Authors have reported no significant difference between the groups at the end of the study (372).

In a randomized controlled clinical trial by ***Khare et al. (2017)***, the retention, caries formation rate and marginal discoloration of fissure sealants applied with the use of four different types of adhesive systems were examined. A total of 208 teeth were used in their study, which included 52 patients aged 6-10 years. The assessments were made at third, sixth and 12th months. Group 1 was determined to be control group. Adper single bond, Scotch-bond universal adhesive, and G-bond self-adhesive were applied to Groups 2, 3, and 4, respectively, following the acid etching procedure and then, fissure sealants were applied. As a result of the 12-month follow-up, the retention rate of fissure sealants in teeth to which Adper single bond and Scotch-bond universal adhesives were applied was found to be the highest (77.1%), whereas the retention rate of fissure sealants in teeth to which g-bond self-etch adhesive was applied was 53.3% and the retention rate of fissure sealants in the control group to which only acid etching was applied was 45.8%. Considering these findings, adhesive systems used before the fissure sealant application had no significant effects on the retention rates (373).

In the present study, it was observed that the use of bonding agent did not create a significant difference between the groups. ***Similar to the present study, in studies by Nirwan et al. (2017),*** , ***MCcafferty et al. (2016)*** and ***Khare et al. (2017)***, bonding agent was used before fissure sealant application and microleakage of fissure sealants was evaluated. The difference between the present study and the aforementioned studies was that those studies were performed under in vivo conditions. They thought that clinical conditions might have affected the outcomes. In an in vitro study by ***Pınar et al. (2015)***, where etch and rinse technique was preferred and Adper Single Bond agent was used similar to the present study, it was concluded that the bonding agent application did not have any significant effect on the microleakage of fissure sealants. Considering all these studies, it has been thought that there is a need for further studies on the use of adhesive systems before fissure sealant application to clearly understand the effect of bonding agent on microleakage.

In the literature, it has been recently reported that there are different opinions about the application of fissure sealant to acid-etched enamel surface following a bonding agent, but there is still no consensus about this issue. Furthermore, the bonding agent has been reported to increase the surface wettability and resin penetration and Adper Single Bond is one of the adhesive systems that can be applied selectively through total-etch or self-etch techniques (374).

Resin extensions with sizes ranging from 10 to 100 microns have been reported to occur due to the leakage of resin contents into the microporosities formed through the acid etching of enamel before the material application. It has been reported that these extensions allow the material to be locked with the enamel surface, increase the mechanical retention of the fissure sealant and reduce the effect of the acids formed by the microorganisms by surrounding the enamel crystals (115,191,338,375).

Self-etch adhesive systems, which do include acid etching-washing-drying steps and provides ease of application in patients who have difficulty in cooperating, have been reported to bind to the enamel weakly because they are not able to solve the aprismatic enamel layer (376). Studies have shown that self-etch adhesives weakly bind to the enamel, increase the microleakage compared to the systems including acid etching step, and significantly improve the resin penetration following the pre-acidification (190,377–379). The etching effect of self-etch adhesives have been reported to lead to an insufficient decalcification in the core section of enamel prisms and negatively affect the enamel bond by forming a thin bonding layer compared to the pre-acidification. This insufficient acidification potential has been explained by the inactivation of the acid in contact with the enamel, resulting in limited decalcification (380). Furthermore, it results in bonding with a low resistance to thermomechanical stresses as a result of not being able to remove dissolved calcium phosphate by the washing process and leads to long-term restoration-tooth gaps (381,382). In the fissure sealant application, the acidification ability of methacryl-modified phosphoric acid esters in self-etch adhesives has been emphasized to be much more superficial than observed in acidification with phosphoric acid (293).

In a study by ***Barutçugil et al. (2013)*** , acid etching application and self-etch application were compared and a statistically significant difference was reported between the acid applied groups and acid-free self-etch groups. The bond strength of the teeth in the acid applied groups was reported to be significantly higher than the acid-free groups (383).

Similar to the study by ***Barutçugil et al. (2013)***, the bonding agent application after acid etching process was found to improve the bond strength in the present study.

In studies by Wagner et al. (2014), ***and Botsalı et al. (2015)***, ***Mena-Serrano et al. (2013)***, and ***Perdigão et al. (2014)***, a bonding agent was applied under the fissure sealant through the self-etch and etch-rinse techniques and no statistically significant difference was observed in the results obtained from the shear bond strength assessment of fissure sealants (213,215,367,384)**.**

In the present study, unlike the other studies reporting that the use of adhesive system had no significant effect on bond, the use of adhesive systems applied through etch-rinse technique was observed to have a positive effect on the bond strength of the fissure sealants, similar to the study by ***Barutçugil et al. (2013)***.

Ülker et al. (2009), ***Youssef et al. (2006)*** and***Symons et al. (1996)*** emphasized that the application of the bonding agent under the fissure sealant was recommended to increase the bond strength and reduce microleakage (222,385,386).

Karaman et al. (2013) and ***Burbridge et al. (2007)*** evaluated the retention of fissure sealants applied using different adhesive systems and reported that the retention rate of the fissure sealants applied through the etch-rinse adhesive system was found to be significantly higher than the self-etch group at the end of one year (387,388).

Similarly, in studies by Venker et al. (2004), ***Sakkas et al. (2013)***, and ***Feigal et al. (2000)***, adhesive agents have been reported to provide long-term stress-relieving effect for fissure sealants on flexible tooth surfaces by increasing the bond strength between the hard tooth structure and resin restorations thanks to their elasticity properties and to increase the marginal integrity and retention of the restoration. They reported that the bond strength of fissure sealants applied through etch-rinse technique was higher than the bond strength of fissure sealants applied after only acid etching process and the bond strength of fissure sealants applied with self-etch adhesive systems was the lowest (127,366,389).

In the present study, the shear bond strength of the group to which Adper Single Bond was applied through etch-rinse technique was found to be significantly higher than the other groups. Similar to the studies by ***Venker et al. (2004)***, ***Sakkas et al. (2013)***, and ***Feigal et al. (2000)***, the higher bond strength values of the bonding agent was attributed to the fact that the acid etching application prior to the adhesive system provided both micromechanical and chemical bonding.

It has been reported that when using adhesive systems with fissure sealants, it is necessary to ensure sufficient dilution of the agent with air after the adhesive is applied to the tooth surface considering that the thickness of the adhesive agent layer may affect the bonding quality (390,391). Low thickness of the adhesive agent layer would reduce the dimensional changes during polymerization (392).

In a study by ***Torres et al. (2005)*** investigating the effect of combined use of fissure sealant and adhesive agent or their individual polymerization on the bond strength, it has been reported that the type of polymerization does not have any significant effect on contaminated surfaces, however, the individual polymerization of the materials significantly has been observed to increase the bond strength on the non-contaminated enamel surfaces (131).

Based on this information, in the present study, the bonding agent was polymerized after being diluted with air in accordance with the manufacturer’s instructions and fissure sealant application was, then, carried out. Considering the findings obtained by ***Torres et al. (2005)***, the bonding agent and fissure sealant were polymerized individually. The results obtained from this study suggested that such application supported the bonding.

Since the present study was carried out under in vitro conditions, there was no problem regarding the saliva and moisture isolation. Dental operating microscope remained in the background for this in vitro study due to its difficulty of use during the treatment. However, it was seen that using dental operating microscope in clinical conditions, particularly in children at the age of 0-12, could be difficult. It can be said that after getting familiar with its use, the dental operating microscope can be used for the treatments under general anesthesia due to the advantages of providing ergonomic working conditions and increasing the field of view. It was observed that the use of bonding agent might be effective after the acid etching step for the purpose of minimizing the microleakage of fissure sealants. Although there was no statistically significant difference in microleakage test results, the use of bonding agent was observed to increase binding. The small number of human third molars meeting the study criteria was seen to affect the microleakage test results. However, it was thought that increasing the number of steps in the fissure sealant application might make the performance of the treatment more difficult under clinical conditions.

The aim of this study is to improve the application technique of fissure sealant material which is a common treatment method used in pediatric dentistry. The effect of fissure sealant application with the guidance of dental operating microscope, one of the newly introduced applications in the dentistry, on the microleakage of fissure sealant was evaluated. Furthermore, the effect of the application of fissure sealant in combination with the bonding agent in addition to the conventional acid etching method on microleakage and bond strength was investigated. It was concluded that the application of bonding agent in combination with the acid etching prior to the fissure sealant application increased the bond strength of the fissure sealants. Dental operating microscope was observed to provide ergonomic working conditions during fissure sealant application and increase the field of view.

6. CONCLUSION

1) The use of bonding agent after acid etching prior to fissure sealant application was found to have no statistically significant effect on the microleakage of fissure sealant.

2) There was no statistically significant difference between the microleakage of fissure sealant applied with the use of dental operating microscope magnification and microleakage of the fissure sealant applied without the help of magnification.

3) A statistically significant difference was found between the samples to which fissure sealant was applied after acid etching process and samples to which fissure sealant was applied following the acid etching and bonding agent application in terms of bond strength. It was observed that the use of adhesive system after acid etching had a positive effect on the bond strength of fissure sealants.

The different conditions that may be encountered in the clinical practice bring together the necessity of using the materials in combination with different methods. As well as supporting the ergonomic posture of the physician while working, dental operating microscope also increases the angle of view and the success of dental treatments. In conclusion, the results obtained from this study shows that the use of a dental operating microscope and adhesive systems cannot completely prevent the microleakage of the fissure sealant and the application of the adhesive system after acid etching prior to the fissure sealant application increases the bond strength. In dentistry, the benefits of magnification and the use of a dental operating microscope are too important to remain in the background. Therefore, such studies need to be carried out with a larger number of samples.

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8. APPENDIX

APPEND 1. ETHICS COMMITTEE DECISION

T.C.
İSTANBUL ÜNİVERSİTESİ
DİŞ HEKİMLİĞİ FAKÜLTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU

Sayı :387 **27.02.2019**

Konu :Doç.Dr.Didem Özdemir Özenen

Sayın Doç.Dr.Didem Özdemir Özenen
Çocuk Diş Hekimliği Anabilim Dalı

İlgi: Yeditepe Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği Anabilim Dalının 20/02/2019 gün ve 12940 sayılı yazısı.

Sorumlu araştırıcılığını üstlendiğiniz 2019/24 dosya nolu "Dental Operasyon Mikroskobu Ve Adeziv Sistemlerin Kullanılmasının Fissür Örtücülerde Makaslama Bağlanma Dayanımı Ve Mikrosızıntı Üzerine Etkilerinin Değerlendirilmesi" başlıklı çalışma kurulumuzun 27/02/2019 tarih ve 81 sayılı toplantısında görüşülerek etik yönden uygun bulunmuş olup, tutanaklar ekte sunulmuştur.

Bilgilerinizi rica ederim.

Prof.Dr. Faruk Haznedaroğlu
İ.Ü. Diş Hekimliği Fakültesi Klinik Araştırmalar Etik Kurul Başkanı

Eki: İ.Ü. Diş Hekimliği Fakültesi Klinik Araştırmaları Etik Kurulu Karar Formu

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Educational Background

Degree	**Branch**	**Graduated From**	**Graduation Year**
Bachelor's Degree/Master's Degree	Dentistry	Yeditepe University Faculty of Dentistry	2015
High School	-	Nuh Mehmet Küçükçalık Anatolian High School	2009

Foreign Languages	**Foreign Language Examination Grade**
English	75.00 (Higher Education Institutions Foreign Language [YÖKDİL] Examination)

Computer Skills

Software	**Ability to use**
Microsoft Office	Good

Papers presented at international scientific meetings and published in the proceedings

The influence of dental operating microscope on the microleakage of composite resin and glass carbomer restorations, an in-vitro study by Kuvvetli S, Salcıoğlu D, Kazandağ M, Sandallı N. *CED-IADR* 2015, Antalya. Poster presentation.
Ectrodactyly-ectodermal dysplasia-clefting sendrom ön tanısı koyulan hastada ağız kuruluğu sebepli oluşan çürüklerin tedavisi ve takibi: Bir olgu raporu (Treatment and follow-up of caries caused by dry mouth in the patient diagnosed with ectrodactyly-ectodermal dysplasia clefting syndrome: a case report) by Salcıoğlu D, Kuvvetli S. *IAPD 2016*, Kuşadası, İzmir. Poster presentation.

Manuscript published in international refereed journals

Büyük Azı Keser Hipomineralizasyonu'na Güncel Bakış: Teşhis ve Tedavi Yaklaşımları (Current View on the Diagnosis and Treatment of Molar Incisor Hypomineralization) by Güner Ş, Salcıoğlu D. *Clin Exp Health Sci* 2016; 6 (1): 28-34.