

Evaluation of gap formation for different adhesive agents in primary teeth with optical coherence tomography

Purpose

This study aimed to evaluate gap formation between the tooth surface and restorative material in terms of microleakage by using optical coherence tomography (OCT) for self-etch and selective-etch applications of two different universal and one self-etch adhesives.

Materials and Methods

Sixty non-carious, primary molar teeth were divided into six groups; self-etch and selective-etch application ways of two different universal and one self-etch adhesive systems (n:10). After Class-V cavities were prepared, every tooth was distributed randomly in groups to apply adhesion procedure and then, all cavities were restored with polyacid-modified composite resin. Microleakage was evaluated by measuring the gap between the tooth surface and restoration by a blind researcher with Image J Software from OCT images. During statistical analysis, the significance level was accepted as $p < 0.05$.

Results

According to the statistical analysis of the measurements obtained by Image J Software, selective-etch groups showed less gap formation than self-etch groups for each tested adhesive ($p < 0.05$), and self-etch adhesive without etching showed significantly highest gap formation among all groups ($p < 0.05$).

Conclusion

Universal adhesives with a selective-etching step might be preferred over self-etch adhesives for long-lasting polyacid-modified composite resin restorations in primary teeth. However, obtained results should be considered with another prospective clinical study for long-term prognosis.

Keywords: Adhesion, Gap formation, microleakage, optical coherence tomography, polyacid-modified composite resin

Introduction

In today's dentistry, Black principles, which means 'expand to protect', have been replaced by minimally invasive treatment principles. In minimally invasive treatment procedure, only caries is removed and remained dental tissue is restored with using adhesives (1). Adhesive systems used today are fourth, sixth and seventh generation systems (2-4). Among these, fourth generation adhesive systems are 'etch & rinse' systems that remove the smear layer with 34-37% orthophosphoric acid applied to both enamel and dentin surfaces. Because of the acid etching step for both enamel and dentin surfaces, fourth generation adhesive systems are also called 'total-etch' systems (5). Sixth generation adhesive systems are self-etch adhesive systems that apply in two-steps and do not require etching step (2-4). These adhesives can be applied in one or two steps depending on whether primer and adhesive are in same or separate bottle (6). Seventh generation adhesive systems are called 'universal' or 'multimode' adhesives, which are

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produced as a single bottle and can be used as both self-etch and total-etch (4). While these systems incorporate features of total-etch and self-etch systems together, their bond strength can be increased due to the 'selective etching' step (7,8). Selective etching means applying shorter time acid-etch to only enamel surface such as 15 seconds of 34-37% orthophosphoric acid to increase enamel bond strength (7,8).

The main bonding mechanism to enamel and dentin in adhesive systems is to provide micromechanical locking and prevent microleakage problem (2,3). Various methods can be used to evaluate the presence of the microleakage between restoration and tooth surface (9). These methods are dye penetration tests (liquid penetrate inspection), radioisotopes, chemical agents, bacterial methods, air pressure method, neutron activation analysis, electrochemical studies, microscopic examination methods and optical coherence tomography (9-12). Optical coherence tomography (OCT) is a non-intraoperative imaging method that was introduced in the early 1990s and recent years, preferred in dentistry. OCT provides a micron-level cross-sectional view of biological tissues so that it has a wide range of uses (10,11). The imaging technique of OCT is a measurement based on the low coherence-centromere logic and correlation of the reflected light from the sample with a reference light (10-12).

This in-vitro research study aimed to evaluate gap formation between the tooth surface and restorative material in terms of microleakage by using OCT to compare self-etch and selective-etch application ways of two different universal and one self-etch adhesive agents in polyacid-modified resin composite restorations applied in primary molar teeth. The tested null hypotheses were the selective-etch application would show the similar gap formation as the self-etch application regarding that the type of adhesive would not be a contributory factor in gap formation.

Materials and Methods

Ethical approval

Extracted non-carious primary molar teeth due to the spontaneous eruption of permanent teeth from healthy patients with no systemic disorders were collected following ethical protocol no: D-KA 18/13 approved by Başkent University Institutional Review Board and included in the study within 6 months after receiving their informed consents.

Study design

Sample size of each group was calculated for binary primary outcome measures as the evaluation of microleakage for primary teeth. So, 10 samples per group with a total number of 60 samples were required to detect a significant difference for a two-sided type I error at 0.05, 0.40 effect size and 90% power. However, after obtaining OCT images, according to the post-hoc analysis, it was decided to evaluate a total of 210 images and 30 images per each group with Image J Software to detect a significant difference at 0.05 error level, 0.40 effect size and 95% power. Besides the power analysis, the study was conducted as double-blind so, the researcher who had done OCT imaging and Image J Software was unaware of group distribution.

Extracted non-carious primary molar teeth due to the spontaneous eruption of permanent teeth from healthy patients with no systemic disorders were collected following ethical protocol no: D-KA 18/13 approved by Başkent University Institutional Review Board and included in the study within 6 months after receiving their informed consents. Also, before preparation, primary molar teeth with any hypomineralized areas on enamel surfaces or restorations on any surfaces were excluded.

Sample preparation

Extracted 60 primary molar teeth were disinfected in 0.5% chloramine and stored in sterile distilled water until all samples were embedded in clear acrylic resin blocks. After the auto polymerization, standard Class-V cavities were prepared on the buccal surfaces of the primary molar teeth. Class-V cavity preparations that do not extend onto root surface were performed on the buccal surface of each tooth using a round diamond instrument, ISO size number 009 (Komet Dental Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) at high speed and air-water spray cooling. Class-V cavities were also standardized by using a periodontal probe as depth 2mm, width 4mm, height 2mm.

All prepared teeth were randomly distributed to the groups with different adhesive agents with self-etch and selective-etch application ways. Distribution of adhesive agents and application ways amongst the groups were given in Table 1. In the self-etch group, there is not any acid-etching step before adhesive application and adhesive agent applied according to the manufacturer's instructions. However, in selective-etch groups, 37% orthophosphoric acid was applied to the only enamel surface for 15 seconds, then washed out for 10 seconds and dried with

Table 1. Groups distribution for the adhesive materials used in this study.

Material	Application Procedure
Scotchbond™ Universal Adhesive, 3M, USA	Group 1: Self-etch application (n=10) Universal adhesive application without acid-etching
Scotchbond™ Universal Adhesive, 3M, USA	Group 2: Selective acid-etch application (n=10) 15 seconds acid-etching + Universal adhesive application
All-Bond Universal™, Bisco, USA	Group 3: Self-etch application (n=10) Universal adhesive application without acid-etching
All-Bond Universal™, Bisco, USA	Group 4: Selective acid-etch application (n=10) 15 seconds acid-etching + Universal adhesive application
Prime&Bond NT, Dentsplay, USA	Group 5: Self-etch application (n=10) Adhesive application without acid-etching
Prime&Bond NT, Dentsplay, USA	Group 6: Selective acid-etch application (n=10) 15 seconds acid-etching + Adhesive application

air for 10 seconds. After etching or without etching, bonding agents were applied to all surfaces of the cavities for 10 seconds by a separate microbrush for each tooth then, dried with 10 seconds and polymerized 20 seconds with LED (Elipar S10, 3M ESPE, Seefeld, Germany) according to the manufacturers' recommendations. All light-curing procedures were performed with the same LED-curing unit operating in a continuous mode while emitting a light-intensity of 1200 mW/cm² with a polymerization distance of 1mm standardized by a curing disc. The output of the LED-curing unit was verified after every three measurements by using a radiometer (Bluephase Meter II; Ivoclar Vivadent, Amherst, New York).

After the adhesive application step, polyacid-modified resin composite was condensed to the cavities and restorations were polymerized for 40 seconds with the same LED device according to the manufacturers' recommendations. After polymerization, all restorations were polished with a pear-shaped finishing bur to finish irregular areas at restoration borders and give final contour to the restoration. Then, abrasive disks were used for final polishing. After restorative procedure, thermal cycle procedure (Thermocycler THE 1100/1200, SD Mechatronik, Westerham, Germany) was carried out for all teeth samples before the microleakage evaluation. Thermal cycles were applied to the samples at 5-55±20C, with a waiting time of 15 seconds and a transfer time of 10 seconds.

After thermal cycle, all teeth samples were evaluated with Optical Coherence Tomography to take images from all borders of the restorations by a second researcher to be blind of group distribution. A set-up was designed for study samples to stand still in front of the OCT device to take appropriate images. In Figure 1 and Figure 2, adjustments made to capture appropriate images can be seen in pseudocolors (Figure 1A and Figure 2A) and grey scale (Figure 1B and Figure 2B), respectively. All obtained images were evaluated with Image J Software Program to take quantitative data to compare different adhesive groups and different application ways of these groups by the same second blind researcher who was unaware of group distribution of all images.

Evaluation with optical coherence tomography

During the OCT (Zeiss Cirrus HD-OCT, Carl Zeiss Meditec, Jena Germany) procedure, restored tooth embedded in acrylic resin blocks were placed in front of the objective at the top of the device. Then, the sample was moved to left-right and up-down by the arm of the device and light beam was orthogonally scanned to the tooth surface and restoration interface in such a way that infrared beam traversed over the tooth surface, the air which was the gap between tooth surface and restorative material and restoration regions, respectively. The scanning probe was positioned at distance of 3cm from the restoration.

The entire tooth-restoration borders were scanned, which means from one approximal surface to the other by taking cavosurface margins guidance. So, after the infrared beam was scanned over tooth-air-restoration surfaces, each scan provided images at per 200µm, which enabled us to determine marginal gaps between tooth surface and restorative material. According to the working principle of OCT, gap ar-

eas reflect in high viscosity colors than tooth and restorative material. These adjustments in the OCT device which had done and the features of the OCT images in pseudocolors and grey scale due to the different viscosities of the tooth and restorative material can be seen in Figure 1 and Figure 2.

Measurement with Image J Software

After images were obtained with OCT, approximately 90 images were taken from every tooth sample and 30 images were randomly chosen from these to take quantitative results with Image J Software (Imaging Processing and Analysis in Java, National Institute of Health, Bethesda, MD). Image J Software was used to measure the gap between tooth surface and restorative material by drawing 'paintbrush tool' with the guidance of different color reflections of the tooth, restorative material, and air between them. All OCT images were taken in pseudocolors to differentiate the borders of the restoration (Figure 1A and Figure 2A), tooth and gap area whereas during the Image J measurements, colors of all images were converted to grey scale to draw the circumference of the gap areas (Figure 1B and Figure 2B). Also, each measurement was repeated three times to prevent or reduce the number of faulty measurements for each image. These quantitative values which were pixel values were saved as excel tables and compared between all the groups.

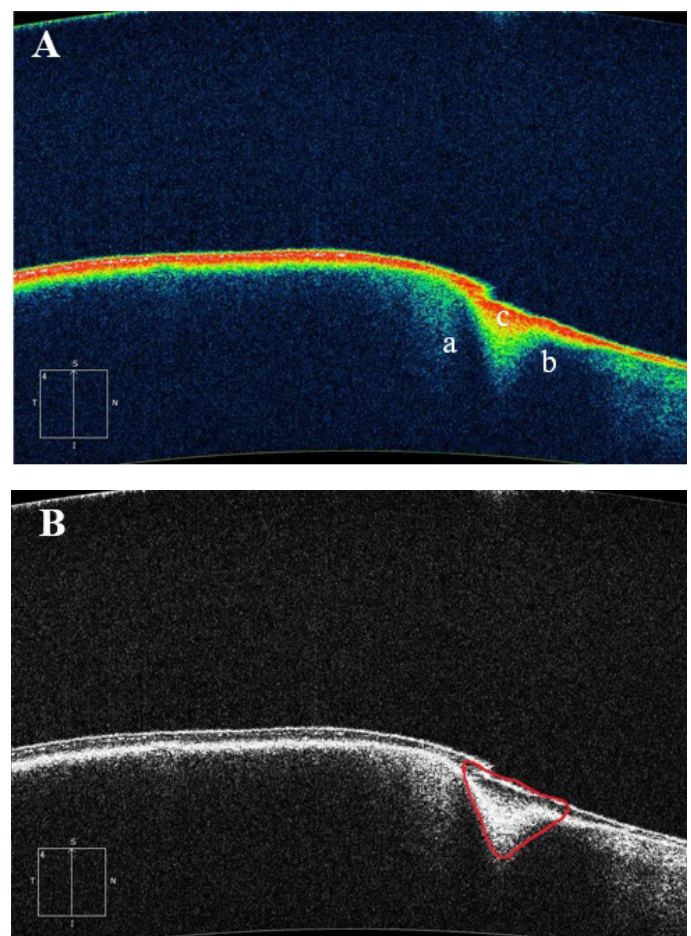


Figure 1. OCT image of one of the teeth samples in self-etch group (Group 5) (A) showing regions in pseudocolors; a: Restoration; b: Tooth; c: Gap area; (B) drawing of the gap area with Image J of the same OCT image in grey scale.

Statistical analysis

SPSS V. 20.0 (Statistical Package for Social Sciences, IBM SPSS, Armonk, NY, USA) software for Windows was used for statistical analysis. Kruskal Wallis test and Mann-Whitney U tests were used, respectively, for multiple and pairwise comparisons. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Results

According to the obtained values from Image J Software, analyzed results showed statistically significant differences between groups in terms of gap measurements between the tooth surface and restorative materials ($p < 0.05$). Table 2 shows the post-hoc analysis of Image J results of the OCT images of

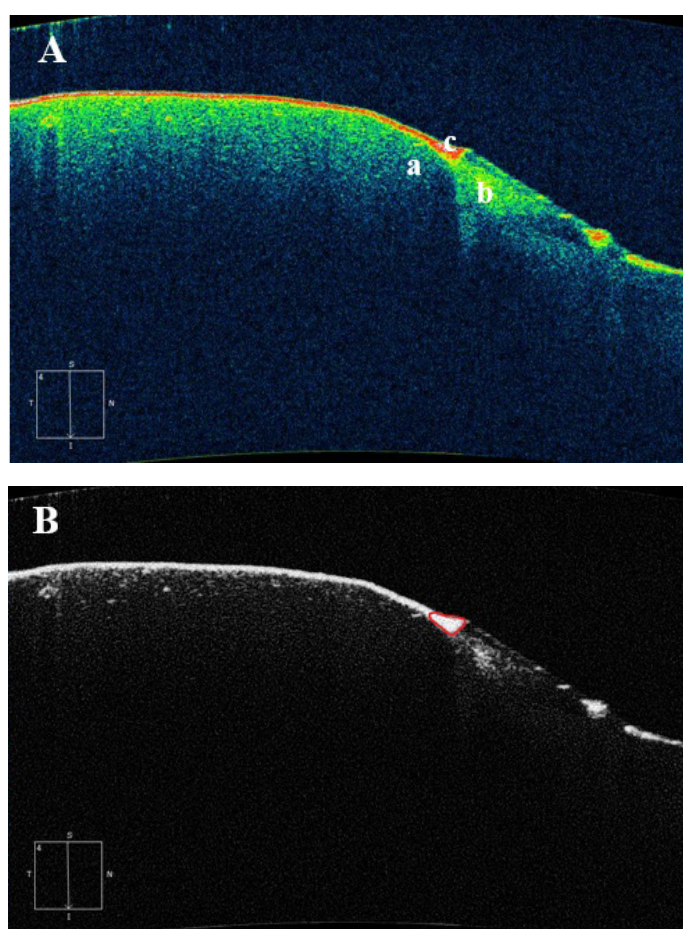


Figure 2. OCT image of one of the teeth samples in Scotchbond Universal Adhesive with selective-etching group (Group 2) (A) showing regions in pseudocolors; a: Restoration; b: Tooth; c: Gap area; (B) drawing of the gap area with Image J of the same OCT image in grey scale.

the enamel/restoration interface, providing means and standard deviation values of the gap formation and statistical differences between groups. According to binary comparisons, Group 5 showed significantly higher gap measurements than all other groups ($p < 0.05$). Group 6 had shown significantly higher gap measurements than Group 1, 2, 3 and 4 ($p < 0.05$). Also, Group 1 showed higher gap measurements than Group 2, 3 and 4 ($p < 0.05$). However, there was no statistically significant difference between Group 2, 3 and 4 ($p \geq 0.05$).

Discussion

Adhesive systems have important technical requirements, particularly in pediatric restorative dentistry (1,13). The most important technical problems during restorative procedures are cavity preparation, saliva isolation, and material adaptation (13). Therefore, developments in adhesive dentistry have primarily aimed to eliminate these issues. According to studies, fourth-generation systems provide higher bond strength to both enamel and dentin than other systems (2-5), and long-term follow-up studies have demonstrated successful results in terms of retention, marginal adaptation, and secondary caries development (2,4,5). As a result, fourth-generation adhesives are currently considered the gold standard when compared to other systems (2-5).

However, longer etching times, especially in primary teeth, can cause more technical problems and decrease the clinical success of restorations. The most significant clinical failure is microleakage between the tooth surface and restorative material due to insufficient acid-etch or isolation problems after the etching step (7,13). Therefore, in recent years, self-etch adhesives have been preferred in pediatric restorative dentistry to decrease chair time and increase the clinical success of restorations by eliminating these technical problems during restorative procedures (14,15).

Studies have shown that there are advantages and disadvantages of self-etch adhesives compared to total-etch adhesives (2-4,7). Self-etch adhesives require less technical precision due to fewer application steps than other generations and can be applied in a shorter chair time (14). While their clinical sensitivity is lower, their bond strength is also lower than total-etch agents (14,16). Additionally, enamel bond strength is not as sufficient as dentin bond strength in self-etch agents, since enamel is more resistant to acids than dentin (2,4,6). To address this issue, recent advancements in adhesive systems have recommended applying a selective-etching step before these adhesives to improve adhesion, increase enamel bonding, decrease marginal microleakage, and increase clinical success. For this purpose, universal adhesives may be an alternative that can be used with or without an acid-etching step in a one-step application.

Table 2. Evaluation of gap measurements formed between enamel and restoration interface amongst all groups.

Measurements	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	P-value
Mean (SD)	0.63(0.12) *G5, G6	0.50(0.10) *G1, G5, G6	0.48(0.08) *G1, G5, G6	0.41(0.10) *G1, G5, G6	1.67(0.46)	1.10(0.33) *G5	0.001
Min-Max Median	0.44-0.97 0.62	0.32-0.76 0.49	0.36-0.68 0.46	0.26-0.56 0.41	0.85-2.44 1.78	0.58-1.78 1.04	

Kruskall Wallis Test; Significance level at $p < 0.05$; sd: Standard deviation; Min: Minimum value; Max: Maximum value; * refers to statistically significant difference between the stated groups in each column according to post-hoc analysis with pairwise comparisons.

However, to our knowledge, there is no study comparing both universal and self-etch adhesives, with or without a selective-etch step, in primary teeth. Therefore, we aimed to compare these adhesive materials and their different application methods before condensing polyacid-modified resin composites in terms of marginal gap formation as another way to determine the risk of microleakage formation. Class-V cavities, also known as cervical cavities, were preferred due to the difficulties in isolation, caries access, and proximity to the gingival margin. In addition, the design of the cavity has a high configuration factor (C-factor), which leads to a high risk for microleakage (17,18). Currently, adhesive systems can provide a favorable marginal seal and decrease marginal microleakage, especially at the cervical margins of the cavity (16,19). In an in-vitro study evaluating microleakage with dye penetration of total-etch and universal adhesives in Class-V restorations, Cirligeriu *et al.* (20) recommended that selective-etch application should be used to improve marginal sealing, especially in cervical restorations and cavities with high C-factors. Therefore, in this study, Class-V cavities were prepared on the buccal surfaces of all teeth and standardized using a periodontal probe to a depth of 2mm, width of 4mm, and height of 2mm.

Polyacid-modified resin composites, which share some similarities with both composite resins and glass-ionomers, have been used for primary teeth restorations for a long time (1,13). Due to their lower polymerization shrinkage and easy condensation compared to composite resins, and similar physical properties with primary teeth, such as erosive tooth wear, pediatric dentists prefer to restore with polyacid-modified resin composites (13). While the material can be used with both self-etch and total-etch adhesives, the literature suggests that application with total-etch adhesives leads to higher clinical success in terms of lower marginal microleakage due to the acid-etching step of the enamel surface (14,16,17).

Long-term microleakage in the oral environment can result in consequences such as discoloration of the tooth or restoration surface, sensitivity, and secondary caries development (18-20). Therefore, in this in-vitro study, all teeth samples were put through thermal cycling to simulate the oral environment and ensure marginal microleakage development before OCT imaging. However, according to Marchesi *et al.* (3), microleakage results for self-etch and total-etch systems did not differ at immediate evaluation or after one year of storage in artificial saliva.

OCT is a method used for diagnosing carious lesions and periodontal diseases, and has also been used to evaluate microleakage localization, continuity, and gap width between composite resin restoration and tooth surface using different adhesives (9). OCT provides quantitative values and does not require additional processes, making it a preferred method for measuring the gap between the tooth surface and restorative material (10-12). Previous studies have shown that OCT evaluation can yield significantly logical results in adhesion studies (11,21-25), and can safely be used to evaluate microleakage in different adhesive techniques (13,26-29). However, OCT has a measurement depth limit of around 2-3mm in many tissues, which might affect results in adhesion studies, although the image resolution is 10-100 times better than ultrasound imaging methods (26-29).

In this study, all cavities were prepared at a depth of 2mm, width of 4mm, and height of 2mm, and the restorative material was polymerized for 40 seconds at one time. However, despite the cavity depth being 2mm, the base of the cavities could not be seen in the images taken with OCT due to the properties of dental tissues and their light transmittance or reflection, which is different from that of soft tissues like the eye.

The study conducted by Haak *et al.* (26) evaluated the selective-etch and self-etch application methods of Scotchbond Universal Adhesive with composite resin restorations in permanent teeth using OCT assessment. The authors reported that the self-etch application method of Scotchbond Universal Adhesive showed significantly higher gap measurements, but there was no significant difference between total-etch and selective-etch applications. Furthermore, the authors concluded that OCT is a sensitive method to evaluate microleakage which cannot be evaluated clinically, and requires lesser time to obtain data and provide bidirectional perspective compared with other evaluation methods (9-12). The authors also noted that aging due to storage time and immediate evaluations did not have any statistical difference in microleakage development.

Another study by Rosa *et al.* (5) reported different bond strength results for total-etch and self-etch application methods of different universal adhesives in permanent teeth. While there was no statistically significant difference in the Scotchbond Universal adhesive groups, there was a statistical difference in the All-Bond Universal adhesive groups. In the present study, the self-etch group without selective-etch application (Group 5) showed significantly higher gap measurement results than all other groups, which suggests a higher microleakage level. This result is supported by the findings of the studies reported by Rosa *et al.* (5) and Haak *et al.* (26). It also supports the general acceptance that selective-etch application before adhesive application during restorative procedures in primary teeth improves enamel bonding and decreases microleakage in terms of marginal gap formation, similar to what is observed in permanent teeth.

In the present study, binary comparisons revealed a significant difference between Group 5 and 6, both of which used the same self-etch adhesive agent, but with and without acid-etch. The lower gap measurements in Group 6 indicated that the acid-etching step effectively decreased gap formation. Furthermore, Group 1, which used Scotchbond Universal Adhesive without selective acid-etch, had statistically higher gap measurements than other universal adhesive groups (Groups 2, 3, and 4). This result supports the findings of Rosa *et al.* (5) and the recommendations of manufacturers that universal adhesive agents can be used with or without selective-etch. However, dentists should opt for adding the selective-etch step to their restorative treatment procedures instead of self-etch to achieve long-term successful prognosis for restorations.

Therefore, selective-etch application could be a better way to increase enamel bonding and reduce marginal microleakage with respect to the adhesive agent. As dentin bonding is easier to handle than enamel bonding, selective etch should be preferred to increase enamel bonding and reduce marginal microleakage, according to the authors' and the present study's results. The first hypothesis was rejected

due to the lower gap formation in the selective acid-etch groups than in the groups without acid-etching, which was verified for both self-etch adhesives and universal adhesives. The second hypothesis was also rejected because the self-etch group, with or without acid-etching, showed statistically significant higher gap measurements than the universal groups. However, it is essential to consider prospective clinical studies to eliminate the technical features of in-vitro studies and evaluate long-term prognosis.

Conclusion

Preferably, selective-etch and universal adhesive applications should be used to restore primary teeth with poly-acid-modified resin composites, as opposed to self-etch adhesives, especially without selective-etch applications. Additionally, OCT may be preferred as a sensitive and minimally invasive evaluation method in adhesive dentistry studies.

Türkçe özet: Süt dişlerinde farklı adeziv ajanlar için mikrosızıntı oluşumunun optik koherens tomografi ile değerlendirilmesi. Amaç: Bu çalışmada, iki farklı universal ve bir self-etch adezivin self-etch ve selektif-etch uygulamaları için optik koherens tomografi (OCT) kullanılarak diş yüzeyi ile restoratif materyal arasındaki boşluk oluşumunun mikrosızıntı açısından değerlendirilmesi amaçlanmıştır. Gereç ve Yöntem: Altmış çürüksüz süt azı dişi, iki farklı universal ve bir self-etch adeziv sistemin self-etch ve selektif-etch uygulama yolları (n:10) şeklinde altı gruba ayrıldı. Sınıf-V kaviteler hazırlandıktan sonra her diş rastgele gruplara dağıtılarak adezyon işlemi uygulandı ve ardından tüm kaviteler poliasit modifiye kompozit rezin ile restore edildi. Mikrosızıntı, kör bir araştırmacı tarafından Image J Yazılımı ile OCT görüntülerinden diş yüzeyi ile restorasyon arasındaki boşluk ölçülerek değerlendirildi. İstatistiksel analiz sırasında anlamlılık düzeyi $p < 0,05$ olarak kabul edildi. Bulgular: Image J Software tarafından elde edilen ölçümlerin istatistiksel analizine göre, test edilen her adeziv için selektif-etch grupları self-etch gruplarına göre daha az boşluk oluşumu gösterdi ($p < 0,05$) ve asitleme yapılmadan uygulanan self-etch adeziv, tüm gruplar arasında önemli ölçüde en yüksek boşluk oluşumunu gösterdi ($p < 0,05$). Sonuç: Süt dişlerinde uzun ömürlü poliasit modifiye kompozit rezin restorasyonları için self-etch adezivlere göre selektif asitlemenin ardından uygulanan universal adezivler tercih edilebilir. Ancak, elde edilen sonuçlar uzun vadeli prognoz için prospektif klinik çalışmalar ile değerlendirilmelidir. Anahtar kelimeler: Adezyon, Boşluk Oluşumu, Mikrosızıntı, Optik Koherens Tomografi, Poliasit Modifiye Kompozit Resin

Ethics Committee Approval: The present study protocol has been reviewed and approved by the Başkent University Institutional Review Board (project no:D-KA 18/13).

Informed Consent: Participants provided informed consent.

Peer-review: Externally peer-reviewed.

Author contributions: DSU, LA, RET participated in designing the study. DSU, LA, RET participated in generating the data for the study. DSU, LA, RET participated in gathering the data for the study. DSU, RET participated in the analysis of the data. DSU wrote the majority of the original draft of the paper. DSU, LA, RET participated in writing the paper. DSU, LA, RET have had access to all of the raw data of the study. DSU has reviewed the pertinent raw data on which the results and conclusions of this study are based. DSU, LA, RET have approved the final version of this paper. DSU guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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