



An in Vitro Study on The Effects of Er,Cr: YSGG Laser on The Enamel and Dentin Morphology and Microleakage

Zeynep Yenen^{1,a,*}, Jale Gorucu^{2,b}

¹ University of Kyrenia, Faculty of Dentistry, Department of Restorative Dentistry, Karakum, Girne, TRNC. Mersin 10 Türkiye.

² Lokman Hekim University Faculty of Dentistry, Department of Restorative Dentistry, Ankara, Türkiye.

*Corresponding author

Research Article

History

Received: 06/04/2024

Accepted: 30/01/2025

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ABSTRACT

Objectives: The evaluation of the enamel and dentin cavity surface morphologies by Scanning Electron Microscopy (SEM) in Class II occlusogingival slot cavities which were prepared and etched by Er,Cr: YSGG (Erbium, Chromium: Yttrium, Scandium, Gallium, Garnet) laser and assessing their effects on the microleakage at composite resin restorations, and also comparing them with those of traditional methods.

Material and Methods: Fifty-five non-carious, sound extracted human molar teeth were divided into five groups: bur preparation + acid etching (Group I), bur preparation + laser etching (Group II), laser preparation + laser etching (Group III), laser preparation (Group IV), laser preparation + acid etching (Group V). The resin-tooth interface was evaluated based on microleakage. SEM examinations of laser irradiated samples (Groups II, III, IV, V) revealed a lack of smear layer, irregular and crater-like surface in enamel. Also, these examinations revealed opened dentinal tubules orifice, step-like flattered surface, and lack of smear layer in dentin.

Results: Statistically significant differences were found among the occlusal ($p=0.003$) and gingival regions microleakage ($p=0.001$) in groups. In Group V at the gingival, microleakage was significantly greater than the other groups ($p<0.05$). In Group II, the microleakage at the occlusal was found significantly greater than Groups I, IV, and V. When the microleakage at occlusal and gingival were compared among each group, only group V showed statistically significant difference ($p<0.05$).

Conclusions: According to the results of this study, the use of Er,Cr: YSGG lasers in cavity preparation and etching processes generally does not negatively affect microleakage, but when acid is applied to laser-prepared teeth in the gingival region where the cavity borders are located in the dentin, leakage can increase.

Keywords: Laser, cavity preparation, etching, marginal leakage, SE.

zyenen@gmail.com

<https://orcid.org/0000-0002-8280-3549>

jale.gorucu@lokmanhekim.edu.tr

<https://orcid.org/0000-0001-7138-5911>

How to Cite: Yenen Z, Gorucu J. (2025) An in Vitro Study on The Effects of Er,Cr: YSGG Laser on The Enamel and Dentin Morphology and Microleakage, Cumhuriyet Dental Journal, 28(1): 36-44.

Introduction

Albeit composite restorations are aesthetic, cavities that occur around them over time are typically seen in the clinic and come to the forefront as a considerable issue. One of the primary causes of this event is marginal microleakage.

Studies have centered on alternative applications to cavity preparation or etching of enamel and dentin to achieve the closest-ideal sealing.

The Food & Drug Administration (FDA) approved the use of erbium lasers in dental hard tissues in 1997.¹⁻³

With the use of lasers in dentistry, attention has centered on the morphological changes that lasers might cause in enamel and dentin, and what kind of effects they have in enhancing the quality of restorations has been a matter of curiosity.

It has been demonstrated that the smear layer does not form during laser etching, does not require washing and isolation as in acid etching, forms a more acid and decay-resistant surface in the enamel and dentin it is

applied and is effective in preventing caries recurrence thanks to its bactericidal effect.⁴

Lasers function by having an impact on the enamel water and Hydroxyapatite (HA). Each laser has a specific absorption coefficient in water and HA. Thanks to the high absorption feature of the erbium laser, the water molecule heats up, the water reaches the boiling point and expands, the surrounding tissue is divided into smaller pieces by micro-explosions and rejected from the cavity. No smear layer is formed during cavity preparation with laser. The laser has a bactericidal effect in the region where it is used.⁵⁻⁷

Lasers can also be used in the etching of enamel and dentin. Various studies in the literature use lasers such as Excimer, CO₂, Nd: YAG, Er, Cr: YSGG, Erbium Yttrium Aluminum Garnet (Er: YAG) in the procedures of etching.⁷⁻¹⁰

Based on the images obtained from the SEM studies, it has been revealed that peritubular dentine remained plainly visible on the dentin surfaces where the laser applied, whereas intertubular dentine was removed by the laser in a greater amount as it has a higher water content. In peritubular dentin, which is less impacted by

laser application, the mouths of the tubules do not expand, and a crater-like protruding tubule opening occurs.¹¹⁻¹⁵

The objective of this in vitro study was to examine the morphological changes caused by the use of Er, Cr: YSGG (Erbium, Chromium: Yttrium, Scandium, Gallium, Garnet) laser in the preparation and etching procedures of Class II occlusogingival cavities on the enamel and dentin surfaces at the boundaries of the cavity via Scanning Electron Microscopy (SEM) images and to assess their effects on marginal microleakage in composite resin restorations by comparing them with traditional methods.

Materials and Methods

In the study, 55 healthy human molar teeth without caries or cracks were used. The samples were stored in 10% formol solution at + 4 ° C until the cavities were opened and then they were cleaned with a posterior scaler. A total of 100 Class II slot cavities were prepared on the mesial and distal surfaces of the 50 teeth, which were divided randomly into five groups of ten, using laser or burs.

Unrestored five teeth were reserved to be used in the SEM study and the mentioned procedures for the experimental groups were applied on the mesial surfaces of these samples. However, a cavity preparation with a bur was also made on its distal surface to be able to view the SEM photograph of the sample in Group I before acid application.

Class II occlusogingival slot cavities were opened with a buccolingual distance of 3 mm, an occlusogingival width of about 5 mm, and a step width of 1.5 mm, provided that the gingival wall is 1 mm below the margin of enamel cement. No beveling was performed on the margins of the cavity surface.

All cavity preparations were performed by the same person and X 2.3 magnifying glasses (Carl Zeiss Jena, GmbH, Germany) were used to see the working area better.

Diamond fissure bur (Diatech 836-0186-ML, Swiss Dental Instruments, SWISS) was used with air and water cooling in the preparation of cavities in groups I and II and was changed after every five cavity preparations. In accordance with the manufacturer's instructions, the cavities in groups III, IV and V were prepared via Er,Cr:YSGG laser in focus mode and using G tip with the parameters of 5 W (250 mJ) in enamel, 4 W (200 mJ) in dentine, 20 Hz, 85% air and 80% water, and placing the head of the instrument perpendicular to the enamel prisms 2 mm away from the tooth surface.

In line with the manufacturer's instructions, etching procedures of the cavities in Group II (Bur + Laser) and III (Laser + Laser) were performed via Er,Cr:YSGG laser at the parameters of 1 W (50 mJ), 20 Hz, 65% air and 55% water and using G tip. During the laser etching procedure, the head of the instrument was kept perpendicular to the tooth surface and 2 mm away from the tooth surface in

focus mode and moved over the surface to be etched for around 30 seconds.

No etching procedure was applied to the cavities in Group IV (Laser).

To assess the surface properties of the cavities that were left unrestored for use in the SEM study, samples were coated with 100 Angstrom (Å) gold-palladium, and they were examined in SEM (JSM, 6400, Tokyo, JAPAN), which is located at Metallurgy Department of Middle East Technical University (METU), at the magnifications of 2000X and 5000X.

Application of Restorations

Acid applications (Gluma Acid Gel) were performed for 20 seconds and washing for 20 seconds, starting from the enamel surfaces, including dentin. A single bottle of light-cured adhesive (Gluma Comfort Bond) was used as adhesive. It was polymerized with the Light-Source Device (Hilux Expert, Benlioglu Dental, Ankara, Turkey) for 20 seconds after the air was sprayed slightly and spread.

In order not to affect the polymerization depth, the same color was used in the study, hence A2 was preferred. During the application of composite resins, the oblique layering technique was used following the matrix (Auto-Matrix, DeTrey GmbH, GERMANY) was applied to the cavities. The light was applied to each layer for 40 seconds, and additional polymerization was performed for 20 seconds after the matrix was removed. The finishing and polishing procedures of all restorations were performed under the water using diamond finishing burs (Diatech 858-012- Swiss Dental Instruments, SWISS) as well as thin and super thin aluminum oxide coated discs in the form of tapering towards the tip, in line with the manufacturer's instructions, and the disc was renewed for every five applications.

All samples, restoration of which were completed, were stored in distilled water at 37 °C for 24 hours. The heat exchange procedure, which ranged between 5-55 °C, was applied 500 times to all samples. The samples were stored in each heat bath for 1 minute. The intervals between hot and cold dips were implemented as 20 seconds.

The root ends of the samples were occluded with composite resin to prevent dye passage, and the regions outside the 1 mm area around the restoration were covered with two layers of nail varnish. All groups were stored in the oven at 37 °C in 0.5% basic fuchsin solution for 24 hours.

The samples were cut in the General Directorate of Mineral Research and Exploration (MRE) on a tooth profile cutting machine (Buehler, Chicago, USA) underwater cooling with a slow rotating diamond disc. Each tooth was first divided into two buccolingual halves, and mesial and distal two halves were obtained. Subsequently, each half was divided into two longitudinally halves in the mesiodistal direction; hence, it could pass through the middle of the restorations, and 200 interfaces were obtained to assess each part separately.

Leakage Grading System

Photographs of the obtained sections were taken (Nikon, COOLPIX 4300, JAPAN) and the leakage formed in the occlusal and gingival walls of the restorations were assessed and recorded by two separate researchers using the double-blind method based on the grading system used by Delfino and Duarte (16) at X 10 magnification on the computer.

In the Occlusal Area;

0=No leakage, 1=Leakage within the enamel margin, 2=Leakage beyond the enamel-dentin margin, 3=Leakage continuing towards the dental pulp.

In the Gingival Area;

0=No leakage, 1=Leakage up to half of the gingival wall, 2=Leakage over half of the gingival wall, 3=Leakage continuing towards the pulp containing the gingival and axial wall (Figure 1).

The obtained data were recorded in the computer and statistical analysis was performed for the comparison of the groups via the Pearson Chi-Square and Fisher's Exact test. The statistical analyzes were performed using the software of SPSS 10.0.

Results

Assessments Regarding SEM

The SEM images, which have been obtained from enamel and dentin surfaces of cavities that were prepared with bur before acid application, are presented in Figure 2A and 2B.

It is seen that the enamel surface is covered with a smear layer and the presence of bur marks parallel to each other is remarkable (Figure 2A). It is observed that the dentin surface is completely covered with a smear layer, and there are large cracks. The length of the cracks ranges between 10-20 μ (Figure 2B).

Group I: SEM images of cavities, which have been obtained from enamel and dentin surfaces after acid application, are presented in Figures 3A and 3B.

On the enamel surface, it is observed that the walls of the prisms are affected primarily, and there is cobblestone-like roughness (Type II) (Figure 3A). The surface is quite retentive and rough compared to the enamel appearance before acid application. Brush-like micro-indentation protrusions that will increase the retention on the surface is remarkable. The smear layer has disappeared.

It is observed in the SEM image, which was obtained from the dentin surface, that the smear layer has completely disappeared, the peritubular dentine has disappeared, the dentinal tubule mouths are cleared, and the calcified matrix is demineralized in some areas, the presence of wide cracks, which are considered to be caused by bur preparations, is remarkable (Figure 3C red arrow).

Group II: SEM images of cavities obtained from enamel and dentin surfaces are presented in Figures 4A and 4B.

It is seen in the SEM image, which has been obtained from the enamel surface (Figure 4A), that the enamel surface is clear and clean, and there is no smear layer. The enamel prisms were split in accordance with the ablation feature of the laser, but a recessed, crater-like surface was formed due to the effect of the pulses. It is seen that it has coarser indentations and protrusions compared to the surface prepared with bur, but the cobblestone-like appearance, which could generate more retention, Superficial irregularities and step-like cascaded surfaces are observed in dentin. The width of the tubule mouths is narrower compared to the tubule mouths on the dentin surface in Group I. It was determined that the number of open tubule mouths was less than Group I. No smear layer is seen. (Figure 4B). Peritubular dentin is more apparent.

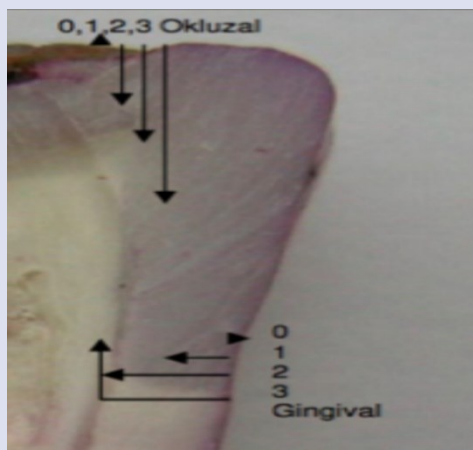


Figure 1: Leakage grades

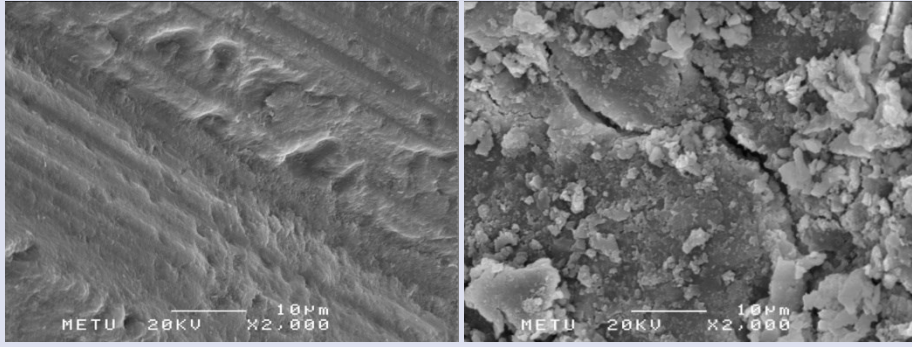


Figure 2A, 2B: SEM images of the cavity prepared by bur before acid application; Enamel; 2000X (A), Dentin; 2000 X (B)

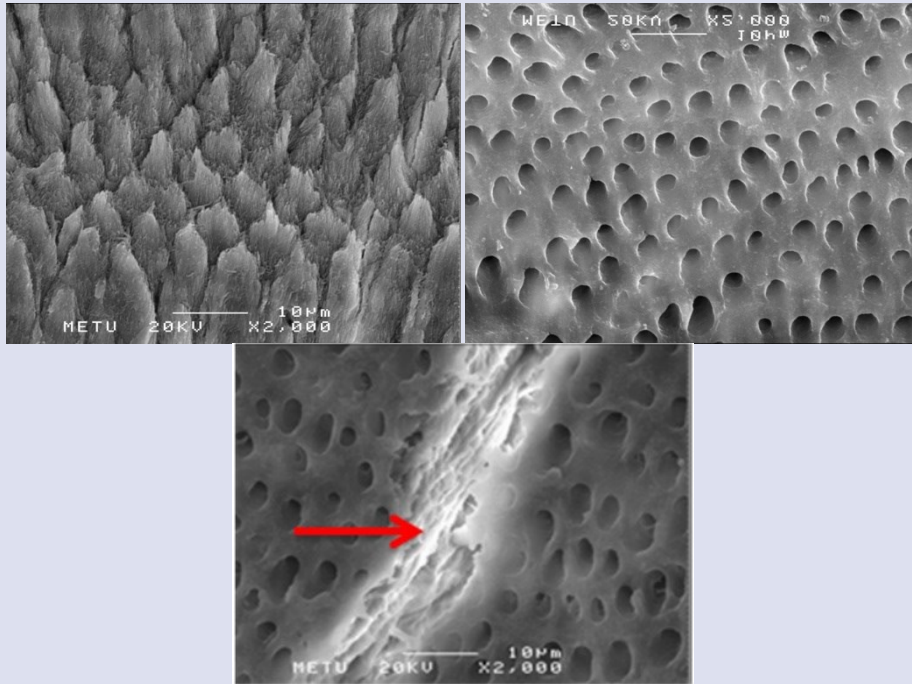


Figure 3A, 3B, 3C: SEM photos of the cavity prepared with bur and etched with acid: Enamel; 2000X (A), Dentine; 2000X (B), Dentine, microcrack; 2000X (C),

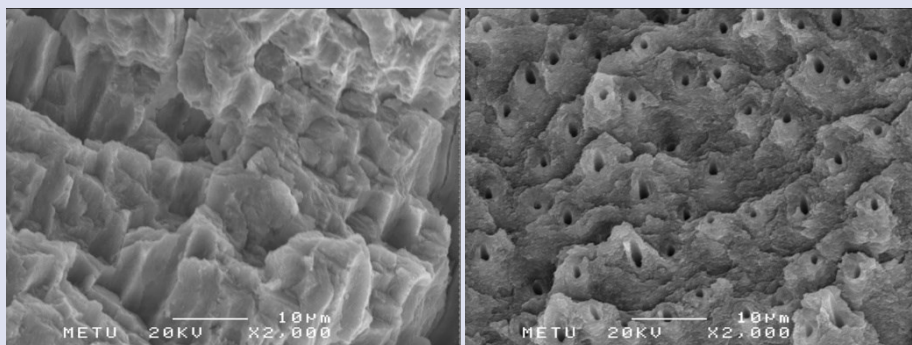


Figure 4A, 4B: SEM images of the cavity prepared with bur and roughened with laser: Enamel; 2000X (A), Dentine; 2000X (B)

Group III: SEM images obtained from the enamel and dentin surfaces of cavities are presented in Figure 5A and 5B.

It is seen in the SEM image, which has been obtained from the enamel surface, that enamel prisms are apparent, as, in Group II, prisms are fractured from

different points, and a protruding crater-like surface is formed. (Figure 5A). Enamel prisms are apparent among micro craters. There is no smear layer. Irregular, finger-like protrusions are seen on the dentin surface (Figure 5B). The fact that Group I cavities have narrower tubule orifices compared to the dentin surface is remarkable. The width of the tubule mouths is small, but their number is higher compared to that of Group II cavities on the dentin surface. Peritubular dentin is more apparent compared to intertubular dentine. The smear layer has disappeared completely.

Group IV: SEM images obtained from the enamel and dentin surfaces of cavities is presented in Figure 6A and 6B.

Apparent indentations and micro craters are noticeable in the SEM image of the enamel surface (Figure 6A). The distinct structure of enamel prisms in larger SEM images and shorter indented protrusions compared to Group III are remarkable. Open tubule mouths and herringbone pointedness are seen on the dentin surface (Figure 6B). The step-like cascaded appearance is less evident compared to the image in Group III. Remarkably, there is no crack in the dentin. It is seen that peritubular dentine is preserved in some areas and peritubular dentin is more apparent than intertubular dentine. The smear layer has disappeared completely.

Group V: SEM images obtained from enamel and dentin surfaces of cavities are presented in Figures 7A and 7B.

It is noteworthy in the SEM image of the enamel surface that the rough indentation protrusions become less apparent, but the micro-indent protrusions increased due to the application of acid (Figure 7A). Retentive micro-indentation protrusions are observed in a form similar to the surface prepared with a bur and etched with acid. It is seen in dentin that the micro-indent protrusions get rounded and become indistinct, and a less retentive surface is formed compared to the surface prepared via bur and etched with acid. The explicitness in peritubular dentin, which has been observed in groups II, III, and IV, is not observed in this group. The smear layer has disappeared completely (Figure 7B). Apparent cracks are

observed in the dentin tubule mouth of this group of cavities (Figure 7B arrow).

Assessment of the leakage:

The number, percentage, and Pearson Chi-Square values of the dye leakage grades, which have been detected in the occlusal and gingival regions of the restorations, are shown in Table 1.

The p values, which have been obtained by comparing the groups with each other in pairs in terms of marginal leakage and comparing their occlusal regions and gingival regions, are shown in Table 2.

Upon comparing the groups in pairs, it was determined that the difference between leaks that occurred in occlusal regions was statistically significant ($p=0.003$). When it was investigated which group caused the difference in the occlusal region, it was determined that there was a statistically significant difference between Group II and Group I, IV, and V ($p<0.05$), but there was no significant difference between the other groups ($p>0.05$). The difference between the groups regarding the leakages, which occurred in the gingival regions, is also significant ($p=0.000$). When it was investigated which group caused the difference in the gingival region, it was found that there was a statistically significant difference between Group V and other groups ($p<0.05$), and the difference among the remaining groups was not significant ($p>0.05$).

Upon comparing the leakage grades of the restorations in the occlusal and gingival regions, it was determined that there is a significant difference between the two regions ($p=0.000$). When the leakage grades of each group in the occlusal and gingival regions are compared; In Group V, the leakage observed in the gingival region was more than the occlusal region, and the difference was found to be significant ($p<0.05$). Moreover, there was no statistically significant difference between the occlusal and gingival regions of the first four groups ($p>0.05$) (Table 2).

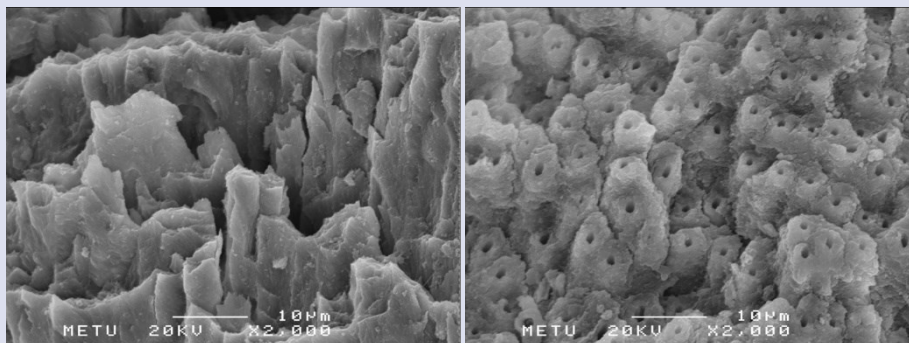


Figure 5A, 5B: SEM images of the cavity that have been prepared and roughed via laser: Enamel; 2000X (A), Dentine; 2000X (B)

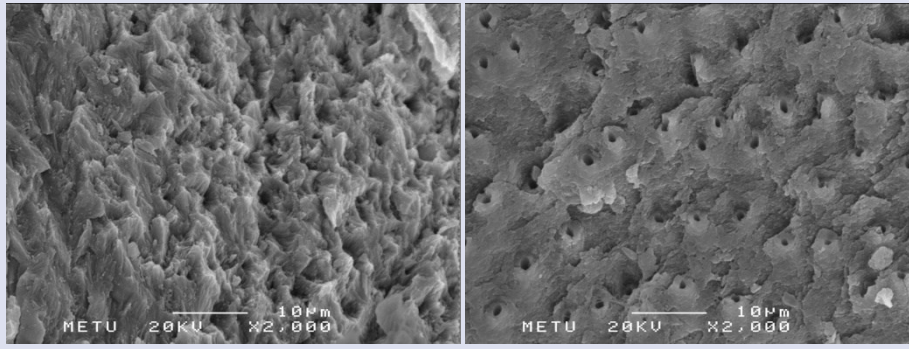


Figure 6A, 6B: SEM images of the cavity prepared with laser and not etched: Enamel; 2000X (A), Dentine; 2000X (B)

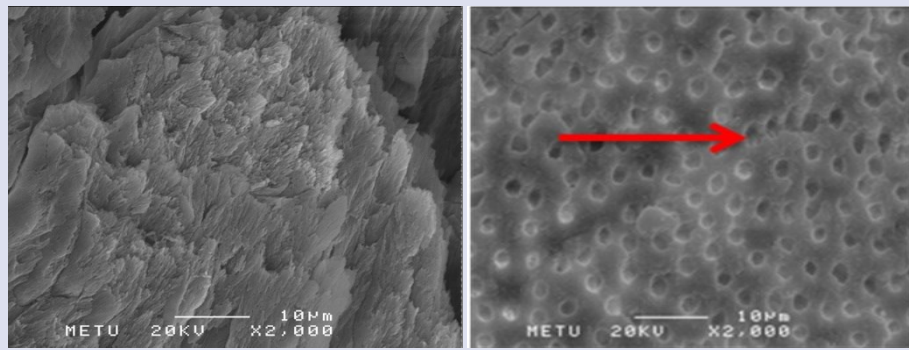


Figure 7A, 7B: SEM images of the cavity prepared via laser and etched with acid: Enamel; 2000X (A), Dentine; 2000X (B)

Table 1: Number, Percentage and Pearson Chi-Square values of Leakage Grades of Groups, (a: occlusal, b: gingival)

Groups	Number of Leakage Grades n (%)				Pearson Chi-Square
	0	1	2	3	
Group 1 a	35(87.5)	5 (12,5)	-	-	3.660
b	28(70)	12 (30,0)	-	-	
Group 2 a	24(60)	16 (40)	-	-	3.810
b	32(80)	8 (20)	-	-	
Group 3 a	31(77.5)	9 (22.5)	-	-	1.119
b	29(72.5)	10 (25)	-	1(2.5)	
Group 4 a	36(90)	4 (10)	-	-	0.125
b	35(87.5)	5 (2.5)	-	-	
Group 5 a	35(87.5)	5 (12.5)	-	-	56.43
b	2(5)	20 (50)	8(20)	10(25)	

Table.2 Statistical Comparison of the Differences between the Detected Leakage Degrees of All Groups, (a: occlusal, b: gingival)

	Group I		Group II		Group III		Group IV		Group V	
	a	b	a	b	a	b	a	b	a	b
Group I a	∅	p>0.05	p<0.05*	∅	p>0.05*	∅	p>0.05°	∅	p>0.05*	∅
b	p>0.05	∅	∅	p>0.05*	∅	p>0.05*	∅	p>0.05*	∅	p<0.05*
Group II a	p<0.05	∅	∅	p>0.05*	p>0.05*	∅	P<0.05*	∅	p<0.05*	∅
b	∅	p>0.05	p>0.05	∅	∅	p>0.05*	∅	p>0.05*	∅	p<0.05°
Group III a	p>0.05	∅	p>0.05	∅	∅	p>0.05*	p>0.05*	∅	p>0.05*	∅
b	∅	p>0.05	∅	p>0.05	p>0.05	∅	∅	p>0.05*	∅	p<0.05

Group IV	a	p>0.05	∅	P<0.05	∅	>0.05	∅	∅	p>0.05°	p>0.05*	∅
	b	∅	P>0.05	∅	p>0.05	∅	p>0.05	p>0.05	∅	∅	p<0.05*
Group V	a	p>0.05	∅	p<0.05	∅	>0.05	∅	p>0.05	∅	∅	p<0.05*
	b	∅	<0.05	∅	p<0.05	∅	p<0.05	∅	p<0.05	p<0.05	∅

Discussion

In our study, the absence of a smear layer and a crater-like surface with indented protrusions in the SEM images, which have been taken from the enamel surfaces of Group II cavities, is remarkable. SEM images that were taken from enamel surfaces, which were prepared via bur and etched via laser, were examined in various studies, and the absence of a smear layer and indented surface features were reported.¹⁷⁻¹⁹ It was observed in the SEM examination of the group II cavities in our study that there were superficial irregularities and a step-like cascaded surface in the dentin, and it was observed that the smear layer disappeared. Peritubular dentin is observed as more apparent. Our study is consistent with the studies of De Munck *et al.*²⁰ and Monghini *et al.*²¹ in terms of the above-mentioned findings and the absence of temperature-related changes and surface features such as the more distinct appearance of peritubular dentin.

SEM images of group IV cavities taken from enamel surfaces are consistent with the findings of Lin *et al.*²² and Freitas *et al.*¹⁴, in terms of not forming smear layer, occurrence of enamel prisms, and no change occurred due to heat. On the other hand, the results of the study regarding the features of dentin surfaces such as open tubule mouths, herringbone pointedness, cascaded and step-like appearance, peritubular dentine being more distinct than intertubular dentine and complete disappearance of the smear layer, the absence of any cracks or burning foci are in line with the studies of Hossain *et al.*¹², and Delme *et al.*¹³

Regarding the SEM images of group V cavities taken from enamel surfaces, it was determined that they have surface features with apparent and clearly indented protrusions, the absence of a smear layer, and a micro retentive appearance due to the effect of acid. Similar results have been revealed in the studies of Delme *et al.*¹² as well. On the dentin surface, it is remarkable that the calcified matrix is demineralized, the indented protrusions become indistinct, the peritubular dentine is not apparent, the smear layer is completely disappeared, there is no burning, melting foci, and the presence of unopened tubule mouths, and similar results have been revealed in the studies of Bertrand *et al.*¹⁵ as well.

As a result of our study, significantly more leakage was detected in the gingival of the samples in Group V compared to the other groups. We consider that the reasons such as cracks and deformations of the tubule mouth, the diminution of the retentive properties of the dentin surface, the loss of calcified matrix, and the occlusion of the dentin tubule mouths in some areas could be the factors that play a role in the finding that the

gingival leakage in group V was statistically greater compared to the other groups.

It has been found out that the acid etching procedure on dentin surfaces reduces the retentive micro-irregularities caused by laser preparation. Many researchers have demonstrated in their studies through the difficulty of the flow of adhesive to the new surface that the hybrid layer either does not form at all or does not form well enough.^{15,23}

In this study, no melting or burning foci was detected in any of the laser applied groups for either preparation or etching. Previous studies have revealed that various factors such as energy level, air-water ratios, application duration, and wavelength of the laser could change the impact of the laser on the target tissue. The absence of thermal effects in our study could be attributed to the fact that the used laser system has adequate air-water support and to the use of appropriate energy levels.

Many studies are examining the effects of using erbium lasers and conventional burs in cavity preparation on marginal leakage.²⁴⁻²⁷ Whereas some of these studies have revealed that lasers give equal or better results with a bur, some have shown that they cause more leakage compared to the conventional technique.²⁸⁻³²

Based on the results of the study by Gutknecht *et al.*³³ lesser marginal leakage was detected in the acid-applied group compared to the only laser-applied group, and acid etching was suggested for laser-prepared cavities as in the conventional technique. According to Gutknecht *et al.*³³, the leakage observed in the gingival region was found to be statistically significant in the group that was prepared with only laser and did not undergo any etching process, contrary to our study. We are of the opinion that the different results obtained in our study compared to the study of Gutknecht *et al.*³³ might be due to the difference in the parameters of the lasers and the applied adhesive and restoration materials.

Hossain *et al.*³⁴ made cavity preparations via Er: YAG (KaVo Key, Germany) laser and bur. They restored the cavities prepared with bur using acid etching procedure, and laser-prepared cavities with composite without applying any etching procedure. It was revealed that there was no statistically significant difference, regarding marginal leakage, between the two groups of restorations. The study of Hossain *et al.*³⁴ is consistent with the statistical results of the similar groups in this study. Our study includes similar groups (Group I and Group IV with laser) as well and there is no statistically significant difference between the leaks observed in the occlusal or gingival areas in Group I and IV.

Delme *et al.*¹³ performed Class V cavity preparation in an in vitro study using human molars. They etched Class V

cavities prepared with bur, and they divided the laser-prepared (Er: YAG Fotona, Slovenia) cavities into three groups and applied the procedures of acid etching, and laser as well as acid etching and laser etching. They revealed that there was no statistically significant difference in marginal leakage between the groups as a result of the study. This study included Groups I, II, and V, where the same procedures were applied as the study groups of Delme *et al.*¹³, and our results match their findings in terms of comparing leaks determined in the occlusal region, but not in terms of marginal leakage in the gingival region.

In the study of Delme *et al.*¹³ occlusal leakage in laser-prepared and laser-etched sample and it was demonstrated that there was a statistically significant difference compared to laser-prepared and acid-etched samples. In our study, statistically significant more leakage was detected only in the gingival regions of Group V compared to the occlusal regions of them. Although leakages in the gingival region are greater than occlusal leaks in groups I, III, and IV, the difference between them is not significant. It was found out that there was greater leakage in the occlusal compared gingiva only in Group II; however, the difference was not significant. This situation suggests that laser etching procedure in enamel after bur preparation impacts the occurrence of leakage adversely.

In our study, the findings we obtained from Groups III and IV reveal that an etching effect occurs while being prepared with laser, this phenomenon is also supported by the images we took with SEM. Remarkably, the cavities in these groups do not have a smear layer on the dentin surface, the dentin tubule mouths are cleared, and the calcified matrix is preserved (Figure 4B, 5B).

It was determined as a result of this study that the use of Er, Cr: YSGG lasers in cavity preparation and etching procedure during the application of composite restorations generally does not impact marginal leakage adversely; however, when the acid application is performed on the laser-prepared teeth in the gingival area where the cavity margins are located in the dentin, the leakage reaches advanced dimensions; plus, it was determined that laser etching of the teeth prepared via bur increased leakage in the occlusal area.

Conclusions

The following results have been obtained in this study: It was determined in the SEM assessment that there was an indented crater-like surface with no smear layer, no smear layer in dentin, a step-like cascaded surface and open tubule mouths among laser applied samples (Group II, III, IV, V) in enamel. As a result of the statistical analyzes, a significant difference was determined between the leaks observed in the occlusal ($p=0.003$) and gingival ($p=0.000$) areas of the groups, as well as between the degrees of leakage in the occlusal region and the gingival region ($p=0.000$). It was found out that the leakage occurred in the gingival region was significantly higher in Group V compared to the other groups ($p<0.05$). Moreover, it was determined that the leakage in the occlusal area was

significantly higher in Group II than in Groups I, IV, and V ($p<0.05$). When the leakage grades of each group in the occlusal and gingival areas were compared, it was observed that there was a statistically significant difference ($p<0.05$) only in Group V; however, the difference was not significant in the other groups ($p>0.05$). As a result, during the application of composite restorations, the use of Er,Cr:YSGG lasers in cavity preparation and etching processes generally does not negatively affect microleakage, but when acid is applied to laser-prepared teeth in the gingival region where the cavity borders are located in the dentin, leakage can increase.

It has also been determined that laser etching of teeth prepared with a bur increases leakage in the occlusal area. This study was performed in vitro conditions on healthy tooth tissues, using specified parameters of Er, Cr: YSGG laser, on a single type of adhesive system and restorative material. No in vitro study can fully simulate the oral environment. Furthermore, many variables such as the wavelength that vary depending on the type of each laser, the difference in the used parameters, and the properties of the restorative materials are significant when making such observations. Considering such variables, we think that more in vivo and in vitro studies should be performed on this issue.

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