Evaluation of Microleakage in Resin Composites Bonded to an Er:YAG Laser and Bur-Prepared Root and Coronal Dentin Using Different Bonding Agents

Farzaneh Shirani1*, Reza Birang2, Elmira Ahmadpour3, Zeynab Heidari4, Rouzbeh Ostadsharif Memar5, Zahra Zarei6, Reza Fekrazad7

1Department of Operative Dentistry, Dental Materials Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
2Torabinejad Dental Research Center, Department of Periodontics, Dental Faculty, Isfahan University of Medical Sciences, Isfahan, Iran
3Department of Endodontics, School of Dentistry, Shahrekord University of Medical Sciences, Shahrekord, Iran
4School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
5University of Toronto Faculty of Dentistry, 124 Edward St, Toronto, Ontario, M5G 1X3 Canada
6Department of Orthodontics, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
7Department of Periodontology, Dental Faculty – Radiation Sciences Research Center, Laser Research Center in Medical Sciences, AJA University of Medical Sciences, Tehran, Iran

*Correspondence to Farzaneh Shirani, Email: f_shirani@dnt.mui.ac.ir

Abstract
Introduction: This study was conducted to assess the microleakage in Er:YAG laser-ablated and bur-prepared root and coronal dentin cavities using self-etch and total-etch adhesive systems.
Methods: Sixty extracted caries-free human third molars were sectioned for dentin exposure. Then, two standard class V cavities were prepared in the root and coronal dentin of each tooth and allocated to one of the following conditioning groups randomly (n = 12/Group): G1: Diamond bur for cavity preparation and single bond (BESB) etch-and-rinse adhesive for bonding, G2: Er:YAG laser (160 mJ, 20 Hz, 29.88 J/cm²) and SB (LESB), G3: Er:YAG laser and SB without acid etching (LSB), G4: Diamond bur and Clearfil SE Bond (BCSE) self-etch system, and G5: Er:YAG laser and Clearfil SE Bond (LCSE). The cavities were filled with Z100 composite resin. Dye penetration was assessed after thermocycling. Data analysis was done by Kruskal–Wallis and Mann–Whitney U tests. Statistical significance was set at P < 0.05.
Results: The results showed there were no statistically significant differences in microleakage between the two preparation methods (bur and laser) or the bonding agents applied (P > 0.05). Regardless of the cavity preparation method, dye penetration was significantly higher in coronal dentin than in root dentin (P < 0.05).
Conclusion: The Er:YAG laser had the same efficacy as the conventional method for cavity preparation, and microleakage did not depend on the bonding agent. Microleakage was significantly higher in coronal restorations than in root restorations.
Keywords: Dentin; Er:YAG laser; Microleakage; Composite Resin; Self-etching primer.

Introduction
The use of composite resins has become very popular in restorative dentistry due to their esthetic features. However, due to polymerization shrinkage and consequently the gaps created at the resin-tooth interface, the use of these materials increases the risk of microleakage, tooth and pulp sensitivity, and recurrent caries.1-4

Population aging has elevated the cases with gingival recession and subsequently the incidence of root caries, which are confined to dentin with no enamel involvement. This has given rise to more research on the restoration of root caries.1 Researchers have always been in search of less traumatizing methods for dental caries removal without vibration, gingival bleeding or pain5,6 and also restorative techniques with minimal postoperative sensitivity and long-term survival.

The Er:YAG laser has been used and confirmed for application in the removal of caries and preparation of cavities based on the fundamentals of conservative and minimally invasive dentistry. The Er:YAG laser has a
wavelength of 2.94 µm, coinciding with the hydroxyapatite absorption peaks in enamel and dentin.\textsuperscript{7,8}

The preparation of tooth substrate by a laser does not produce a smear layer and creates a cavity surface with properties more desirable than the cavities prepared by conventional techniques.\textsuperscript{9,10} Rough surfaces, for instance, are not demineralized and show patent dentinal tubules, which may elevate micromechanical retention.\textsuperscript{11} However, literature available on the microleakage of composites bonded to lased tooth substrates is controversial. Some studies have reported lower microleakage of restorative materials to laser-conditioned dentin than to acid-etched conventionally-prepared dentinal surfaces,\textsuperscript{12,13} while others have reported significantly higher microleakage\textsuperscript{8-10} or no significant difference.\textsuperscript{14-16}

The basis of bonding systems to the conventionally prepared surfaces is the infiltration of bonding agents into the etched dentinal surfaces. There are no separate bonding agents designed so far for use on laser-prepared cavity preparations. This has caused debate about whether the current bonding agents act similarly on the laser-prepared surfaces as they do on conventionally prepared cavities. The optimum etching technique for laser preparation of dentin surfaces is also disputable. Some have suggested acid etching with total-etch adhesive systems while others believe the self-etch adhesive systems would generate the optimal bonding strength following laser ablation.\textsuperscript{17,18}

Given that the composition of coronal dentin is different from that of the root dentin, there is little information on the effectiveness of these adhesive systems in bonding to root dentin. Therefore, this study aimed to assess the microleakage of composite resin bonded to Er:YAG laser-ablated and bur-prepared root and coronal dentin by Clearfil SE Bond self-etch and Single Bond total-etch adhesive systems. The null hypothesis is that there is no difference in the microleakage of composite resin restorations placed with different bonding systems following conventional or laser cavity preparation in both coronal and root dentins.

**Materials and Methods**

**Cavity Preparation**

Sixty healthy human third molars extracted were kept in 0.2% thymol and cleaned by a scaler and dental prophylactic cups using pumice/water slurry. Based on the surface preparation technique and adhesive system, the teeth were allocated to five groups randomly (n = 12) (Figure 1). Two millimeters of the buccal surface of the enamel and 1 mm of the buccal surface of the root were ground under a water cooling system using a polishing machine (Politriz Struers A/S, Copenhagen, Denmark) and 320–400-grit silicon carbide abrasive papers (Buehler Ltd, Lake Bluff, IL, USA). This process eliminated the superficial enamel and cementum and provided a uniform superficial dentin surface. Wedge-shaped cavities were prepared in the coronal third of the root surfaces and the middle third of the buccal surface of crowns. Two aligned class V cavities with equal dimensions (1×2×3 mm) were prepared in each tooth: one in the exposed coronal dentin and another in the root dentin.

In G1 and G4, wedge-shaped cavities were prepared by means of a diamond barrel bur (G811, Coltene/Whaledent AG, Switzerland) and a high-speed handpiece, operating at 120,000 rpm under air and water spray. In G2, G3 and G5, the Fidelis Plus (Fotona, Ljubljana, Slovenia) Er:YAG laser device was used at a 2940 µm wavelength. The laser settings were 160 mJ pulse energy and a 20 Hz repetition rate (frequency) and a 29.88 J/cm\textsuperscript{2} energy density for

![Figure 1. The Experimental Groups Based on the Type of Cavity Preparation and Dentin Bonding Agent Applied.](image-url)
Microleakage of resin composite to Er:YAG laser and bur-prepared dentin cavity preparation. A barrel bur was used as a gauge for standardization of the sizes of cavity preparations with laser techniques. Then laser conditioning was performed for G3 by the same laser device but with 35 mJ energy and 10 Hz frequency. The laser beam was applied in a contact mode (0.5 mm distance from the tooth surface) under a water cooling system (7 mL/min). The RO7 handpiece with a removable fiber tip (0.9 mm diameter) was used.

**Bonding Procedures**

According to the manufacturer’s instructions, Single Bond (3M Dental Product, St. Paul, MN, USA) or Clearfil SE Bond (Kuraray Medical Inc., Osaka, Japan) was applied over the dentin surface after cavity preparation. In G1 and G2, the samples were acid-etched by 37% phosphoric acid (Gel Etchant, Kerr SPA, Salerno, Italy) for 15 seconds, irrigated with water for 15 seconds and dried slightly with absorbent paper to obtain a lightly wet dentin surface. Next, in G1, G2 and G3, a layer of adhesive was applied gently by a microbrush for 15 seconds and spread as a thin layer using gentle dry air spray. Another layer of adhesive was then applied without rubbing, thinned by gentle air spray and light-cured by a Coltolux light-curing unit (Coltolux 75, Colten/Whaledent Inc, Mahwah, NJ, USA) for 20 seconds at 500 mW/cm² light intensity. Prior to light-curing, the unit light intensity was checked using a radiometer (Demetron LED, Radiometer, Kerr Dental, USA).

In Clearfil SE Bond groups (G4 and G5), the self-etch primer was rubbed on the dentin surface by a disposable microbrush and the excessive primer was air sprayed to obtain a thin film. Next, the bonding agent was utilized, which was thinned by air spray and light-cured for 10 seconds.

All teeth were restored using Z100 composite resin (3M Dental products, St. Paul, MN, USA); the A1 shade of composite was applied in three increments. The first two layers were applied at sides against the occlusal and cervical walls and the last increment was placed to fill the cavity. Each layer was light-cured for 40 seconds.

**Microleakage Test**

Following the completion of the restoration, the teeth were stored in an incubator (Behdad, Tehran, Iran) at 37°C without vibration for 24 hours. They were then polished by composite polishing burs (Brasseler, Savannah, GA, USA) and Sof-Lex discs (3M Dental Products, St. Paul, MN, USA) based on the manufacturer’s guidelines. This was followed by 1000 cycles of thermocycling at 5-55°C (Sanati Vafaei Company, Tehran, Iran) with 30-second dwell time and 5-10-second transfer time. After thermocycling, the samples were waterproofed with two thin coats of nail varnish, up to 1 mm around the restoration margins, to inhibit dye penetration. Further, the root apices were sealed with sticky wax (L. D. Caulk Co., Milford, USA) in order to prevent dye penetration through the pulp chamber. The specimens were then submerged in 0.5% basic fuchsin solution (Merck, Darmstadt, Germany) at 37°C for 24 hours. After washing with tap water, the samples were singly fixed in auto-polymerized acrylic resin (Acro-pars, Tehran, Iran) in a PVC cylinder with the coronal and root restorations facing upward. The teeth were then sectioned along their long axis by a cutting disk (D&Z, 914-220, Diament, Paranaque, Philippines) under cold water.

The maximum dye penetration degree was registered for each section. The dye penetration degree was scored under a stereomicroscope (MGC-IO, N9116734, Russia) by three examiners in a blinded manner at ×40 magnification using the following standardized scoring system:

0: No dye penetration; 1: Dye penetration up to half of the cavity wall; 2: Dye penetration to more than half of the cavity wall; 3: Dye penetration beyond the full depth of the cavity

(Figure 2A-2D). Scores for coronal and root cavities were recorded separately and analyzed by the Kruskal-Wallis and Wilcoxon tests. The level of significance was set at $P$ value < 0.05.

**Results**

Descriptive data including the mean, standard deviation (SD), and median values of the dye penetration in all groups based on the bonding agent and cavity preparation method are presented in Table 1. No statistically significant differences were found between the dye penetration in coronal ($P=0.86$) and root dentin study groups ($P=0.58$). Table 2 shows the frequency distribution of dye penetration in the crown and root dentin. Intra-group comparison of dye penetration in coronal and root dentin in groups 1 to 5 yielded the following $P$ values: 0.01, 0.78, 0.04, 0.02 and 0.41 respectively. Moreover, the Wilcoxon

![Figure 2. Stereomicroscope Image (×40) of Dye Penetration scores (A) 0, (B) 1, (C) 2, and (D) 3.](image)
Based on the results, the null hypothesis of the study was confirmed for the most part. It was found that there is no significant difference in the microleakage of cavity preparations prepared and restored with different techniques and bonding agents. However, we found that regardless of the method of cavity preparation and the bonding agents used, the dye penetration into the coronal dentin-restoration interface is significantly higher when compared to the root dentin-restoration interfaces.

Considering the numerous studies conducted on the complexity of dentinal bonding\(^8\) and the fact that root caries is often confined to dentin with no enamel protection, this in vitro experimental study focused on dentin and its behavior. For this purpose, the enamel of all teeth was removed completely and cavities were prepared in dentin to assess dye penetration into the dentin surfaces. Thermocycling was also performed for the aging of the restorative materials, taking into account the coefficient variations of the thermal expansion of dentin and materials in order to better simulate the clinical setting.

An important factor in anticipating the clinical success of bonding is the extent of microleakage at the dentin/restoration interface.\(^9\) Studies aiming to decrease the microleakage at the composite-dentin interface have extensively assessed the methods of cavity preparation. Considering the advantages of lasers compared to conventional dental burs for cavity preparation, such as the significantly lower prevalence of post-operative tooth hypersensitivity,\(^8\) we used the Er:YAG laser to prepare the teeth and examined the dye penetration degree at the interface of composite and laser-prepared cavity walls. Since the effect of tooth preparation instruments on the bonding strength of various bonding systems to dentin has been documented in the literature, we made a comparison between two commonly used dentin bonding systems, namely total-etch and self-etch, in cavities prepared by a laser, a conventional bur and a handpiece.

Significant histological differences exist between the structure of coronal and root dentin. Due to increased life expectancy, improved oral hygiene and tooth survival, cases of root caries are more commonly seen.\(^9\) Therefore, the quality of the bond of tooth-colored restorations to

| Group | Dentin | Mean | SD | P Value |
|-------|--------|------|----|---------|
| 1 (BESB) | C | 1.41 | 1.31 | 0.01* |
| | R | 0.33 | 0.49 | |
| 2 (LESB) | C | 0.58 | 0.59 | 0.78 |
| | R | 0.50 | 0.67 | |
| 3 (LSB) | C | 2.08 | 0.99 | 0.04* |
| | R | 0.58 | 0.51 | |
| 4 (BCSE) | C | 1.33 | 1.37 | 0.02* |
| | R | 0.25 | 0.86 | |
| 5 (LCSE) | C | 0.66 | 1.15 | 0.41 |
| | R | 0.33 | 0.88 | |
| Total | C | 1.21 | 1.26 | 0.86 |
| | R | 0.40 | 0.69 | 0.58 |

C means coronal and R means root.

*Significant difference between two groups at \(P \leq 0.05\)

### Discussion

The current study aimed to compare the cavities prepared and restored in root and coronal dentin with different preparation techniques and bonding systems with regard to their microleakage properties. The hope is that the findings would specifically guide us in clinical situations to achieve optimal bonding where the caries is limited to the root or coronal dentin, as found in the margins of crowns intended for replacement and root caries. At the same time, we hope to shed light on the suitability of conventional bonding agents to bond to laser-prepared cavities, which is the method of choice for cavity preparation in elderly patients with recession and accessible root caries. The present research indicated no significant differences in the microleakage of various dentin bonding agents in coronal and root restorations. Based on the results, the null hypothesis of the study

### Table 1. Dye Penetration Descriptive Data of the Studied Groups Based on the Type of Bonding Agent and Method of Cavity Preparation

| Groups | 1 | 2 | 3 | 4 | 5 |
|--------|---|---|---|---|---|
|        | C | R | C | R | C | R | C | R | C | R |
| 0      | 4 (33.33) | 8 (66.66) | 8 (66.66) | 7 (58.33) | 0 (0) | 5 (41.66) | 5 (41.66) | 11 (91.66) | 8 (66.66) | 10 (83.33) |
| 1      | 3 (25) | 4 (33.33) | 2 (16.66) | 4 (33.33) | 5 (41.66) | 7 (58.33) | 2 (16.66) | 0 (0) | 2 (16.66) | 1 (8.33) |
| 2      | 1 (8.33) | 0 (0) | 1 (8.33) | 1 (8.33) | 1 (8.33) | 0 (0) | 1 (8.33) | 0 (0) | 0 (0) | 0 (0) |
| 3      | 4 (33.33) | 0 (0) | 1 (8.33) | 0 (0) | 6 (50) | 0 (0) | 4 (33.33) | 1 (8.33) | 2 (16.66) | 1 (8.33) |
| Total  | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |

C = Coronal dentin, R = Root dentin
cervical root dentin must be evaluated. For this purpose, cavities of the same size were prepared in the coronal and root dentin to compare the bonding behavior of coronal and root dentin.

In the current study, Class V cavities were prepared for dye penetration testing due to their high C-factor and easier restoration. Also, 0.5% aqueous basic fuchsin was used as the dye. This method is simple and affordable for the evaluation of microleakage. Currently, there are discrepancies with regard to the evaluation of the microleakage of composite resin restorations. Some studies oppose the use of dye penetration techniques due to the vast variability of test results, validation of methods not being possible, and no correlation with clinical findings. This is presumably due to the dye being composed of very small particles (< 1 nm), which causes penetration through any marginal discrepancy that may be present. Obviously, such a penetration pattern and penetration depth are clinically irrelevant. Some studies still advocate the use of the dye penetration technique for assessing microleakage due to its convenience, low cost, accessibility and ability to detect even the smallest levels of dye penetration between the dental tissues and restorative materials, which is not possible with silver particles. Therefore, we decided to use the dye penetration method in this study for evaluating the microleakage.

Despite the elimination of enamel, lack of polymerization shrinkage towards the enamel and incremental application of composite, our findings showed that dye penetration and microleakage still existed in all experimental groups. Our results showed that none of the two-cavity preparation techniques or bonding agents had any superiority over the other, which is in accord with the findings of previous studies. SEM observations have shown that lased cavities are irregular and also without a smear layer. Laser irradiation produces thermo-mechanical ablation and water vaporization in dental hard tissues, leading to water content micro-explosions and irregularity in the hydroxyapatite matrix. The resultant porosities would facilitate the penetration of primer and adhesive in the absence of a smear layer. The main principle in decreasing the penetration of fluid at the tooth-restoration interface is the infiltration of resin into adequate exposure of the collagen network. In laser-prepared cavities, this is achieved, leading to a suitable and strong adhesion. This phenomenon has also been reported in former studies making use of the Er:YAG laser.

Some studies have demonstrated greater microleakage in laser-prepared cavities. For instance, Synarellis et al examined the microleakage of Er,Cr:YSGG laser-prepared Class V resin restorations (2780 nm wavelength, 4 W power, 20-Hz frequency,) and showed that cavities prepared by the conventional method had lesser microleakage. They believed that increased microleakage in laser-treated cavities is due to the lesser exposure of collagen and lack of demineralization, which together compromise the development of a hybrid layer. The results of this study showed no greater microleakage in cavities prepared and restored with the laser technique. This suggests that bonding agents are equally effective regardless of the method of cavity preparation.

In general, conventional etching is recommended after laser conditioning. Bertrand et al reported that the pretreatment of irradiated surfaces with acid made the development of a hybrid layer possible (5–6 μm thick) and highly elevated the penetration of resin tags. In this study, laser-prepared cavities without applying acid etchant showed the highest degree of microleakage in both the coronal and root dentin, even though there was no statistically significant difference. Therefore, our results further confirm the validity of recommendations regarding acid-etching lased cavity preparations before bonding.

Bonding agents have been designed for surfaces prepared by rotary tools to remove or change the smear layer. Since laser preparation of dentin does not generate a smear layer, it is assumed that the bonding agents produced for the dentin prepared mechanically have less effect on the dentin prepared by a laser. This suggests that bonding agents need to be specifically designed for laser-irradiated substrates. However, our results indicate that bonding agents can still perform equally well on lased cavity preparation.

Although direct correlations of microleakage with cavity size and C factor have been proven, we observed differences in microleakage between similar-sized cavities prepared in the coronal and root dentin. The differences in microleakage can be related to the microscopical structure of dentin, its mineral content and different effects of bonding agents on two distinct substrates. Studies have shown that during developmental stages, root dentin is formed slightly later than coronal dentin with a slower rate of deposition. Coronal dentin is deposited at a rate of 4 microns per day while the root dentin has a slightly lower rate. In addition, it is believed that the product of odontoblasts in the root is different from that in the crown in terms of structure, composition and orientation of collagen fibers. The amount of phosphoserine and its mineralization in root dentin are less than those in coronal dentin. Dentinal tubules follow an S-shaped path in coronal dentin and a straight path in root dentin. The number and diameter of dentinal tubules are greater in the crown, although their terminal branches are much more in the root. Coronal dentin is more permeable than root dentin due to structural differences. It seems that the available bonding agents designed for bonding to coronal dentin have different performances when used on root dentin. Elucidation of their exact mechanism requires further investigations using electron microscopy.
As described, the different characteristics of the root and coronal dentin due to the reasons explained can lead to different bonding results when different bonding agents are used under standard conditions. In the current study, different bonding systems displayed the same performance in terms of microleakage. This result may be attributed to the method of application, environmental conditions and irradiation parameters. A rise in pulse energy has been shown to result in a deeper crater pattern on the surface of teeth. This, in turn, might affect the adaptation of the restorative materials to the cavity walls. In the end, it is noteworthy that in vitro studies can never truly simulate an oral environment due to their innate limitations.

Conclusion
Overall, we found that even though there were no significant differences between the microleakage of restored cavities with different preparation techniques, the laser-prepared cavity preparations in both the coronal and root dentin had lower microleakage. There was also no significant difference between the microleakage of laser-prepared cavities in the root and coronal dentin. In other groups, where the cavities were prepared conventionally or the etch-and-rinse technique was not used, the dye penetration was significantly higher in coronal dentin when compared to root dentin.

The Er:YAG laser and bur had the same efficacy in cavity preparation irrespective of the kind of bonding agent applied. This conclusion regarding the efficacy of Er:YAG is only valid under the variables of this study. It is possible to see different results if parameters for assessing the efficacy of Er:YAG in cavity preparation are changed.

Ethical Considerations
This article contains no studies on human participants or animals carried out by any of the authors.

Conflict of Interests
No conflicts of interest are declared in this study.

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