Effect of erbium-doped: yttrium-aluminum-garnet laser preparation on resin-cavity interface using a universal adhesive evaluated by swept source optical coherence tomography

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Purpose: The purpose of this *in vitro* study was to evaluate cavity adaptation between erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser irradiated cavity and composite resin using a universal type adhesive with different application by means of a swept source optical coherence tomography (SS-OCT).
Materials and Methods: A cylindrical cavity was prepared on the labial surface of extracted bovine incisor by either rotary cutting instrument or Er:YAG laser. The cavity was applied by either two-step self-etching adhesive (Clearfil SE Bond, CS) or universal type adhesive (Schotchbond Universal, SU), as self-etching or selective enamel etching, and then was restored with a flowable composite resin. Gap formation of enamel and dentin interface was evaluated by an SS-OCT.
Results: At the enamel interface, there are no statistical differences between bur preparation and laser preparation in each group. For bur prepared SU groups, selective enamel etching showed less gap formation than self-etching group. At the dentin interfaces, there are no statistical differences between bur preparation and laser preparation. Selective enamel etching did not affect gap formation at dentin. On lased dentin, CS showed better adaptation compared to SU.
Conclusion: It was concluded that the universal type adhesive showed comparable adaptability to tooth substrate prepared by bur and laser, and selective enamel etching improved adaptation to bur prepared enamel in case of a universal type adhesive.

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Key Words: Er:YAG laser, gap formation, selective enamel etching, SS-OCT, universal type adhesive

Introduction

The erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser with a wavelength of 2.94 μm was introduced for dentistry in 1989[1] and then, the Food and Drug Administration of United States cleared for marketing the first Er:YAG laser for use in preparing human dental cavities in 1997.[2] The Er:YAG laser is able to remove caries lesion in tooth structures together with sound enamel and dentin with minimal thermal effects in the adjacent hard and soft tissues.[2] Although the cavity preparation using Er:YAG laser takes more time, compared to rotary cutting instruments,[3,4] that preparation method advantages include low noise and vibration, eliminating, in most cases, the need for local anesthesia.[5] A clinical study suggested that the application of the Er:YAG laser system was a more comfortable alternative or adjunctive method to conventional mechanical cavity preparation.[4]

After removal of caries by the Er:YAG laser, the cavity is generally restored with an adhesive material, mainly composite resin. Indications for composite resin restoration have been widely expanded as improving the various properties of the adhesives and the composite resins. Clinical trials demonstrated that composite resin restorations were acceptable for long period.[6,7] As a result, directly placed composite resins serve as standard materials in restorative and esthetic dentistry. Recently, new adhesive systems, so called "universal type" adhesives have been launched, which allow self-etching, selective enamel etching and total etching protocols for bonding. However, there are no study on the effect of universal type adhesive on Er:YAG laser irradiated cavity.

For longevity of the restoration, bonding and adaptability between cavity and filling material should be critical. Adaptation at the resin-cavity interface and bond strength have often been investigated by *in vitro* evaluation of the restorative materials and techniques.[8-26] Among them, there are many research concerning the bond strength and the adaptation between restoration and cavity prepared by Er:YAG laser.[8,9,12,13,15,18,19,21,24,26] Some researches showed the comparable bond strength[8,18] and adaptability[26] of lased tooth and bur-prepared tooth, and other reported different results.[9,12,13,15,19,21,24]

For evaluation of the resin-cavity interface, various methods have been employed. Recently, a new evaluation method using optical coherence tomography (OCT) was proposed.[17,27-30] OCT was developed for noninvasive cross-sectional imaging in biological systems which were transparent and turbid in 1991.[31] The OCT technology was first applied for ophthalmology and then, was expanded for many medical fields. In 1998, OCT was applied for oral soft and hard tissues including tooth, and it was suggested the potential of the OCT for diagnosis of periodontal disease, detection of caries, and evaluation of dental restorations.[32] There have been many OCT studies about evaluation of resin cavity interface with various cavity shapes, adhesives, filling materials and restorative techniques.[17,27-30] However, there is no research about cavities prepared by the Er:YAG laser.

The purpose of this *in vitro* study was to evaluate cavity adaptation between Er:YAG laser irradiated cavity and composite resin using a universal type adhesive with different application by means of a swept source OCT (SS-OCT).

Materials and Methods

Materials

The materials used in this study, their manufacturers, code and ingredients were listed in Table 1. Clearfil SE Bond (CS, Kuraray Noritake Dental, Tokyo, Japan) is a two-bottle, two-step self-etching adhesive and Scotchbond Unversal (SU, 3M ESPE, St.Paul, MN, USA) is a single-bottle “universal type” adhesive which can be used as self-etching, total etching or selective enamel etching. In this study, SU was evaluated as self-etching and selective enamel etching using a phosphoric acid gel (K etchant GEL, Kuraray Noritake Dental) which contains 40% phosphoric acid. Estelite Flow Quick (Tokuyama Dental, Tokyo, Japan) is a light-curing flowable composite resin. Their chemical compositions showed in Table 1 were information disclosed by the manufacturers.

Cavity preparation

Forty-eight extracted bovine lower incisors were used in this study. The teeth were stored frozen after extraction and were thawed by running tap water. The crowns were cleaned by removing soft tissues using a scalpel, then crowns and roots were separated by a slow-speed diamond saw. The labial enamel was ground using 600 grit silicon carbide paper (SiC) to obtain flat surface. Then surface was polished up to 1,200 grit SiC. In order to keep 1 mm thickness of remaining enamel, the amount of removal enamel was determined by a pilot study.

The bovine samples were divided in two groups of each of 24 specimens. In one group (bur preparation), a cylindrical cavity with round-shape line angle surrounding cavity floor was prepared in the center of polished surface with approximately 2.5 mm in depth and 4 mm in diameter using a flat-end tapered diamond bur (207R, Shofu, Kyoto, Japan) and high-speed air-turbine handpiece under water splay coolant.

In another group (laser preparation), a slight smaller size of cavity than that of bur-preparation group was prepared with same method, then all cavity walls were finished by irradiating an Er:YAG laser (Erwin AdvErl, J.Morita, Kyoto, Japan) in which the wavelength (λ) is 2.94 μm. A curved quarts tip (C600F, J.Morita) was used and irradiation condition was 80 mJ/pulse of output power at the end of the fiber at 20 Hz. Total irradiation energy to each cavity was estimated approximately 133 J. Finally, same shape and size cavities were achieved in both groups.

Table 1. Materials used in this study

Material type	Materials, Manufactures, Code	Ingredients
Adhesive	Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan, CS	Primer HEMA, MDP, hydrophilic aliphatic dimethacrylate, *dl*-camphorquinone, *N,N*-diethanol-*p*-toluidine, water, dyes Bond Bis-GMA, HEMA, MDP, hydrophobic aliphatic methacrylate, silanated colloidal silica, *dl*-camphorquinone, *N,N*-diethanol-*p*-toluidine
Adhesive	Scotchbond Universal, 3M-ESPE, St.Paul, MN, USA, SU	MDP, dimethacrylate resins, HEMA, copolymer, filler, ethanol, water, initiators, silane
Etchant	K-etchant GEL, Kuraray Noritake Dental	phosphoric acid 40%, colloidal silica, water, dyes
Flowable composite resin	Estelite Flow Quick Tokuyama Dental, Tokyo, Japan	UDMA, 2,2'-(methylimino)diethanol, Bis-MPEPP, camphorquinone, dibutyl hydroxy toluene, mequinol, TEGDMA, silicazirconia filler, silica-titania filler

Abbreviations: Bis-GMA, bisphenol A diglycidylmethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; Bis-MPEPP, Bisphenol A polyethoxy methacrylate; UDMA, 1,6-bis(methacryloyloxyethoxycarbonylamino)trimethylhexane; TEGDMA, triethyleneglycol dimethacrylate

Cavity treatment and restoration

Prepared teeth of each group were further divided in four subgroups in each of six samples (n=6). The used adhesive and cavity treatment in each group were shown in Table 2. Each cavity was treated by Clearfil SE Bond (CS) or Scochbond Unversal (SU) as self etching (SLF) or selective enamel etching (SEE) as following procedures.

Table 2. Experimental group

Group	Preparation	Adhesive	Selective etching
B-CS-SLF	Bur	CS	Self-etching
L-CS-SLF	Laser	CS	Self-etching
B-CS-SEE	Bur	CS	Selective enamel etching
L-CS-SEE	Laser	CS	Selective enamel etching
B-SU-SLF	Bur	SU	Self-etching
L-SU-SLF	Laser	SU	Self-etching
B-SU-SEE	Bur	SU	Selective enamel etching
L-SU-SEE	Laser	SU	Selective enamel etching

CS, Clearfil SE Bond; SU, Scotchbond Universal

For B-CS-SLF and L-CS-SLF groups, Primer of CS was applied by a disposable small brush. After leaving for 20 s without rinse, the cavity was dried with mild air from an air-water syringe. Bond of CS was coated by a small brush distributing evenly with gently air flow and then, light cured for 10 s by a halogen light-curing unit (Optilux 501, SDS Kerr, Middleton, WI, USA) with intensity of 900 mW/cm^2. For B-CS-SEE and L-CS-SEE groups, K-etchant GEL was applied on the enamel in the cavity with a disposable small brush. After 60 s, the cavity was washed thoroughly and dried with an air-water syringe. After selective enamel etching, the cavity was treated by CS as same as the cavities of CS-SLF groups. For B-SU-SLF and L-SU-SLF groups, cavities were treated by SU as self-etching. SU was applied in each cavity by a small brush. After 20 s, the cavity was dried by a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent was evaporated completely, then light cured for 10 s by a light-curing unit. For B-SU-SEE and L-SU-SEE groups, each cavity was selective enamel etched by K-etchant GEL as same as the cavities of CS-SEE groups. Then, cavities were treated with SU as same as SU-SLF groups.

After treatment by CS or SU, a flowable composite resin (Estelite Flow Quick) was directly filled from the syringe into the cavity and photo cured for 20 s. After storage in tap water for 24 hours at 37°C, sample was analyzed by an SS-OCT.

Image analysis by SS-OCT

The SS-OCT system (IVS-2000, Santec, Komaki, Japan) used in this study was a frequency-domain OCT which had the same setup components as previous reports.[33,34] The light source of a high-speed frequency swept external cavity laser sweeps the wavelength between 1,260 nm to 1,360 nm (central wavelength 1,319 nm) at 20 kHz sweep rate. The probe power is less than 5 mW, which is less than safety limit defined by American National Standards Institute. The axial resolution of this SS-OCT system is 7 um for tooth and the lateral resolution is 17 nm. Cross sectional images which were grayscale images with 2,001×1,019 pixels were synthesized from A-scan data which is direction of light source and B-scan data which is direction of scan. Each B-scan corresponded to an image, 8×6.6 mm in x, z dimensions (2,001×1,019 pixels), obtained in approximately 100 ms.

Each sample was positioned and fixed on the stage perpendicularly to the scanning probe of the SS-OCT and tomographic images were taken by the SS-OCT. The 2-D raw tomograms cross-cut through the center of the restoration were obtained. Six images were obtained rotated 30° in each on x-y dimension. Finally, six image through the center of the restoration with different direction were taken. Percentages of gap-formed interface of enamel (GIE) and dentin (GID) were calculated from each OCT image by following procedure.[27,30] Obtained crosscut images were imported to an image analyzing software (ImageJ 1.45, NIH, Bethesda, MD, USA) and a median filter was applied to decrease background noise. The grayscale OCT image was converted to the binary image (black and white image) based on a threshold determined automatically using an algorithm. On the binary image, the length of each bright cluster along the resin-enamel interface and the resin-dentin interface at the cavity wall was calculated by means of counting bright pixels. GIE and GID were calculated according to the following equation:

GIE or GID (%) = (total length of bright clusters at enamel or dentin) × 100 / (length of the enamel or dentin cavity wall at that slice)

Statistical analysis

The data were subjected to analysis of normality to select a parametric test. Average GIE and GID of each group were then statistically analyzed using three-way and one-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey HSD correction. All the analyses were performed using the statistical package software (IBM SPSS Statistics 21.0, IBM, Armonk, NY, USA).

Results

The average and standard deviation of GIE and GID in each experimental group were shown in Table 3 and 4 respectively. Typical OCT images of each group were shown in Figs. 1 and 2.

Table 3. Gap-formed interface of enamel (GIE)

	Bur	Laser
CS-SLF	18.2 (4.4) [a,b]	15.4 (3.8) [a,b]
CS-SEE	11.5 (4.8) [a]	15.0 (4.4) [a,b]
SU-SLF	26.4 (4.9) [c]	19.9 (2.4) [b,c]
SU-SEE	13.9 (4.4) [a,b]	16.0 (4.8) [a,b]

average (%) and SD
Same superscripts showed no statistical differences.

Table 4. Gap-formed interface of dentin (GID)

	Bur	Laser
CS-SLF	8.6 (5.3) [a]	18.4 (9.2) [a]
CS-SEE	12.6 (7.5) [a]	18.0 (14.5) [a]
SU-SLF	22.6 (18.1) [a,b]	44.5 (11.8) [b,c]
SU-SEE	47.7 (16.3) [b,c]	54.6 (21.2) [c]

average (%) and SD
Same superscripts showed no statistical differences.

At the enamel interface, there were no statistical differences between bur preparation and laser preparation groups. Three way ANOVA revealed the statistical differences between self-etching groups and selective enamel etching groups (p=0.000) and between adhesives (p=0.002). B-SU-SEE showed statistical less gap formation than B-SU-SLF. At enamel interface, gap formation was found at both cavo-surface marginal area and the deeper area close to dentino-enamel junction.

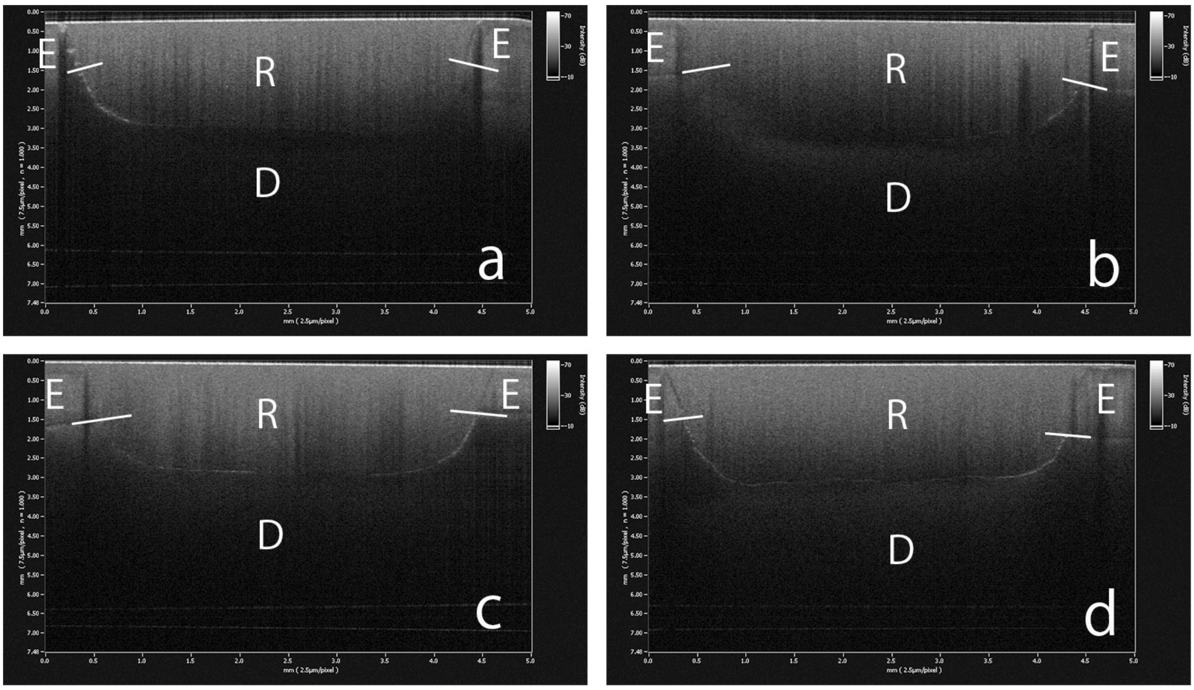

Fig. 1 Typical SS-OCT images of CS groups
a, Bur-prepared CS-SLF group; b, Laser-prepared CS-SLF group; c, Bur-prepared CS-SEE group; d, Laser-prepared; CS-SEE group; E, enamel, D, dentin; R, composite resin

At the dentin interfaces, statistical differences could not find between each bur preparation and laser preparation group and between each SLF and SEE group by Tukey HSD correction. Comparing adhesive groups, CS groups showed lower GID score than SU groups except between B-CS-SLF and B-SU-SLF groups. At dentin interface, gap formation was more often observed at pulpal walls, as compared to lateral walls.

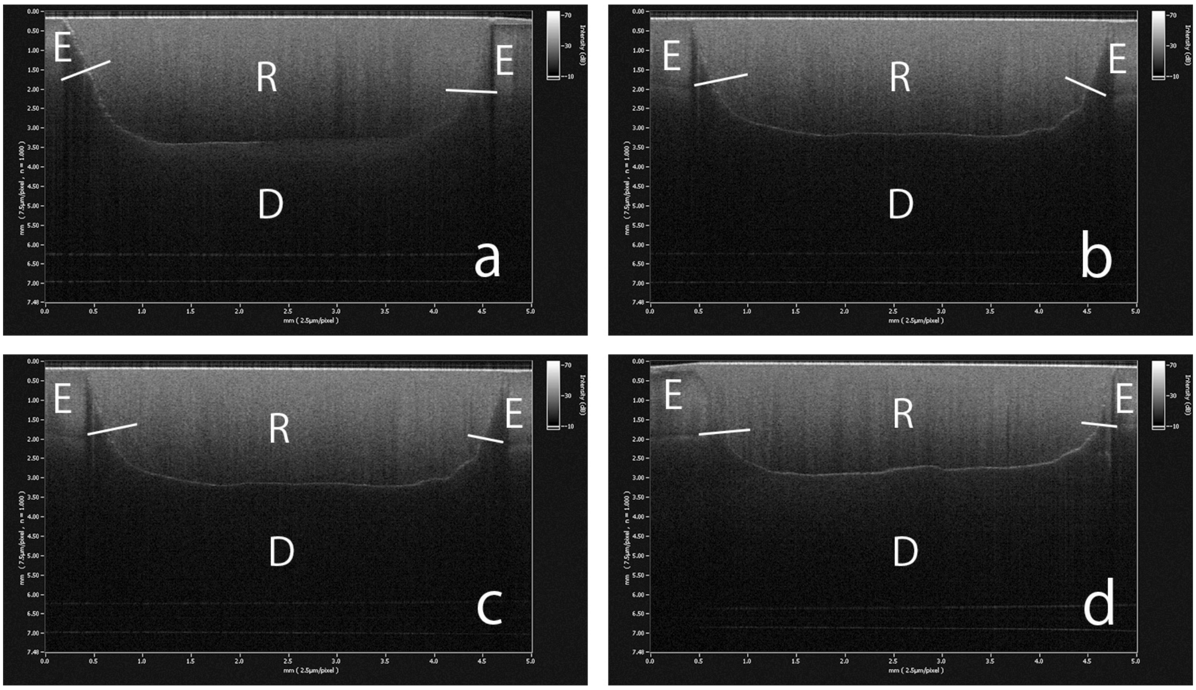

Fig. 2 Typical SS-OCT images of SU groups
a, Bur-prepared SU-SLF group; b, Laser-prepared SU-SLF group; c, Bur-prepared SU-SEE group; d, Laser-prepared SU-SEE group; E, enamel; D, dentin; R, composite resin

Discussion

The mechanism of dental hard tissue removal by the Er:YAG laser is quite different from that by a rotary cutting instrument. The wavelength of Er:YAG laser (λ=2.94 μm) is well absorbed by water and hydroxyapatite. When the Er:YAG laser is irradiated and the molecules of the water in the tooth become superheated, “microexplosion” is occurred dividing the surrounding tissue into very small pieces and blowing them apart.[35] Also, the morphologies of prepared enamel and dentin surfaces are different between rotary cutting instrument and Er:YAG laser ablation. The cut surfaces of enamel by rotary cutting instruments are varied by the types of used burs.[14] In this study, bur prepared groups used a regular-grit diamond bur. The enamel prepared by the regular-grit diamond bur exhibited as rough and small enamel fragments were observed by a light microscope.[14] The enamel surface irradiated by Er:YAG laser was chalky and irregular.[16] Micromorphology of the Er:YAG laser-treated enamel depicted a retentive pattern similar to acid etched enamel and the anatomical features of enamel rods were preserved.[35] The dentin surfaces prepared by a rotary cutting instrument was covered with a smear layer and the thickness of smear layer affected the bonding.[11] The dentin surfaces irradiated by Er:YAG laser were irregular, scaly or flaky and dentinal tubules were opened without smear layer.[36] Vaporization of intertubular dentin is greater than that of peritubular dentin, showing a protrusion of the dentinal tubules with a cuff-like appearance.[35] Although the dentin surface irradiated by Er:YAG laser seems to increases restorative material retention,[37] this surface and subsurface contained acid resistant layer and acid vulnerable layer.[36] Those characteristic surface and subsuraface layer of prepared enamel and dentin would affect the bonding to resin, consequently gap formation. Comparing bur prepared and laser prepared groups, there were no statistical difference. In this study, laser preparation did not show improving nor interfering adaptability of resin cavity interface.

In laser prepared groups, cavity was prepared by diamond bur followed by Er:YAG laser. Because it was very difficult to prepare cylindrical cavity using laser alone. Since the laser was irradiated sufficiently, the ablated cavity surface was thought to be same as that by prepared using laser alone. In this study, relatively large size of cavities were prepared because of technical reason. Consequently, the gap formation might be emphasized. The cavities were restored with a flowable composite resin. Flowable composites can be easily inserted into small cavities and are expected to demonstrate better adaptation to the internal cavity wall compared to the conventional restorative composites which are more viscous.[38] Because low viscosity is well contact with cavity wall and the gap formation by technical error is reduced. In this study, samples were stored without any loading, such as thermal stress or mechanical loading which might affect the results of the experiment. Although it was reported that the thermal cycling had no deleterious effect on the bonding efficacy of SU,[22] further study should be necessary about the effect of those loading stress on the results. Enamel and dentin have quite different property and they showed different bond strength and adaptation to resin restorations. In this study, gap formation was evaluated in enamel and dentin cavity walls individually. It was seemed to be well adopted the purpose of this study. For evaluation of sealing and adaptability between cavity and restoration, dye penetration tests with optical microscope,[14,16,26,38,39] observation by scanning electron microscope (SEM)[20,25] or confocal scanning laser microscope (CSLM)[27,28,30] were often used. For these methods, the section of samples is necessary and damage is inevitable during the process of sample preparation. The SS-OCT used in this study showed a remarkable capability in detection and quantifying microgaps under the restorations non-invasively.[27]

An SS-OCT was employed in this study. The early OCT imaging system was time-domain OCT (TD-OCT) in which a reference mirror is translated to match the optical path from reflections within the sample.[31,40] Afterwards, several researchers used different types of OCT systems for research and diagnosis of dental diseases, including periodontal diseases and early caries lesions.[33,34,41,42] Since Fourier domain OCT (FD-OCT) is no need for moving parts to obtain the axial scans, image scan speed of FD-OCT is faster than TD-OCT with better sensitivity and less noise.[40,43] FD-OCT has two types; Spectral domain OCT (SD-OCT) and Swept source OCT (SS-OCT). Although SD-OCT is better at stability of signal, SS-OCT is faster than SD-OCT in scan speed, which means less effectiveness against motion artifact, signal loss in depth is less. The SS-OCT is one of the most recent implements of the spectral discrimination, using a wavelength-tuned laser as the light source and providing improved imaging resolution and scanning speed.[40] Although an axial resolution of SS-OCT is 11 μm, the gap formation with a few micrometers can be detected. Because the Fresnel reflections at the gaps were detected even when the gaps were as small as half a micrometer in height, which is well below the SS-OCT axial optical resolution (11 μm) and vertical dimension of each image pixel (6.48 μm).[17,27,42] In this study, the bright clusters along the interface in OCT image was recognized as the gap formation. The observation of the resin-cavity interface by OCT is relatively new technique. Many previous study already reported relationship between the bright clusters and the gap formation comparing the image of crosscut surfaces by CSLM.[17,27,28,30] Those studies examined the tooth-resin restoration interface at the cavity floor of the restored teeth by SS-OCT and compared the findings with CLSM. Increased SS-OCT signal intensity along the interface corresponded well to the interfacial gaps detected by CLSM. Since the gap detection technique by OCT is thought to be established, another method was not used and compared in this study. SS-OCT is a high-resolution, cross sectional imaging technique that permits instant non-invasive imaging of the underlying defects in a biological system without any hazards, which makes it safe for the patients clinically.[27] The SS-OCT used in this study has a potential of clinical usage. For the future, gap formation of restoration will be able to be detected by SS-OCT clinically and it will improve quality of resin restoration in operative dentistry.

Bond strength studies on Er:YAG-lased tooth substrate reported in the literature are often confusing and contradictory.[35] Some studies reported the advantage of Er:YAG laser irradiation for bonding to dentin.[9,19,24] Also, Er:YAG laser was likely to improve the resistance of resin dentin interface to acid-base challenge.[44] However, others reported that the Er:YAG laser irradiation showed significantly lower bond strengths to dentin[12,13,15,21] or bond strength values obtained in bur-prepared samples were similar to those of Er:YAG laser values in terms of initial periods of evaluation.[8,18] Although the total-etch adhesive bonded significantly less effectively to lased than to bur-cut enamel and laser conditioning was clearly less effective than acid etching,[12,13] the self-etch adhesive performed equally to lased and bur-cut enamel surfaces.[12] There are many factors which affect bond strength and gap formation of lased enamel and dentin in the cavities; e.g. condition of laser irradiation (wavelength, output power, irradiation energy, and water supply), used adhesive and composite resin, restorative technique, and experimental design. The differences of those factors might result in varied results.

Two different types of two-step adhesive systems were developed from three step systems including etching, priming and bonding. One is the total etching adhesive combined priming and bonding and another is the self-etching adhesive system combined with etching and priming. Self-etching systems were further simplified to one-bottle one-step systems combined self-etching and bonding. Self-etching adhesive systems have been often argued about bonding to enamel.[7,10] Generally, the etching property of self-etching bonding systems is milder compared to total etching systems. CS was categorized as a “mild” self-etching adhesive[7] with pH 2.0 by the manufacture’s information. When bonding to enamel, an etch and rinse approach is preferred, with micro-mechanical interaction to achieve a durable bond to enamel. When bonding to dentin, a mild self-etching approach is superior.[7] In 8-years clinical study, CS showed excellent result using as selective enamel etching. Altogether, when bonding to both enamel and dentin, selective etching of enamel followed by the application of the 2-step self-etching adhesive to both enamel and dentin currently appears the best choice to effectively and durably bond to tooth tissue.[7] However, the manufacturer of CS does not recommend selective etching. Because the functional monomer; 10-methacryloyloxydecyl dihydrogen phosphate (MDP) contributes for both enamel and dentin with a strong chemical bond with the hydroxyapatite. The acidity of the self-etching primer is designed for the simultaneous treatment of both enamel and dentin layers in one step. Generally, etching to uncut enamel is necessary for self-etching primer adhesives.[10] In this study, there were no statistical differences between GIEs of CS-SLF and CS-SEE in both bur-prepared and laser prepared groups.

Recently, another type of one-bottle adhesive was launched, called “universal type” which can be applied as total etching, self-etching and selective enamel etching. In this study, one universal type adhesive was evaluated as self-etching and selective enamel etching comparing to an established two-bottle two-step self-etching adhesive system. SU is categorized as “ultramild”[7] with pH 2.7 by manufacture’s information. The SU demonstrated an uneven hybrid layer with clearly demineralized collagen bundles at dentin-resin interface by using a focused ionbeam milling and transmission electron microscope and was validated in terms of its capability in dentin adhesion.[25] Although etching did not affected the dentin bond strength of SU,[22,23] preliminary etching of enamel significantly increased bond strength for this new one-step multimode adhesive SU and two-step self-etching adhesive CS.[20] B-SU-SEE group showed less GIE than B-SU-SLF group. It is suggested that the selective enamel etching is recommended for the universal type adhesive. Although there were relatively large differences of mean value of GID in some groups, statistical difference was not found between GIDs of SLF and SEE groups. From those results, selective enamel etching would not hinder the bonding to dentin. Because of simple procedure and multi usage, universal type adhesives may be clinically useful and convenient, which should induce low technique sensitivity. It is noteworthy that only one product was evaluated in this study and it is difficult to generalize the result for all universal adhesives. Further study should be necessary about other universal type adhesives.

Within the limitation of this study, it could be concluded that a universal type adhesive showed comparable adaptability to tooth substrate prepared by bur and laser, and selective enamel etching improved adaptation to enamel in case of a universal type adhesive. A two-step self-etch adhesive showed better adaptation on lased dentin.

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