Comparison of the micro-tensile bond strengths of four different universal adhesives to caries-affected dentin after ER:YAG laser irradiation

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INTRODUCTION

The most common pathological change of dentin is dental caries. According to a previous study, carious dentin is typically identified by the presence of external (infected) and internal (affected) layers¹. The external layer, called the caries-infected layer, is spongy and infected by bacteria. This layer consists of a collagenous matrix, which is necrotic and non-remineralizable. The internal layer, which contains caries-affected dentin (CAD), shows little bacterial infection and is composed of a collagen matrix, with a regular cross-banded ultrastructure¹¹. This demineralized dentin layer can be rebuilt by remineralization. CAD is softer than unaffected dentin, as it is partly demineralized. CAD also has larger tubules than normal dentin, and these are obturated by mineral crystals³⁵. Nevertheless, these are bacteria free.

Although caries-infected dentin layers can be treated, CAD layers cannot³⁴. Thus, a huge area of the cavity floor, which is prepared for adhesive restoration, consists of CAD. Caries removal is generally performed by hand excavation, with or without low-speed burs. This type of caries removal technique can cause patient discomfort. Although pain may be reduced by local anaesthesia, fear of the needle and of the noise and vibration of mechanical preparation remain causes of anxiety. Moreover, the high and low rotating speed drills, used to achieve a complete decayed dentine removal, might lead to caries overexcavation with an increased risk (when caries involves the deeper dentine layers) of pulpal dental exposure or damage, or both.

Many methods of caries removal, such as chemical-mechanical removal and laser irradiation, have been introduced to try to avoid the aforementioned problems. The erbium:yttrium-aluminium-garnet (Er:YAG) laser has a wavelength of 2.94 mm, which is highly absorbed by water and hydroxyapatite. The absorption (i.e., transformation of thermal energy) and refraction properties of the Er:YAG laser results in minimal infiltration of dental tissue, with no underlying heating, as all the radiation is absorbed by the water surface⁹. Numerous laboratory and clinical studies have shown that the Er:YAG laser completely ablated hard dental tissue, with minimal harm to pulp and neighbouring structures. In addition to the efficient removal of dental tissue, research demonstrated that the use of the Er:YAG laser enhanced patient comfort⁵.

Cavities prepared with Er:YAG laser show irregular margins and rugged cavity walls and a rough floor, that differs from the essential principles of cavity preparation found by Black⁶. The surface asymmetry of laser-irradiated dentin appears well suited to adhesive restorations, although some reports have cast doubts on the bonding capacity of irradiated dentin⁷-⁹. Since the approval by the U.S. Federal Drug Administration in 1997 of the use of the Er:YAG laser for caries removal, in addition to cavity preparation and conditioning of tooth texture, there have been many reports on its use, in combination with composite materials⁹-¹².

Recently, a new type of one-step self-etch adhesive, classified as 'universal' or 'multi-mode', has been...
introduced that can be used with either etch-and-rinse or self-etch procedures\textsuperscript{10}. The dentist can use these universal adhesives with the so-called selective enamel etching method, which combines the benefits of the etch-and-rinse procedure on enamel with the simplified self-etch technique on dentin. This method provides additional chemical bonding on remnant carbonated apatite crystallites in those bonding substrates.

Adhesive bonding to CAD has been well studied\textsuperscript{14}. However, few studies have assessed the bond strengths of universal adhesives to CAD prepared with lasers\textsuperscript{15,16}. The aim of this in vitro study was to compare the microtensile bond strengths (micro-TBSs) of four different universal adhesive systems to CAD after Er:YAG laser irradiation. The null hypothesis tested was there would be no difference between four universal adhesives used for bonding to CAD with etch-and-rinse or self-etch procedures.

MATERIALS AND METHODS

Tooth selection

Twenty-four freshly extracted human molars with occlusal dentin caries were used in this study. The inclusion criteria were teeth with coronal caries lesions continuing at least half of the distance from the enamel-dentin junction to the pulp chamber. The lesions had to be surrounded by enough sound dentine to be used as control bonding sites. These characteristics were determined by radiological inspection. The extracted teeth were cleaned thoroughly to remove both hard and soft deposits. They were then stored at 4°C in a saline solution containing some crystals of thymol until used.

Laser irradiation

The carious tissue was removed using an Er:YAG laser system (Fotona, Ljubljana, Slovenia), with a laser wavelength of 2.94 µm. The power output was 3.5 W, the pulse period was 300 µs (short pulse mode), and the pulse repetition rate was 10 Hz. The irradiation beam was focused and operated at a distance of 1 mm (energy density: 44 J/cm²). For dentin irradiation, cylindrical quartz with a diameter of 1 mm was mounted on the R14 hand piece.

The treated area was constantly cooled using an air and water spray system. Caries tissue was irradiated until soft, and the demineralized dentin was removed. To distinguish caries-infected dentin from CAD, the dentin was removed using both visual and palpable examinations (probing with an explorer) and staining with a caries disclosing solution (Caries Detector, Kuraray, Osaka, Japan). Dark pink to red-colored dentin was considered caries-infected dentin. CAD was defined as dentin that was colorless to light pink, firm and opaque. The exposure of the CAD substrate, subsequent bonding and testing of all the specimens in this study were all performed by one operator to avoid interoperator variability. After the preparation of the samples, all the teeth were washed with distilled water and air dried.

Adhesive systems

The teeth were randomly assigned to four groups (n=20) according to the bonding system used: All-Bond Universal (Bisco, Schaumburg, IL, USA); Clearfil Universal Bond (Kuraray Noritake Dental, Tokyo, Japan); Prime & Bond One Select (Dentsply DeTrey, Konstanz, Germany); and Single Bond Universal Adhesive (3M Deutschland, Neuss, Germany). The composition, batch numbers and manufacturers of the adhesive systems are listed in Table 1. Each group was divided into two subgroups according to the bonding technique performed: the

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### Table 1  Materials used in the study

| Adhesive, batch, manufacturer | Classification according to pH | Composition of adhesive | Composite resin, batch |
|------------------------------|--------------------------------|-------------------------|------------------------|
| All-Bond Universal -1200011536 | Ultra mild (pH=3.1) | 10-MDP, BPDM, Ethanol, Bis-GMA, HEMA, Water, Initiators | AELITE All-Purpose Body 1400006253 |
| Bisco, Schaumburg, IL, USA | | | |
| Clearfil Universal Bond (500009) | Mild (pH=2.3) | 10-MDP, HEMA, Camphorquinone, Hydrophilic dimethacrylate, Water,silane | Clearfil Majesty Posterior 7H0009 |
| Kuraray Noritake Dental Products, Tokyo, JAPAN | | | |
| Prime&Bond One SELECT -1410000577 | Mild (pH=2.5) | Dipentaerythritol penta acrylate monophosphate, Polymerizable dimethacrylate and trimethacrylate resin, Diketon, Organic Phosphin Oxide, Stabilizers, Cetylamine hydrofluoride, Acetone, water | Ceram X Universal nano-ceramic 1507000529 |
| Dentsply DeTrey, Konstanz, Germany | | | |
| Single Bond Universal Adhesive -569482 | Mild (pH=2.7) | 10-MDP, HEMA, Dimethacrylate resins, Methacrylate-modified polyalkenoic acid copolymer, Filler, Ethanol, Water, Initiators, Silane | Filtek Z550 nanohybrid N477299 |
| 3M Deutschland, Neuss, Germany | | | |
self-etch technique or etch-and-rinse technique. The adhesives were applied to the cavities according to the manufacturers’ instructions and after restored with the selected manufacturers’ recommended resin composites materials [Aelite All-Purpose Body (Bisco), Clearfil Majesty Posterior (Kuraray Noritake Dental), Ceram X Universal nano-ceramic (Dentsply DeTrey), and Filtek Z550 (3M ESPE)]. All procedures and curing times were performed according to the manufacturers’ instructions (Table 1). All the teeth were then subjected to thermocycling for 2,500 cycles at a temperature of 5 and 55°C, with a dwell time of 10 s (THE-1100, SD Mechatronik, Feldkirchen-Westernham, Germany).

**Micro-TBS tests**
After the aging procedure, the teeth were sectioned into multiple 0.9×0.9-mm beams, using a low-speed saw (Mecatome T180 Presi, Eybens, France), with a diamond blade under water cooling. The micro-tensile test used the ‘non-trimming’ method. Two teeth were used for each bonding system. Ten beams were tested for each subgroup and each bonding agent. The composite-resin-dentin sticks that were obtained were subjected to tensile forces using a universal testing machine (MTD-500 plus, SD Mechatronik) at a crosshead speed of 1 mm/min until failure. A digital calliper (Model CD-6BS, Mitutoyo, Tokyo, Japan) was used to measure the cross-sectional area at the section of failure to the adjacent 0.01 mm. The micro-TBS was then calculated and recorded in MPa.

After the micro-TBS testing, to evaluate the type of failure, the fracture surfaces of all the specimens were inspected at ×50 magnification, using a stereomicroscope (SMZ-1000, Nikon, Tokyo, Japan). Failures were classified as adhesive (interfacial failure); cohesive dentin failure, cohesive resin failure (including failures either within the resin composite or adhesive layer); or mixed.

**Scanning electron microscopy (SEM) observation of resin-dentin interfaces**
Ultrastructural observations of representative resin-dentin interfaces were examined by a SEM. Since teeth with similar caries depths were choosen according to the radiographic images when choosing the teeth for the study, 1 sample image supporting the test results of micro-TBS among 3 SEM analysis samples was selected for each group. Bonded specimens of each group were stored in distilled water at 37°C for 24 h, embedded in self-curing acrylic resin (Panacryl, Inci Dental, Istanbul, Turkey), and stored at 37°C for a further 24 h.

Teeth to be subjected to SEM analysis were sectioned vertically using a water-cooled low-speed diamond blade on a cutting machine (Mecatome T180, Presi). Each of the bonded cross-sections along the cut surface were polished with P1200, P2000, and P2400 SiC paper using a grinder-polisher (Minitech 233, Presi) The teeth were sonicated in distilled water for 30 s to remove any superficial debris created during the cutting and polishing procedures. The cut surfaces were exposed to 5 N HCl for 30 s followed by rinsing with distilled water to partially remove the mineral part of the dentin. They were then immersed in 5% NaOCl solution for 5 min and washed with distilled water to dissolve all collagen dentinal tissue. Finally samples were dehydrated with increasingly concentrated ethanol solutions, mounted on metallic stubs, and sputter-coated with gold. They were inspected under SEM at ×1,500 magnification.

**Statistical analysis**
Statistical analyses were performed using Sigma Plot Software (Ver. 11.0, SPSS, Chicago, IL, USA). The micro-TBS data of the tested groups were statistically analysed using Kruskal-Wallis and Mann-Whitney U nonparametric tests. In all the tests, the level of statistical significance was considered as $p<0.05$.

**RESULTS**
The results of the micro-TBS test in different groups are shown in Fig. 1. There were statistically significant differences in the micro-TBS values between the groups.
Comparing the tested adhesive systems, in the etch-and-rinse procedure, Clearfil Universal Bond yielded the highest bond strength, whereas All Bond Universal yielded the lowest bond strength. In the self-etch procedure, the highest value was obtained using Single Bond Universal, and the lowest value was obtained using Prime & Bond One Select. The bond strength values of the Prime & Bond One Select and Single Bond Universal systems were similar in both etch-and-rinse and self-etch applications.

The fracture modes of the groups are shown in Fig. 2. There were significant differences in the fracture modes of the study groups (p<0.05). Most of the fractured samples showed a mixed failure type. Minimal dentin
cohesive failure was observed in all the groups.

Figure 3 presents the SEM assessment of the interaction pattern between dentin and resin after cavity configuration using Er:YAG laser followed by application of different universal adhesives [Clearfil Universal Bond (CU), All-Bond Universal (AU), Prime&Bond One Select (PU) and Single Bond Universal (SU)] in different bonding techniques. For AU, when AU applied in both etch-and-rinse and self-etch procedures, we can not notice signs of hybridization and the thickness of the hybrid layer was irregular along the bonding interface. There were gap formations between resin and dentine as well (Figs. 3A, a). A hybrid layer was rarely detected at the resin-dentine interface when adhesive was applied to the Er:YAG-irradiated dentine for CU. The cavity surfaces exhibited irregularities due to Er:YAG laser irradiation. Gap formation was also visible at resin-dentine interfaces when CU applied in both etch-and-rinse and self-etch procedures (Figs. 3B, b). For PU adhesive when applied in self-etch procedure, there was a gap formation between resin-dentine interface. Fractured resin tags and irregular thickness of hybrid layer were also observed (Fig. 3C). On the other hand, when PU applied in etch-and-rinse procedure, the resin tags were directly connected with the composite resin and there was no visible hybrid layer. Irregular dentin surface due to laser irradiation was seen (Fig. 3c). For SU in self-etch procedure, funnel shaped configuration of the resin tags at their base were visible and the resin tags were directly connected with the composite resin. No hybrid layer was seen (Fig. 3D). It was possible to verify an irregular dentin appearance, suggesting characteristics of ablation and melting for SU in etch-and-rinse procedure. There was gap formation between resin and dentine but no resin tag and hybrid layer formations were observed (Fig. 3d).

**DISCUSSION**

This in vitro study compared the micro-TBSs of four universal adhesives to CAD, following caries removal using an Er:YAG laser. The results of this study did not support the hypothesis that there would be no differences among the bond strength values of universal adhesives when applied using etch-and-rinse and self-etch strategies. The null hypothesis was rejected because there were statistically significant differences between the adhesive systems tested.

With regard to caries removal, dentin is a living tissue, which responds to changes in its environment. Reactions to conventional cavity preparation using burs can cause local neurogenic inflammation of pulp. To prevent this problem, clinicians require alternative caries removal techniques. As the Er:YAG laser is presently accepted as the preferred method for cavity preparation, the present study applied irradiation using an Er:YAG laser. A previous study demonstrated that the Er:YAG laser could be safely applied to dental pulp and nearby tissues as the irradiated dentin area showed little heat increase (<5.5°C)\(^{17}\). In addition, research showed that the irradiation had a disinfectant effect on dental tissues\(^{19}\). Irradiation by Erbium lasers was also shown to alter the chemical configuration of the dental structure, producing acid-resistant surfaces and reduced susceptibility to secondary caries. Tachibana et al.\(^{19}\) prepared the sample as flat dentin surface containing a central zone of caries-infected dentine surrounded by sound dentine, then they have polished with wet 600-grit silicon carbide paper. Contradictory to them, in our study, the carious tissue was removed using an Er:YAG laser to fully reflect clinical conditions. Therefore, a control group is not needed in our study.

According to previous research, the application of the Er:YAG laser produced changes in the configuration and content of the organic matrix at the dentin surface, culminating in limited collagen degradation and 3–5 µm of denatured dentin at the subsurface\(^{20}\). The same study reported that a hybrid layer formed in the presence of collagen preservation and that the monomer infiltration would not occur in the absence of such preservation\(^{20}\).

Another study reported that in the presence of denatured collagen fibrils and no cross-banding, insufficient resin diffused into the inter-fibrillar collagen spaces, thus compromising the bond strength\(^{21}\). As indicated by Cardoso et al.\(^{22}\), noticeable irregularities on a lased dentin surface appeared to decrease the bond strength by preventing homogeneous stress distribution at the adhesive-dentin interface. Furthermore, the existence of irregularities on the dentin surface resulted in non-uniform thickness of the adhesive layer, thereby reducing the effectiveness of the bonding. In the present study, as shown by the results of the SEM analysis, irregularities in the dentin surface were observed after the preparation of the cavity with the Er:YAG laser (Fig. 3). These may explain why the bond strength values obtained were low and compatible with those in the study by Cardoso et al.\(^{22}\). The results of in vitro studies of dentin bonding agents applied to sound dentin can generally be considered reliable. However, the findings of in vivo studies of CAD may be unreliable. As reported earlier, changes in the chemical and morphological characteristics of CAD can also explain lower bond strengths, with the bond strength to CAD significantly lower than that of normal dentin\(^{23}\).

The micro-tensile test was selected for the bond strength test in this study. Because, in micro-tensile test, further specimen processing or the actual preparation of the micro-specimens is required after the bonding procedure. Advantages are that it involves better economic use of teeth (with multiple micro specimens originating from one tooth), the better control of regional differences (e.g. peripheral versus central dentin) better stress distribution at the true interface, ability to test irregular surfaces and very small areas and facilitates microscopic examinations of the failed bonds due to smaller areas\(^{24}\).

In the present in vitro studies, the efficiency of the universal adhesives was dependent on the adhesive technique used. All the tested bonding systems could readily be used with both the etch-and-rinse and self-
etch strategies. The dissimilarities in the composition of the adhesives might explain their difference in their bond strengths, as determined by the in vitro studies. Other than the different application strategies, the components of the adhesives might play a critical role in the bond strength of the dental material (Table 1). Materials containing 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) bond chemically to dentin \(^{25}\). Yoshida et al.\(^{26}\) reported that a chemical interaction between MDP and hydroxyapatite resulted in the formation of a durable nano-layer, which resulted in stronger bonding at the adhesive interface, thereby increasing the mechanical strength of the adhesive interface. Studies also reported that MDP-Ca salt deposition, together with nano-layering, resulted in high bond stability \(^{26,27}\). The latter was proven in both in vitro and in situ experiments \(^{28-30}\).

In this in vitro study, the Er:YAG laser was applied for caries removal. Following the application of universal adhesives to CAD, the bond strength values were calculated. There were statistically significant differences among the tested groups. In the etch and rinse groups, the highest value was obtained using the Clearfil Universal Bond adhesive, and the lowest value was obtained using the All-Bond Universal. Due to their ethanol and acetone contents, the micro-TBS values of other universal bonding agents may be lower than those of the Clearfil Universal Bond adhesive when applied using the etch and rinse procedure. Unlike the other three universal adhesives, Prime Bond Select does not contain 2-hydroxyethyl methacrylate (HEMA) and contains acetone as solvent. Water is comparatively easy to remove with the use of an acetone-based adhesive because acetone contains more water and has a high vapour pressure. Luque-Martinez et al.\(^{31}\) concluded that entrapment of residual water in the resin-dentin interface compromised the performance of universal adhesives and that water elimination might be improved by extended solvent evaporation times. In the present study, as the surface of the irradiated CAD was more irregular than that of sound dentin, the residual water may not have been completely removed. The latter may have resulted in lower micro-TBS values (Figs. 3C, c). The low bond strength values could also be explained by the formation of gaps in the adhesive area, as observed in the SEM findings, in addition to irregular dentin formation (Figs. 3a, C). Moreover, the hybrid layers in CAD are thicker than those of normal dentin because CAD is more exposed to acid etching due to partial demineralization, resulting in the formation of a deeper demineralized zone \(^{21,32,33}\).

Prime Bond Select contains acetone as solvent, whereas both All Bond Universal and Single Bond Universal Adhesive contain ethanol. Tezvergil et al.\(^{34}\) concluded that acetone- or ethanol-based adhesive mixtures resulted in increased solvent retention (between 4.9 and 13.2%) due to the increased hydrophilicity of adhesive monomers and that the retention increased further (26.4–41.6%) when water was added to simulate wet bonding conditions. Another study demonstrated that these residual solvents could further compromise cross-linking reactions during polymerization, affecting the ultimate tensile strength of the resin, as well as that of the hybrid layer \(^{35}\). The aforementioned factors may explain the low micro-TBS values in the present study.

Single Bond Universal Adhesive also contains polyalkenoic acid copolymers. Both HEMA and polyalkenoic acid copolymers reportedly compete with 10-MDP for calcium coordination sites on the surface of apatite crystallites, resulting in markedly reduced nanolayering of 10-MDP calcium salts within the resin-dentin interface \(^{36}\). In the present study, the bond strength of the Single Bond Universal Adhesive did not differ according to the bonding technique. These results are consistent with those of our pilot study. In the pilot study, CAD was prepared with a laser, and Single Bond Universal Adhesive was used as a universal adhesive. In the study, there was no difference in the bonding strength when the adhesives were applied using different procedures \(^{37}\). The results obtained using the Single Bond Universal Adhesive were also compatible with those of Munoz et al.\(^{38}\). Munoz et al. assessed the immediate bonding strength in sound dentin following the application of the Single Bond Universal Adhesive using the same two procedures. Based on these results, it can be suggested that Single Bond Universal Adhesive can be applied to both sound dentin and CAD irradiated using a laser.

According to in vitro studies \(^{39-42}\), prior acid etching of mild universal adhesives improved the bond strength to enamel but not to dentin. All-Bond Universal Adhesive was shown to provide better dentin bonding when applied using the etch-and-rinse technique, and it was reported to be ultra-acidic. In a previous study, All-Bond Universal was the only universal adhesive that resulted in an improvement in bond strength when applied using the etch-and-rinse technique \(^{43}\). The improved bond strength was probably due to the ultra-mild acidity (pH=3.1) of the adhesive, which was unable to ‘condition’ and ‘prime’ the dentin substrate \(^{44}\). However, in the present study, the bonding strengths of the All-Bond Universal (ultra-mild) and Single Bond Universal (mild) adhesives to dentin were lower when applied using the etch-and-rinse technique than when using self-etch technique. In a systematic review, Oliveira da Rosa et al. demonstrated that prior acid etching did not influence the dentin bond strength of universal, mildly acid adhesives. However, the acidic monomer content can affect the bond strength. Thus, to ensure bond stability, a mild self-etch adhesive is currently recommended for adhesion to dentin \(^{45}\).

In the present study, the dentin bond strength of the universal adhesives, which are classified as mild adhesives, did not differ according to the bonding technique that was used (i.e. etch-and-rinse vs. self-etch). The relatively superficial interaction of these adhesives with the dentin substrate without prior phosphoric acid etching might reduce the risk of post-operative sensitivity and the possibility that collagen fibrils will
undergo degradation, which could compromise the bond stability over time.\textsuperscript{46}

Bertrand et al.\textsuperscript{17} reported that when the dentin surface was irradiated using an Er:YAG laser and then etched with orthophosphoric acid before bonding, surface demineralization allows a hybridization process. These surface treatments remove a small layer from the surface, so they may eliminate the possible drawbacks related to Er:YAG laser surface alterations. In the present study, the SEM results showed that the smear layer was removed and that a hybrid layer formed. However, the thickness of the hybrid layer was irregular along the bonding interface. The results are incompatible with the previous study.

According to previous in vitro studies\textsuperscript{13,48,49}, the performance of universal adhesives depended on the adhesive technique. All new adhesive systems can be used with both etch-and-rinse and self-etch approaches. The differences in their compositions might explain the differences in their bond strengths, as proposed in earlier in vitro studies\textsuperscript{13,48,49}. The results of this in vitro study of Er:YAG laser irradiated CAD suggested that the performance of the adhesives depended on the composition of the adhesives and the application methods. The findings are consistent with those in the literature.

Future studies are required to analyse the effect of long-term water storage on the in vitro performance of universal adhesives. Additionally, studies of different substrates, such as carious dentin, and in vivo tests are needed to assess the long-term clinical behavior of these new universal adhesives.

CONCLUSION

Within the limitations of the present study, there is no control groups because in this study we cleaned the caries with laser without creating flat dentin to simulate completely clinical practice and as a minimally invasive procedure, the cavity was confined to only the bruised area. So, it was hardly possible to get sound dentin with CAD after cleaning the caries in the same cavity. We conclude that the caries removal method and universal adhesives applied in etch-and-rinse and self-etch technique, affected the bonding strength to CAD. Further studies with a greater number of samples are needed to confirm the results of the present study. After the caries irradiated with laser, universal adhesives containing MDP can be preferred as bonding agents since they have higher bonding strength. The findings of this study can aid the selection of bonding systems for individual patients and dental practices.

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