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Er:YAG Laser in Operative Dentistry: Keys for successful treatment

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Dedicated to the light shining in my life, my young family,

my beloved wife Dr. Mona Aly Abbassy

and to my little Angel my beloved daughter

Farida Ahmed Bakry

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Preface

This work is based on the original research works by the authors, to which the following articles refer:

Chapter 1: Bakry AS, Sadr A, Takahashi H, Otsuki M, Tagami J.Analysis of Er:YAG lased dentin using attenuated total reflectance Fourier transform infrared and X-ray diffraction techniques. Dent Mater J. 2007 May;26(3):422-8.

Chapter 2: Bakry AS, Sadr A, Inoue G, Otsuki M, Tagami J. Effect of Er:YAG laser treatment on the microstructure of the dentin/adhesive interface after acid-base challenge. J.Adhes Dent. 2007 Dec;9(6):513-20.

Chapter 3: Bakry AS, Nakajima M, Otsuki M, Tagami J. Effect of Er:YAG Laser on Dentin Bonding Durability Under simulated Pulpal Pressure. J.Adhes Dent. Accepted

INTRODUCTION

Different types of lasers have been applied in dentistry for cavity preparation and caries removal, however, Er:YAG Laser has proved its feasibility to be used for dental cavity preparation when compared to other types of lasers. The use of Er:YAG laser with proper parameters for cavity preparation causes no harmful effects on the pulp tissue, however, literature provide contradicting results regarding the quality of dentin resulting from Er:YAG laser ablation. Moreover the interaction between the cavities' walls prepared by Er:YAG laser and the restorative materials raises many questions regarding the reliability of this technique for cavity preparation.

Chapter 1 is going to discuss the basic chemical changes induced by different parameters of Er:YAG laser on the dentin, moreover the most suitable Er:YAG laser parameter associated with minimal chemical changes is going to be recommended.

Chapter 2 is adopting the Er:YAG laser parameters recommended in chapter 1 and is going to use the acid/base challenge to test the durability of the interface between Er:YAG lased dentin and SE:Bond by using different durations of challenge.

Chapter 3 is going to investigate the effect of pulpal pressure on the durability of the interface between the Er:YAG lased dentin and SE-Bond.

Chapter 4 is going to give some general conclusions based on the aforementioned chapters.

CHAPTER 1

ANALYSIS OF ER:YAG LASED DENTIN USING ATTENUATED TOTAL REFLECTANCE FOURIER TRANSFORM INFRARED AND X-RAY DIFFRACTION TECHNIQUES

Introduction:

Different types of lasers have been applied in dentistry for cavity preparation and caries removal, however, the use of some types of laser like CO_2 and Nd:YAG lasers for cavity preparation has long been plagued by undesired effects, such as damage of hard tissue and necrosis of tooth pulp. These effects are a result of excess heat production during laser treatment.[1-3]

Erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser — with a wavelength of 2.94 μm can remove dental caries together with sound enamel and dentin[4]. This laser is efficiently absorbed by the intrinsic water and hydroxy group of apatite mineral in dental hard tissues. The heating expands and builds up pressure until a micro-explosion occurs, and then a small portion of tissue is removed. This phenomenon is called thermally induced mechanical ablation[5].

Er:YAG laser was found to cause no significant effect on the tensile bond strength of a self-etching system to enamel[6]. Moreover, Er:YAG laser showed less penetration in dentin and had less effects on the ultimate tensile bond strength of dentin when compared to diode laser or CO_2 laser[7]. At this juncture, it must be pointed out that although

available literature has reported on various aspects and parameters about Er:YAG laser, it remains to be clarified whether the application of Er:YAG laser is really effective in improving adhesion to bonded restorations[8].

A previous research work[9] showed that Er:YAG laser treatment could induce some chemical changes on treated dentin surface, depending on the duration time of Er:YAG laser pulse. It was also suggested that there is a need to develop a new class of restorative materials with optimal interaction properties with laser-treated dentin surface[9]. To develop such restorative materials and to determine the most suitable parameters for Er:YAG laser application on dentin, an extensive research work should be carried out analyzing the chemical compounds and changes in crystalline structure of dentin after Er:YAG laser irradiation.

The attenuated total reflectance Fourier transform infrared (FT-IR/ATR) spectroscopy technique is often applied for the chemical characterization of restorative materials[10-13] and dentin[14], since it is an effective nondestructive technique. Besides, X-ray diffraction (XRD) is considered as one of the most important tools for investigating the crystalline structure of solid materials[15]. The purpose of this study, therefore, was to investigate the chemical characteristics of dentin after Er:YAG laser irradiation, using various output energies and under wet or dehydrated condition, by means of FT-IR/ATR and XRD.

Materials and Methods:

Dentin tablets preparation

Ten freshly extracted, non-carious, third molars were used in this study. They were hand-scaled, cleaned, and stored in saline solution at 4°C. Disks of 3 mm thickness were obtained from mid-coronal dentin by cutting parallel to the occlusal surface of the teeth using a water-cooled diamond saw (1600 Microtome, Leitz, Wetzlar, Germany). The enamel of each disk was removed using a plain-cut tungsten carbide fissure bur. Each disk was then cut into two halves: one half was dehydrated in ascending grades of ethanol (25%, 50%, 75% for 20 minutes, 95% for 30 minutes, and 100% for 60 minutes)[16], and then left to dry in a desiccator. The other half was stored in distilled water.

All specimens were crushed and then pulverized into powder using a mortar and pestle. Hydrated and dehydrated dentin powders were set apart and labeled, and then each powder was pressed into a plastic mold under a load of 245 N for 30 seconds to form 15 tablet-shaped specimens of hydrated and dehydrated dentin with a diameter of 10 mm and a thickness of 5 mm. For FTIR/ATR analysis, the hydrated dentin tablets were distributed randomly into Groups I, II, and III, while the dehydrated dentin tablets were distributed randomly into Groups IV, V, and VI, with five tablets in each group.

Table 1: Er:YAG irradiation conditions on dentin

Condition	Control	Hydrated specimens			Dehydrated specimens		
		Group I	Group II	Group III	Group IV	Group V	Group VI
Laser output power (mJ)	–	100	200	250	100	200	250
Water irrigation	–	+	+	+	–	–	–

Laser treatment of dentin tablets

The dentin tablets were subjected to laser treatment using an Er:YAG laser system (Elfine 400, Osada, Tokyo, Japan) with wavelength of 2.94 μm, pulse energy of 10–400 mJ, pulse frequency of 1–25 pps, and pulse duration of 300 μsec. The contact probe had a sapphire tip of 0.63 mm diameter, and the contact tip was applied perpendicular to the dentin tablets in a sweeping motion with the lased dentin spots overlapping each other. This was to ensure effective application of Er:YAG laser to all parts of each lased dentin tablet. Irradiation conditions of the dentin tablets are summarized in Table 1. Any signs of thermal damage, *i.e.*, carbonization[17] or change of dentin powder color, were recorded by the operator.

FT-IR/ATR analysis

In-order to obtain the Infrared spectra of the Er:YAG irradiated and the non irradiated (control) dentin powders, a small amount of the dentin powder was collected before the Er:YAG irradiation and served as the control. As for the Er:YAG irradiated specimens a very small amount of the remaining irradiated dentin tablet was scraped gently using the

tip of a scalpel blade to avoid contaminating the sample with non-irradiated dentin powder particles[18].

Infrared spectra of the control and Er:YAG laser irradiated dentin powders mentioned above were obtained using a FT-IR spectrometer (FTIR-8300, Shimadzu, Kyoto, Japan). This was done by pressing the collected dentin powder onto the face of a diamond of an ATR attachment (DuraSamplIR II Smiths Detection, Danbury, CT, USA). Spectra were obtained under the following conditions: multiple reflections, 650–4,000 cm^{-1} range, 4 cm^{-1} resolution, and entrance angle of 45°.

Spectra of the samples after Er:YAG laser irradiation were checked for appearance of new bands and changes in the height of band peaks. For each group of powder, the intensity peak of amide I that represents one of the organic contents peaks was compared to the carbonate intensity peak which is one of the inorganic components of dentin (*i.e.*, amide I/carbonate intensity ratio), and the carbonate peak will be compared to the phosphate peak intensity which is one of the major inorganic components of dentin (*i.e.*, carbonate/phosphate intensity ratio). These two intensity ratios were observed to identify possible changes that might take place in the chemical structure of dentin after Er:YAG laser irradiation.

XRD analysis

Crystalline phases of dentin powder before (control) and after Er:YAG irradiation were examined using an X-ray diffractometer (RAD IIA,

Rigaku Denki, Tokyo, Japan) with CuKα radiation and Ni filter. Scanning range was 20° to 50°, with a scanning speed of 4° per minute.

Statistical analysis

A comparison was made among the average intensity ratios of amide I/carbonate and carbonate/phosphate before and after Er:YAG irradiation. All data were analyzed using one way ANOVA, and Dunnett's t-test was used to compare the control group (non-irradiated dentin) against all the other Er:YAG laser-irradiated groups ($p \leq 0.05$).

Results:

FT-IR/ATR analysis

The infrared spectrum of Er:YAG laser-irradiated dentin powder showed no extra peak formation at all irradiation conditions used in this study when compared to the control (non-irradiated) dentin powder. The IR spectrum of the control (non-irradiated dentin) is shown in Fig. 1(a): orthophosphate group peak — which is one of the major inorganic components of dentin — was observed at 1,000 cm^{-1} (between 1,030 and 1,150 cm^{-1}), amide I peak at 1,650 cm^{-1} (between 1,680 and 1,600 cm^{-1}), amide II peak at 1,550 cm^{-1} (between 1,580 and 1,480 cm^{-1}), amide III peak at 1,250 cm^{-1} (between 1,300 and 1,200 cm^{-1}), carbonate peak which is one of the minor inorganic components of dentin was observed at 1,400 cm^{-1} (between 1,560 and 1,410 cm^{-1}), and hydroxy group (OH) peak at 3,400 cm^{-1} (between 3,600 and 2,400 cm^{-1}).

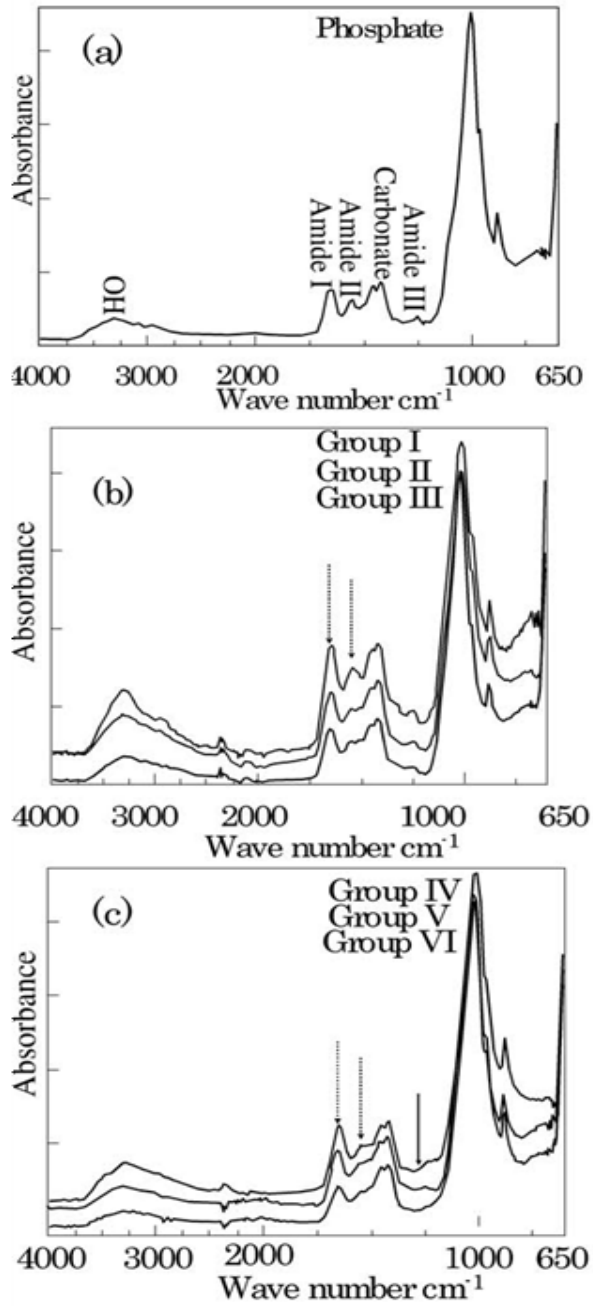

Fig. 1-1 FTIR spectroscopy profiles of: (a) Control; (b) Groups I, II, and III; (c) Groups IV, V, and VI. Dotted arrows show weakening of the relative intensities of Amide I and II peaks, while solid arrow shows disappearance of Amide III peak especially in Group VI.

The IR spectra of Er:YAG laser-irradiated hydrated dentin powders of Groups I, II, and III are shown in Fig. 1(b). Amide I peak was well defined in specimens of Group I (Fig. 1(b)), while relative intensity peaks of amide I and II decreased slightly in specimens of Groups II and III (Fig. 1(b)).

Figure 1(c) shows the IR spectra of Er:YAG laser-irradiated dehydrated dentin powders of Groups IV, V, and VI. The relative intensity peak of amide I slightly decreased in Group IV, *versus* a significant decrease in the relative intensity of amide I peak in Group V. Group VI showed a more accentuated decrease in amide I peak, with a severe depletion in the relative intensities of amide II and III. Signs of thermal damage, *i.e.*, carbonization, started to appear on the irradiated dentin powder of Group IV, and increased gradually on the irradiated dentin powders of Groups V and VI.

Ratios of the peak intensities of the different dentin components — amide I/carbonate and carbonate/phosphate — were compared among the control and test groups to evaluate the effects of Er:YAG laser irradiation conditions on dentin, as shown in Fig. 2. One-way ANOVA and Dunnett's test revealed that there were no statistically significant differences among the ratios of the peak intensity of carbonate/phosphate, although there were some deviations in the observed results which might be attributed to variation in the degree of mineralization of the samples used. However, the ratios of amide I/carbonate for Groups V and VI were significantly lower than that of control.

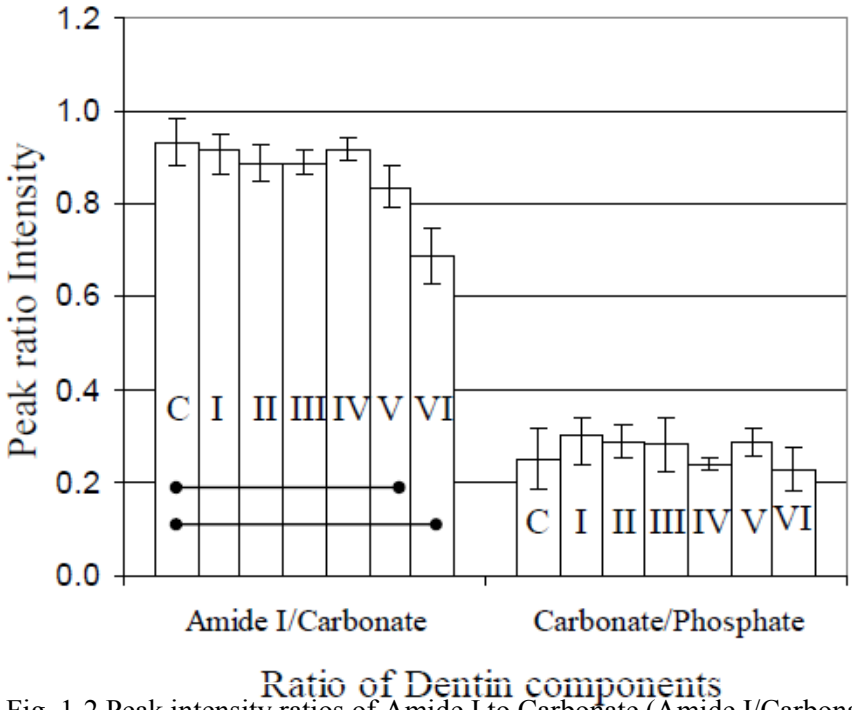

Fig. 1-2 Peak intensity ratios of Amide I to Carbonate (Amide I/Carbonate) and Carbonate to Phosphate (Carbonate/Phosphate), where: C—Control; I—Group I; II—Group II; III—Group III; IV—Group IV; V—Group V; and VI—Group VI. Connected columns are significantly different statistically ($p<0.05$).

XRD analysis

Figure 3 shows the XRD patterns of control (non-irradiated) and Er:YAG laser-irradiated dentin powders. Apatite peaks of (002) and (112) lattice planes were located at $2\theta=25.8°$ and $2\theta=32.2°$ [15] respectively. After irradiation, there were minor changes in the peaks (38.1º, 40º, 44.4º, 46.5º, 49.5º), together with a very slight shifting of the (112) peak. However, the XRD patterns of β-$Ca_3(PO_4)_2$ were not clearly observed among the XRD patterns of all specimens —

regardless of Er:YAG laser output energy used or dehydration condition.

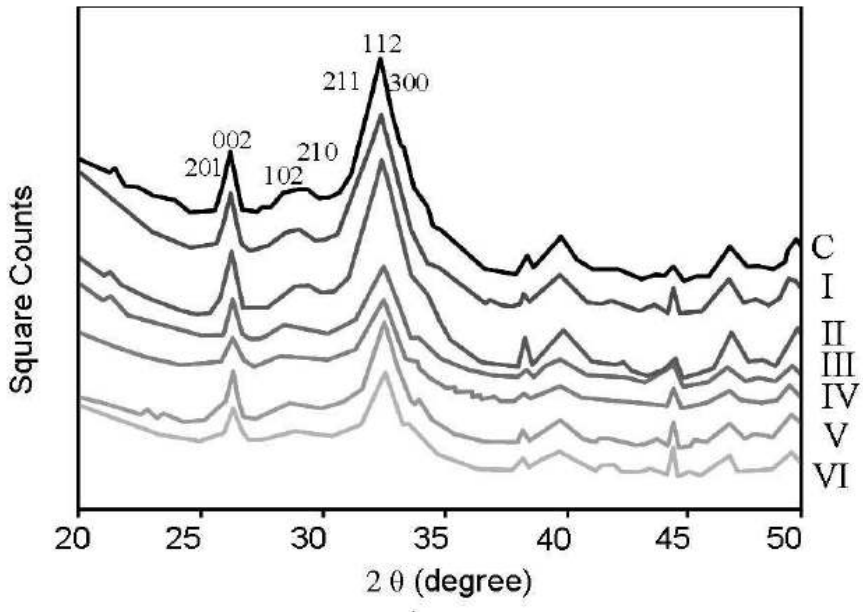

Fig.1-3 X-ray diffraction patterns of: C—Control; I—Group I; II—Group II; III—Group III; IV—Group IV; V—Group V; and VI—Group VI.

Discussion:

The complementary use of FT-IR/ATR with XRD technique in the present study yielded important information on the chemical interactions occurring in dentin structure when irradiated with Er:YAG laser. The FT-IR/ATR technique can provide information on the organic and inorganic molecular structures of laser-irradiated hard tooth structure[19], while XRD is the most important tool for characterizing changes in the solid phase[15]. With increase in output energy and when

water irrigation was not used, intensities of the organic peaks of dentin weakened. However, no obvious phase changes were noticed between the XRD pattern of control dentin powder and those after Er:YAG laser irradiation.

With FT-IR/ATR technique, the infrared beam of the spectrometer penetrates a very short distance beyond the diamond on which the specimen is pressed. This penetrating beam is called the evanescent wave and its penetration depth typically reaches a few micrometers. Since the intensity of the infrared beam is reduced (attenuated) by the tested sample, it is necessary to ensure the close adaptation between sample and diamond ATR of the spectrometer[20], which presented a difficulty in the current experiment, since Er:YAG lased specimens possess a highly rough surface. However, since FT-IR/ATR can easily provide the infrared spectra of powdered samples, dentin powder was alternatively tested in this experiment. Against this background, dentin powder was compressed into tablets to prevent the powder from rising up or scattering during Er:YAG laser irradiation. In this manner, it was easy to scrap off the remaining Er:YAG laser-irradiated dentin powder for FT-IR/ATR analysis.

Moreover, it was previously reported that the size and roughness of test particles influenced the peak heights of the infrared spectra[21],and since it was difficult to standardize the size of test particles in the current study, direct quantitative comparison based on the height of each peak was not reliable. Against this background, a relative comparison between peak ratios was carried out instead.

Infrared spectra of specimens in Group I did not show obvious signs of thermal damage, hence there were no statistically significant differences in their peak ratios compared to the control group. These findings could be explained by the fact that the heat induced in the tissue by Er:YAG laser was not high due to the relatively low output energy, coupled with the use of a water-cooling spray in Group I[22]. These findings agreed with those of Sasaki *et al.*[18], whereby it was reported that the infrared spectra of dentin irradiated by Er:YAG laser using an output energy of 40 mJ/pulse were similar to those of non-irradiated dentin. Further, the results of the present study agreed with those of Shigetani *et al.*[23], whereby laser scanning microscope observation revealed that the appropriate laser output for dentin caries removal was 100 mJ/pulse.

When the output energy of Er:YAG laser was increased, intensity of amide II peak in Groups II and III weakened. These results complemented the micro-Raman spectroscopy findings of a previous study[9], whereby it was reported that dentin collagen components were modified when Er:YAG laser was used at 350 mJ/pulse coupled with short pulse duration and water irrigation.

For groups without water irrigation, *i.e.*, Groups IV, V, and VI, signs of thermal damage such as carbonization of dentin powder increased gradually with increase in output energy, thereby suggesting an increase in the temperature of dentin powder in these groups. Moreover, the increase of output energy affected the organic dentin portion — whereby this effect was observed as a slight decrease of

amide I, II, and III peaks. It should be noted that amide I peak might be overlapped by OH peak at 1630 cm^{-1} [24]. However, control dehydrated samples in this experiment did not show weakening of the peak intensity at 1,650, but rather at 3,400 cm^{-1}. Further, previous research work[25] showed that amide III peak of bovine dentin was affected by an increase in the output energy of Er:YAG laser irradiation and with no water irrigation.

Changes detected in the organic components in this experiment might be explained as follows. Absorbed Er:YAG laser radiant energy in the hydroxyapatite crystals of dentin increased because of the absence of water irrigation. As a result, dentin temperature might have been raised higher than 60°C a temperature at which collagen transforms from highly ordered helices to amorphous gelatin[26].

Previous research work[27] showed that using of Er:YAG laser with parameters of 30 mJ/pulse and 10 pps the root temperature increased under dry condition to 66.5 ^{o}C, while that under wet condition remained at 28.6^{o}C. Moreover, it was reported that the calcified collagen molecules in dentin might break down at approximately 175^{o}C, however changes in collagen structure, which might occur with such heating and dehydration, can be reverted to natural conformation after rehydration provided that the surface temperature did not increase beyond 175^{o}C[28]. The increased temperature together with the dehydrated condition of dentin might have been the cause of the more detectable changes in the organic component of specimens in groups IV,

V and VI compared to groups I, II and III, that were using the same output energies with water irrigation.

Statistical analysis of the peak ratios showed a significant decrease of amide I/carbonate peak ratio in Groups V and VI compared to the control (non-irradiated dentin). This decrease was probably not due to the increase of carbonate content, but rather due to the decrease of amide content[18]. Moreover, none of the groups showed a statistically significant difference in peak ratio of carbonate/phosphate compared to the control group. Therefore, it was speculated that heat effect induced by Er:YAG laser in Groups V and VI was high enough to cause a significant decrease in the amide I component of dentin. However, this occurred without affecting the carbonate band which has been reported to show significant loss from dentin at 700°C and totally vanish from dentin at 1,000°C[29].

It has been suggested that correlation between the results of such *in vitro* experiments and the results obtained *in vivo* needs discerning interpretation and discrete comparison. This is because when dentin tissue is irradiated *in vivo*, the maximum temperature rise in the superficial and sub-superficial layers would be lower than that during *in vitro* irradiation. This lower temperature arises from the large thermal dissipation that occurs in the tissue when the tooth is whole and inserted in the oral cavity. By inference, the effects observed in this work which were a function of the temperature produced by laser irradiation would happen in the same form but with a lower intensity *in vivo*, assuming that a major thermal dissipation occurs *in vivo*[25].

X-ray diffraction analysis was used in this study to detect any phase change in the crystalline structure of dentin. The selected range of observation was from 20° to 50°, and which included the strong peaks of 002, 211, 112, 300, and 202, as well as the weaker 201, 102, and 210 reflections of hydroxyapatite crystals. However, due to the poorly crystalline structure of dentin apatite, only the peaks of 002 and 211 were clearly identified and used to compare the phase changes of dentin before and after Er:YAG irradiation[15]. There were minor changes in peaks (38.1º, 40º, 44.4º, 46.5º, 49.5º) after irradiation, together with a very slight shifting of (112) peak. These changes might be attributed to the rough Er:YAG lased surface of the samples, which might have caused such minor changes in peak positions.

However, there was no clear change in the crystalline structure of dentin after Er:YAG irradiation in any group. These results were in agreement with the IR results that no detectable change in inorganic peak ratio was found for any group. Carbonated hydroxyapatite in dentin changes into the less soluble hydroxyapatite at 350°C. It recrystallizes to β-$Ca_3(PO_4)_2$ from 650°C to 1,100°C, and if heated above 1,100°C recrystallization into α-$Ca_3(PO_4)_2$ will occur[30, 31]. In the present study, the peaks of β-$Ca_3(PO_4)_2$ were not clearly observed. Therefore, it could be suggested that the temperature of Er:YAG lased dentin did not reach 650°C. As a result, the crystalline structure of dentin was stable in all the test groups.

CONCLUSIONS

Within the limitations of this *in vitro* study, it was concluded that the intrinsic water content of dentin together with extrinsic water irrigation played an important role in achieving the desired outcome of Er:YAG ablation to coronal dentin during conservative tooth treatment. The laser output of 100 mJ/pulse with water irrigation did not cause any detectable chemical change on the remaining irradiated dentin. On this score, these irradiations conditions of 100 mJ/pulse laser output with water irrigation were recommended to be used for dentin ablation by Er:YAG laser.

CHAPTER 2

EFFECT OF ER:YAG LASER TREATMENT ON THE MICROSTRUCTURE OF THE DENTIN/ADHESIVE INTERFACE AFTER ACID-BASE CHALLENGE

Introduction:

Cavity preparation and maintaining patient comfort are important challenges in dental practice. In the attempt to find a method to remove diseased and healthy dental hard tissues without the negative stimuli associated with dental handpieces, laser ablation is being considered as a potential alternative[32]

In 1989, Keller and[4] demonstrated that the erbium- doped yttrium aluminum garnet (Er:YAG) laser (wavelength: 2.94 μm) could remove carious tissues together with sound enamel and dentin. This laser is efficiently absorbed by the intrinsic H2O and the hydroxy groups (-OH) in the apatite mineral of dental hard tissues[5, 33-36] heating dentin and producing water vapor, which expands and builds up pressure until a micro-explosion occurs and a small portion of tissue is removed[37].

It became the first dental laser approved to be used for hard tissue ablation by the US Food and Drug Administration (FDA) in 1997.[38] However, there is still controversy regarding the effect of Er:YAG on the resistance of hard tooth structure to decalcification.[39-41]

Recurrent caries occurring along the restoration margins or under the restorative materials over time is considered one of the main causes for failure of restorations.[32, 42] A number of mechanisms have been shown to be responsible for recurrent caries, including microleakage. The

contraction stress that occurs during the polymerization of the resin based restorative materials may result in an early marginal breakdown, facilitating microleakage.[43] Acidic bacterial byproducts may infiltrate not only the interface, but also the dentin tissue at the periphery, creating a marginal demineralizing zone, and thus rapidly promote recurrent caries.[43] In this regard, it is speculated that an increased resistance of the dentin-resin interface to demineralization by acid may retard the progression of recurrent caries. Other key factors in preventing recurrent caries are marginal integrity of the restoration,[42] durable adhesion,[44] quality of the hybrid layer,[45] physical properties of the restorative materials, and oral hygiene[46].

Using the scanning electron microscope, Tsuchiya et al[45] demonstrated the existence of an acid-base resistant zone beneath the hybrid layer on conventionally prepared dentin bonded to composite using an adhesive system (SE Bond), suggesting that this zone may retard the progression of recurrent caries. Inoue et al[47] described the morphological and the mechanical characteristics of the acid-base resistant zone. The purpose of the present study was to evaluate the effect of the Er:YAG laser on the microstructure of the dentin/adhesive interface after acid-base challenge in vitro.

The null hypothesis tested was that the thickness of the acid resistant zone formed on dentin underneath the adhesive restoration does not differ significantly between Er:YAG-laser ablated and conventionally prepared (ground) dentin.

Materials and Methods

The experimental procedures are schematically illustrated in Fig 1.

Dentin Disk Preparation

Thirty-two freshly extracted non carious third molars were used in this study. They were hand scaled, cleaned, and stored in saline solution at 4°C until the experiment began.

Disks 2 mm in thickness were obtained from mid coronal dentin by cutting parallel to the occlusal plane of the tooth using a water-cooled diamond saw microtome (1600 Microtome, Leitz; Wetzlar, Germany). The dentin disks were distributed into 4 groups (Table 1) with 8 disks in each group.

Treatment of the Dentin Surfaces

One half-disk of each pair was treated using an Er:YAG laser system (Elfine 400, Osada; Tokyo, Japan). The Er:YAG laser parameters were: wavelength 2.94 μm, pulse energy 10 to 400 mJ, and pulse frequency 1 to 25 pps, pulse duration 300 μs, having a contact probe with a sapphire tip of 0.63 mm diameter. The output energy levels used to irradiate these dentin specimens were 100 mJ per pulse, 1 pps with water irrigation, with a contact tip applied perpendicularly to the dentin surfaces in a sweeping motion with lased dentin spots overlapping each other to ensure sufficient irradiation on all parts of the lased specimens. The other half of the dentin disk was ground using 600-grit silicon carbide (SiC) abrasive paper under wet conditions to create a standardized smear layer, and served as control (conventionally prepared dentin) (n = 8).

Bonding Procedures

All specimens in groups I, II and III were treated with the self etching primer of Clearfil SE-Bond (Kuraray Medical; Tokyo, Japan) according to the manufacturer's instructions (Table 2). The bonding resin of Clearfil SE-Bond was then applied to the surface and photocured using a light-curing unit (Optilux 501, Kerr; Orange, CA, USA). The surfaces of the specimens in group IV were coated with the bonding resin of Clearfil SE-Bond and photocured for 10 s; the self-etching primer was omitted. After photocuring the bonding resin, a restorative composite resin (Clearfil AP-X, shade A3, KurarayMedical) was placed between the treated surfaces of the disk halves in each pair and photocured for 40 s to form dentin-resin sandwiches[48].

Acid-Base Challenge

Each bonded pair was then embedded in a self-curing epoxy resin (Epoxycure resin, Buehler; Lake Bluff, IL, USA). The resin/dentin interface was ground with SiC abrasive papers down to 1000-grit to obtain a smooth surface.[48] The specimens were then stored in water at 37°C for one week, and the dentin surfaces 1 mm away from the bonding interface were covered with two coats of protective nail varnish to leave a treatment window exposed.[45] A buffered demineralizing solution, containing 2.2 mmol/L of calcium chloride (CaCl2), 2.2 mmol/L of sodium dihydrogen phosphate (NaH2PO4), and 50 mmol/L of acetic acid (CH3COOH) adjusted to pH 4.5, was prepared and used to create artificial recurrent caries.[45] The embedded specimens were stored in the demineralizing solution for 20, 60 or 180

min in groups I, II, and III, respectively, at room temperature. In group IV as well, the specimens were stored in the demineralizing solution for 20 min. After acid treatment, all specimens were then immersed in 3.7 mmol/L (5%) sodium hypochlorite (NaOCl) for 20 min in order to remove collagen fibrils of demineralized dentin, and then rinsed with running water for 30 s. The specimens were subjected to the acidic and basic solutions in a beaker with a magnetic stirrer to ensure complete access of acid to all of the exposed surfaces. Super Bond C&B (SunMedical; Kyoto, Japan) was applied to the top of the sample after the acid-base challenge as a standard procedure (as described by Inoue et al[47]) to prevent wear of the SE Bond adhesive material during the following polishing procedures.

Scanning Electron Microscope Analysis

The specimens were then sectioned perpendicularly to the treated surface and the interface to yield 1.5-mm-thick slabs. The surfaces lateral to the treatment window were ground and then polished with diamond pastes down to 0.25 μm. The polished surfaces of the slabs were etched using an ion shower milling system (EIS-IE, Elionix; Tokyo, Japan) for 7 min at an accelerating voltage of 1 kV and an ion current density of 0.2 mA/cm2. After gold sputter coating, the morphological changes to the dentin and its relation to the bond and composite surfaces were evaluated using a scanning electron microscope (SEM JSM-5310LV, JOEL; Tokyo, Japan). The thickness of the layer that resisted the acid-base challenge was recorded.

Statistical Analysis

One-way ANOVA was used to investigate the effect of acid-base

challenge duration on the thickness of the acid-base resistant zone between control (conventionally prepared) specimens of groups I, II, and III, and between lased specimens of groups I, II, and III. Whenever there was significance, Tukey's test was applied for comparison of the means. The paired t-test was used to compare the results between the control and lased specimens in each group. All statistical analyses were performed at a 5% significance level (n = 8) using SPSS software version 10 (SPSS; Chicago, IL, USA).

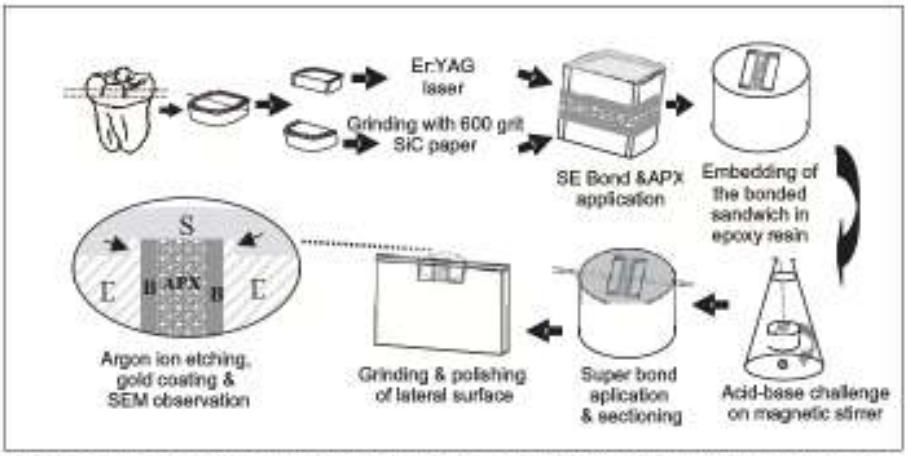

Fig 1 Experimental procedures. Arrows point to the acid-base resistant zone. (D) dentin, (APX) Clearfil AP-X composite, (B) SE-Bond, (S) Super Bond.

Table 1 Summary of groups

Group	Primer	Bond and composite	Acid application duration (min)	Base application duration (min)
I	applied	applied	20	20
II	applied	applied	60	20
III	applied	applied	180	20
IV	not applied	applied	20	20

Table 2 Materials used in this study

Material, manufacturer, batch no.	Composition	Procedure
Clearfil SE-Bond; Kuraray, Osaka, Japan; batch no. 011391	Primer: MDP, HEMA, water, PI, accelerators, CA Bond: MDP, HEMA, MFM, PI, accelerators, CA, microfiller	Apply self-etching primer 20 s Apply adhesive, gently air dry, light cure 10 s
Clearfil AP-X; Kuraray; batch no. 1117AA	Bis-GMA, TEG-DMA, barium glass filler (85 wt%), PI, accelerators	Apply and light cure for 40 s

Abbreviations: HEMA: 2-hydroxyethyl methacrylate; bis-GMA: bisphenyl glycidyl methacrylate; MDP: 10-methacryloxydecyl dihydrogen phosphate; TEG-DMA: triethylene glycol dimethacrylate; MFM: multifunctional methacrylate; PI: photoinitiator; CA: catalyst.

Results

The SEM evaluation showed dentin surface demineralization (the peripheral lesion) after the acid-base challenge in all groups. The dentin region underneath the hybrid layer remained after the acid-base challenge, i.e., an acid-base resistant zone was found for all specimens, except those of group IV, which debonded completely (Figs 2 to 5). The exposed surfaces of all specimens were observed in their entirety with SEM, and it was obvious that all the surfaces in groups I to III showed a uniform decalcification throughout, except those adjacent to the bonding agent, which showed resistance to decalcification, as illustrated in Fig 6.

Figure 7 shows the means and standard deviations of the thickness of the acid-base resistant zones in different groups. There was a statistically significant difference in the thickness of the acid-base resistant zone between group I specimens and those of groups II and III for both control and Er:YAG-irradiated surfaces. No statistically significant difference was found in the thickness of the acid-base resistant zone between groups II and III for either control or Er:YAG

irradiated surfaces. The paired t-test analysis showed that a significant difference existed between the mean thickness of the acid-base resistant zone of the Er:YAG irradiated surfaces and that of their corresponding control surfaces in group I. There were no statistically significant differences between the control and the lased surfaces in either groups II or group III ($p < 0.05$).

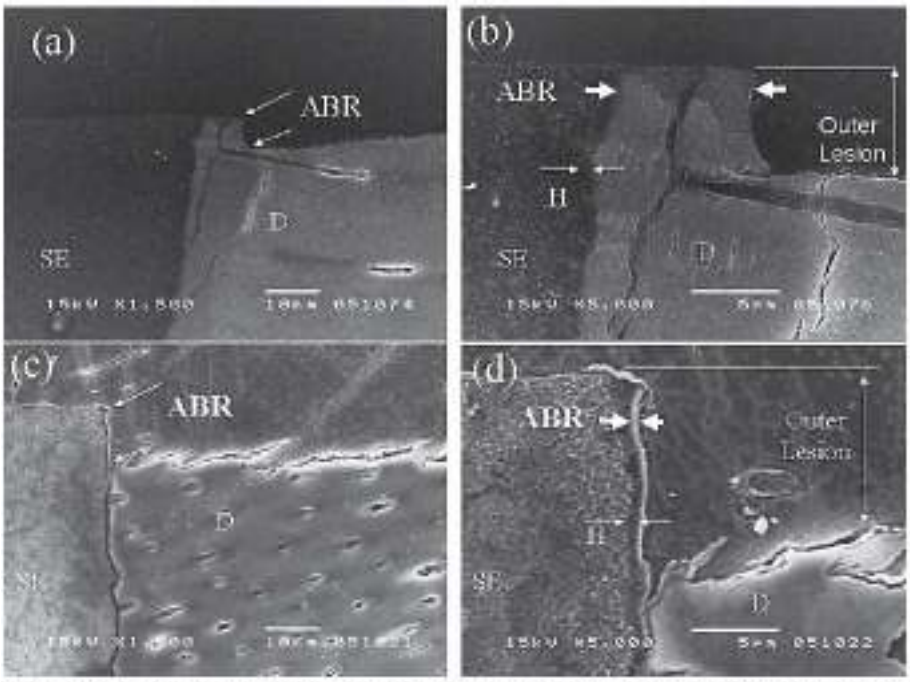

Fig 2a Specimens of group I after 20 min acid application. On the Er:YAG-lased dentin surface, SE Bond (SE) was not damaged by the acid-base challenge. The adjacent dentin (D) was lost due to the acid-base challenge. An acid-base resistant zone (ABR) was observed.
Fig 2b A magnified view of the top surface. An acid-base resistant zone (ABR, thick white arrows) adjacent to SE Bond (SE) was observed between Er:YAG-lased dentin and SE Bond beneath the hybrid layer (H).
Fig 2c On control (conventionally prepared) dentin surfaces, SE Bond (SE) was not damaged by the acid-base challenge. The adjacent dentin (D) was lost due to the acid-base challenge.
Fig 2d A magnified view of the top surface. An acid-base resistant zone (ABR, thick white arrows) was observed between conventionally prepared dentin and SE Bond (SE) beneath the hybrid layer (H, thin white arrows). The adjacent dentin (D) was lost due to the acid-base challenge.

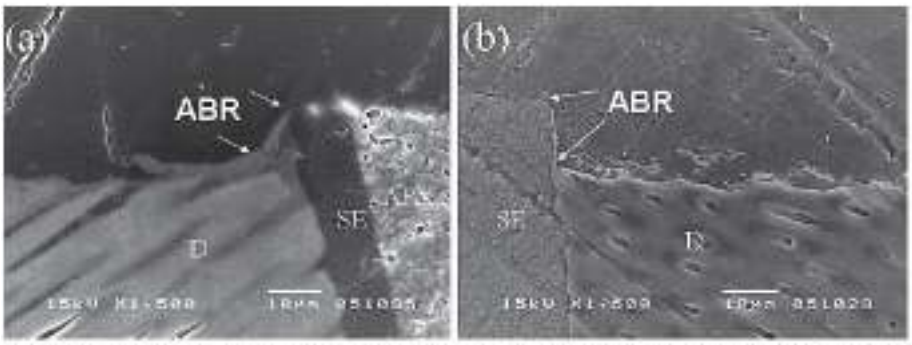

Fig 3a Specimens of group II after 60 min acid challenge. On Er:YAG-lased dentin surfaces, an acid-base resistant zone (ABR, white arrows) was observed between Er:YAG-lased dentin on one side and SE Bond (SE) and Clearfil AP-X (APX) composite on the other side. The adjacent dentin (D) was lost due to the acid-base challenge.
Fig 3b On control (conventionally prepared) dentin surfaces, an acid-base resistant zone (ABR, white arrows) was observed between dentin and SE Bond (SE). The adjacent dentin (D) was lost due to the acid-base challenge.

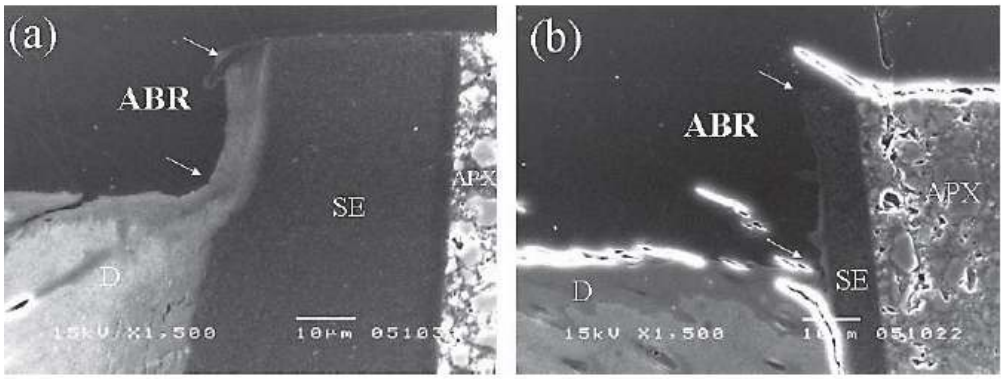

Fig 4a Specimens of group III after 180 min acid application. On Er:YAG-lased dentin, an acid-base resistant zone (ABR) was observed between Er:YAG lased dentin on one side and SE Bond (SE) and Clearfil AP-X (APX) composite on the other side. The adjacent dentin (D) was lost due to combined effects of acid and base.

Fig 4b On control surfaces, an acid-base resistant zone (ABR) was observed between dentin on one side and SE Bond and Clearfil AP-X (APX) on the other side. The adjacent dentin (D) was lost due to combined effects of the acid-base challenge.

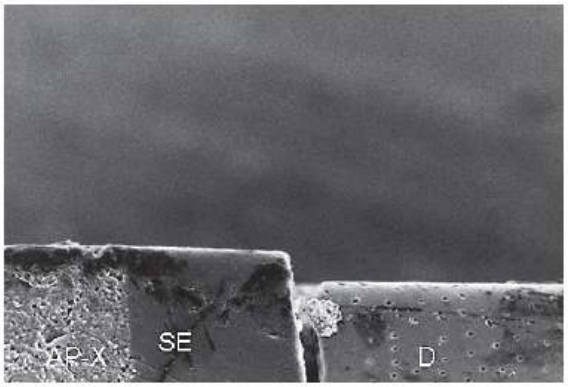

Fig 5 Specimens of group IV after 20 min acid application. There was decalcification of dentin (D) together with bond failure between Er:YAG-lased dentin and SE Bond (SE) and Clearfil AP-X (APX) composite.

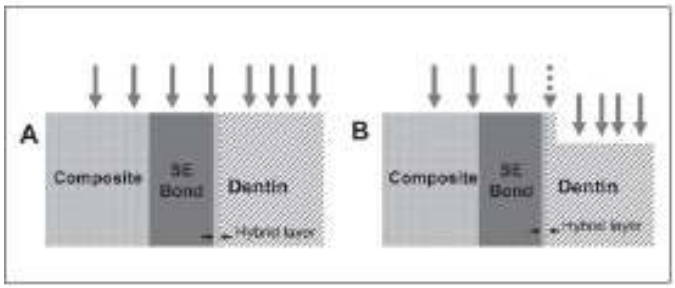

Fig 6 Schematic illustration showing the specimens before (A) and after (B) acid-base challenge. Solid (gray) arrows indicate the direction of application of the acid and base solutions. The dotted (gray) arrow points to the acid-base resistant zone.

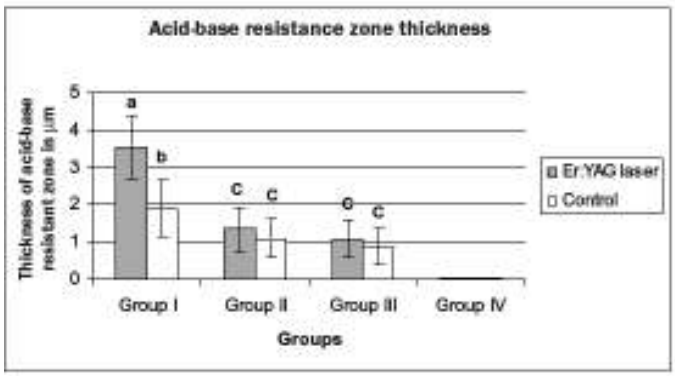

Fig 7 Effect of acid-base challenge duration on the thickness of the acid-base resistant zone. Groups marked by same letters are not statistically different ($p < 0.05$).

Discussion

Recurrent caries is a major cause of restoration failure. It has been suggested that the formation of acid-base resistant dentin beneath the bonding layer may be an important factor in retarding the progression of recurrent caries.[47] Tsuchiya et al[45] observed a zone that resisted the acid-base challenge, beneath the hybrid layer formed between non-fluoride releasing bonding agents and dentin; they hypothesized that penetration of the monomers beyond the hybrid layer would form this zone. This zone was termed the acid-base resistant zone.[45] In the current study, a demineralizing solution was used in order to determine how well this adhesive material would be able to protect the Er:YAG lased dentin from acid attack, compared to the conventionally prepared dentin. In group IV, the self-etching primer was not used. The SEM images revealed that the dentin adjacent to the resin was dissolved to the same extent as the other parts of the exposed surface, showing a complete bond failure in all specimens (Fig 5). This observation confirmed that the dentin remained unscathed beneath the hybrid layer not because the resin physically covered the dentin surface and blocked acid demineralization, but rather because of increased acid resistance of dentin thanks to the penetration of monomers and proper hybridization with the bonding agent. Specimens of group I showed an acid-base resistant zone after 20 min of acid-base challenge. This finding was in agreement with previous studies that reported an acid-base resistant zone formed on dentin treated with SE Bond[45, 47].

This layer should be different from the “inhibition zone” produced

by fluoride-releasing materials,[49] since the material used is a fluoride-free bonding system. SE Bond is a two-step self-etching adhesive system that contains the acidic monomer 10-methacryloxydecyl dihydrogen phosphate (MDP) in both self-etching primer and bonding resin agents.

The self-etching primer partially removes the smear layer and demineralizes underlying, conventionally prepared dentin, resulting in a mild surface etching[50] that leaves some of the hydroxyapatite crystals partially attached to dentinal collagen. These remaining crystals interact with the monomers upon application of the bonding agent[51] which penetrates into the prepared dentin. The SEM micrographs in the current study showed the resistance of the bonding layer to the acid-base challenge (Figs 2 to 4). Previous research suggested that the methacrylate monomers particularly MDP together with the microfillers abundant in SE-Bond would improve the physical properties of the bonding resin.[45]

The hybrid layer[42] formed by SE bond was reported to be acid resistant.[52] This concurs with the results of the present study, in which the hybrid layer was found to be resistant to the acid-base challenge. Argon-ion beam etching was used prior to SEM examination of the interface between adhesive resin and dentin surface. It is believed that the hybrid layer, which has low resistance to argon-ion bombardment, becomes highly distinguishable from the argon-ion resistant adhesive resin layer and resin tags when the resin-dentin interface is etched with an argon-ion beam.[48, 53, 54] However, some previous studies suggested that argon-ion etching of the specimens prior to scanning electron

microscope examination might have some drawbacks,[55] which include the production of some artifacts due to the inadvertent use of a rather strong argon ion etching procedure. This might have caused the incomplete observation of the entire thickness of the hybrid layer in the present study.

The intent of increasing durations of the acid-base challenge in this study was to observe the microstructural changes taking place at the dentin-restoration interface, and determine the extent of interface resistance to the acid challenge. The persistence of the acid-base resistant zone in the specimens exposed to the acid-base challenge for longer than 20 min (ie, groups II and III) confirms the capacity of this zone to resist extended invasion by the acid.

This phenomenon may be explained by the dentin's hydroxyapatite crystals being protected by a stable adhesion to MDP, as suggested by previous research.[56] The decrease in the thickness of the acid-base resistant zone of specimens in groups II and III for both conventionally prepared and Er:YAG-laser irradiated surfaces may be attributed to increasing the duration of the acid-base challenge.

There was a significantly thicker acid-base resistant zone formed under the hybrid layer in Er:YAG-lased surfaces than in the conventionally prepared dentin in group I. The Er:YAG laser acted on the specimen's dentin surface by thermo-mechanical ablation. This mechanism involves the absorption of water molecules in dentin components and structures, mainly the intratubular fluid and collagen network.[57] Such a mechanism leaves the dentinal tubules opened and ablates the intertubular dentin to a greater extent than the peritubular dentin, due to

the high water and hydroxyapatite content of the former.[33, 35, 36, 40] Er:YAG laser does not widen the tubule entrance.[38] Moreover, it does not affect the inorganic components of dentin when applied using the parameters of the current study.[58] It has been reported that when the dentin is ablated by the Er:YAG laser with water irrigation, it may become slightly more acid resistant compared to the conventionally prepared dentin.[41] Such an increase in the acid resistance could be attributed to the chemical changes due to reduction in carbonate and water content in the mineralized tissue as result of heating.[35] However, the heat induced by the low output energy of Er:YAG laser is not sufficient to cause fusion zones, melting, or cracks.[59] Moreover, the dentin prepared by Er:YAG laser is not covered by any smear layer,[59]which is likely to allow a better penetration of the bonding resin into the dentin, especially when combined with dentin conditioning after dentin irradiation.[60] It was suggested that increased permeability of dentin to monomers may result in an improvement in the mechanical properties of the acid-base resistant zone compared to the underlying deeper dentin.[47] These speculations might explain the significant difference between the thickness of the acid-base resistant zone formed on the Er:YAG irradiated dentin surfaces compared to the conventionally prepared surfaces in group I.

However, there is still a controversy over the bonding performance of adhesive restorations to Er:YAG-lased dentin in the literature. On the one hand, some authors have shown a decrease in the bond strength of Er:YAG-irradiated dentin to bonding agents,[57, 61] which suggested less receptivity of irradiated dentin to bonding. On the other hand, a

recent study[62] showed that the shear bond strength of a two-step self-etching bonding system was not affected by Er:YAG irradiation of the dentin surface compared to conventionally prepared dentin. Moreover, the shear bond strength to an all in one self-etching bonding system was improved when the dentin surface was irradiated by Er:YAG laser.[62] Other techniques were suggested to improve the bond strength of the Er:YAG lased dentin to composite resin, such as the use of flowable composite resin as a liner.[8] The different results reported by the studies mentioned above may be attributed to the different bonding techniques or materials used in conjunction with the Er:YAG-lased dentin or to the different parameters of the Er:YAG laser beam used.

Conclusion:

The null hypothesis of the study was rejected. The existence of an acid-base resistant zone following the application of the SE Bond on both conventionally prepared and Er:YAG lased human dentin, regardless of the acid-base challenge duration, was confirmed. Er:YAG laser is likely to slightly improve the resistance of resin-dentin interface to acid-base challenge.

CHAPTER 3

EFFECT OF Er:YAG LASER ON DENTIN BONDING DURABILITY UNDER SIMULATED PULPAL PRESSURE

Introduction

Effective bonding to tooth tissues using tooth colored restorative materials is an absolute necessity for clinical success. There are many factors that may affect the bond strength of resin composite to dentin; these include types of bonding systems, and the method of cavity preparation.[63-65] Among the techniques used for cavity preparation is the use of Er:YAG laser. It was demonstrated that the erbium-doped: yttrium aluminum-garnet (Er:YAG) laser (wave length: 2.94μm) which was introduced as a potential alternative method for cavity preparation that can remove carious tissues together with sound enamel and dentin.[5] It has been reported that after dentin ablation by Er:YAG laser the dentinal tubules are opened but not widened, and the intertubular dentin is ablated to a greater extent than the peritubular dentin, due to the high water and hydroxyapatite content of the former.[1] Moreover, Er:YAG laser when used with the proper parameters does not affect the chemical composition of dentin.[58] However, when improper parameters of the Er:YAG laser are used to ablate dentin some modifications may be noticed in the dentin amide components which is the main component of the dentin collagen[58], that plays an important role in the hybridization of the two step self etching primers to dentin and thus may negatively influence the integrity of the dentin-bond

interface. Other factors contributing to the difficulty of bonding to dentin include the presence of the smear layer formed immediately after cavity preparation, and continuous moist conditions due to presence of dentinal fluid and pulpal pressure,[63, 66, 67] which is the outward flow of dentinal fluid through the dentinal tubules. Whereas, it was previously reported that dentin wetness from tubular fluid lowers both bond strength and the ability of the bonding agents to seal dentin[68, 69], moreover it was previously reported the existence of fluid movement across the resin-dentin interface during and after bonding.[70]

However, it was previously suggested that the tubular occlusion caused by the remaining smear layer prior to the bonding procedure might affect the bond strength and sealing ability of adhesive restorations placed under the simulated pulpal pressure.[71] At this juncture, it must be pointed out that the dentin surface after conventional cavity preparation is covered by a smear layer while after Er:YAG laser ablation is not covered by any smear layer.[1]

One other factor that may play a role in the effective bonding of the self etching adhesive systems to the cavity walls is the occurrence of "nanoleakage". This term was introduced by Sano et al [72, 73] and represents micro porosities less than 50nm in size which are located between the unaltered dentin and the resin infiltrated collagen-rich fibrous network, and should have been penetrated by resin to reinforce the demineralized dentin structures, these porosities might increase by time and thus negatively affecting the integrity of the dentin bonding agent interface causing the failure of the bonded restoration.[74] The

nanoleakage test was introduced to detect these nanometer-sized defects and analyze them by field emission scanning electron microscope (FE-SEM)[44, 75-77] or transmission electron microscopy (TEM).[71, 78, 79]

Current literature provides little information about the durability of the Er:YAG lased dentin bonded to the available two step self etching bonding system under various simulated clinical situations, thus the present study aimed at investigating the micro tensile bond strength and the nanoleakage of dentin-adhesive interface after either conventional dentin preparation or Er:YAG laser dentin ablation under simulated pulpal pressure. The null hypotheses were that: 1- The micro tensile bond strength of a two step self etching primer bonding system would not be significantly affected when bonded to Er:YAG lased dentin under simulated pulpal pressure. 2- There will be same nanoleakage expression associated with bonding the two step self etching primer bonding system to Er:YAG lased dentin under simulated pulpal pressure.

Materials and methods:

The Experimental procedures are schematically illustrated in Fig 1.

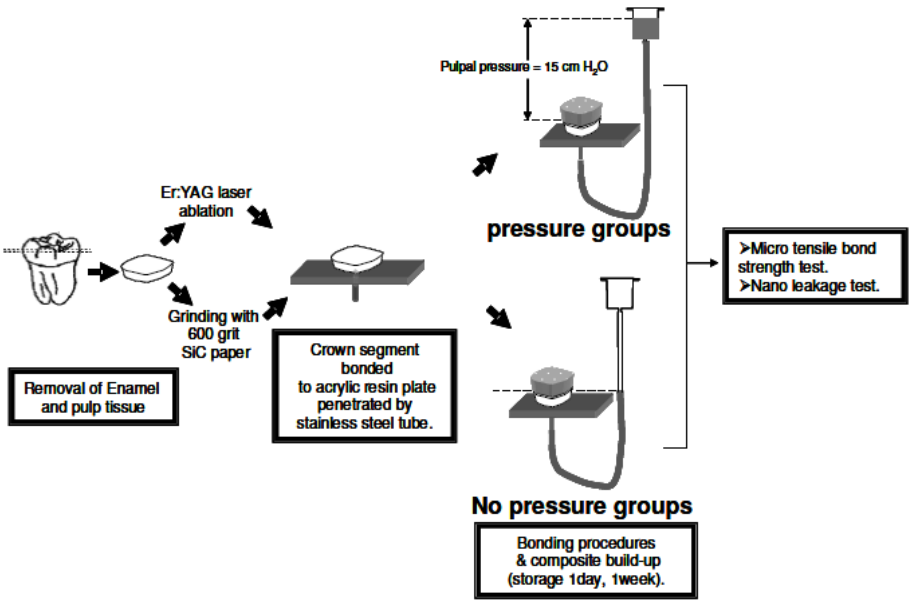

Fig 1 Experimental procedures

Specimens preparation

Forty eight freshly extracted non-carious third molars were used in this study. They were hand-scaled, cleaned and stored in saline solution at 4^{o}C until experiment time. The occlusal surfaces of the teeth were ground under a stream of running water to expose the mid-coronal dentin using a water-cooled diamond saw microtome (1600 Microtome, Leitz, Wetzlar, Germany). The teeth were randomly divided into eight groups (Table 1) with twenty four teeth used for the micro tensile bond strength test and twenty-four teeth used for the nanoleakage test.

Treatment of the dentin surfaces

Specimens of groups I, II, III, and IV were ground using 600-grit silicon carbide (SiC) abrasive paper under wet conditions to create a standardized smear layer and served as control. Specimens of groups V, VI, VII, and VIII were treated by an Er:YAG laser system (Elfine 400, Osada, Tokyo, Japan) wave length of 2.94 μm, pulse energy of 10-400 mJ, and pulse frequency 1-25 pps, pulse duration 300 μsec, having a contact probe with a sapphire tip of 0.63 mm in diameter. The output energy levels used to irradiate these dentin specimens were 100 mJ per pulse, 1 pps with water irrigation, with a contact tip which was applied perpendicular to the dentin surfaces in a sweeping motion with lased dentin spots overlapping each other to ensure sufficient irradiation on all parts of the lased specimens.

Preparation of samples for bonding

Summary of the procedure as previously described by Nakajima et al[69] is schematically illustrated in Fig 1. Direct communication to the pulp chamber was created by cutting at a level approximately 1 mm below the cemento-enamel junction, and parallel to the occlusal surface using a water-cooled diamond saw (Isomet, Buehler Ltd, Ill, USA). The remaining pulp tissue was carefully removed with endodntic files, without touching the walls of the pulp chamber. The pulp chambers were irrigated with 2.5% sodium hypochlorite solution (NaOCl) for 30 seconds followed by immersion in distilled water for 30 minutes to remove the residue of NaOCl. Each crown segment was luted with cyanoacrylate (Zapit, DVA, Anaheim, CA, USA) to a Plexiglas plate (Altuglas International, Arkema, PA, USA) through which an 18-gauge

stainless steel tube had been inserted. This tube permitted communication with the pulp chamber and was attached to 20 ml plastic syringe barrel by a Tygon tube (US Plastic, Lima, OH, USA).

The dentin surfaces of groups I, II, V, and VI were exposed to a simulated pulpal pressure of 15 cm H2O by filling the syringe barrel and the Tygon tube with distilled water (Fig. 1). Specimens of groups III, IV, VII and VIII were exposed to a simulated pulpal pressure of 0 cm H2O by keeping the barrel empty and water level equal to that of the dentin surface.[69]

Bonding procedures

The primer of the SE Bond (Kuraray Medical, Tokyo, Japan) was applied on all of the treated areas and air blown followed by SE Bond application and light curing according to the manufacturer instructions (Table 2). Half number of the specimens was stored for 1 day and the remaining specimens were stored for 1 week and were exposed to either 0 cm H2O or 15 cm H2O (Table 1).

Micro tensile bond strength (μTBS)test

After storage, four bonded specimens in each group were serially sectioned into 0.7mm thick slabs using a low speed diamond saw under water cooling to produce 12 dentin slabs in each group (n=12). The slabs were trimmed into an hourglass shape with a 1mm cross-sectional area, using a superfine diamond bur (SF114, Shofu, Kyoto, Japan) mounted in a high-speed turbine handpiece under copious water spray.

The specimens were subjected to the microtensile bond strength test using a tabletop testing machine (EZ Test, Shimadzu, Kyoto, Japan) with a cross head speed of 1 mm/min. The micro tensile bond strength data were subjected to three-way ANOVA analysis (the three factors were the method of dentin preparation, the pulpal pressure storage condition and the storage duration). Two way ANOVA was used to compare the μTBS values of the specimens stored under pulpal pressure or stored with no pulpal pressure. The t-test was used to compare the μTBS values of specimens ablated by Er:YAG laser to the conventionally prepared samples either stored with/without pulpal pressure for 1 day or 1 week, moreover the μTBS values of conventionally prepared or Er:YAG ablated specimens were compared. All statistical analyses were performed at a 5% significance level using SPSS software version 10 (SPSS, SPSS Inc., Il, USA). Analysis of the failure modes was done using a scanning electron microscope (SEM JSM-5310LV, JOEL, Tokyo, Japan). Bond failures were classified as cohesive failure within dentin, cohesive failure within resin, mixed adhesive/cohesive failure at resin dentin interface and adhesive failure.

Table 1 Summary of groups

	Er:YAG Laser	Pulpal pressure	Storage duration
Group I	Not applied	Not applied	1 day
Group II	Not applied	Applied	1 day
Group III	Not applied	Not applied	1 week
Group IV	Not applied	Applied	1 week
Group V	Applied	Not applied	1 day
Group VI	Applied	Applied	1 day
Group VII	Applied	Not applied	1 week
Group VIII	Applied	Applied	1 week

Table 2 Materials used in this study

Materials		Composition	Procedures
Clearfil SE Bond (Kuraray Medical ,Tokyo, Japan) Batch No.: 011391	Primer:	MDP,HEMA ,Water ,PI, accelerators, CA.	Apply self-etching primer(20 s)
	Bond:	MDP,HEMA ,MFM ,PI, accelerators, CA. microfiller	Apply adhesive, gently air dry, light cure (10 s)
Clearfil AP-X (Kuraray) Batch No.: 1117AA		Bis-GMA,TEG-DMA barium glass filler (85 wt%), PI, accelerators	Apply and light cure for (40 s)

HEMA= 2-Hydroxyethyl methacrylate; bis-GMA=bisphenyl glycidyl methacrylate; MDP=10-methacryloxydecyl dihydrogen phosphate ; TEG-DMA=Triethylene glycol dimethacrylate; MFM=Multifunctional methacrylate; PI=Photoinitiator; CA=Catalyst

Nanoleakge evaluation

The remaining four bonded specimens in each group were vertically sectioned with a diamond saw (Isomet, Buehler Ltd, Ill, USA) under water lubrication, through the composite buildups and the dentin, into approximately 1mm thick slabs. Two central slabs were chosen from each tooth, forming a total of eight specimens per group. Bonded slabs were ground and polished using wet #1000 silicone-carbide paper, then coated with two layers of fast-drying nail varnish applied up to within 1mm of the bonded interfaces. The slabs were not allowed to dry completely prior to immersion in the tracer solution for 18 h. Ammoniacal silver nitrate was prepared according to the protocol

previously described by Tay et al.[80] Tooth slabs were placed in the ammoniacal silver nitrate in total darkness for 18 h, rinsed thoroughly and immersed in photo developing solution (Kodak, NY, USA) for 6h under a fluorescent light to reduce silver ions into metallic silver.

FE-SEM analysis

The silver stained resin-bonded specimens were lightly polished down to a size of 0.25 µm and sonicated for 5min to remove the superficial silver adsorption.[76] The polished surfaces of the slabs were etched using ion shower milling system (EIS-IE, Elionix, Tokyo, Japan) for 7 minutes at an accelerating voltage of 1 kV and an ion current density of 0.2 mA/cm.[76] After gold-sputter coating, the specimens were observed under FE-SEM (S-4500, Hitachi, Hitachinaka, Japan) by means of yttrium–aluminium–garnet (YAG) backscattered electron images at 2000×.

Results

The mean and standard deviation values for the micro tensile bond strength test are shown in Table 3. Failure modes are shown in Fig 2. Three way ANOVA test showed that the method of dentin preparation and the pulpal pressure storage condition significantly affected the µTBS, while the storage duration did not significantly affect the µTBS ($p<0.05$). Moreover, there was a significant interaction between the method of dentin preparation and the pulpal pressure storage condition ($p<0.05$). Two way ANOVA showed that the method of dentin

preparation had an effect on the μTBS when the pulpal pressure was applied on the samples' dentin surfaces, while the storage duration had no significant effect, but on the other hand the method of dentin preparation and the storage duration had no significant effect on the μTBS when the pulpal pressure was not applied ($p<0.05$). The t-test showed that there was no significant difference between the μTBS values of groups I and III when compared to specimens of groups II, IV, V and VII, while groups VI and VIII were significantly lower than specimens II, IV, V and VIII ($p<0.05$). The predominant failure mode recorded for the conventionally prepared dentin was the adhesive mode of failure, while the predominant failure mode recorded for the conventionally prepared dentin was the mixed adhesive/cohesive failure mode. YAG backscatter images for groups I, II, III, and IV. Fig 3 showed minimal silver particles infiltration limited to the hybrid layer zone, while YAG backscatter images for groups V, VI, VII, and VIII. Fig 4 showed more heavily infiltration of the hybrid zone by the silver particles that extended beyond the hybrid layer into the adhesive layer in specimens of group VIII (Fig 4d).

Fig 2: Bar chart showing the percentage of fracture modes for each experimental group.

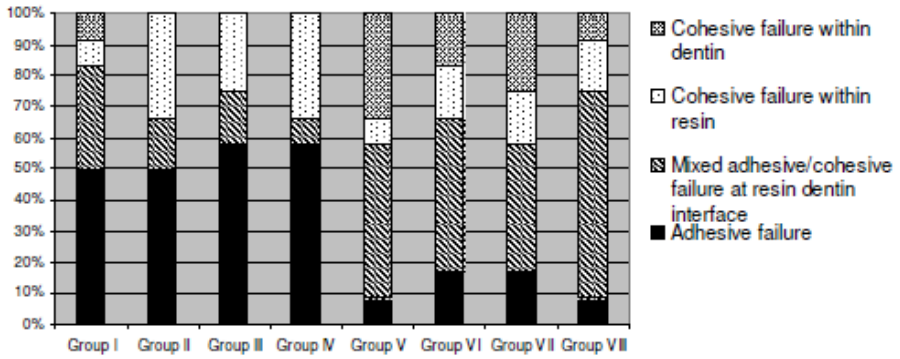

Table 3. Microtensile bond strength results

		No pulpal pressure	Pulpal pressure
Conventional prepared dentin	1 day	Group I	Group II
		43.15±10.1	37.42±6.68[a]
	1 week	Group III	Group IV
		41.2±8.7	37.1±8.5[b]
Er:YAG lased dentin	1 day	Group V	Group VI
		40.9±7.76[c]	24.4±6.8[a,c]
	1 week	Group VII	Group VIII
		39.04±5.22[d]	17.5±7[b,d]

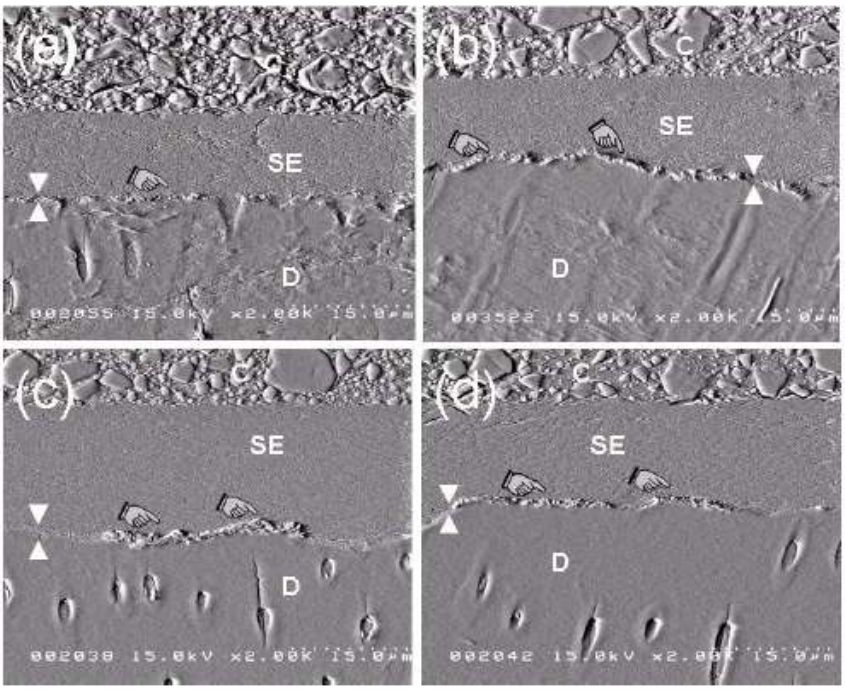

Fig 3: YAG Backscattered FE-SEM images of nanoleakage at the resin dentin interface of the conventionally prepared dentin 2000x. (a) Representative image for specimens of group I. Silver nitrate particles (Finger pointer) within the hybrid layer were hardly detected. (b) Representative image for specimens of group II. More deposition of silver nitrate particles (Finger pointer) within the hybrid layer was detected. (c) Representative image for specimens of group III. Scattered silver nitrate particles (Finger pointer) within the hybrid layer were detected. (d) Images of group III. Scattered silver nitrate particles (Finger pointer) within the hybrid layer were detected. (C: composite, SE: SE Bond, D: Dentin, The area between the white arrows is the hybrid layer).

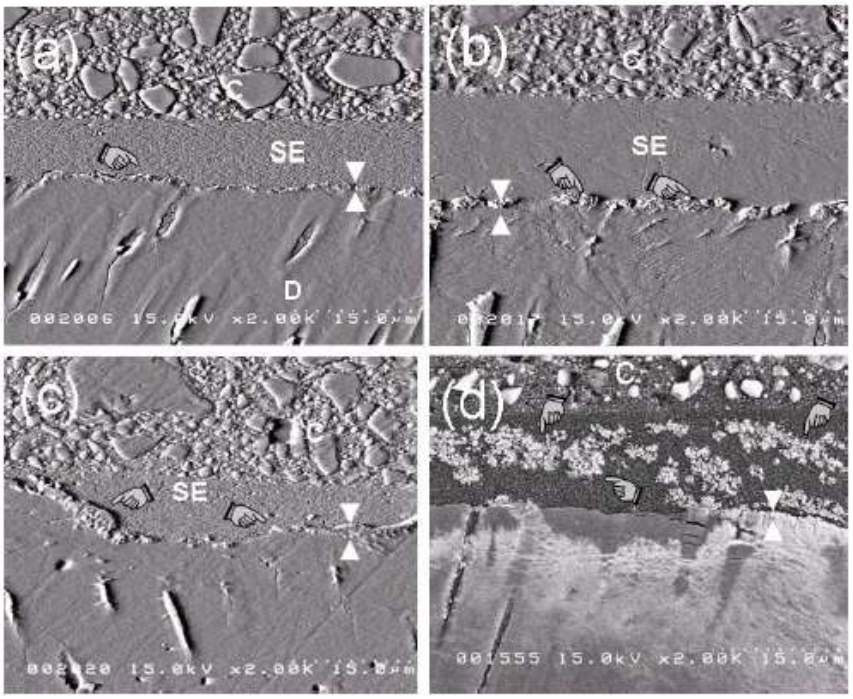

Fig 4: YAG Backscattered FE-SEM images of nanoleakage at the resin dentin interface of the conventionally prepared dentin 2000x. (a) Representative image for specimens of group V. Scattered silver nitrate particles (Finger pointer) within the hybrid layer were detected. (b) Representative image for specimens of group VI. The whole thickness of the hybrid layer was infiltrated by silver nitrate particles (Finger pointer). (c) Representative image for specimens of group VII. The whole thickness of the hybrid layer was heavily infiltrated by silver nitrate particles (Finger pointer). (d) Images of group VIII. The whole thickness of the hybrid layer was infiltrated by silver nitrate particles (Finger pointer). Extension of the silver nitrate particles was noticed within the adhesive layer itself. (C: composite, SE: SE Bond, D: Dentin, The area between the white arrows is the hybrid layer).

Discussion:

The wetness of dentin surfaces and the presence of pulpal pressure are extremely important variables during bonding procedures to conventionally prepared dentin,[70] however, there was minimal

information about the influence of these factors when dentin is ablated by Er:YAG laser. The results showed the significant influence of the pulpal pressure on the μTBS of SE Bond bonded to Er:YAG lased dentin. In this study simulated pulpal pressure was applied to increase the convective fluid movement from the dentinal tubules to the dentin surface in order to simulate the in vivo condition[69] and thus to be able to observe any possible deteriorating effect on the μTBS of SE Bond bonded to either conventionally prepared or Er:YAG lased dentin. Moreover, the existence of any water-channels resulting from the seep of water under pulpal pressure from the dentinal tubules was detected which might be potential sites of hydrolytic degradation that may adversely affects the longevity of the dentin-bond interface.[68, 69] To facilitate the detection of these water channels, ammoniacal silver nitrate solution was used as tracer solution which has the ability to penetrate the microporous zones beneath or within the hybrid layer without causing artefactual microporosities which may be associated with the use of the acidic silver nitrate solution (pH=3.4).[79] The samples were not allowed to dry completely after nail varnish application in order to avoid the creation of artefactual submicron hiati beneath the resin-dentin interface that could be mistaken for nanoleakage.[79]

The YAG backscattered electron mode of SEM which is a widely used method employed for detection of nanoleakage was utilized in the current study because of its ability to produce a material's contrast image that has an element atomic number dependent feature, and thus

be able to decrease the erroneous interpretations due to electron microscopic edge effects.[44, 73, 76, 81]

The surfaces of the specimens were either treated by Er:YAG laser with a contact probe or by grinding using 600 grit SiC paper to create a standard smear layer simulating the clinical situation.[76] The Er:YAG laser used in this experiment acts on dentin by thermo-mechanical ablation, where the incident radiation is highly absorbed by molecules in dentin components and structures mainly the intra-tubular fluid and collagen network, heating dentin and producing water vapor, which expands and builds up pressure until a micro-explosion occurs and a small portion of tissue is removed.[5] Moreover, the dentin prepared by Er:YAG laser is not covered by any smear layer.[1] The parameters used for the dentin ablation by the Er:YAG laser was previously studied and it was found that these parameters do not affect the interface integrity between SE Bond and Er:YAG lased dentin even after acid-base challenge.[82]

To determine the effect of pulpal pressure on the μTBS of the conventional and Er:YAG ablated dentin a two-step self etching primer adhesive system that contains an acidic monomer (10-methacryloxydecyl dihydrogen phosphate: MDP) in both self etching primer and bonding resin was used in this experiment to bond the composite to the treated dentin surfaces.

The results showed that the μTBS bond strength values were not affected by the method of dentin preparation when no pulpal pressure

was applied on the bonding dentin surfaces which do not agree with Ceballos et al [34] where in their experiment the Er:YAG parameters used to ablate dentin were 180 mJ and 2 Hz repetition rate under water irrigation, these parameters might have negatively affected the dentin collagen leading to a deteriorating effect on the bond strength of SE Bond to Er:YAG laser ablated dentin, as it was previously shown using the FTIR/ATR technique that the amide II peak which is one of the components of dentin collagen was negatively affected when the Er:YAG laser parameters were 200 mJ with 1 Hz repetition pulse[58] which is very near to the parameters used in the previously mentioned experiment, while no detectable chemical changes were observed when the parameters adopted in the present experiment were used.[58] On the other hand, the current bonding test results agree with the findings of Celik et al[62] although higher Er:YAG laser output energy was used in their study which may be attributed to the high volume of water spray cooling 15ml/min which would have dissipated the heat associated with use of the high output energy.

The FE-SEM images of specimens stored with no pulpal pressure either conventionally prepared or Er:YAG laser ablated showed some silver particles deposition in the hybrid layer zone, this minimal silver particles deposition is less likely to result from the residual water contained in the SE Bond system because it is composed of a primer which is a hydrophilic aqueous solution that should be air-dried to remove the water and solvent content before adhesive application. Moreover, the SE adhesive contains a hydrophobic resin with no water

or solvent[83], these minimal silver particles deposition are more likely attributed to some minor discrepancies between the depth of demineralization and the depth of resin infiltration that might have occurred in these samples. However, these minor effects were probably limited by the mild self etching property of the SE primer that has the ability to preserve some hydroxyapatite crystals within the hybrid layer which was reported to serve as a receptor for additional chemical bonding which may provide a hydrolytically stable interface.[56]

When the simulated pulpal pressure was applied in the conventionally prepared groups the μTBS was not affected in agreement with previous results[69, 84] which may be attributed to the short period of storage adopted in the current experiment and by the mild self etching effect exerted by the SE Bond on the standardized smear layer prepared which can preserve the smear plugs occluding the dentinal tubules and limit the seep of water to the bonding interface.[71]

On the other hand, the Er:YAG laser ablated specimens stored for one week showed serious deterioration in the μTBS values, moreover there were distinct silver particles depositions in the hybrid layer region when the pulpal pressure was applied for 1 day, these silver particles [56]deposition extended more deeply in the adhesive layer itself when the pulpal pressure was applied for 1 week. These deteriorating effects may be explained as follows. The Er:YAG ablated dentin surface free from any smear layer under positive pulpal pressure may have lead to the seep of water from the dentinal tubules to the ablated dentin surface ready for bonding rendering it wet and thus it is speculated that these

residual water might have diluted the concentration of the SE Bond monomer[67, 85], moreover the highly hydrophilic 2- hydroxyethyl methacrylate (HEMA) present in the SE Bond residual primer might have been absorbed this excess water causing the decrease of the degree of conversion of the bonding agent double bonds[86]and some phase separation[87] in the SE bonding agent stored under the above condition. Additionally, this residual water might have interfered with the proper infiltration of the bonding agent that was subsequently applied after the application of the primer causing improper hybridization between the dentin collagen and the bonding agent.[85] These sites of improper hybridization were suggested to be sites in which the interfacial degradation begins[56], and thus it is speculated that in the current study this lead to the deterioration of the μTBS of Er:YAG lased specimens bonded to SE Bond that was associated with more heavy silver particles deposition in the dentin-SE bond interface when stored under pulpal pressure for one week. It was previously advocated that bonding to the carious affected dentin (conventionally prepared) even under a simulated pulpal pressure provided an excellent seal along the bonded interfaces when compared to sound dentin[71] although there was a concomitant decrease in the bond strength.[88] However according to the results of the current study it may be recommended that the dentin ablation by Er:YAG laser should involve the caries affected dentin areas and avoid unnecessary deep removal of sound dentin to avoid the drastic seal deterioration of the dentin-bond interface concomitant with the serious bond strength decrease.

Conclusion

The μTBS of Er:YAG laser ablated dentin bonded to SE Bond was adversely affected by the simulated pulpal pressure. There were different nanoleakage patterns observed in the dentin-SE Bond interface when the dentin was either conventionally prepared or ablated by Er:YAG laser under simulated pulpal pressure. Both null hypotheses formulated at the beginning were rejected

Clinical relevance

The cavity preparation should be designed to diminish the effect of pulpal pressure when using Er:YAG laser for cavity preparation .

CHAPTER 4

GENERAL CONCLUSIONS

According to the results obtained from the presented studies it is obvious that the Er:YAG Laser is a very promising and reliable tool for cavity preparation provided it is used with proper knowledge of its mechanism of action and thorough background about the previous research work done using it in literature.

Chapter 1 recommended the 100mJ energy output with 1 pulse per second with water irrigation as optimum parameters for Er:YAG laser ablasion of dentin. In this experiment the FTIR/ATR and the XRD techniques did not detect any obvious chemical changes either in the organic or the inorganic dentin components which may have been induced by the Er:YAG laser. However, the afore mentioned parameters may cause less efficiency in dentin ablasion and consequently may prolong the treatment session when using this Laser for cavity preparation, so, it is recommended to use parameters not more than 200mJ with water irrigation for cavity preparation and use the 100 mJ energy output for finishing of the cavity walls.

Chapter 2 showed that the acid-base resistant zone can be observed in the cavity wall-restorative material interface when the dentin surface was prepared using Er:YAG laser. This confirms the results obtained from chapter 1 which showed the stability of the dentin chemical composition when using the 100 mJ output energy.

Chapter 3 concluded that the surface morphology of the dentin after Er:YAG laser ablasion may have a deteriorating effect on the durability of the laser ablated dentin-restoration interface when exposed to pulpal pressure. It is emphasized in that chapter to ablate the carious lesions leaving enough areas of caries affected dentin which may have a role in diminishing the detrimental effect of pulpal pressure on cavities prepared by Er:YAG Laser.

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