

# Adhesion after erbium, chromium:yttrium-scandium-gallium-garnet laser application at three different irradiation conditions

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**Abstract** The aim of this study was to investigate whether distinct cooling of low fluence erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser irradiation would influence adhesion. Main factors tested were: substrates (two), irradiation conditions (three), and adhesives (three). A 750  $\mu\text{m}$  diameter tip was used, for 50 s, 1 mm from the surface, with a 0.25 W power output, 20 Hz, energy density of 2.8 J/cm<sup>2</sup> with energy per pulse of 12.5 mJ. When applied, water delivery rate was 11 ml/min. The analysis of variance (ANOVA) showed that laser conditioning significantly decreased the bond strength of all adhesive systems applied on enamel. On dentin, laser conditioning significantly reduced bond strength of etch-and-rinse and one-step self-etch systems; however, laser irradiation under water cooling did not alter bonding of two-step self-etching. It may be concluded that the irradiation with Er,Cr:YSGG laser at 2.8 J/cm<sup>2</sup> with water coolant was responsible for a better adhesion to dentin, while enamel irradiation reduced bond strength, irrespective of cooling conditions.

**Keywords** Er,Cr:YSGG laser · Dental hard tissue · Water cooling · Bond strength

## Introduction

Erbium lasers have been indicated for cavity preparation as the thermo-mechanical ablation process occurs. Furthermore, these lasers are painless and do not involve vibration or heat, making them highly attractive for routine use [1].

Specifically, erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser is emitted at a wavelength of 2.79  $\mu\text{m}$  and has energy interaction with water at the tissue interface [2], being therefore termed a hydrokinetic system [2]. It has been shown to be effective for soft-tissue mucous membrane and cutaneous surgery, as well as for cutting enamel, dentin and bone [3, 4] without thermal damage to the pulp [5] and also without creating smear layers [6].

Exclusively, the application of erbium lasers for caries prevention, with no intention of ablation or melting, aims only to change the chemical composition of dental structure in order to achieve acid-resistant surfaces and consequent reduced susceptibility to secondary caries [7]. Some authors [8] have claimed that, even when erbium lasers are used with energies below ablation threshold, reduced demineralization was observed, and also, restorations would be less susceptible to secondary caries [8, 9].

Effects caused by laser irradiation depend on the fluence applied on the surface, as well as the focal distance, beam spot size, repetition rate, structural properties of target tissue, amount of water during irradiation, and pulse duration, which can have a strong influence on erbium laser interaction with dental hard tissues, avoiding thermal damage and helping the ablation process [10]. Er,Cr:YSGG

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laser activity is highly influenced by water present at the dental surface. Cooling with water spray is highly recommended in clinical applications, so that damage is avoided, such as overheating and consequent pulpal injury, micro-fissures, mineralization defects, carbonization and dehydration of the tissues [10, 11].

Previous studies on the effects of erbium laser irradiation on bonding procedures are somewhat controversial. It has been reported that Er,Cr:YSGG bonding was more effective when associated with acid etching [12]. Even with improved adhesive systems, adhesion to enamel is predictable [13], and adhesion to dentin is more challenging, which may be related to the morphological differences of these tissues as well as to their water content. Thus, studies on how interaction between different adhesive systems and irradiated dental tissue occur have become an important subject for modern operative dentistry.

The aim of this in vitro study was to evaluate whether Er,Cr:YSGG laser irradiation at low fluence, under different water cooling conditions, prior to the use of etch-and-rinse and self-etching bonding systems, has any influence on adhesion to enamel and dentin.

## Materials and methods

### Samples

The crowns of 216 extracted bovine teeth were stored in distilled water for no longer than 7 days and embedded in acrylic resin. A flat buccal bonding surface was prepared by wet grinding with a sequence of 180-grit and 400-grit silicon carbide papers, using an automatic polishing machine (Ecomet 6/Automet, Buehler, IL, USA). The teeth were ground to expose a 5-mm diameter of enamel/dentin to accommodate bonding material. A standard smear layer was prepared over the bonding surface using 600-grit silicon carbide paper for 1 min [14], and an area of 5 mm diameter on each tooth was delimited by an acid-resistant nail varnish. Specimens were randomly divided into 18 groups ( $n=12$ ), as shown in Table 1.

### Laser and settings

Laser irradiation was performed with an Er,Cr:YSGG hydrokinetic system (Millenium, Biolase Inc., CA, USA), with a 140- $\mu\text{m}$  pulse duration and fixed repetition rate of 20 Hz. The irradiation procedure was performed by manual scanning in rows [15] over a demarcated area with an S75 tip (750  $\mu\text{m}$  diameter) for 50 s at a standard distance of 1 mm from the surface. Power output was set at 0.25 W, yielding an energy density of 2.8 J/cm<sup>2</sup> and energy per pulse of 12.5 mJ. In specimens irradiated with air–water spray, water delivery rate was adjusted to 11 ml/min (50% air and 20% water), while, for specimens irradiated without air–water spray, the device was set at 0% air and water.

### Bonding procedure

Three commercial adhesives were used in this study: an etch-and-rinse, water/ethanol-based system [Adper Single Bond 2 (SB), 3M ESPE, St. Paul, MN, USA]; a two-step self-etching system [Clearfil SE Bond (SE), Kuraray, Osaka, Japan], and a one-step self-etching system [One Up Bond F (OU), Tokuyama, Kyoto, Japan]. Application of the adhesives for all groups followed the manufacturers' instructions, which are detailed in Table 2, and were carried out by the same operator.

### Tensile bond strength test

After application of the adhesive, the specimens were mounted in a tensile test apparatus composed of a polytetrafluoroethylene matrix, with an inverted truncated cone shape of 3 mm in diameter at the adhesive interface, developed at Houston Biomaterials Research Center [16]. Filtek Z250 composite, shade A2 (3M ESPE) was added in two increments, each light-cured for 20 s with an Astralis 3 unit (Ivoclar Vivadent, Amherst, NY, USA) at 600 mW/cm<sup>2</sup> in order to compose specimens that were immersed in distilled water at 37°C for 24 h. Tensile bond tests were performed, using a universal testing machine (Instron Model 4440, Norwood, MA, USA) at a crosshead

**Table 1** Experimental design of the study considering as main factors: adhesive systems (three), substrates (two) and irradiation conditions (three)

Irradiation conditions	Adhesive systems					
	Adper Single Bond (SB) (etch-and-rinse)		Clearfil SE Bond (SE) (two-step self-etch)		One Up Bond F (OU) (one-step self-etch)	
	Enamel (E)	Dentin (D)	Enamel (E)	Dentin (D)	Enamel (E)	Dentin (D)
Unlased (a)	ESBa	DSBa	ESEa	DSEa	EOUa	DOUa
Laser with coolant (b)	ESBb	DSBb	ESEb	DSEb	EOUb	DOUb
Laser without coolant(c)	ESBc	DSBc	ESEc	DSEc	EOUc	DOUc

**Table 2** Batch numbers, compositions and application techniques for the adhesive systems used in this study (*BIS-GMA* bisphenylglycidyl-methacrylate, *HEMA* 2-hydroxyethyl methacrylate, *MAC*-10 10-methacryloyloxydecyl dihydrogen phosphate, *MDP* 10-methacryloyloxydecyl dihydrogen phosphate)

Bonding system	Batch number	Composition	pH	Application technique
Adper Single Bond 2	4KC	Etchant: 35% phosphoric acid Adhesive: Bis-GMA, HEMA, ethanol, water, polyalkenoic acid copolymer	0.6	Etch substrate for 15 s, rinse with water spray and dry gently Apply adhesive layer, air-thin, light cure for 10 s
Clearfil SE Bond	00330A	Primer: MDP, HEMA, water, hydrophilic dimethacrylate	1.9	Apply primer and allow to act for 20 s; briefly air-dry
	00422A	Bond: MDP, BIS-GMA, HEMA, water, hydrophilic dimethacrylate, microfiller		Apply bonding resin, spread with gentle air stream, and light-activate for 10 s
One Up Bond F	1068M	Bonding agent A: MAC-10, water, methacryloxyalkyl acid phosphate	1.3	Mix equal drops of bonding agents A and B until pink, homogeneous liquid mixture is obtained. Apply to dental substrates and leave undisturbed for 20 s; Do not rinse; do not further air-dry, and light-activate for 20s
	594M	Bonding agent B: HEMA, MMA, water, submicron fluoroaluminosilicate glass		

speed of 0.5 mm/min [16]. Bond strengths were expressed in MPa.

#### Statistical analysis

Bond strength data were separately analyzed for each tissue (enamel and dentin) by two-way analysis of variance and then by Tukey's test (Minitab 14, Minitab Inc., Pennsylvania, USA) for pairwise comparisons among groups ( $\alpha=0.05$ ).

## Results

Mean bond strengths for the different groups were calculated, together with standard deviations (Table 3). The data from the two substrates tested (enamel and dentin) were analyzed separately, due to their intrinsic distinct morphological and ultra-structural characteristics.

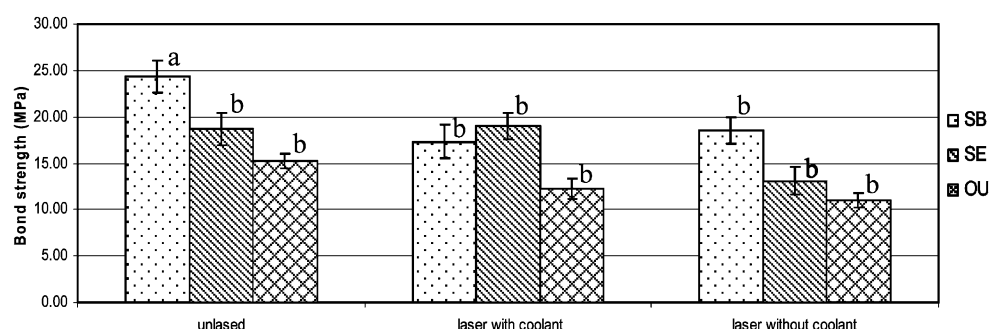
The two-way analysis of variance (ANOVA) for enamel indicated that tensile bond strength was significantly affected by adhesive system (SB, SE, OU) ( $P<0.000$ ) and irradiation condition [unlased (a), with coolant (b) and without coolant (c)] ( $P<0.000$ ). However, there was no statistical significance in the interaction between the two main factors ( $P=0.062$ ). Higher bond strengths were obtained for unlased samples than for samples with (b) and without (c) water coolant. However, no statistically significant difference was found between the bond strengths of irradiated samples with (b) and without (c) water coolant. When the data from the adhesive systems were tested, statistical differences were found in the comparisons between SBxOU and SExOU, with higher adhesive performance for SB and SE. However, no statistically significant bond strength means were observed when SB and SE were compared (Fig. 1).

The two-way ANOVA of dentin substrate showed that tensile bond strength was significantly affected by the main

**Table 3** In vitro tensile bond strengths (MPa) and standard deviations of composite bonded to enamel and dentin with three adhesive systems and three irradiation conditions

Irradiation condition	Adhesive systems					
	Adper Single Bond (SB)		Clearfil SE Bond (SE)		One Up Bond F (OU)	
	Enamel (E)	Dentin (D)	Enamel (E)	Dentin (D)	Enamel (E)	Dentin (D)
Unlased (a)	ESBa 24.38 ( $\pm 5.90$ )	DSBa 20.99 ( $\pm 4.64$ )	ESEa 18.68 ( $\pm 5.90$ )	DSEa 16.08 ( $\pm 4.52$ )	EOUa 15.24 ( $\pm 2.48$ )	DOUa 14.55 ( $\pm 2.43$ )
Lased with coolant (b)	ESBb 17.34 ( $\pm 6.35$ )	DSBb 11.90 ( $\pm 3.13$ )	ESEb 19.03 ( $\pm 5.01$ )	DSEb 16.28 ( $\pm 2.93$ )	EOUb 12.31 ( $\pm 3.76$ )	DOUb 11.80 ( $\pm 3.27$ )
Lased without coolant (c)	ESBc 18.60 ( $\pm 4.87$ )	DSBc 5.59 ( $\pm 1.42$ )	ESEc 13.10 ( $\pm 5.20$ )	DSEc 14.55 ( $\pm 2.43$ )	EOUc 10.95 ( $\pm 2.79$ )	DOUc 8.52 ( $\pm 1.99$ )

**Fig. 1** Means of tensile bond strength (MPa) for enamel groups. Error bars correspond to standard errors. Means followed by distinct letters are statistically different by the Tukey test ( $P<0.05$ )



factors adhesive system (SB, SE, OU) ( $P<0.000$ ) and irradiation condition [unlased (a), with coolant (b) and without coolant (c)] ( $P<0.000$ ), as well as by their interaction ( $P<0.000$ ). No statistically significant difference was found between the bond strengths of SB and OU when irradiation was performed with (b) or without (c) water spray. Additionally, when dentin was unlased (a), SB presented better bond strength values than SE and OU that did not show statistically differences when compared with each other (Fig. 2).

## Discussion

The Er,Cr:YSGG laser device has been shown to create precise hard-tissue cuts by laser energy interaction with water at the tissue interface. When dental hard tissues were irradiated by Er,Cr:YSGG laser with water coolant, the temperature rise was reduced [5, 17], and no inflammatory pulpal response was detected [18]. On the other hand, when Er,Cr:YSGG was applied without coolant in fluences below the ablation threshold, no deleterious increase in pulp chamber temperature was reported [19].

However, there is some controversy about the amount of water and laser efficiency. While some authors [2] have shown that the amount of water (0.8 ml/s or 3 ml/s) is not really important for Er,Cr:YSGG as it is for erbium:yttrium-aluminum-garnet (Er:YAG) laser, under 43 J/cm<sup>2</sup> of energy density for enamel irradiation, Fried et al. [10] showed that the cutting efficiency is correlated with the amount of water content, as well as pulse duration. Both authors agree that, for ablation to occur, water must be present over dental

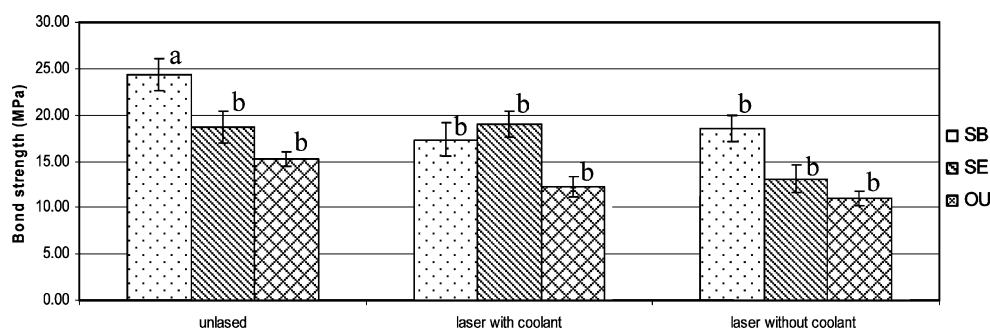
surfaces, due to the higher absorption coefficient of Er,Cr:YSGG laser in water than in enamel [10].

Kang et al. [20] showed that 2 J/cm<sup>2</sup> is required to induce a vapor channel in the spray to deliver the remaining laser pulse, and only beyond this threshold would material removal occur. In this study an energy density of 2.8 J/cm<sup>2</sup> was used, with the objective of removing superficial debris from the dental surface prior to the use of different adhesive systems. It is important to point out that it is well known that this fluence is not able to promote either ablation of enamel [20] or laser etching [21]. Also, considering that some authors [11] observed no increased resistance of laser-treated enamel to demineralization when irradiation was performed with 104 J/cm<sup>2</sup> under 33 ml/min of water coolant, we supposed that adhesive systems would still adequately permeate dental enamel.

In this study results showed that the irradiation of enamel at 2.8 J/cm<sup>2</sup>, despite the cooling parameter used, impaired bond strength of etch-and-rinse and self-etching tested systems. Differences between groups that were irradiated under water coolant and without water spray were not detected, but both irradiated groups presented a lower bond strength than that of the group without laser irradiation (unlased).

Fried et al. [10] stated that, during high intensity laser irradiation, marked chemical and physical changes may be induced in the irradiated dental enamel. These changes can have profound effects on the laser ablation process, leading to a reduction in the ablation rate and efficiency, increase in peripheral thermal damage, and even lead to stalling, without further removal of tissue with subsequent laser pulses. Moreover, thermal decomposition of the mineral can

**Fig. 2** Means of tensile bond strength (MPa) for dentin groups. Error bars correspond to standard errors. Means followed by distinct letters are statistically different by the Tukey test ( $P<0.05$ )



lead to changes in the susceptibility of the modified mineral to organic acids in the oral environment. Morphological changes may also result in the formation of loosely attached layers of modified enamel that can delaminate, leading to failure during bonding to restorative materials [10].

SEM studies [21, 22] have shown that enamel, after irradiation with cooled Er,Cr:YSGG laser, differs according to the energy used, while 2 W irradiation [21, 22] has disclosed that the surface of enamel resembles a type III etching pattern, characterized by a more random pattern in which adjacent areas of the tooth surface corresponding to types II and I were present, mixed with regions where the pattern could not be related to prism structure [23]. 1 W Er,Cr:YSGG irradiation produced the more preferred type I etching pattern, with a honeycomb appearance, while no significant enamel surface etching was obtained by 0.5 W laser irradiation [22].

Authors [6, 22, 24] performing adhesion tests in enamel observed similar bond strength when comparing acid and laser etching, always using high energy densities of Er,Cr:YSGG, and these result differed from our findings. In accordance with our findings, Usumez et al. [21] performed irradiation with a low parameter and observed that bond strength was so low that they did not perform SEM analysis.

Based on SEM results reported in the literature [21, 22], and considering that our findings indicated that irradiated enamel, even at low energy density ( $2.8 \text{ J/cm}^2$ ), reduced bond strength, we understand that only the morphological evaluation cannot explain bonding performance of the adhesive systems tested.

Cehreli et al. [25] related that laser irradiation with 3.5 W could significantly alter the chemical composition of superficial enamel, leading to the formation of metastable crystalline products, such as  $\beta$ -tri-calcium phosphate ( $\beta$ -TCP), resulting in a reduction of previously available surface  $\text{Ca}^{2+}$ . There is no evidence that this alteration occurs at  $2.8 \text{ J/cm}^2$ , but, based on the results obtained, the main goal is to detect the ideal energy and amount of water coolant that, with no harm to the pulp, promotes morphological and chemical alterations in enamel in order to increase acid resistance and, at the same time, does not impair bonding.

Our findings showed that specimens without laser irradiation (a) presented higher bond strengths than irradiated groups, despite enamel cooling condition (b, c), because it did not have its chemical and morphological surface altered, being a discussion based only on the interaction of enamel and bonding systems.

According to this study, the strength of the bond to enamel of etch-and-rinse (SB) and two-step self-etching (SE) systems were similar and higher than that obtained for the one-step self-etching system (OU).

When enamel is conditioned with phosphoric acid, inter-prismatic demineralization occurs that produces free spaces for infiltration of the adhesive, which forms around  $1 \mu\text{m}$  long resin tags. On the other hand, with self-etch systems, thinner hybrid layers and shorter resin tags (between  $0.6 - 0.7 \mu\text{m}$ ) are formed [26], but adequate strength of bonding to the enamel is achieved [27].

Despite some authors claiming that self-etch systems present a deficient etching pattern [28], others relate that the acidic primer is able to promote irregularities on the enamel surface [29], promoting efficient adhesion [30], explained by additional adhesion promoted by the MDP monomer (10-methacryloyloxydecyl dihydrogen phosphate) that presents chemical adhesion with Ca ions of the hydroxyapatite [30, 31]. This could explain the similar performance of SE and the etch-and-rinse system (SB), this result being in accordance with those of other authors [26, 28, 29].

Because enamel bond strength depends on the composition of the adhesive system [32], a one-step self-etching system (One Up Bond F) could compromise the individual performance of each component [33]. Studies in the literature [29, 34] have reported results similar to ours, relating lower adhesive resistance to one-step self-etching systems in enamel.

Dentin presented higher values of bond strengths for unalased samples (a), followed by irradiation with water coolant (b), while the worst adhesion performance was observed when Er,Cr:YSGG laser was used without water coolant (c). This result can be easily explained by the fact that this laser is more absorbed by  $\text{OH}^-$  of water than by  $\text{OH}^-$  mineral of dental tissues. When water is not present, laser caused only superficial melting and deeper carbonization zones [2] that would probably impair primer infiltration.

Authors [2] have already shown that, without an external water spray, no ablation will occur in dentin, but it is important to point out that the fluence employed in this study was not meant to ablate dentin, just to clean its surface, removing the smear layer. In our understanding, when laser is used, despite energy density, water spray must be added to prevent thermal side effects.

Laser irradiation of dentin promotes a rough and clean surface, with no smear layer, and the dentinal tubules are wide open [24]. Also, after acid etching, the peritubular dentin protrudes from the surrounding intertubular dentin, due to its higher mineral and lower water contents [35]. These characteristics might let one think that this dentin should be more prone to adhesion than bur-cut dentin, covered by a smear layer.

Er,Cr:YSGG laser irradiation, even with water coolant, is able to promote micro-structural alterations as well as micro-rupture of collagen fibers [36]. Ceballos et al. [37] reported that there existed  $3-4 \mu\text{m}$  of dentin subsurface that is denatured, with cross-banding lost, in Er:YAG-irradiated

dentin, decreasing interfibrillar spaces that would impair primer infiltration. Lee et al. [35] suggest that the same mechanism may occur for Er,Cr:YSGG, although they state that the effect of Er,Cr:YSGG on collagen fiber has not yet been clarified. At the same time it is well known that a hybrid layer will be adequately formed only in the presence of preserved collagen structure [38], otherwise monomer permeation would not occur.

In accordance with the above-mentioned statements, adhesion studies [36, 39, 40] have shown a decrease in bond strengths of laser-treated surfaces compared with bur-cut preparations. Other studies [6, 35] have shown no difference in bond strengths of laser-irradiated samples.

Dentin without laser irradiation (a) presented higher bond strength values for etch-and-rinse (SB) than for self-etching systems (SE and OU), which were considered similar when compared with each other. This result is in accordance with those in the literature [41, 42], explained by the fact that, for the etch-and-rinse system, dentin etching with phosphoric acid promotes the demineralization of peri- and inter-tubular dentin, widening the apertures of the dental tubules, dissolving hydroxyapatite crystals, and exposing the collagen network to a depth of 3–5  $\mu\text{m}$  [43] or 7  $\mu\text{m}$  [44]. As this system is ethanol and water based, it permeates the demineralized dentin supported collagen network that will be infiltrated by monomers and form a hybrid layer, as well as resin tags [38, 44], which could be responsible for the higher adhesive resistance observed in the unlased group.

On the other hand, self-etch systems (SE and OU) presented pH values higher than that of phosphoric acid, leading to a less pronounced depth of dentin demineralization, due to the presence of calcium and phosphate ions that acted as a buffer [45]. Both systems present water as a solvent, methacrylate monomers and an inorganic phase. In this way, hybrid layers are thinner, with shorter resin tags, than etch-and-rinse, leading to lower adhesion to dentin [46].

When Er,Cr:YSGG laser was used with (b) or without water spray (c), the two-step self-etching system (SE) showed higher bonding to dentin than SB and OU did, which presented similar adhesive resistance when compared with each other.

The two-step self-etching system (SE) works not only on micro-mechanical retention, but also by MDP monomer chemical interaction with calcium in hydroxyapatite [30], and this could be the reason why SE bonded to irradiated dentin. Even when cooling was not employed, the increased surface area with fixed calcium originated from superficial melting and recrystallization of irradiated dentin could be another explanation for the best results observed with SE.

Alterations in the collagen of irradiated dentin [36] can support our findings that Er,Cr:YSGG irradiation impaired the bond strengths of etch-and-rinse and one-step self-

etching, because they do not present chemical interaction with hard tissues. The worst performance of ethanol/water (SB) and water (OU) based adhesives may be also related to the lower interfibrillar spaces in the collagen network [37], impairing monomer permeation and the adequate formation of hybrid layers [47].

It was concluded that Er,Cr:YSGG laser irradiation reduces the strength of bonding to enamel, irrespective of the adhesive system used and the irradiation conditions. Furthermore, it was observed that the best adhesive system to be used in association with Er,Cr:YSGG laser-irradiated dentin was the two-step self-etching one (SE).

As the use of Er,Cr:YSGG laser is a new technology for restorative dentistry, it is suggested that further investigation be conducted to establish a better understanding of bonding to lased surfaces.

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