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Effect of Er: YAG Laser Pulse Width on Shear Bond Strength Between Layered Ceramic and Zirconia Substructure

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Abstract
Introduction: This study aimed to investigate the influence of different erbium-doped yttrium aluminum garnet (Er: YAG) laser pulse widths (pulse durations) on the shear bond strength (SBS) of layered ceramic to zirconia substructures.
Methods: A total of 60 zirconia blocks (5 mm radius and 10 mm height) were divided into 6 groups. Group C received no treatment, while Group S was treated with sandblasting using aluminum oxide (Al_2O_3). Groups L1, L2, L3, and L4 were irradiated with Er: YAG lasers with pulse widths of 50 µs, 100 µs, 300 µs, and 600 µs, respectively. The layered ceramics were applied using a custom index and then sintered. The specimens were mounted in acrylic resin and subjected to a SBS test at a speed of 0.5 mm/min using a universal testing machine until the bonding surface developed cracks. The mode of failure was observed under a microscope.
Results: The highest SBS was observed in the L4 and L2 groups, followed by the L3, L1, and S groups. Group C showed the least SBS. However, no significant difference was found between laser groups (L1, L2, L3, and L4) and group S.
Conclusion: In conclusion, this study highlights the importance of surface treatment in improving the bond strength of zirconia restorations. The use of Er: YAG laser irradiation can produce a rougher surface, which is beneficial for adhesion. These findings provide valuable insights for clinicians seeking to improve the durability and longevity of zirconia-based restorations.
Keywords: Dental materials; Laser therapy; Surface properties; Dental bonding; Yttria stabilized tetragonal zirconia.

Introduction

All-ceramic restorations are used by many dentists as a primary option due to their superiority in tooth-like esthetics, biocompatibility, affordability, and relatively acceptable mechanical properties, leading to their replacement with traditional materials such as gold or porcelain fused to metal (PFM).[1] The tooth-colored zirconia-based ceramics, stabilized with 3 mol% yttria (3Y-TZP), have become the leading alternative to other alumina-based ceramics due to better flexural strength and fracture toughness.[1,2] Zirconia, a solid polycrystalline structure, does not have a glassy phase to mask its opaque look. Therefore, veneering with feldspathic porcelain (VZir) is recommended to mimic the aesthetic perfection of the natural tooth.[3] Nevertheless, compromising the integrity of the zirconia structure with porcelain veneer is its major drawback because it will result in a higher failure rate and clinical complications.[3,4] These failures include technical failures such as catastrophic fracture of the substructure, loss of retention, veneer chipping, marginal discrepancies, and biological complications such as pulpitis and root fracture.[5] This is because of differences in the coefficient of thermal expansion, insufficient structural strength, bond strength, and porcelain support.[6] Among all the mentioned failure modes, veneer chipping or delamination is the most common.[5,7] Roughly 25% of all veneered zirconia restorations are delaminated when subjected to fatigue within the first 5 years.[8] The primary aim of overcoming this problem is to increase the bond between the zirconia substructure and the veneered ceramic (Z-C bond). The Z-C bond depends on several factors, such as the Van der Waals force, surface wetting, coefficient of thermal expansion, phase transformation of zirconia, and, above all, mechanical clamping, or material

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interlocking, as the main factor.[9,10] To minimize such errors, previous studies have suggested using compatible materials, professionalizing the processing technique and the design of the substructure, decelerating the cooling phase, using liner materials, and modifying the surface texture of zirconia accordingly.[6,9,11-13]

The relation between surface roughness, material clamping, and wetting plays an important role in achieving a better and stronger Z-C bond.[11,14-18] Surface modification on zirconia to increase surface roughness has shown promising outcomes. Different conventional methods for such a procedure include acid-etching,[19,20] machining and grinding,[21] atmospheric pressure plasma,[22] aluminum oxide sandblasting,[23,24] and tribochemical silica coating.[25,26] Each method has its limitations and complications, such as physical damage, geometry deformation, unfavorable phase transformation of zirconia (tetragonal to monoclinic), and micro-cracking, which all lead to lowering mechanical strengths. Laser irradiation, however, is an innovative method for surface texturing with minimum damage and unfavorable changes.[27,28] Various lasers, particularly those used for medical purposes such as Co2, have been studied before, and they have not shown any positive results. Negative effects such as microcracks or surface melting points have been observed.[29,30]

Many researchers recommend Er: YAG as a beneficial method for increasing surface roughness.[31,32] The Er: YAG laser has several clinical applications, such as tissue management, endodontics, and restorative dentistry,[33] making it a great candidate for this study. Many factors and parameters of the laser determine the final result of effectiveness, such as the source of radiation material, frequency, irradiation time, the movement algorithm, pulse length and pulse width, energy, output power, and distance. The established protocol for ultimate optimization has not yet been found.[34] Previous studies have demonstrated that the laser surface treatment of zirconia can heighten the shear bond strength (SBS) between the zirconia substructure and cement.[32] However, there remains a gap in understanding how this treatment impacts the SBS when a ceramic layer is applied to the zirconia substructure.[35] One parameter that clinicians can adjust is pulse width. Therefore, the objective of this study is to evaluate the effect of different Er: YAG laser pulse widths on the SBS of the zirconia substructure.

Methods

Zirconia Substructure Preparation

Sixty zirconia disks (Amann Girrbach) (5mm radius and 10 mm height) were milled and sintered according to the manufacturer's (KooshaFanPars) recommendation. The specimens were then gently cooled down to prevent thermal shock, which would negatively interfere with the results.

The disks were divided randomly into 6 groups:

- Group C→ the Control group (left untreated); with no surface treatment (n = 9)
- Group S→ the Sandblasted group: the zirconia-ceramic interface (Z-C interface) received Al2O3 sandblast with 2 bar pressure (Renfert, Germany) (n = 9)
- Group L1→ Er:YAG Laser irritation with 50µs pulse width (n = 11)
- Group L2→ Er:YAG Laser irritation with 100µs pulse width (n = 9)
- Group L3→ Er:YAG Laser irritation with 300µs pulse width (n = 13)
- Group L4→Er:YAG Laser irritation with 600µs pulse width (n = 9)

Other laser parameters, such as frequency, power, time, distance, water, and air cleaning, remained the same for the L1 to L4 groups.

Customized Index (Mold) Design and Fabrication for Ceramic Application

The specimen's dimension was transferred to CAD software (Blender 2021) to design a mold for ceramic application later in progress. The design was in two matching parts, covering the whole specimen with exceeding walls with a hole inside (1mm radius x 2mm height) to preserve the space for ceramic powder. The design was exported as Standard Triangle Language (STL) and sent to the dental laboratory for printing (Frozen 8K, Sony, Japan). The mold was checked randomly with 20 specimens to ensure its adaptation (Figure S1).

Laser Radiation

For 10 minutes, the L1–L4 specimens were ultrasonically cleaned in a medical ethanol solution, and then dried for 5 minutes at room temperature before radiation. Each Z-C substructure surface was subjected to Er:YAG laser radiation (Pulser, Italy) using the device probe at a right angle and marked for later reference. Laser irradiation was performed with identical parameters in all groups (frequency: 20 Hz; energy: 150 mJ; power: 3.0 W; water spray: 30%; distance: 1 mm), with the only variable being the pulse width, which was set at 50, 100, 300, or 600 µs for groups L1, L2, L3, and L4, respectively.

Profilometer Analysis (Roughness Analysis)

All specimens' surface roughness was evaluated using a profilometer device (TR200). Average roughness (Ra) and root mean square roughness (Rq) were observed for later evaluation.

Ceramic Application

All specimens were cleaned ultrasonically with 90% ethanol, and each specimen was put into the fabricated mold. Then the ceramic powder (Zr-fs, GC, Japan) was mixed with special build-up liquid according to the manufacturer's recommended ratio, and the ceramic slurry was achieved. This slurry was put and pressed into the mold hole using a dental brush (Renfert, Layart), and the excess liquid was driven out using paper tissue. The mold was taken away, and the specimen and the ceramic slurry, now in cylindrical shape on the top of the substructure, were put into the dental ceramic furnace (Programmat, Germany) according to the manufacturer's recommended temperatures (Table S1). Preheat and the cooling phase time of the zirconia and ceramic firing schedule were set with extra care to eliminate the chance of ceramic slurry explosion.

After the ceramic application was done, the specimens were placed in 37° centigrade distilled water for 24 hours and then subjected to a thermocycling process (5000 cycles, 5°-55° centigrade, 15 seconds transfer time between cycles, and 30 seconds remained in each cycle). After the thermocycling process, the specimens were kept in distilled water for storage to mimic the intra-oral environment.

Scanning Electron Microscope Evaluation

One specimen from each group (6 specimens in total) was cut vertically to enable the SEM evaluation of the Z-C junction. Several features of the specimens were evaluated using field emission scanning electron microscopy (FE-SEM) (sigma VP, ZEISS, Germany).

Shear Bond Strength Test

Fifty-four specimens (excluding the SEM ones) were fixed in self-cure acrylic resin (Polymethyl methacrylate) to prevent displacement in the SBS testing machine. The specimens were subjected to shear bond test and the shear bond strength peak was calculated (Figure S2).

The failure mode was observed under a stereoscope and categorized into Z-C junction fracture, ceramic layer fracture, zirconia substructure fracture, and mixed fracture (Figure 1). Surface irregularities were assessed under a SEM.

Results

Surface Roughness Analysis

Table 1 presents the descriptive statistics (mean ± SD) for the various roughness parameters in each study group. According to the ANOVA test, the mean values of the five parameters of surface roughness (Ra, Rq, Rt, Rsk, and Rp) showed statistically significant differences between the six groups of the study ($P < 0.05$; Table 2).

According to the results of the Tukey Post hoc test, the control group and the L4 group showed significant differences in five parameters of surface roughness: Ra ($P = 0.022$), Rq ($P = 0.022$), Rz ($P = 0.044$), Rt ($P = 0.032$), and Rsk ($P = 0.043$). The L1 group and the L4 group showed a significant difference in Rsk ($P = 0.011$). The L1 group and the control group showed a significant difference in Rp ($P = 0.020$). In general, the L4 group showed the maximum surface roughness (Ra, Rq, Rz, Rt, and Rv).

Shear Bond Strength Analysis

ANOVA analysis was used to compare the groups' mean SBS. There was no significant difference in the mean SBS between the six groups (F (5, 60) = 1.63 and $P = 0.17$). However, the L4 group showed slightly more SBS,

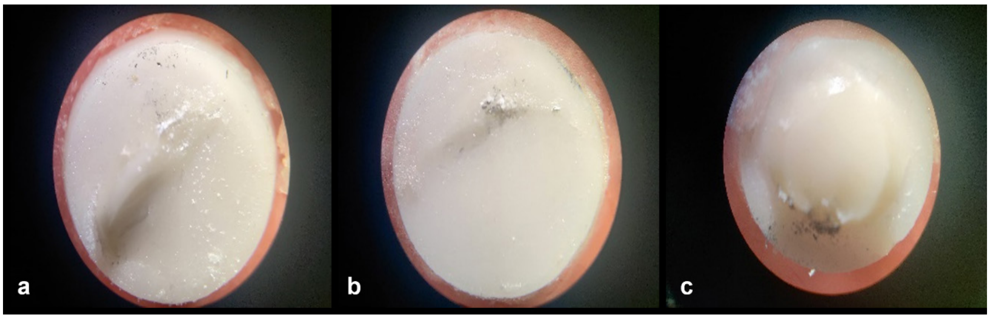

Figure 1. Stereoscope Analysis of (a) Mixed Fracture, (b) Zirconia-Ceramic Junction Fracture, and (c) Ceramic Layer Fracture

Table 1. Mean ± SD of Different Parameters of Roughness in Each Study Group

Roughness parameters	Control group (n = 9)	Sandblast group (n = 9)	L1 group (n = 11)	L2 group (n = 9)	L3 group (n = 13)	L4 group (n = 9)	Total (N = 60)
Ra	0.33 ± 0.03	0.78 ± 0.29	1.07 ± 0.16	0.71 ± 0.128	0.70 ± 0.39	1.87 ± 1.08	0.91 ± 0.64
Rq	0.42 ± 0.04	0.95 ± 0.36	1.36 ± 0.22	0.89 ± 0.15	0.89 ± 0.46	2.32 ± 1.34	1.14 ± 0.80
Rz	1.88 ± 0.16	4.13 ± 1.56	5.55 ± 0.83	4.03 ± 0.53	4.05 ± 1.68	6.98 ± 3.68	4.44 ± 2.22
Rt	2.56 ± 0.37	5.07 ± 1.52	11.02 ± 4.48	5.35 ± 0.450	6.08 ± 2.92	20.82 ± 14.04	8.48 ± 8.12
Rv	0.90 ± 0.08	2.36 ± 1.04	2.91 ± 0.51	2.24 ± 0.41	2.43 ± 1.08	4.49 ± 2.81	2.56 ± 1.56
Rsk	0.36 ± 0.29	-0.06 ± 0.29	1.67 ± 2.31	-0.11 ± 0.35	-0.52 ± 0.47	-5.06 ± 4.06	-0.62 ± 2.70
Rp	0.97 ± 0.09	1.77 ± 0.52	2.97 ± 0.95	1.79 ± 0.13	1.62 ± 0.64	2.49 ± 0.87	1.94 ± 0.84

followed by the L2 group. The sandblast group showed the most varied numbers (Table 3, Figure S3).

Mode of Failure

The mode of failure of specimens after being subjected to the SBS universal machine is shown in Table 4.

Scanning Electron Microscope Evaluation Analysis

SEM analysis showed different irregularities in terms of number, size, and penetration of ceramic into these irregularities in different groups. L4, followed by L2, showed the most numerous and largest size of irregularities (Figure 2).

Table 2. Results of ANOVA Analysis Regarding Different Parameters of Roughness

		Sum of squares	Degree of freedom	Mean Square	F	*P* value
Ra	Between groups	4.182	5	0.836	3.472	0.036
	Within groups	2.890	12	0.241		
	Total	7.073	17			
Rq	Between groups	6.338	5	1.268	3.445	0.037
	Within groups	4.416	12	0.368		
	Total	10.754	17			
Rz	Between groups	44.024	5	8.805	2.666	0.076
	Within groups	39.639	12	3.303		
	Total	83.663	17			
Rt	Between groups	662.832	5	132.566	3.480	0.036
	Within groups	457.069	12	38.089		
	Total	1119.901	17			
Rv	Between groups	20.315	5	4.063	2.301	0.110
	Within groups	21.192	12	1.766		
	Total	41.507	17			
Rsk	Between groups	79.658	5	15.932	4.286	0.018
	Within groups	44.607	12	3.717		
	Total	124.265	17			
Rp	Between groups	7.363	5	1.473	3.734	0.029
	Within groups	4.732	12	0.394		
	Total	12.095	17			

Discussion

This study evaluated the effect of different Er: YAG laser pulse widths on the surface roughness and the SBS between zirconia and veneering ceramics. The L4 group (with a pulse width of 600 µs) demonstrated the highest surface roughness parameters, which can enhance micromechanical interlocking and potentially contribute to long-term durability of the bond in clinical settings. This could help explain why the L4 group showed the highest mean SBS, despite the lack of statistical significance. This improvement in surface topography may be particularly beneficial under real clinical conditions where mechanical and thermal stresses are present over time. In addition, it provides guidance on the most effective laser settings to use for the surface treatment of zirconia. While the created surface roughness can be beneficial to SBS, other factors also play a role in determining the overall bond strength. The chemical composition of the resin cement, the type of adhesive used, and the curing process may contribute to the final bond strength.[36]

The failure mode analysis revealed that adhesive failure at the zirconia–ceramic interface was dominant in all groups, consistent with previous studies indicating this interface as a weak link in layered restorations.[37] Interestingly, the L4 group, exhibiting the highest surface roughness parameters, was the only group among the laser-treated samples that showed a mixed failure. This may suggest a stronger interfacial bond due to enhanced micromechanical interlocking, shifting the failure pattern from purely adhesive to partially cohesive. While this

Table 3. Statistical Values Related to Shear Bond Strength in Each Group

	Mean (±Standard deviation)	Standard error	95% Confidence interval for mean	Minimum	Maximum
Control group	15.03 (±8.14)	2.71	8.77, 21.29	5.56	29.15
Sandblast group	16.28 (±11.06)	3.69	7.77, 24.78	4.88	30.49
L1 group	19.42 (±5.64)	1.70	15.63, 23.21	12.64	27.34
L2 group	23.62 (±7.07)	2.35	18.19, 29.05	14.99	31.25
L3 group	19.97 (±8.56)	2.37	14.79, 25.14	11.05	39.02
L4 group	23.43 (±8.91)	2.97	16.58, 30.28	14.79	40.33
Total	19.64 (±8.54)	1.10	17.43, 21.85	4.88	40.33

Table 4. Frequency of Different Types of Failure Modes in Each Study Group

	Control group	Sandblast group	L1 group	L2 group	L3 group	L4 group
Adhesive failure (Z-C junction)	9	8	10	9	8	9
Cohesive failure in ceramic	1	1	0	1	2	0
Mixed failure	0	1	0	0	0	1
Cohesive failure in zirconia	0	0	0	0	0	0

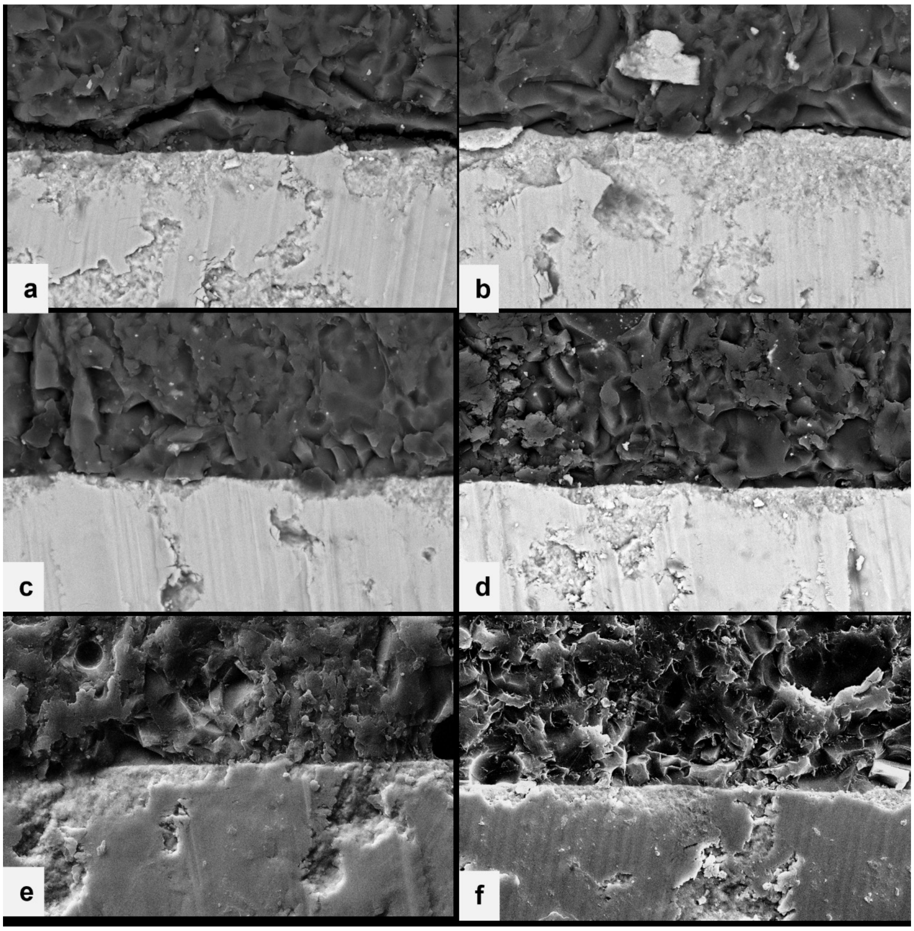

Figure 2. Scanning Electron Microscope Images at 1.00 kX Magnification and 10µm Scale Bar. a-f images are related to the control group, sandblast group, and laser groups with 5 0µs, 100 µs, 300 µs, and 600 µs pulse widths

trend was observed in only one specimen, it highlights a potential correlation between increased surface roughness and improved bonding quality.

Dental technicians use different lasers, including semiconductors, Er: YAG, and Nd: YAG lasers. The majority of them lack protection against thermal cooling. However, the Er: YAG laser is better at controlling the range of heat penetration in deep tissue and blowing off water to offset the temperature increase during irradiation.[38] The Er: YAG laser mechanism works by converting sufficient laser energy into heat energy and pressure, which destroys the local surface of ceramics and forms a suitable mechanical retaining structure on it to influence physical adhesion.[39] For the adhesive effect, lasers must have the proper parameter values, which include irradiation time, power, and pulse energy. If the parameters are too high, the laser irradiation may severely damage the structure of the ceramic surface. In contrast, too small parameters will not have any effect on the ceramic surface or will just create a shallow structure that hinders retention.[39]

Navarro et al. assessed the effect of different Er: YAG laser pulse widths on the composite microleakage in dental cavities. Four groups with different pulse widths were compared with each other. Microleakage was unaffected by the laser irradiation with varying pulse widths. Compared to cavities made conventionally, the SEM morphology examination of laser-created cavities revealed more conservative patterns with uneven enamel borders and dentinal internal walls.[40] Hou et al evaluated how Er: YAG laser irradiation affected self-glazed zirconia (SZ) ceramics' SBS and microleakage. The groups of the study were the control group, standard grid processing group, and Er: YAG laser group with different power energies (100, 200, 300, 400, 500, and 600 mJ). The Er: YAG laser with 600 mJ power showed the highest SBS and the least microleakage depth. Regarding the fracture mode, the control group mainly showed adhesive failure, suggesting that the SBS between untreated ceramics and resin was not particularly high. In contrast, cohesive failure and mixed failure began to dominate the fracture mode following various surface treatments using the laser as the pulse energy increased. This indicates that these groups had higher SBSs than ceramics or resin. While Hou et al. observed the positive effect of laser irradiation on the SBS at the zirconia-ceramic layer interface, our study did not confirm the direct effect of the laser on it.[39] However, in Hou et al.'s study, the varied parameter was the "power output" of the irradiation, and other parameters, such as pulse duration (pulse width), were not evaluated. The relationship between laser energy and pulse duration ($E = PPEAK \times t$) proves that increasing pulse width is one of the parameters involved in increasing laser energy. The core parameter for increasing the output energy in the study by Hou et al. is not clearly explained, and therefore, even with favorable results, it cannot be assumed to be a specified method for establishing the optimal protocol for laser irradiation. Also, the discrepancy highlights the need for further investigation and a deeper understanding of the underlying mechanisms involved in laser treatment for zirconia-ceramic bonding.

Improvements in the use of ytterbium laser surface treatment on zirconia were found by Toyoda et al. Their results showed that this treatment increased the SBS between zirconia and 4-META/MMA-TBB resin cement, as well as composite resin cement. They also found that the surface roughness of the zirconia substructure was significantly higher than that of the non-treated control group, followed by the alumina sandblasted group, which is consistent with our findings.[35]

In a study reported by Nguyen et al, the effect of scanning speed and acid-etching on the adhesion strength of laser-treated enamel and dentin surfaces was assessed. They examined 25 and 50 mm/s laser beam scanning rates. A single plane shear-bond test was used to assess the adhesive strength of the dental composite to laser-treated enamel and dentin. The binding strength was not significantly affected by the scan rate.[41] A study using femtosecond lasers demonstrated that the surface shape and beam angle significantly influence surface roughness and SBS to zirconia. The pulse power, wavelength, pulse duration, and scanning speed of the laser were 700 mW, 800 nm, 900 fs, and 30 mm/sec. The highest values were achieved with spiral and square patterns applied at a 30° angle, highlighting the importance of laser configuration in optimizing adhesion.[42] A study on enamel bonding found that the laser irradiation distance significantly affects SBS, with optimal adhesion achieved at closer distances (1–2 mm). Specifically, Er: YAG and Er,Cr: YSGG lasers at 1 mm produced results comparable to conventional acid etching.[43]

Ghoveizi et al investigated the use of Nd: YAG laser and reported no significant difference in SBS between laser-treated groups at different power levels. However, both sandblasting and laser treatment significantly increased surface roughness. Additionally, the authors highlighted that heat generation becomes a major drawback when laser power is increased and could cause negative damage to the surface and decrease its roughness.[30] On the other hand, Hatami et al. found that Er: YAG laser irradiation showed equal surface roughness to sandblasting and increased the SBS of zirconia cementation. They discouraged the use of Nd: YAG and CO_2 lasers due to the overheating effect.[32] According to Mahmoodi et al, the surface layer of zirconia disks may thermally deteriorate when exposed to an Nd: YAG laser. Air cooling was used during laser irradiation to minimize overheating and partially mitigate this adverse consequence.[44]

Assery et al assessed roughness and surface alteration following treatment with an Er: YAG laser on titanium and zirconia discs. In contrast to their study, our results showed a significant difference in average surface roughness after using Er: YAG laser irradiation on the zirconia substructure.[45] A systematic review and meta-analysis study revealed no statistically significant improvement in bond strength between zirconia and ceramic using Er: YAG. This finding aligns with the results of the current study, suggesting that other lasers, such as Nd: YAG, improve the bond strength to zirconia.[46] The reason that Er: YAG is chosen in many similar studies, as well as ours, is its versatility and wide range of applications in clinical dentistry.[47,48] One limitation of the current study was the use of a clinical laser device. Future studies should benefit from exploring the effects of more industrial lasers, such as blue diode lasers. It is also worth noting that this field of study is of great interest to the selective laser sintering (SLS) industry, particularly due to its wide-ranging applications in dentistry, such as implantology and maxillofacial prosthetics.[49]

From a clinical standpoint, the application of lasers must balance efficacy with practicality. The substantial initial costs—alongside expenses for maintenance, training, and dedicated workspace—may limit accessibility for general dental practices. Despite the limitations of the current study, we found that varying laser pulse widths had no statistically significant effect on the SBS of the zirconia-ceramic junction. However, additional factors and parameters remain to be investigated. The authors recommend that future studies be conducted to evaluate the effect of laser beam diameter and movement speed. Furthermore, the use of clinical lasers requires skilled operation by a trained clinician to ensure uniform laser application. Further data and analysis may determine whether an algorithm that accounts for irregularities would be more appropriate to improve ceramic penetration during the stacking or application stage. The proper laser irradiation protocol has not yet been definitively established, and many practitioners continue to rely on preset parameters. However, these preset parameters can lead to variable and sometimes conflicting results. The optimal zirconia surface roughening protocol may increase the cohesive bond strength between zirconia and other dental restorative materials such as CAD-CAM composites. For more detailed microscopic analysis, the authors suggest using atomic force microscopy (AFM), in addition to FESEM. AFM is a valuable tool for high-resolution examination of surface topography.[50] Analyzing the surface view of zirconia ceramic with both SEM and

XDR (X-ray diffraction (XRD) patterns) instruments can provide even more detailed data[50] compared to the front view presented in this article. By examining the material from different angles, more insights can be gained into its microstructure and grain boundaries, as well as possible microcracks. This comprehensive approach enhances our understanding of zirconia ceramic properties.

Conclusion

Based on the findings, Er: YAG laser irradiation at a 600 µs pulse-width produced the highest surface roughness among all tested groups. While differences in SBS were not statistically significant, the 100 µs and 600 µs Er: YAG laser-treated groups exhibited slightly higher mean values. These results highlight the importance of surface treatment, particularly using specific Er: YAG laser parameters, in improving the bond strength of zirconia restorations. The use of Er: YAG laser irradiation can produce a rougher surface, which is beneficial for adhesion. These findings provide valuable insights for clinicians seeking to improve the durability and longevity of zirconia-based restorations. The surface roughness observed with the 600 µs setting highlights its promise for standardizing laser conditioning protocols in clinical practice to optimize bonding outcomes.

Authors' Contribution

Conceptualization: Somayeh Allahyari, Nasim Chiniforush, Reza Masaeli.
Data curation: Pouria BoroujerdiKarimi, Negar Ebrahimi.
Formal analysis: Negar Ebrahimi.
Investigation: Pouria BoroujerdiKarimi, Negar Ebrahimi.
Methodology: Somayeh Allahyari, Nasim Chiniforush, Reza Masaeli.
Project administration: Somayeh Allahyari.
Resources: Nasim Chiniforush, Reza Masaeli.
Software: Pouria BoroujerdiKarimi.
Supervision: Somayeh Allahyari.
Validation: Somayeh Allahyari.
Visualization: Pouria BoroujerdiKarimi.
Writing–original draft: Pouria BoroujerdiKarimi, Negar Ebrahimi.
Writing–review & editing: Negar Ebrahimi, Somayeh Allahyari, Nasim Chiniforush, Reza Masaeli.

Competing Interests

None.

Ethical Approval

This in vitro study did not involve human participants or animal subjects, and no patient data or biological samples were used. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.DENTISTRY.REC.1401.128).

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Supplementary Files

Supplementary file 1 contains Table S1 and Figures S1-S3.

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