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Investigating the Effect of Different Surface Treatments on Vickers Hardness and Flexural Strength of Zirconium and Lithium Disilicate Ceramics

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Abstract

Purpose: To investigate the effect of different surface treatments on Vickers hardness and flexural strength of zirconia and lithium disilicate ceramics.
Materials and Methods: 50 zirconia and 50 lithium disilicate ceramic specimens were used. After identifying one group as the control, grinding, sandblasting, CoJet, and Er:YAG laser were applied on the surface of specimens (n = 10). After applying Vickers hardness test to specimens, flexural strength test was performed. The data were analyzed with two-way ANOVA and Duncan's multiple comparisons tests.
Results: There were statistically significant differences found between surface treatment groups in terms of Vickers hardness of zirconia and lithium disilicate ceramics ($p < 0.001$), but there were no statistically significant differences found between surface treatment groups in terms of flexural strength ($p > 0.05$).
Conclusions: Surface treatments could affect the hardness of ceramics. Therefore, was zirconia found to be harder and more durable than lithium disilicate ceramics.

In dentistry, restoration of missing teeth and tissues with esthetic, resistant, and biologic materials has been researched for many years. Fixed prosthetic restorations are an important part of prosthodontics. Metal ceramic restoration is the preferred system for fixed prosthetic restoration, because of its resistance and long-term clinical success; however, the popularity of tooth-colored restorations has grown due to an increase in esthetic requirements and allergic and other reactions to the toxic materials used in some alloys.[1] Zirconia and lithium disilicate glass-ceramic restorations are limited to use in the posterior region and for long-span fixed restorations because of their low resistance to fracture; however, they have superior esthetic properties and biocompatibility.[2] Thus, they are applied successfully in the anterior region. Manufacturers have developed new ceramic systems and materials, using all-ceramic systems with improved mechanical properties allowing the materials and to be applied to the posterior region.[3-6]

Different substructure materials are used in all-ceramic restorations. Zirconium is used due to its physical properties.[7] In recent years, manufacturers have developed lithium disilicate ceramics. The physical properties of this compound make it especially useful in anterior region restorations, which need to be thinner and more durable. Thus, lithium disilicate ceramics have become a common alternative to zirconia ceramics. The esthetic qualities of all-ceramic restorations are excellent, but their lack of strength affects their long-term use. Different surface treatments affect the surface hardness of all-ceramic materials, but the treatments do not affect the strength. It may be effective to apply the surface treatments together to increase the strength of the materials. The aim of this study was to investigate the effect of different surface treatments on the Vickers hardness and flexural strength of zirconium and lithium disilicate ceramics. It was hypothesized that different surface treatments would decrease the Vickers hardness and flexural strength of both zirconia and lithium disilicate ceramics.

Materials and methods

One hundred rectangular specimens ($25 \times 4 \times 2$ mm^3) were used. Specimens were prepared from zirconium (Prettau

Zirconia; Zirkonzahn GmbH, Gais, Switzerland) and lithium disilicate ceramic (IPS e.max Press; Ivoclar Vivadent AG, Schaan, Liechtenstein). In this in vitro study, two power analyses were performed using the G*Power software program (v3.0.10) to obtain the highest power level with the smallest sample size. The analysis showed that at least 10 specimens were required for the highest power level (power = 80, $\alpha = 0.05$).

Zirconium specimens were prepared from Prettau Zirconia blocks using CAD/CAM and sintered at 1600°C for 8 hours in a sintering oven (Zirkonofen; Zirkonzahn GmbH). Then, glaze porcelain (Glaze Plus; Zirkonzahn GmbH) was applied to all specimens. Lithium disilicate ceramic specimens were prepared from IPS Empress wax (Elastiwac; Ivoclar Vivadent AG) and were placed investment rings (IPS e.max Universal Paper Ring; Ivoclar Vivadent AG). After hardening, the wax was eliminated according to the manufacturer's instructions, and the pressing was completed. Then, glaze porcelain (IPS e.max Press Ceram Glaze Paste; Ivoclar Vivadent AG) was applied to all specimens. Dimensions of all specimens were checked using a digital caliper. Specimens were divided into five groups according to surface treatment:

- Group 1: No surface treatment
- Group 2: Specimen surfaces were ground with diamond burs (Blaudent, Tallinn, Estonia) with a particle size of 50 μm using handpieces (Marathon 600; Meta Dental Corp., Glendale, NY). A digital caliper was used to ensure that 0.1 mm of material was removed from the surfaces of all specimens.
- Group 3: Specimen surfaces were sandblasted with Al_2O_3 (Airsonic; Hager & Werken, Duisburg, Germany) with a particle size of 50 μm under 4-bar pressure for 15 seconds from a distance of about 10 mm.
- Group 4: Specimen surfaces were sandblasted with silanized Al_2O_3 (CoJet Sand; 3M ESPE, St. Paul, MN) with a particle size of 30 μm under 2.8-bar pressure for 15 seconds from a distance of about 10 mm using CoJet (CoJet Prep; 3M ESPE).
- Group 5: The first step was to apply graphite powder to the surfaces of zirconia specimens for absorption. Then, an Er:YAG laser (Fotona D.D., Ljubljana, Slovenia) was applied to the surfaces of all specimens. This was done in short pulse mode (300 mJ, 10 Hz) with a water-cooled 3 kW power output for 40 seconds from a distance of about 1 mm.

To ensure standardization, one investigator applied all surface treatments. After surface treatments, scanning electron microscope (SEM) images were taken of one specimen from each group. Then, the Vickers hardness test was performed on specimens with a force of 9.8 N for 15 seconds using a Vickers hardness tester (TMTech HV 1000B; TMTeck Manufacturing Ltd., Beijing, China), and Vickers hardness values (HV) were recorded. To ensure standardization, three measurements were performed from the left, right, and central regions of specimens, and these values were averaged. After the Vickers hardness test, the flexural strength test was performed on specimens with a 20 mm distance between steel bars with a universal testing machine (0.5 mm/min) (Model 2519-106; Instron Corp., Norwood, MA).

Statistical analysis comprised a two-way ANOVA and the Duncan multiple comparison test. IBM SPSS Statistics (SPSS v20.0; SPSS Inc., Chicago, IL) software package was used.

Results

The results of the two-way ANOVA and Duncan multiple comparison tests for the hardness of materials are presented in Table 1. The results of the two-way ANOVA showed that there were statistically significant differences between surface treatments ($p < 0.001$), the Vickers hardness of ceramic materials ($p < 0.001$), and surface treatments on the Vickers hardness of zirconia and lithium disilicate ceramic ($p < 0.05$). The highest Vickers hardness value for zirconia ceramics were in group 2 (grinding), while the lowest mean Vickers hardness value was in group 1 (control). Of the lithium disilicate ceramic specimens, the highest Vickers hardness value was found in group 4 (CoJet), while the lowest was found in group 1 (control).

The results of the two-way ANOVA test for strength of material are presented in Table 2. Results showed that there were statistically significant differences between the flexural strength of ceramic materials ($p < 0.001$), but there were no statistically significant differences between surface treatments ($p > 0.05$) nor of surface treatments on the flexural strength of zirconia and lithium disilicate ceramic ($p > 0.05$). Of zirconia ceramic specimens, the highest flexural strength value was found in group 2 (grinding), while the lowest was found in group 4 (CoJet). Of lithium disilicate ceramic specimens, the highest flexural strength value was found in group 3 (sandblasting), while the lowest was found in group 2 (grinding).

Images from the SEM (1000×) of zirconia specimens from all groups have been presented in Figure 1. Zirconia specimens from group 2 showed signs of bur use, while those in group 3 showed many irregular retentive regions. Although the surfaces of group 4 (CoJet) zirconia specimens appeared similar to the control group, they showed a regular and intensive surface. Finally, there were many irregular retentive regions and microspaces in zirconia specimens from group 5, which were subject to the Er:YAG laser.

Images from the SEM (1000×) of lithium disilicate specimens from all groups have been presented in Figure 2. In lithium disilicate specimens from group 2 (grinding), regular structure was decayed, and intensive cracks had occurred, filling the microspaces in the material's surface. The sandblasting of group 3 lithium disilicate specimens changed the structure of microspaces in the material's surface, and irregular retentive regions were observed. Although group 4 (CoJet) lithium disilicate specimens showed similar surfaces to group 3 specimens, their retentive regions were more regular. Finally, lithium disilicate specimens from group 5, which were subject to the Er:YAG laser, showed similar surfaces to control group specimens.

Discussion

The hypothesis was that different surface treatments would decrease the Vickers hardness and flexural strength of both

Table 1 Mean hardness values of the materials

Materials	Surface treatments	Mean (HV)	Standard deviation	Max. (HV)	Min. (HV)
Zirconia	Control	1219.83[a]	94.11	1477.51	962.15
	Grinding	2150.75[c]	988.24	2408.43	1893.07
	Sandblasting	1940.63[b,c]	458.38	2198.31	1682.95
	CoJet	2100.84[c]	350.5	2358.52	1843.16
	Er:YAG Laser	1567.17[a,b]	200.94	1824.85	1309.49
	Total	1795.84	613.95	1911.08	1680.60
Lithium disilicate	Control	656.30[a]	163.82	913.98	398.62
	Grinding	857.35[a,b]	151.79	1115.03	599.67
	Sandblasting	889.94[a,b]	199.37	1147.62	632.26
	CoJet	944.22[b]	167.19	1201.90	686.54
	Er:YAG Laser	761.78[a,b]	453.85	1019.46	504.1
	Total	821.92	264.61	937.16	706.68

Values with different superscript letters are statistically significant (Duncan multiple comparison test).

Table 2 Mean flexural strength values of the materials

Materials	Surface treatments	Mean (MPa)	Standard deviation	Max. (MPa)	Min. (MPa)
Zirconia	Control	640.59	200.32	720.94	560.24
	Grinding	692.31	178.55	772.66	611.96
	Sandblasting	676.50	119.19	756.85	596.15
	CoJet	615.57	209.15	695.92	535.22
	Er:YAG Laser	629.33	165.08	709.68	548.98
	Total	650.86	172.42	686.79	614.93
Lithium disilicate	Control	182.14	34.33	262.49	101.79
	Grinding	155.36	18.48	235.71	75.01
	Sandblasting	212.24	47.49	297.59	136.89
	CoJet	182.96	42.39	263.31	102.61
	Er:YAG Laser	205.39	28.21	285.74	125.04
	Total	188.62	40.44	224.55	152.68

zirconia and lithium disilicate ceramics. The results of this study suggest that the null hypothesis should be rejected. This study found that surface treatments increased the Vickers hardness of zirconia and lithium disilicate specimens; however, they did not affect the flexural strength of both ceramic materials.

Hardness is a special mechanical property that affects the success of clinical materials.[8] Materials with a lower surface hardness may deteriorate easily, and the deterioration of a surface causes fatigue to the material, which decreases its survival rate.[9] Baharav et al[10] applied a 1 kg force to alumina porcelains for 20 seconds and obtained values between 530 and 540 HV for hardness testing of all-ceramic materials. Konishi et al[11] applied a 500 g force to ceramic discs for 15 seconds and obtained values between 531 and 537 HV for hardness testing of all-ceramic materials. These findings were used to guide the present study, in which the Vickers hardness values of specimens were measured by a force of 1 kg applied for 15 seconds.

Roy et al[12] compared the surface hardness of aged and non-aged yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramics, and they reported that non-aged Y-TZP ceramics had higher hardness values. Al-Radha et al[13] investigated the surface properties and bacterial adhesion of zirconia and titanium specimens, and they reported that titanium-blasted zirconia specimens had the highest hardness values. In the present study, surface treatments affected the surface hardness of zirconia and lithium disilicate ceramics ($p < 0.05$). Grinding produced the highest hardness values for zirconia ceramics, and this supports previous studies,[14,15] which suggested that polishing could affect the surface hardness of zirconia. The highest hardness values of lithium disilicate ceramics were produced by CoJet application. Sandblasting increased hardness values for both ceramics, while Er:YAG laser had a similar effect to the control group. There is a lack of research on the effect of surface treatments on the hardness of ceramic materials. The findings of this study will contribute to the literature.

Strength is described as the maximum stress that can be applied to a material before it breaks, and this is used as a mechanical property to identify the clinical success of materials.[16] Flexural strength tests often prefer to evaluate the effect of different factors on the strength of materials. According to these tests, restorations that use materials of the highest flexural strengths have more strength against forces.[17] The International Organization of Standardization (ISO 6872) and the American Dental Association (ADA) have reported that the three-point bending test is a suitable and reliable method for the evaluation of the flexural strength of dental ceramics.[18-20] Thus, the three-point

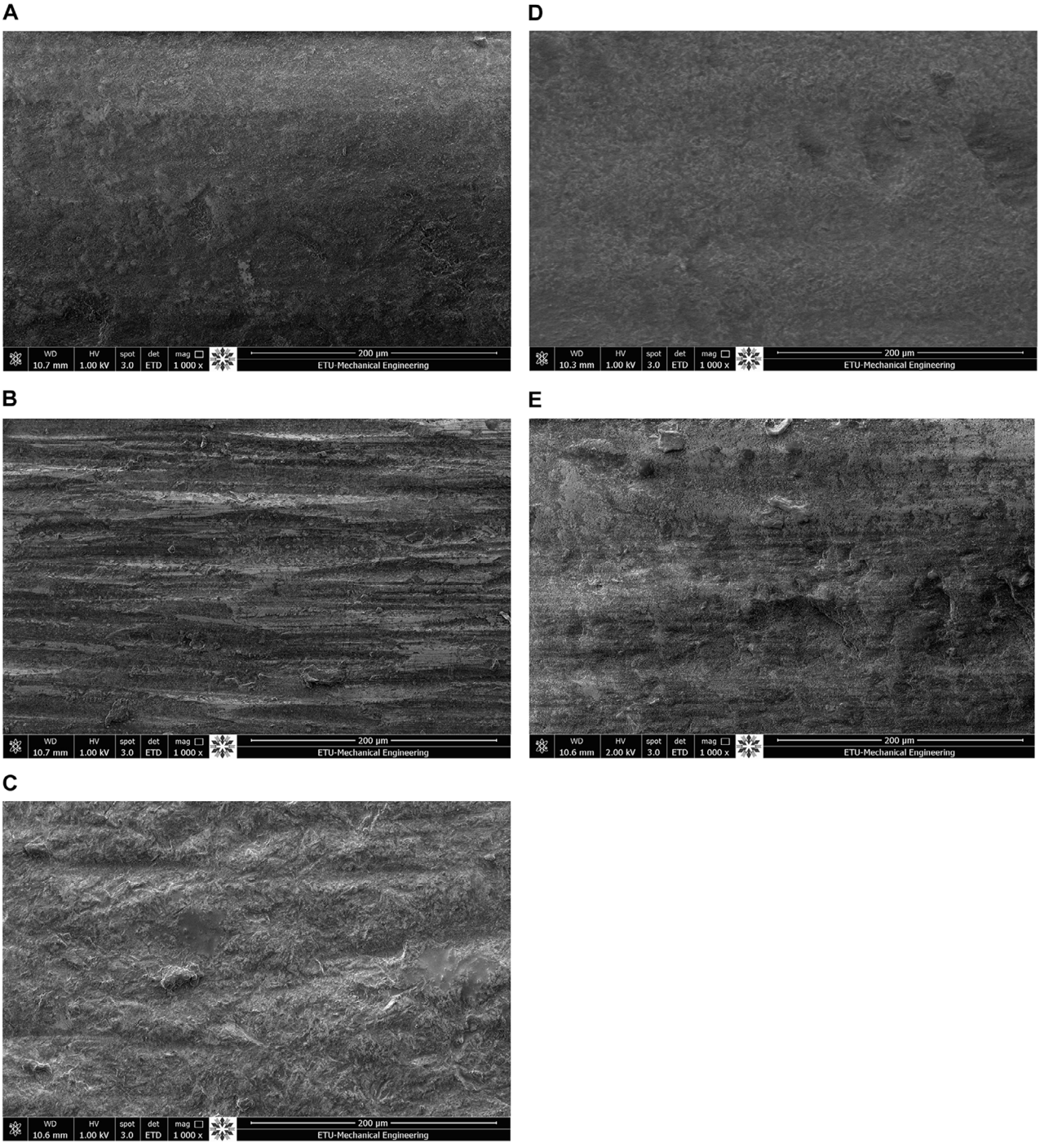


Figure 1 A) SEM images (1000×) of zirconia control group. (B) SEM images (1000×) of zirconia grinding group. (C) SEM images (1000×) of zirconia sandblasting group. (D) SEM images (1000×) of zirconia CoJet group. (E) SEM images (1000×) of zirconia Er:YAG laser group.

bending test was used in the present study. Previous studies[21-25] have shown that zirconia has the highest flexural strength compared to other ceramic materials. Mean values found in this study (Prettau Zirconia 650 MPa, IPS e.max Press 188 MPa) support this.

Different surface treatments may change the structure of materials, and they may affect the mechanical properties of materials. Many studies have investigated the effect of surface treatments on zirconia.[14,26-28] For example, Denry and Holloway[29] ground and polished Y-TZP ceramics with

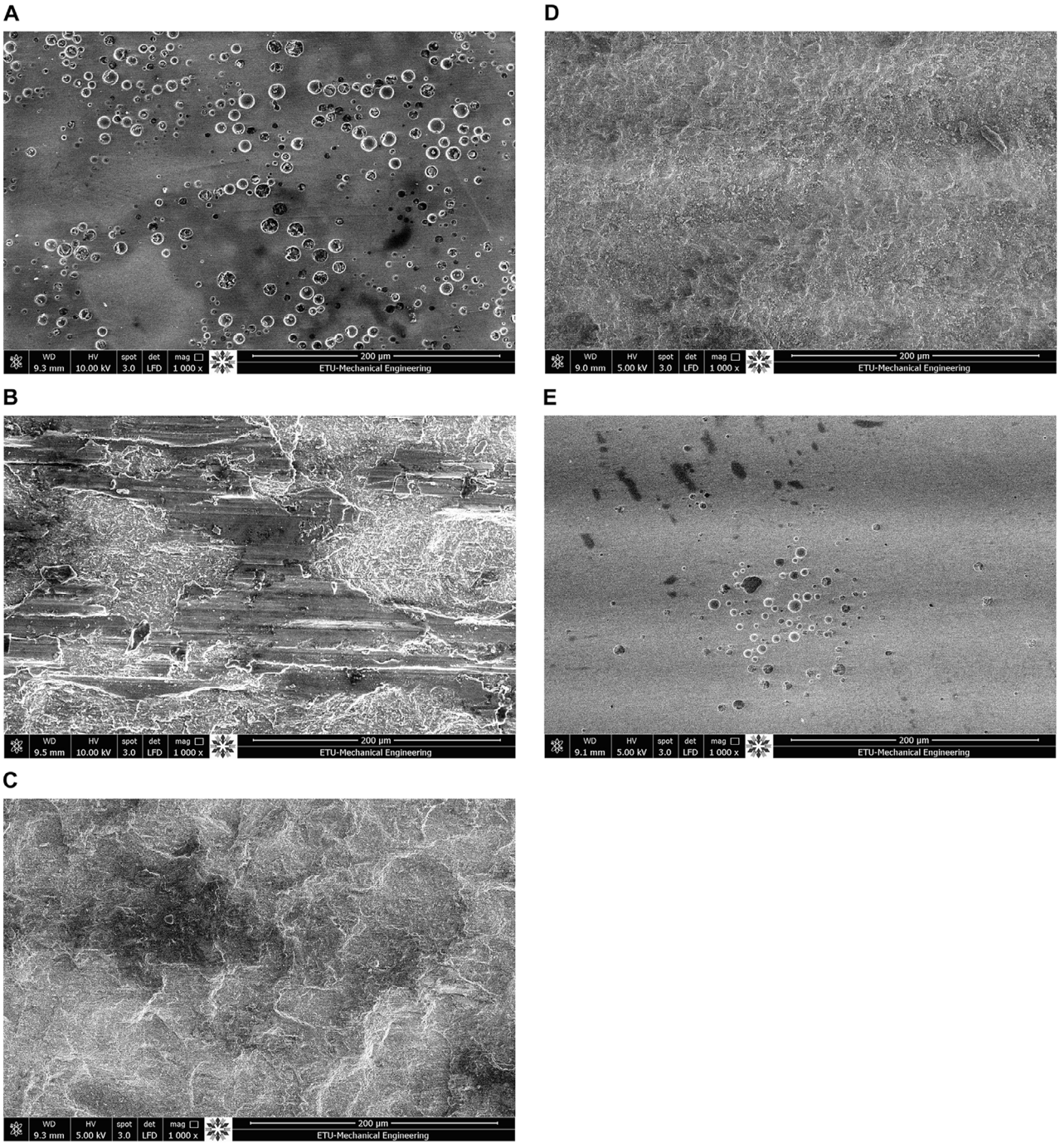


Figure 2 A) SEM images (1000×) of lithium disilicate control group. (B) SEM images (1000×) of lithium disilicate grinding group. (C) SEM images (1000×) of lithium disilicate sandblasting group. (D) SEM images (1000×) of lithium disilicate CoJet group. (E) SEM images (1000×) of lithium disilicate Er:YAG laser group.

diamond burs with a particle size of 30 to 40 μm. They reported that ground Y-TZP ceramics showed more flexural strength than the control group. Curtis et al[27] reported that grinding Y-TZP ceramic with large particle sizes under water cooling decreased its flexural strength, and grinding with small particle sizes under water cooling did not yield a statistically significant effect. Guazzato et al[26] and Qeblawi et al[30] showed that grinding and sandblasting increased the flexural strength of Y-TZP ceramics as measured by the three-point bending test. In the present study, grinding with 50 μm diamond burs increased

the flexural strength of zirconia compared to the control group; however, it decreased the flexural strength of lithium disilicate compared to the control group, though these changes were not statistically significant.

Papanagiotou et al[31] applied low-temperature aging, sandblasting, and polishing to Y-TZP ceramics. They found that low-temperature aging and polishing decreased flexural strength but that sandblasting increased the flexural strength of Y-TZP ceramics. Wang et al[32] showed that sandblasting with Al_2O_3 with a particle size of 50 μm increased its strength; however, increasing the particle size to 120 μm decreased its strength as measured by the three-point bending test. Karakoca and Yilmaz[33] reported that sandblasting increased the flexural strength of different ceramic materials, yet grinding decreased it. In the current study, zirconia and lithium disilicate ceramics sandblasted Al_2O_3 with particle size of 50 μm increased the flexural strength of both, but these increments were not statistically significant. This difference in the literature may be related to the focal distance and duration used in the sandblasting process.

Yamaguchi et al[34] reported no statistically significant differences in the flexural strengths of Y-TZP ceramics sandblasted with Al_2O_3 with a particle size of 70 μm and the same material sandblasted with silanized Al_2O_3 with particle sizes of 30 μm and 110 μm. Qeblawi et al[30] stated that sandblasting Y-TZP ceramic with silanized Al_2O_3 with a particle size of 30 μm under 3-bar pressure did not influence its flexural strength significantly. In the present study, sandblasting zirconia with silanized Al_2O_3 with a particle size of 30 μm under 2.8-bar pressure decreased its flexural strength compared to the control group, but this was not statistically significant. Applying the same process to lithium disilicate did not change its flexural strength much compared to the control group. These findings supported Yamaguchi et al[34] and Qeblawi et al.[30] Cavalcanti et al[35] reported that melting, fracture, and loss of material occurred on the surface of Y-TZP ceramics after the application of an Er:YAG laser at 4 W and 6 W. Gökce et al[36] stated that the application of an Er:YAG laser at 3 W provided enough roughness on lithium disilicate ceramic's surface. Thus, the Er:YAG laser was applied at 3 W in the current study. There is a lack of research on the effect of Er:YAG laser application on the hardness and flexural strength of ceramic materials.

The present study has some limitations. For example, as this study was prepared in vitro, intraoral factors were not present. Therefore, whether the data can be applied to mechanical properties in the oral cavity is not fully known. Also, surface roughness must be examined in order to know the effect of superstructure porcelain connection to the infrastructure material. The surface roughness is also an important factor in good bonding between materials. In addition to the strength of the materials, the bond strength should also be known for the evaluation of the clinical success of materials.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1. Zirconia had a harder surface and was more durable than lithium disilicate ceramic.
2. Different surface treatments had a statistically significant effect on the surface hardness of both materials, but they did not have a statistically significant effect on the flexural strength of either material.
3. Grinding and CoJet application significantly increased the surface hardness of zirconia; however, only CoJet application significantly increased the surface hardness of lithium disilicate.
4. Grinding and sandblasting increased the flexural strength of zirconia; however, CoJet, sandblasting, and the application of an Er:YAG laser increased the flexural strength of lithium disilicate; however, these differences were not statistically significant.
5. This study shows that the external processes applied to the material can make a change in the physical properties of the material.
6. Different and combined surface preparations may contribute to the development and clinical success of the materials in the future, and studies of this type are planned for the future.

References

1. Craig RG, Powers JM: Restorative Properties in Restorative Dental Materials. New York, Mosby, 2002, pp. 551-592
2. Hobo S, Shillingburg HT, Jr: Porcelain fused to metal: tooth preparation and coping design. J Prosthet Dent 1973;30:28-36
3. Phillips RW: Skinner's Science of Dental Materials (ed 11). Philadelphia, W.B. Saunders, 1991, pp. 505-553
4. Wohlwend A, Strub JR, Scharer P: Metal ceramic and all-porcelain restorations: current considerations. Int J Prosthodont 1989;2:13-26
5. White SN, Caputo AA, Vidjak FM, et al: Moduli of rupture of layered dental ceramics. Dent Mater 1994;10:52-58
6. Jones DW: Development of dental ceramics. An historical perspective. Dent Clin North Am 1985;29:621-644
7. Luthardt RG, Holzhuter M, Sandkuhl O, et al: Reliability and properties of ground Y-TZP-zirconia ceramics. J Dent Res 2002;81:487-491
8. Fischer H, Marx R: Fracture toughness of dental ceramics: comparison of bending and indentation method. Dent Mater 2002;18:12-19
9. Rawls KJ: Mechanical properties of dental materials In Anusavice KJ (ed): Phillips' Science of Dental Materials (ed 11). St. Louis, W.B. Saunders, 2003, pp. 69-143
10. Baharav H, Laufer BZ, Mizrachi A, et al: Effect of different cooling rates on fracture toughness and microhardness of a glazed alumina reinforced porcelain. J Prosthet Dent 1996;76:19-22
11. Konishi J, Watari F, Kawamoto C, et al: Effect of sphered particles on the firing contraction of porcelain inlay processed by cold isostatic pressing. J Biomed Mater Res B Appl Biomater 2003;66:553-558
12. Roy ME, Whiteside LA, Katerberg BJ, et al: Phase transformation, roughness, and microhardness of artificially aged yttria- and magnesia-stabilized zirconia femoral heads. J Biomed Mater Res A 2007;83:1096-1102
13. Al-Radha AS, Dymock D, Younes C, et al: Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. J Dent 2012;40:146-153

14. Kosmac T, Oblak C, Jevnikar P, et al: The effect of surface grinding and sandblasting on flexural strength and reliability of Y-TZP zirconia ceramic. Dent Mater 1999;15:426-433
15. Pittayachawan P, McDonald A, Young A, et al: Flexural strength, fatigue life, and stress-induced phase transformation study of Y-TZP dental ceramic. J Biomed Mater Res B Appl Biomater 2009;88:366-377
16. Albakry M, Guazzato M, Swain MV: Biaxial flexural strength, elastic moduli, and x-ray diffraction characterization of three pressable all-ceramic materials. J Prosthet Dent 2003;89:374-380
17. Sunnegardh-Gronberg K, Peutzfeldt A, van Dijken JW: Flexural strength and modulus of a novel ceramic restorative cement intended for posterior restorations as determined by a three-point bending test. Acta Odontol Scand 2003;61:87-92
18. International Organization for Standardization: ISO 6872:1995, Dental ceramic. Geneva, ISO, 1995
19. Ersu B, Yenigül M, Tulunoğlu I: Three point bending strength of in-ceram core material sinterized with colloidal alumina. HÜ Diş Hek Fak Der 2007;31:71-78
20. Seghi RR, Sorensen JA: Relative flexural strength of six new ceramic materials. Int J Prosthodont 1995;8:239-246
21. Yilmaz H, Aydin C, Gul BE: Flexural strength and fracture toughness of dental core ceramics. J Prosthet Dent 2007;98:120-128
22. Guazzato M, Albakry M, Ringer SP, et al: Strength, fracture toughness and microstructure of a selection of all-ceramic materials. Part II. Zirconia-based dental ceramics. Dent Mater 2004;20:449-456
23. Chai J, Chu FC, Chow TW, et al: Chemical solubility and flexural strength of zirconia-based ceramics. Int J Prosthodont 2007;20:587-595
24. Guazzato M, Albakry M, Swain MV, et al: Mechanical properties of in-ceram alumina and in-ceram zirconia. Int J Prosthodont 2002;15:339-346
25. Itinoche KM, Ozcan M, Bottino MA, et al: Effect of mechanical cycling on the flexural strength of densely sintered ceramics. Dent Mater 2006;22:1029-1034
26. Guazzato M, Quach L, Albakry M, et al: Influence of surface and heat treatments on the flexural strength of Y-TZP dental ceramic. J Dent 2005;33:9-18
27. Curtis AR, Wright AJ, Fleming GJ: The influence of surface modification techniques on the performance of a Y-TZP dental ceramic. J Dent 2006;34:195-206
28. Kosmac T, Oblak C, Jevnikar P, et al: Strength and reliability of surface treated Y-TZP dental ceramics. J Biomed Mater Res 2000;53:304-313
29. Denry IL, Holloway JA: Microstructural and crystallographic surface changes after grinding zirconia-based dental ceramics. J Biomed Mater Res B Appl Biomater 2006;76:440-448
30. Qeblawi DM, Munoz CA, Brewer JD, et al: The effect of zirconia surface treatment on flexural strength and shear bond strength to a resin cement. J Prosthet Dent 2010;103:210-220
31. Papanagiotou HP, Morgano SM, Giordano RA, et al: In vitro evaluation of low-temperature aging effects and finishing procedures on the flexural strength and structural stability of Y-TZP dental ceramics. J Prosthet Dent 2006;96:154-164
32. Wang H, Aboushelib MN, Feilzer AJ: Strength influencing variables on CAD/CAM zirconia frameworks. Dent Mater 2008;24:633-638
33. Karakoca S, Yilmaz H: Influence of surface treatments on surface roughness, phase transformation, and biaxial flexural strength of Y-TZP ceramics. J Biomed Mater Res B Appl Biomater 2009;91:930-937
34. Yamaguchi H, Ino S, Hamano N, et al: Examination of bond strength and mechanical properties of Y-TZP zirconia ceramics with different surface modifications. Dent Mater J 2012;31:472-480
35. Cavalcanti AN, Pilecki P, Foxton RM, et al: Evaluation of the surface roughness and morphologic features of Y-TZP ceramics after different surface treatments. Photomed Laser Surg 2009;27:473-479
36. Gokce B, Ozpinar B, Dundar M, et al: Bond strengths of all-ceramics: acid vs laser etching. Oper Dent 2007;32:173-178