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Effects of surface treatment methods on shear bond strength of ceramic to cast, milled and laser-sintered titanium frameworks

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ABSTRACT

Aims: This study determined the effect of three different surface treatment methods [sandblasting (SB), tribochemical silica coating (TSC), and ytterbium fiber laser (YFL) roughening] on surface roughness and titanium-ceramic shear bond strength using specimens obtained using casting (CST), milling (ML) and selective laser melting (SLM).

Methods: In this *in vitro* study, we obtained 32 cylindrical titanium specimens for each fabrication method and subjected them to each surface treatment method. Nine experiments (n=11) were conducted. One specimen was randomly selected from each group for scanning electron microscope analysis. Surface roughness was examined using a profilometer device (n=10). Ceramic was applied to titanium specimens. A universal testing machine was used to determine shear bond strength in megapascal (MPa).

Results: Surface roughness of CST/YFL (1.254±0.058 µm), SLM/SB (1.294±0.054 µm), and SLM/YFL (1.208±0.057 µm) groups were significantly higher than other groups (CST/SB, CST/TSC, ML/SB, ML/TSC, ML/YFL, and SLM/TSC, $p<0.01$). Shear bond strengths of CST/YFL (20.28±6.97 MPa), SLM/SB (21.9±8.06 MPa), and SLM/YFL (29.92±5.67 MPa) were significantly lower than other groups ($p<0.01$). Shear bond strength of the ML/SB group (42.40±7.52 MPa) was highest but there were no significant differences between ML/SB and CST/SB (33.04±7.62, p=0.101), CST/TSC (35.38±4.15, p=0.426), ML/TSC (40.03±6.42, p=0.998), ML/YFL (39.43±9.24, p=0.991) and SLM/TSC (37.05±7.84, p=0.766).

Conclusions: This study showed that the production and surface treatment method impact shear bond strength. Excessive roughness affects the bonding strength. The highest shear bond strength was identified in the ML group.

Introduction

Metal-ceramic restorations (MCRs) have been used to combine the high resistance of metal with the high aesthetic features of ceramic since 1960s (1). Many types of metals and metal alloys have been tried in the fabrication of MCR frameworks in the last five decades. Due to costs, base metal alloys have become the most frequent alternatives (2). However, base metal alloys, especially nickel-chrome in MCRs, may have some biological effects (2-4). Commercially, pure titanium and titanium alloys (Ti) are alternative MCR frameworks because of their excellent biocompatibility with lower density and thermal conductivity, good corrosion, and fatigue resistance (5).

Ti has a strong tendency to oxidize. When Ti contacts with oxygen, a thick titanium oxide (TiO or TiO_2) layer, known as α-case, is formed on its surface. The surface TiO_2 layer shields the alloy to corrode (6). However, some disadvantages of Ti casting (CST) such as a strong tendency to oxidize and increased chemical reactivity at high temperatures, limit its use

(7,8). Porosity and incomplete CST, especially in the margins of the restoration, can also occur (7). Therefore, using an arc melting pressure and a high-speed centrifugal CST machine with an argon atmosphere are recommended to improve the castability of Ti (9). Also, stable oxides like magnesia, alumina, and zirconia, refractory materials of investment, are used to control the thickness of the surface TiO_2 layer, which weakens the Ti-ceramic bond (10,11). Because of CST difficulties of Ti, milling (ML), spark erosion, laser welding, and selective laser melting (SLM) emerged as alternative techniques for the fabrication of more predictable Ti frameworks than CST (6,12-14).

ML is a subtractive method that reduces the oxide formation on the surface of Ti frameworks. However, disadvantages of this procedure, such as a significant amount of wasted material, time spent on production, limited production of complex specimens and the need for manual finishing after fabrication limit its use (15). Laser sintering is a relatively new alternative additive technique developed for the fabrication of metal frameworks. A high-power laser beam melts the alloy powder to form a thin solid layer (0.02-0.06-mm thickness) on a metal bed. The fabrication is completed by repeating the path layer by layer (16). This procedure enables a high degree of accuracy of the framework's manual finishing or wasted material (17).

However, the production of mechanically and chemically consistent frameworks does not guarantee the clinical success of MCRs. The clinical success of MCR is related to the bond strength between the framework and the ceramic (17). The thickness of the TiO_2 layer on the Ti surface and the mismatch of coefficients of thermal expansion Ti and ceramics reduce the bond strength (18). To minimize the mismatch of coefficients of thermal expansion, veneering Ti substructures with low-fusing ceramics were recommended (15).

To improve the bonding strength between Ti-low fused ceramic, surface treatment techniques such as airborne particle abrasion, acid etching, pre-oxidation, tribochemical silica coating (TSC), and laser etching (LE) applied on the adherent surface is recommended for clinical success (19,20). Sandblasting (SB) is the most common ST. During the procedure, the size of Al_2O_3 particles and applied pressure affect Ti-ceramic bond strength. Al_2O_3 particle size from 110 μm to 250 μm can be used to achieve an adequate bond strength between Ti and ceramic (21). However, surface contamination with Al_2O_3 can reduce Ti-ceramic bond strength (22,23).

LE is an acceptable alternative without the risk of Al_2O_3 contamination. It easily modifies the surface properties of materials and increases the metal-ceramic bond strength (24). It was reported that LE with Nd: Yg improved Ti-low fused ceramic bonds as strongly as SB (25,26) and was better than acid etching (25). Another method used to improve Ti-ceramic bond strength is TSC. The scientific basis of TSC is that SB with silica-coated alumina powder forms a silicate layer on an adherent surface (27). It was reported that TSC improved Ti-ceramic bond strength (28,29).

Although several studies have evaluated Ti-ceramic bond strength, to the best of our knowledge, limited studies have evaluated the effect of the production and surface treatment methods on Ti-ceramic bond strength. Therefore, this study aimed to examine the effects of different production methods such as CST, ML, and SLM and surface treatment methods such as SB, TSC, and ytterbium fiber laser (YFL) etching methods on Ti-low-fused ceramic bond strength. The null hypothesis was that various production methods and surface treatments do not affect the surface roughness of Ti specimens and the Ti-ceramic bond strength.

Methods

Specimen preparation

A total of 99 Ti specimens, cylindrical in shape (10 mm in diameter and 15 mm in height) were prepared: CST (n=33), ML (n=33) and SLM (n=33).

CST specimens were manufactured using the lost-wax technique. A cylindrical metal mold of 10 mm diameter and 15 mm height was prepared. Inlay wax (774 Inlay wax, Dental Direct, Spenge, Germany) was melted and poured into the mold. Then, the wax specimen was positioned in the silicone CST ring. Phosphate-bonded investment (Rematitan Plus, Dentaurum, Germany) was vacuum mixed according to the manufacturer's recommendations (liquid/powder ration: 40 mL/250 gr) and poured into a silicone CST ring. The ring was placed in a pre-heating furnace and wax was eliminated (900 °C for 50 min.). Grade 1 commercially pure titanium (Tritan, Dentaurum, Germany) (Lot no: 161) was melted (1668 °C for 40 sec.) and CST was prepared using a CST device (Rematitan, Dentaurum, Germany) according to the manufacturer's instructions. After the removal of the investment, CST specimens were trimmed with a carbide bur. The α-case layer was removed using a universal grinding machine (FV-315-V/2, Tak-San, Turkey) in all CST specimens.

For the production of Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) specimens, 10 mm in diameter and 15 mm in height, were designed with CAD software (RapidForm XOR3; 3D Systems Inc). The stereolithography file was transferred to the 5 Axis Milling Machine (Deckel Maho HSC 20 Linear, Pfronten, Germany) for the ML groups and an SLM unit (M2, Concept Laser; Hoffmann Innovation Group) for the SLM groups. ML specimens were prepared using grade 5 Ti blank (CopraTi-5, Whitepeaks Dental Solutions GmbH & Co. KG, Wessel, Germany) (98.3 mm in diameter and 15 mm in height, lot no: 0483). A 200 W fiber laser beam melted and fused-grade 5 Ti powder (CL 41 Ti Eli, Concept Laser GmbH, Lichtenfels, Germany) (Lot no: UK1058) into 40 μ layers until the completion of the production. According to the manufacturer's instructions, the SLM specimens were transferred to a sintering furnace (Protherm Furnaces ACF, Ostim, Ankara, Turkey). The furnace temperature was adjusted to 850 °C for four hours. The specimens were exposed to this temperature for two more hours.

Ti specimens were subjected to SB, TSC, and YFL roughening. A total of 9 experimental groups (n=11) were assigned according to production and ST (groups CST/SB, CST/TSC, CST/YFL, ML/SB, ML/TSC, ML/YFL, SLM/SB, SLM/TSC, and SLM/YFL). Airborne particle abrasion with 250 µm aluminum oxide (Al_2O_3) particles (Korox® BEGO, Bremer Goldschlägerei Wilh. Herbst GmbH & Co. Bremen, Germany) was applied to bonding surfaces of CST/SB, ML/SB, and SLM/SB for 10 seconds at 0.2 MPa pressure and from a distance of 10 mm from the surface using an airborne particle abrasion device (Meliodent, Heraeus Kulzer GmbH, Hanau, Germany). For TSC, the bond surfaces of CST/TSC, ML/TSC, and SLM/TSC specimens were cleaned and activated by blasting 110 µ pure aluminum sand (Rocatec Pre, 3M ESPE, St. Paul, MN, USA) with a sanding device (Junior Blasting Module 3M ESPE, St. Paul, MN, USA) at 0.2 MPa pressure from a distance of 10 mm for 10 secs. Then, the TSC was applied to the bonding surfaces of the specimens by sanding with 30 µm silica-coated Al_2O_3 (Rocatec soft, 3M ESPE, St. Paul, MN, USA). TSC procedure was performed according to manufacturer instructions. CST/YFL, ML/YFL, and SLM/YFL specimens were treated using a YFL (SCANLAB, Puchheim, Germany) (7 W, 140 mJ with 50-Hz frequency with 300 µsecs pulse duration) (YFL). The bonding surfaces of the specimens were irradiated for 10 seconds by the linear movement of a glass fiber of the YFL laser, positioned 17 cm away from the bonding surface. After applying surface treatments, all the specimens were ultrasonically cleaned in an ultrasonic bath (Mercury Ultrasonic Cleaner, Sozer Machine Co, Turkey) with distilled water for 10 mins.

Determination of bonding surface property

One specimen for each group was randomly selected and the microstructural analysis was performed using a scanning electron microscope (SEM) (Carl Zeiss SMT, EVO® 40 Series, Oberkochen, Germany), an area of 10 µm^2 was examined at x1000 magnification. The surface roughness values (Ra) for all specimens (n=10) were measured using a profilometer device (time TR 100, Surface Roughness Tester, PHYNIX GmbH & Co. KG, Germany). Roughness measurements (0.25-mm cut-off length, 0.05-10.0 µm measuring range, and 6 mm tracing length) on two perpendicular measuring lines of radius length on the treated surface of each specimen were made. A mean of measurement was calculated and the Ra for each specimen was obtained.

Veneering procedure

A silicone mold was prepared at 10 mm in diameter and 15 mm in height with openings of 4 mm in height and 6mm in diameter to standardize the size of the ceramic with the manual layering technique. Low-fusing ceramic (Vita Titankeramik, Vita Zahnfabrick, Bad Säckingen, Germany) was applied to the Ti specimens with the use of a silicone mold. Binder, opaque, dentin, and glaze layers were fired using a ceramic furnace (Programat P 300 Ivoclar Vivadent AG Schaan, Liechtenstein) according to the manufacturer's instructions (Table 1). After firing procedures, a micro-measuring device (Alpha-Tools Digital Caliper, CA, USA) with a minimum reading of ±0.01 was used to measure the exact size of bonded ceramic before the shear bond strength (SBS) test.

SBS test

An SBS test was performed using a universal testing machine (Instron 1195, Instron Corp, Canton, MA) at a crosshead speed of 1 mm/min. The loading jig was positioned 1mm away from the ceramic-metal joint line at an angle of 10 degrees. SBS at failure was measured at Newton. The measured values were divided by the bonding surface area of the specimens to calculate the SBS in megapascal (MPa).

Fracture modes were classified as cohesive failure within the veneering porcelain, adhesive failure between titanium and porcelain, or a combination of both (13) and were identified by a trinocular invert metal microscope (SOIF XJP-6A Boeco, Hamburg, Germany) at x40 magnification.

Table 1. Firing schedules of the veneering porcelain according to manufacturers' instructions

		Start temperature (°C)	Rate of temperature rise (°C/min)	End of temperature (°C)	Vacuum
Titankeramic	Bonder	600	65	795	+
	1st opaque	600	65	795	+
	2nd opaque	600	65	785	+
	1st dentin	600	55	755	+
	2nd dentin	600	55	755	+
	Glaze	600	55	755	-

min: Minimum

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 22.0 software package (IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was used to analyze the effects of production methods and surface treatment methods on surface roughness and SBS. A one-way ANOVA was used to analyze the effects of surface treatment methods on surface roughness and SBS of production method groups. Comparisons among the roughness and SBS values of production method groups were made using the post-hoc Tukey's honestly significant difference test. A p-value less than 0.05 was considered statistically significant.

Results

The SEM images of the specimens after applying surface treatments are presented in Figure 1. In SB specimens, heterogeneous wide craters were imaged on bonding surfaces in parallel with the size of the Al_2O_3 particles (Figure 1A-1C). Surface contamination with Al_2O_3 particles was seen in the SB groups. On TSC surfaces, homogeneous, narrower, and shallow craters were imaged with partially silicated micropits (Figure 1D-1F). In the YFL groups, molten Ti marks compatible and smooth surfaces extending linearly between them were observed (Figure 1G-1I).

Roughness results of the two-way ANOVA determined that there were significant differences between production methods and surface treatment techniques ($F_{(2,81)}=196.538$, $p<0.01$). The mean Ra and standard deviations are summarized in Table 2. For the CST groups, there was no significant difference between CST/SB and CST/YFL groups (p=0.176) that was significantly higher than the CST/TSC group (p<0.01). For the ML groups, there were significant differences between all groups (p<0.01). For SLM groups, there was no significant difference between SLM/SB and SLM/YFL groups (p=0.062) which was significantly higher than the SLM/TSC group (p<0.01).

SBS results of the two-way ANOVA determined that there were significant differences between production methods and surface treatment techniques ($F_{(2,81)}=9.594$, $p<0.01$). The mean SBS values and standard deviations are summarized in Table 3. For the CST groups, no difference was found between the mean SBS of CST/SB and CST/TSC (p=0.697) which were significantly higher than the mean SBS of CST/YFL (p<0.01). For the SLM groups, the mean SBS of SLM/SB and SLM/YFL was significantly lower than SLM/TSC (p<0.01). No difference

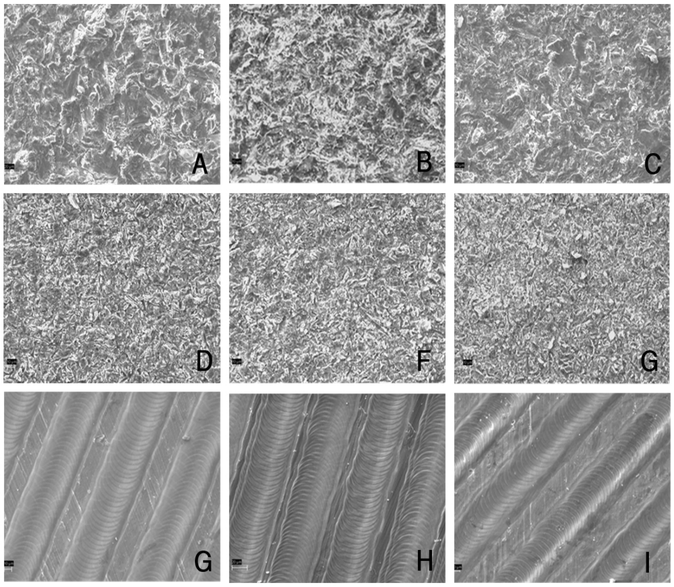

Figure 1. SEM images of titanium surfaces at x1000 magnification: CST/SB group (a), CST/TSC group (b), CST/YFL group (c), ML/SB group (d), ML/TSC group (e), ML/YFL etching group (f), SLM/SB group (g), SLM/TSC group (h), SLM/YFL etching group (i)

SB: Sandblasting, CST: Casting, ML: Milling, SLM: Selective laser melting, YFL: Ytterbium fiber laser, TSC: Tribochemical silica coating

Table 2. Results of surface roughness values (Ra) of test groups

	Sandblasting	Tribochemical silica coating	Laser etching	F	p
Casting, µm, mean (SD)	1.069 (0.05)	0.628 (0.058)	1.254 (0.058)	336.24	**<0.01***
Miling, µm, mean (SD)	1.149 (0.058)	0.687 (0.056)	0.916 (0.05)	191.40	**<0.01***
Selective laser melting, µm, mean (SD)	1.294 (0.054)	0.917 (0.05)	1.208 (0.057)	135.144	**<0.01***

*Indicates significant changes for intragroup comparison (one-way ANOVA).
µm: Micrometer, SD: Standard deviation

Table 3. Shear bond strengths of test groups

	Sandblasting	Tribochemical silica coating	Laser etching	F	p
Casting, MPa, mean (SD)	33.04 (7.62)	35.38 (4.15)	20.28 (6.97)	15.997	**<0.01***
Miling, MPa, mean (SD)	42.40 (7.52)	40.03 (6.42)	39.43 (9.24)	0.404	0.671
Selective laser melting, MPa, mean (SD)	21.90 (8.06)	37.05 (7.84)	29.92 (5.67)	11.512	**<0.01***

*Indicates significant changes for intragroup comparison (one-way ANOVA).
MPa: Megapascal, SD: Standard deviation

was found between SLM/TSC and SLM/YFL (p=0.09). However, surface treatment methods on ML specimens did not affect the mean SBS (p>0.05).

The distribution of failure modes of groups is provided in Table 4. No cohesive-type fracture was observed in any specimen. In the SLM/SB group, all failures were in the combined mode. In the CST/SB, CST/TSC, ML/YFL, and SLM/YFL groups, all failures were in the adhesive mode.

Discussion

The main purpose of the study was to evaluate the various production (CST, ML, and SLM) and surface treatments (SB, TSC, and YFL) on surface roughness and the bond strength of ceramic to the Ti specimens. Surface roughness and the bond strength results revealed that the production and surface treatment methods affected both roughness and SBS values; therefore, the null hypothesis was rejected.

Metal-ceramic bonding is a crucial factor that affects the clinical performance of MCRs. According to ISO 9693-2019 (30), a minimum acceptable value of 25 MPa calculated using a three-point bending test was defined for reliable clinical MCRs. However, the stress distribution at the interface between the ceramic and metal makes the 3-point bending test method difficult for interpreting the bond strength measurement (25). Smaller variations in the SBS test were identified rather than in the three-point bending test on measuring bond strength (23). Therefore, the SBS test was used to evaluate bond strength between Ti and low-fusing ceramic in this study.

The mismatch of thermal expansion coefficients of Ti and ceramics (18) and the strong tendency of Ti alloys to oxidize limited bonding strength between Ti and ceramics. Low-fusing ceramics with compatible thermal expansion coefficients were recommended for the veneering of Ti frameworks (18). The silicon dioxide (SiO_2) concentration on the Ti-ceramic interface also affects the Ti-ceramic bond strength (31,32). To obtain the highest concentration of SiO_2, a ceramic border was applied to the Ti-ceramic interface. The highest SiO_2 concentration was achieved with the Titankeramik border (32). In this study, low fused ceramic with a Ti border was used to eliminate the incompatibility of the thermal expansion coefficient, to prevent the oxidation of the Ti-ceramic interface during firing cycles, and to obtain the highest SiO_2 concentration on the Ti-ceramic interface.

Ti-ceramic bonding strength also depends on various factors such as the production methods of Ti specimens (7) and surface treatment procedures (19). In research (13,20,33,34) including the presented research, different SBS values were obtained in Ti specimens produced with different production methods. Mohsen (35) demonstrated that the composition differences in Ti alloys affect the bonding strength. It can be concluded that not only the production method and surface treatment procedures but also Ti composition (35) affects SBS values.

Ra demonstrated that surface treatment methods had different effects on the surface roughness of CST, ML, and SLM specimens. SLM induces rougher surfaces before surface treatment due to the “balling phenomenon”, which is a partial fusion of isolated powder particles during SLM (33). The partially melted particles loosely attached to the Ti specimens cause rougher surfaces (36). SB reduces the roughness of SLM specimens while increasing ML ones (37). The Ra of SLM specimens is also affected by material composition, powder particle size, layer thickness, laser type, and power (36). The Ra of the SLM/SB specimens differed from those of recent studies (34,37). The reason for this difference in results may be the differences in Ra measurement methods, as well as the factors that affect the Ra of SLM Ti specimens, as mentioned above.

It is generally considered that Ra is imported for mechanical locking on the metal-ceramic interface and enlarges the chemical bonding surface (19). Excessive roughness affects the bonding strength adversely by reducing the wetting of ceramics (37,38). In the SLM/SB group, the main factor for lesser bonding strength was due to excessive roughness. The surface contamination with Al_2O_3 particles can also be considered a factor for loosening bonding strength (22,23). The combined mode of fracture pattern

Table 4. Failure modes distribution

Production method of specimen	Surface treatment method	Adhesive failure	Combined failure
Casting	SB	10	-
	TSG	10	-
	YFL	9	1
Milling	SB	9	1
	TSC	9	1
	YFL	10	-
Selective laser melting	SB	-	10
	TSC	7	3
	YFL	10	-

SB: Sandblasting, TSC: Tribochemical silica coating, YFL: Ytterbium fiber laser etching in all samples supports Al_2O_3 contamination. However, residual Al_2O_3 particles were observed on the surface in all SB groups in SEM imaging, and a dramatic decrease in SBS was observed only in the SLM/SB group. The main reason for the difference between the SBS values obtained in the SLM/SB group and the other SB groups can also be considered as due to the content of the Ti powder (35) and the production method (7).

LE with YFL (140 mJ, 50 Hz, 7 W, 300 μ secs) was also used as a surface treatment in this study. LE has become current for avoiding Al_2O_3 contamination on the Ti-ceramic interface with SB (17). It was mentioned that LE enhances the SBS between Ti and ceramics (17,26). In this study, however, the lowest SBS values were obtained in the YFL groups. The higher pulse energy of the YFL was evaluated to produce increased Ra and may cause material deterioration (25,35). The adhesive failures, that support the implication, were observed in almost all CST/YLF and SLM/YFL specimens. However, the element distribution on the Ti-ceramic interface was not examined for material deterioration in this study which could be considered a limitation. However, similar to the results of this study, Kim and Cho (25) reported that laser-etched ML specimens demonstrated no significant difference in the bond strength compared to ML/SB. So it can be concluded that the YFL procedure could be applied to milled Ti substructures to optimize the surface texture for the wetting ability of low-fused ceramics.

SB also causes different Ra and bonding strengths on commercially pure Ti. In previous studies (18,35,38), Ti surfaces were polished for standardization before SB treatment. However, in this study, to imitate clinical practice, only the α-case layer was removed and no polishing process was applied. SB decreased the roughness of the bonding interface of CST and other groups. Therefore, the obtained Ra differed from those of previous studies (35,38). The bond strength between sandblasted commercially pure Ti and low-fused ceramics with bonded (Titankeramik) was reported to be between 25.2 MPa (39) and 28.78 MPa (20). However, these values were obtained using the 3-point bending test. Iseri et al. (13) evaluated bonding strength with SBS between the same Ti alloy and ceramic materials and determined lower SBS values in the CST/SB group than in this study. The difference between these two studies is in the SB process. Although Iseri et al. (13) did not compare the Ra, it can be considered that this difference in SBS values may be due to the difference in sample roughness difference between the two studies.

Mohsen (35) and Fukuyama et al. (40) stated that TSC could significantly improve SBS between commercially pure Ti and ceramics. In this study, the mean SBS of CST/TSC was slightly higher than CST/SB. Contrary to Fukuyama et al. (40), no significant difference was observed between the mean SBS of CST/TSC and CST/SB. As in the CST group, the SBS values of ML/TSC do not statistically differ from those obtained using ML/SB. However, the highest SBS values in SLM groups were determined in the SLM/TSC group in this study. TSC seemed to be an effective method on SLM specimens to form a silicate layer like CST specimens (27). Considering the results of this study, effective SBS values can be obtained by treating SLM specimens with TSC. Although TSC is considered an effective method among the surface treatment processes applied on the Ti-ceramic interface, it was reported that a significant decrease in SBS values in TSC groups was determined in Ti specimens with artificial aging compared to SB groups (35). In this study, artificial aging was not applied.

Study Limitations

This study has several limitations. Only a single brand of ceramic was applied in this study. Therefore, the results of this study cannot be extrapolated to similar low-fused ceramics with a different chemical composition. Artificial aging, which may affect SBS values, was also applied. The relationship between the compositions of Ti alloys and ceramics requires further studies. And, clinical studies are necessary to assess the long-term performance of titanium-ceramic fixed dental prostheses.

Conclusion

Within the limitations of the study, the production and the surface treatment method affected the SBS between titanium and low-fusing ceramic. The highest SBS values could be achieved by ML. Excessive roughness affects the bonding strength adversely. The highest surface roughness was identified in the YFL etc. CST group and airborne particle abraded SLM group. In these groups, the fewest SBS values were identified.

Ethic

Ethics Committee Approval and Informed Consent: Since there's no data used referring to any living thing in this research, it is not necessary to provide an Ethics Committee Approval Form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Y., Concept: H.Y, C.O.S., Design: H.Y., C.O.S., Data Collection, or Processing: H.Y., Analysis, or Interpretation: H.Y., B.E., Literature Search: H.Y., B.E., Writing: H.Y., B.E., C.S.

Conflict of Interest: The authors declare they have nothing to disclose regarding the conflict of interest concerning this manuscript.

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