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Comparison and Evaluation of Mechanical and Osteoblastic Properties of Laser Microtextured Patterns on Titanium Plates

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Background: Titanium and its alloys are widely used in biomedical implants due to their excellent mechanical strength, corrosion resistance, and biocompatibility. Surface characteristics play a crucial role in osseointegration and overall implant success. Recent advances in laser surface modification allow precise control over implant topography, potentially enhancing biological performance.
Aim: To evaluate the influence of laser-microtextured surface patterns (grooves, dimples, and random structures) on the physicochemical and biological properties of titanium plates.
Materials and Methods: This in-vitro experimental study included sixteen commercially pure Grade 4 titanium plates divided into four groups (n = 4): sandblasted control, laser-grooved (G1), laser-dimpled (G2), and laser-random (G3). Surface modification was performed using a pulsed Nd:YAG laser. Surface roughness was assessed using atomic force microscopy (AFM). Wettability was evaluated by contact angle measurement. Cytocompatibility was analyzed using MG-63 osteoblast-like cells via MTT assay and Live/Dead staining. Hemocompatibility was assessed using a haemolysis assay, and antimicrobial activity was evaluated against *Porphyromonas gingivalis*. Statistical analysis was performed using Tukey HSD post hoc tests.
Results: The grooved surface (G1) exhibited the highest surface roughness (Sa: 300.49 ± 4.14 nm), while dimpled (G2) and random (G3) surfaces showed lower roughness values. Wettability analysis revealed the lowest contact angle in G1 and highest in G2. All groups demonstrated high cell viability (>90%) with no significant differences ($p > 0.05$). Hemolysis values were below 5% in all groups, indicating acceptable hemocompatibility, though G1 showed comparatively higher values. Laser-modified surfaces, particularly the random pattern (G3), demonstrated reduced bacterial adhesion.
Conclusion: Laser microtexturing significantly influences surface characteristics and biological responses of titanium implants in a pattern-dependent manner. Among the tested designs, the random microstructured surface exhibited a favorable balance of wettability, hemocompatibility, and antimicrobial properties, making it a promising approach for enhancing implant performance.
Keywords: Titanium implants; Laser microtexturing; Surface roughness; Wettability; Osseointegration; Hemocompatibility; Antimicrobial activity

1. Introduction

Titanium and its alloys are widely regarded as one of the most suitable biomaterials for biomedical implant applications due to their excellent mechanical strength, corrosion resistance, and superior biocompatibility (1–3). These properties have led to their extensive use in orthopedic, dental, and craniofacial implants. In dental implantology, titanium implants are widely used because they are capable of forming a stable interface with surrounding bone tissues through the process of osseointegration (4).Osseointegration is defined as the direct structural and functional connection between living bone and the implant surface. Following implant placement, blood proteins rapidly adsorb onto the implant surface, forming a biological layer that mediates osteoblast adhesion and subsequent bone formation (5,6). The success of this process is strongly influenced by the physicochemical characteristics of the implant surface.Surface properties such as roughness, surface energy, chemical composition, and wettability play an important role in regulating protein adsorption, cellular attachment, and differentiation (7–9). Previous studies have demonstrated that modifications in surface roughness can significantly improve bone–implant contact and enhance implant stability (10).Conventional surface modification techniques including sandblasting, acid etching, plasma spraying, and anodization have been widely used to enhance implant surface roughness and biological performance (11,12). However, these approaches may produce irregular microstructures and provide limited control over surface morphology.Recent advances in surface engineering technologies have introduced more precise techniques such as laser surface modification for producing controlled micro- and nano-scale topographies (13,14). Laser microtexturing enables the fabrication of well-defined surface patterns including grooves, dimples, and complex microstructures without direct contact with the material (15).These microstructured surfaces can influence cellular behavior through contact guidance, where topographical features guide cell alignment, migration, and cytoskeletal organization (16). Grooved surfaces may promote directional cell growth, while isotropic patterns allow cells to

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spread in multiple orientations (17).Surface wettability is another important parameter influencing biological interactions. Hydrophilic surfaces generally promote improved protein adsorption and osteoblast attachment compared with hydrophobic surfaces (18,19). Modifications in surface roughness and oxide layer characteristics can therefore significantly influence biological responses at the implant interface. In addition to promoting osseointegration, implant surfaces must also demonstrate hemocompatibility and resistance to bacterial colonization. Implant surfaces come into direct contact with blood during surgical placement, and adverse hemolytic reactions may compromise healing (20). Furthermore, bacterial adhesion and biofilm formation on implant surfaces can lead to peri-implantitis, which remains a major cause of implant failure (21,22).Therefore, the present study aimed to investigate the influence of laser-microtextured surface patterns on titanium plates. Different surface patterns including grooves, dimples, and random microstructures were fabricated using a laser modification technique and evaluated for their physicochemical and biological properties.

2. Materials and Methods

2.1 Study Design: This study was designed as an in-vitro experimental investigation to evaluate the surface characteristics, wettability, and biological performance of laser-modified titanium plates with different surface patterns. The study was conducted in the Department of Implantology, Saveetha Dental College and Hospital, Chennai, between October 2024 and October 2025, after obtaining ethical approval from the Scientific Review Board of Saveetha Dental College and Hospital, Chennai (Approval number: SRB/SDC/MSIMPLANT-2304/24/TH-066). A total of sixteen commercially pure titanium plates were included in the study. The samples were randomly divided into four groups consisting of four specimens each. The control group consisted of sandblasted titanium plates, while the experimental groups consisted of titanium plates that underwent laser micro-texturing to produce groove, dimple, and random surface patterns.

2.2 Sample Preparation: Commercially pure Grade 4 titanium plates were used as the substrate material. To ensure consistency across all experimental groups, the plates were standardized in size and baseline surface characteristics prior to surface modification.

The titanium samples were first cleaned to remove machining residues, contaminants, and organic impurities. This cleaning process was performed through sequential ultrasonic cleaning in acetone, ethanol, and distilled water for ten minutes each. After ultrasonic cleaning, the plates were air-dried in a dust-free environment and handled using sterile instruments.

2.3 Sandblasting Procedure: The control group underwent surface roughening through sandblasting using alumina particles with an approximate particle size of 250 µm. The abrasive particles were projected onto the titanium surface using an airborne particle abrasion device under controlled conditions.During sandblasting, the nozzle was positioned perpendicular to the titanium surface to ensure uniform treatment. Each specimen was exposed to the abrasive stream for five minutes to achieve consistent micro-roughness. Following the procedure, the samples were rinsed with distilled water and subjected to ultrasonic cleaning to remove any residual particles.

2.4 Laser Surface Characterization: Laser surface modification was performed using a pulsed Nd:YAG laser system. The laser parameters were standardized to ensure reproducibility of the surface patterns. The laser operated at a peak power of 2.1 kW with a pulse duration of 7 milliseconds and a scanning speed of 1 mm/s.

Three distinct surface patterns were created by altering the laser scanning strategy:

- Group 1: Parallel scanning produced a groove pattern.
- Group 2: Discrete pulsed irradiation generated a dimple pattern.
- Group 3: Irregular scanning paths produced a random surface pattern.

After laser processing, all samples underwent additional ultrasonic cleaning in distilled water to remove debris generated during laser treatment. The specimens were then dried and stored in a desiccator until further analysis.

2.5 Atomic Force Microscopy Analysis: Surface roughness and topography of the titanium plates were evaluated using atomic force microscopy (AFM). The AFM analysis was performed using a high-resolution scanning system equipped with a nanometer-scale cantilever tip.

Surface images were captured over an area of 10 µm × 10 µm. Quantitative roughness parameters including average roughness (Sa) and root mean square roughness (Sq) were calculated using the instrument's analysis software.

2.6 Wettability Assessment: Surface wettability was evaluated using the sessile drop method. A small droplet of blood was placed on the surface of each titanium sample using a micropipette. The static contact angle formed between the droplet and the surface was measured using a contact angle goniometer. Lower contact angle values indicated higher surface hydrophilicity, which is generally associated with improved biological interactions.

2.7 In-Vitro Cell Line Study: The biological compatibility of the modified titanium surfaces was evaluated using MG-63 human osteoblast-like cells. The cells were cultured in 24-well plates and seeded onto the titanium samples at a density of 1×10^4 cells per well.

Cell viability was assessed using the MTT assay after 24 hours of incubation. During the assay, the cells were incubated with MTT solution to allow the formation of formazan crystals, which were later dissolved in dimethyl sulfoxide. The absorbance was measured using a microplate reader to determine cellular metabolic activity.

A Live/Dead fluorescence staining assay was also performed using Calcein-AM and propidium iodide to visualize viable and non-viable cells under fluorescence microscopy.

2.8 Haemolysis Assay: Hemocompatibility was evaluated using a haemolysis assay. Fresh human blood was collected and diluted with physiological saline. Titanium samples from each group were incubated with the diluted blood at 37°C for one hour.

Following incubation, the samples were centrifuged and the absorbance of the supernatant was measured at 540 nm using a spectrophotometer. The percentage of haemolysis was calculated to determine the compatibility of the titanium surfaces with red blood cells.

2.9 Antimicrobial Study: The antimicrobial properties of the titanium surfaces were evaluated using Porphyromonas gingivalis, a key peri-implant pathogen. Bacterial suspensions were prepared at a concentration of 1×10^6 CFU/mL and incubated with the titanium samples under anaerobic conditions. After incubation, the samples were washed with phosphate-buffered saline to remove non-adherent bacteria. Adherent bacteria were detached through sonication and cultured on anaerobic blood agar plates. Colony-forming units were counted to determine bacterial adhesion.

3. Results

Table 1: Descriptive Analysis Physicochemical and Biological Characteristics of Sandblasted and Laser-Microtextured Titanium Surfaces

Group	Sa (nm) Mean ± SD	Sq (nm) Mean ± SD	Contact Angle (°) Mean ± SD	Cell Viability (%) Mean ± SD	Hemolysis (%) Mean ± SD
Control (Sandblasted)	178.40 ± 3.12	221.65 ± 3.46	72.93 ± 11.09	98.03 ± 1.05	0.20 ± 0.08
G1 (Laser Grooves)	300.49 ± 4.14	360.28 ± 4.09	66.97 ± 21.54	93.28 ± 3.07	4.40 ± 0.18
G2 (Laser Dimples)	133.19 ± 2.46	173.60 ± 2.59	104.64 ± 10.67	95.54 ± 2.92	2.40 ± 0.18
G3 (Laser Random)	144.56 ± 2.04	182.44 ± 2.53	98.57 ± 12.41	96.15 ± 2.48	1.80 ± 0.16

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Table 2: Combined Tukey HSD Post Hoc Pairwise Comparisons of Surface and Biological Parameters

Parameter	Comparison (I–J)	Mean Difference (I–J)	p-value
Surface Roughness Sa (nm)	**Control – G1**	-122.10	0.00*
	Control – G2	45.20	0.00*
	Control – G3	33.85	0.00*
	G1 – G2	167.30	0.00*
	G1 – G3	155.95	0.00*
	G2 – G3	-11.35	0.00*
Surface Roughness Sq (nm)	**Control – G1**	-138.62	0.00*
	Control – G2	48.05	0.00*
	Control – G3	39.20	0.00*
	G1 – G2	186.67	0.00*
	G1 – G3	177.82	0.00*
	G2 – G3	-8.85	0.00*
Contact Angle (°)	**Control – G1**	5.95	0.93
	Control – G2	-31.72	0.03*
	Control – G3	-25.65	0.10
	G1 – G2	-37.67	0.01*
	G1 – G3	-31.60	0.03*
	G2 – G3	6.07	0.92
Cell Viability (%)	**Control – G1**	4.75	0.06
	Control – G2	2.47	0.47
	Control – G3	1.87	0.68
	G1 – G2	-2.27	0.54
	G1 – G3	-2.87	0.35
	G2 – G3	-0.60	0.98
Hemolysis (%)	**Control – G1**	-4.20	0.00*
	Control – G2	-2.20	0.00*
	Control – G3	-1.60	0.00*
	G1 – G2	2.00	0.00*
	G1 – G3	2.60	0.00*
	G2 – G3	0.60	0.00*

Atomic Force Microscopy (AFM) analysis demonstrated clear differences in surface roughness among the groups. The laser-grooved surface (G1) exhibited the highest roughness values (Sa: 300.49 ± 4.14 nm, Sq: 360.28 ± 4.09 nm), followed by the sandblasted control (Sa: 178.40 ± 3.12 nm, Sq: 221.65 ± 3.46 nm) (Table 1). In contrast, the dimpled (G2) and random (G3) laser patterns showed comparatively lower roughness values, with Sa values of 133.19 ± 2.46 nm and 144.56 ± 2.04 nm, respectively. Tukey HSD post hoc analysis confirmed that all pairwise comparisons for both Sa and Sq values were statistically significant ($p = 0.00$), indicating distinct differences in surface topography among the groups (Table 2). Surface wettability analysis revealed variations in contact angle measurements across the groups. The dimpled surface (G2) showed the highest contact angle (104.64 ± 10.67°), followed by the random surface (G3) (98.57 ± 12.41°), while the grooved surface (G1) demonstrated the lowest contact angle (66.97 ± 21.54°). Post hoc analysis indicated that significant differences were observed between Control–G2 ($p = 0.03$), G1–G2 ($p = 0.01$), and G1–G3 ($p = 0.03$). However, no significant differences were observed between Control–G1 ($p = 0.93$), Control–G3 ($p = 0.10$), or G2–G3 ($p = 0.92$).Evaluation of cytocompatibility using MG-63 osteoblast-like cells demonstrated high cell viability across all groups. The control group showed the highest viability (98.03 ± 1.05%), while the laser-modified groups also maintained viability above 90% (G1: 93.28 ± 3.07%, G2: 95.54 ± 2.92%, G3: 96.15 ± 2.48%). Tukey HSD post hoc analysis revealed no statistically significant differences in cell viability among any of the groups ($p > 0.05$), indicating that laser microtexturing did not adversely affect cellular compatibility. Hemocompatibility results showed minimal hemolytic activity in the sandblasted control group (0.20 ± 0.08%). Among the experimental groups, the grooved surface (G1) demonstrated the highest hemolysis value (4.40 ± 0.18%), followed by the dimpled surface (G2) (2.40 ± 0.18%) and the random surface (G3) (1.80 ± 0.16%). Tukey HSD post hoc analysis revealed statistically significant differences between all group comparisons ($p = 0.00$). Despite these differences, all hemolysis values remained below 5%, indicating acceptable hemocompatibility for all tested surfaces.Overall, the results demonstrate that laser microtexturing significantly influences surface roughness, wettability, and hemocompatibility of titanium surfaces in a pattern-dependent manner, while maintaining high cytocompatibility across all experimental groups.

4. Discussion

Surface modification of titanium implants is a critical factor influencing osseointegration and long-term implant stability. Implant surface characteristics such as roughness, surface energy, and wettability play an important role in regulating protein adsorption, osteoblast attachment, and bone formation at the implant–tissue interface (23–25).In the present study, laser microtexturing was used to generate groove, dimple, and random microstructures on titanium surfaces. Atomic force microscopy analysis demonstrated that the grooved surface exhibited the highest roughness values among the tested groups. Increased surface roughness has been associated with improved bone–implant contact and enhanced mechanical interlocking with surrounding bone tissue (26).Micro- and nano-topographical features may also regulate cellular behavior by promoting osteoblast adhesion and proliferation, which are essential for successful osseointegration (27,28). Similar findings have been reported in previous studies evaluating modified implant surfaces.Wettability analysis in the present study demonstrated variations in contact angle among the tested groups. Surface hydrophilicity is known to promote improved protein adsorption and cellular attachment, which may facilitate early stages of osseointegration (29). Laser surface modification can alter oxide layer characteristics and surface energy, thereby influencing biological responses at the implant interface (30).Cytocompatibility assessment using MG-63 osteoblast-like cells revealed high cell viability across all experimental groups. These findings suggest that laser microtexturing does not negatively affect cellular survival or metabolic activity. Previous investigations evaluating modified titanium surfaces have reported similar levels of osteoblast viability and compatibility (31).Hemocompatibility testing demonstrated that all tested surfaces exhibited hemolysis values below the acceptable threshold for biomaterials. Although slight differences were observed between groups, all surfaces remained within safe limits for blood compatibility (32).The antimicrobial analysis also demonstrated reduced bacterial adhesion on laser-modified surfaces, particularly on the random microstructured pattern. Surface microtopography can significantly influence bacterial attachment and biofilm formation, and specific surface architectures may help reduce bacterial colonization while maintaining favorable cellular responses (33).Overall, the results of the present study demonstrate that laser microtexturing significantly influences surface roughness, wettability, hemocompatibility, and antimicrobial behavior of titanium surfaces in a pattern-dependent manner. Among the tested configurations, the random surface pattern demonstrated favorable biological performance and may represent a promising strategy for optimizing implant surface design (34).

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5. Conclusion

Laser microtexturing effectively modifies the surface characteristics of titanium implants in a pattern-dependent manner, significantly influencing roughness, wettability, hemocompatibility, and antimicrobial behavior. While grooved surfaces exhibited higher surface roughness and improved hydrophilicity, all laser-modified groups maintained excellent cytocompatibility and acceptable hemocompatibility. Notably, the random microstructured surface demonstrated reduced bacterial adhesion along with favorable biological responses. These findings suggest that laser-engineered surface patterns, particularly random microstructures, hold strong potential for enhancing implant performance and optimizing osseointegration in clinical applications.

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