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Article

Comparative Effects of Fine and Conventional Shot Peening on Surface Morphology, Topography, Wettability, and Antibacterial Activity of Biomedical Ti6Al4V Alloy

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Abstract

Interest in textured surfaces for biomaterials and implants is increasing, with shot peening emerging as a promising surface modification method. This study investigates the influence of conventional and fine shot peening on the surface morphology, topography, wettability, and antibacterial properties of biomedical-grade Ti6Al4V alloy. Peening was conducted using a custom-built, fully automated system, employing fine (100-200 µm) and coarse (700-1000 µm) shots using wellcontrolled sets of parameters. Both treatments introduced severe plastic deformation on the surface, resulting in increased roughness. Conventionally shot-peened samples exhibited deeper and wider dimples compared to finely peened ones. Surface wettability shifted from hydrophilic (contact angle: 4.1°, untreated) to hydrophobic, reaching contact angles of 91.13° and 99.76° for fine and conventional shot peening, respectively. Despite these topographical changes, both treatments preserved the intrinsic antibacterial activity of the alloy. Quantitative assays revealed high bacterial reduction rates after 6 hours of incubation: 99.58% (E. coli) and 92.54% (S. aureus) for fine shot peening, and 98.98% (E. coli) and 99.2% (S. aureus) for conventional shot peening. Both methods were effective in modifying the surface without compromising antibacterial performance. However, fine shot peening provided a smoother and more uniform surface topography, and thus potentially offers better control over bacterial adhesion and surface-cell interactions in biomedical applications.

Keywords: antibacterial properties; biomaterials; implants; surface metrology; surface roughness; surface wettability; titanium alloys

1. Introduction

Titanium alloys have been preferred materials in various biomedical applications for a long time due to their good mechanical properties, including biocompatibility, strong acid and alkali resistance, toughness, and fatigue and yield strength [1–6]. However, these alloys are rarely employed in engineering disciplines particularly associated with tribology due to certain disadvantages that include low surface hardness, an elevated coefficient of friction, and inadequate wear resistance [3,4]. Their tribological, corrosion, and biological characteristics, including wear and corrosion resistance, antibacterial activity, osteointegration, are directly influenced by the surface properties of these materials [3]. Thus, there is a substantial demand for enhancements in their surface properties (such as roughness, wettability, and morphology), mechanical properties (including hardness, wear resistance, corrosion resistance, fatigue behavior, and toughness), and antibacterial properties of titanium alloys. Roughened surfaces featuring patterns are generally more useful than smooth, patternless surfaces in numerous respects, particularly when considered for biomedical properties [7]. Patterned surfaces are typically achieved through the deposition of coatings or by processing the material's surface at the sub-micrometer or micrometer scale [8,9].

Methods employed for surface modifications of titanium alloys in biomedical applications include: laser surface texturing (LST) [10,11], laser peening (LSP) [12], electro-spark surface texturing (ESST) [13], electro-erosion (EDM), chemical reactive ion etching (RIE) [14], lithography and anisotropic etching (LIAE) [15], abrasive jet machining (AJM) [16], and physical vapor deposition (PVD) [17–19], among others [3,6]. The predominant technique for surface patterning seems to be LST. Liu et al. [4] found that laser processing of a titanium alloy to create cell-like patterns enhanced surface microhardness while decreasing the friction coefficient and wear volume. Milles et al. [20] generated patterns at varying depths by fabricating hexagonal honeycomb structures on the aluminum surface using a laser processing technique, concluding that these patterns enhanced surface wettability. Shivakoti et al. [3] developed pit and pillar surface patterns on titanium and zirconia alloys employed in the biomedical sector through laser processing, concluding that the corrosion and wear resistance, and cell adhesion, increased after the patterning process. Erdoğan et al. [9] employed a laser method to process dot and straight-line patterns on Ti6Al4V, concluding that the quality of cell adhesion improved following the patterning procedure. Numerous studies have demonstrated that laser oxidation can enhance the microhardness of titanium surfaces, refine grain size, improve micro morphology, and enhance corrosion resistance [6]. Nevertheless, the development of cracks on both the surface and subsurface of the material, with crack size directly proportional to the processing duration, has also been documented [21]. Even though laser treatment has proven to be an effective method for producing surface patterns on titanium alloys and improving the surface properties, it also often leads to undesirable surface and subsurface defects. Therefore, to present a relatively more robust method, researchers have recently focused on using mechanical surface treatment methods (e.g., shot peening) to modify surface properties of titanium alloys and to obtain specific surface patterns for biomedical applications [6,22-24]. Among these, there has been a particular interest in utilizing shot peening methods to tailor the surface properties of titanium alloys for biomedical applications.

In the shot peening process, peened material in the form of spheres impacts the surface at high velocities, resulting in plastic deformation and grain refinement in both the surface and subsurface regions [25]. Grain refinement enhances the mechanical properties of the material, including its hardness and toughness. Furthermore, shot peening notably modifies the surface characteristics of the treated alloy [26], such as topography, morphology, and wettability, particularly in relation to the applied shot peening parameters. Shot peening offers several potential advantages over other surface processing techniques including reduced equipment requirements, adjustable surface and subsurface characteristics based on adjustable process parameters, lower energy consumption, shorter processing durations, higher efficiency, cost-effectiveness, and the capability to treat more complex-shaped components with ease [27]. Nevertheless, suboptimal shot peening conditions may also result in detrimental surface effects including excessive roughening, microcrack initiation, and particle

embedding [28], which can negatively influence mechanical integrity and in vivo performance. A rigorous optimization and comprehensive evaluation of shot peening parameters are imperative to maximize its functional benefits while mitigating potential surface damage. There are some well-documented mechanical benefits of shot peening; however, research is also required to elucidate the specific effects of both conventional and fine shot peening on surface roughness, wettability, and biological responses of titanium alloys. These surface-related attributes are critical particularly for biomedical applications where cell adhesion, protein adsorption, and antibacterial performance are highly sensitive to micro- and nano-scale surface features.

Recent studies addressing the influence of shot peening parameters, especially in relation to surface morphology, topography, and biofunctionality, are critically discussed as follows to provide a clearer understanding of current research trends and remaining knowledge gaps: Wang et al. [29] applied fine particle shot peening (FPSP) to Ti6Al4V, generating half-dimpled surfaces that improved tribological performance; however, assessment of wettability or antibacterial effects were not conducted. Zhu et al. [30] showed that ultrasonic shot peening (USP) on commercially pure Ti largely enhanced hydrophilicity, directly linking surface roughness modifications to greater antibacterial behavior. Hsiao et al. [31] combined conventional shot peening (CSP) with HF/HNO3 etching to achieve Ra ≈ 0.9 µm on Ti6Al4V, promoting osteoblast adhesion and spreading. Uanlee & Khantachawana [32] applied FPSP to Ti6Al4V, resulting in higher surface hardness and improved fibroblast viability, though an assessment of wettability and antibacterial effect were not provided. Khandaker et al. [33] used laser peening to create microgrooves on Ti surfaces that notably boosts osteoblast adhesion, proliferation, and differentiation. Shen et al. [34] combined shot peening with Si/Cu-doped micro-arc oxidation coatings (MAO) coatings to achieve >95% antibacterial efficacy against S. aureus and stimulate osteogenic marker expression. Li et al. [35] implemented copperpowder ultrasonic shot peening (C-USSP) on cp-Ti, demonstrating important antibacterial effects directly due to peening-induced surface changes. Finally, Zheng et al. [36] applied shot peening and gas nitriding to TC4 and found that, although the mechanical properties were substantially improved, antibacterial effectiveness rates did not change much-highlighting the complexity of peening vs. antimicrobial surface behavior. Altogether, these studies confirm that shot peening, especially when combined with additional treatments or optimized as USP/C-USSP, has the potential to substantially modulate antibacterial responses and cell behavior via surface topography and wettability control. At the same time, not all peening variants largely enhance antibacterial performance, indicating the need for careful parameter optimization and comprehensive biological testing to identify protocols that consistently benefit implant integration and microbial resistance.

This study aims to systematically investigate the effects of conventional and fine shot peening techniques on the surface morphology, topography, wettability, and antibacterial performance of biomedical-grade Ti6Al4V alloy. Using a fully automated, custom-designed shot peening system, samples were treated under controlled parameters by using coarse and fine media to generate distinct surface textures. Through a comparative approach, the study mainly explores how shot size influences biologically relevant surface features and bacterial adhesion behavior. The outcomes are expected to advance the development of biofunctional titanium implant surfaces by promoting antibacterial efficacy while maintaining desirable wetting and morphological characteristics.

2. Materials and Methods

Conventional and fine shot peening were conducted on biomedical Ti6Al4V alloy samples utilizing fine (100-200 μ m) and coarse shots (700-1000 μ m) under regulated conditions with a custom-designed fully automated peening system. Subsequently, optical profilometry, scanning electron microscopy (SEM), and contact angle measurements were performed to characterize the surface properties of fine-peened and shot-peened samples. The antibacterial efficacy of the samples against *S. aureus* and *E. coli* was assessed.

2.1. Materials

In this study, Ti6Al4V alloy (grade 5) samples with equiaxed microstructure were commercially supplied in rod form (Ø20 mm x 1000 mm) by TIMET, Türkiye. These were cut into slices that were 10 mm thick using a semi-automatic band saw. Before shot peening, the samples were ground using sandpapers of 360, 600, 1000, and 2000 mesh and polished sequentially with diamond solutions of 9 μ m, 3 μ m, and 1 μ m. The alloy's nominal chemical composition, provided in the material certification by the supplier (TIMET, Türkiye), is presented in Table 1.

Table 1. Chemical composition of Ti6Al4V alloy.

Chemical Composition (wt. %)					
V	Al	Fe	Sn	Si	Ti
2.769	5.629	0.089	0.006	0.052	91.455

2.2. Shot Peening of Ti6Al4V Alloy

Shot peening treatments were carried out using a CNC-controlled, custom-designed shot peening system with the configuration and operating principles previously described in detail and illustrated schematically in our earlier work [24]. The samples subjected to shot peening at an applied pressure of 5 bar for 2.5 minutes from a distance of 40 mm, with the peening parameters detailed in Table 2. Two distinct sizes of stainless-steel shots were utilized in the shot peening processes. The morphology and chemical composition of the stainless-steel shots were examined by SEM coupled with energy-dispersive X-ray spectroscopy (EDS). As shown in Figure 1a,b, the coarse (700–1000 μ m) and fine (100–200 μ m) shots exhibited spherical morphology with surface texture features. EDS spectra revealed that both shot types primarily consisted of Fe, Cr, Ni, Mn, and Si, in agreement with their stainless-steel composition.

Table 2. Shot peening parameters used for peening of the Ti6Al4V alloy.

Parameter	Value	
Shot type	Stainless steel shots	
Shot hardness	460 HV	
Shot size	100-200 μm (Fine shot peening)	
	700-1000 μm (Conventional shot peening)	
Acceleration pressure	5 bar	
Duration	2.5 min	
Standoff distance	40 mm	
Nozzle diameter	4.76 mm	

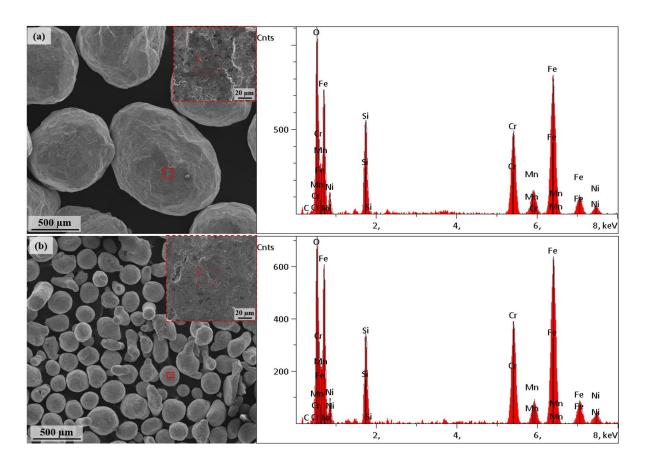


Figure 1. SEM images and EDS analysis of stainless-steel shots used in the present study; (a) coarse shots (700-100 μ m); (b) fine shots (100-200 μ m).

2.3. Surface Characterisation of Shot-Peened Alloy

Figure 2 illustrates the methodology employed for characterizing surface roughness of the peened samples. First, the three-dimensional topographies of the sample surfaces were acquired utilizing an optical profilometer (Huvitz, Gyeonggi, Republic of Korea). Then, the surface roughness characteristics (i.e., surface topography, areal roughness parameters, roughness profiles) of the samples were analyzed by Mountains® 9 software (Digital Surf, Besançon, France).

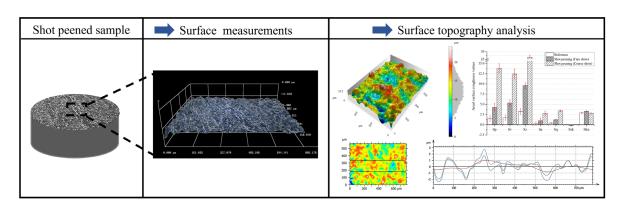


Figure 2. Representation of surface topography analysis of shot-peened Ti6Al4V alloy.

Subsequent to the characterization of surface roughness, the surface morphologies of the shot-peened samples were analyzed utilizing an SEM (Jeol JSM-6060, Tokyo, Japan) coupled with an energy dispersive spectroscopy (EDS) detector (Oxford Instruments, Oxford, UK). The wettability of the unpeened, fine-peened, and shot-peened samples was assessed using an optical contact angle

measurement system (Kruess GmbH, Germany). Contact angle tests were conducted using distilled water, maintaining a constant droplet volume of 3 ml, with each specimen tested five times.

2.4. Antibacterial Activity of Shot-Peened Surface

To perform antibacterial tests of samples before and after shot peening, *S. aureus* strain ATCC 29213 and *E. coli* strain ATCC 25922 (Mueller-Hinton Agar-Merck, Germany) were commercially supplied as testing microorganisms. Nutrient broth was prepared by dissolving 34 g of Mueller Hinton Agar (MHA) in 1,000 ml of distilled water and then sterilized at 121 °C for 15 min. The resulting solution was spread into Petri dishes and then allowed to cool under sterile conditions at room temperature. *S. aureus* and *E. coli* were grown in the nutrient broth at 37 °C to a concentration of 10⁸ CFU ml⁻¹. Then, the broth was diluted 10-fold with phosphate buffered solution (PBS) to a concentration of 10⁴ CFU ml⁻¹ (bacterial suspension). 0.4 ml of bacterial suspension was dropped onto the samples and then the Petri dishes were incubated at 37 °C for 18-24 hours under 90% humidity. After incubation, the solutions on the surface of the samples were collected in sterile tubes containing 3.6 ml of PBS solution. The samples were also placed in the same tubes and then 0.02 ml of the solution was taken and cultured in MHA with glass rods and incubated at 37 °C for 18-24 hours. After incubation, the number of active bacteria was determined by manually counting the colony-forming units (CFUs) on each plate using a standard colony counter.

3. Results and Discussion

3.1. Surface Morphology After Shot Peening

Figures 3 and 4 display low and high magnification SEM micrographs of the Ti6Al4V alloy surfaces subjected to conventional and fine shot peening, respectively. Both treatments induced severe plastic deformation, with characteristic surface features directly influenced by the size and kinetic energy of the impacting media. The surfaces exhibit dense impact craters and material pileups, typical of shot peening-induced topographical modifications [24,37,38]. In the conventionally shot-peened sample (Figure 3), the morphology is dominated by larger and deeper dimples, associated with high-energy impacts from coarse shots (700-1000 µm). The surface shows pronounced ridges and valleys with irregular crater geometry and evidence of micro-cutting or plowing, suggesting intense localized plastic deformation. Similar morphological features have been reported in Ti-based substrates processed by high-intensity peening, where the severity of plastic flow correlates with surface work hardening and increased roughness [24,39]. In contrast, the surface treated with fine shot peening (Figure 4) reveals more uniform and shallower dimples, attributed to the lower kinetic energy of fine shots (100–200 µm). The resulting surface layer thus exhibits a more homogeneous texture, with finer-scale peak-valley features and less severe surface distortion. This microtopography reflects a more controlled peening action, capable of refining the surface without extensive surface damage or excessive roughening, a behaviour that is consistent with prior observations on fine shot or ultrasonic peening of titanium alloys [32,40–43]. No significant cracks, microcracks, fractures, or particle dislodgement were seen on either surface, demonstrating the absence of harmful surface degradation. This observation corroborates the efficacy of the employed process parameters in attaining advantageous surface modification while preserving material integrity.

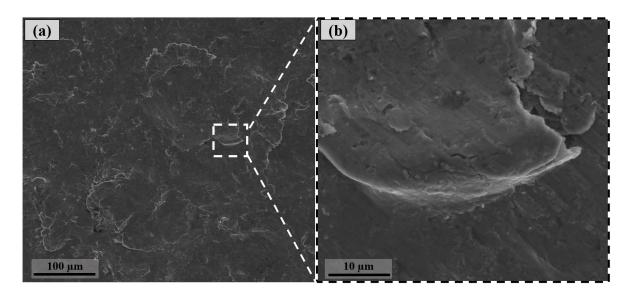


Figure 3. (a) Low magnification and (b) high magnification SEM images of the surface of conventionally shot-peened Ti6Al4V.

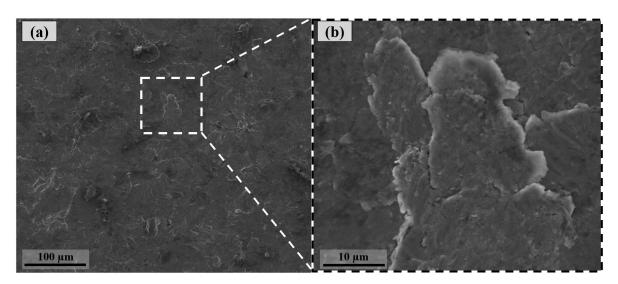


Figure 4. (a) Low magnification and (b) high magnification SEM images of the surface of fine shot-peened Ti6Al4V.

From a mechanistic standpoint, the microstructural refinement and surface texture variation stem from shot-induced strain localization. The higher dimple density and smoother contours in the fine shot-peened sample suggest a more uniform energy transfer, whereas coarse shot impacts lead to heterogeneous strain zones and larger deformation footprints. These distinct morphological features are known to critically influence surface properties such as protein adsorption, wettability, and cellular interaction, particularly in biomedical applications [32–35]. Moreover, the differences in morphology align with surface roughness measurements (Figures 5 and 6), wherein conventionally peened surfaces exhibit greater roughness amplitudes. The fine peening technique offers a desirable balance between surface modification and preservation of topological integrity, supporting its suitability for biofunctional surface engineering of Ti6Al4V implants.

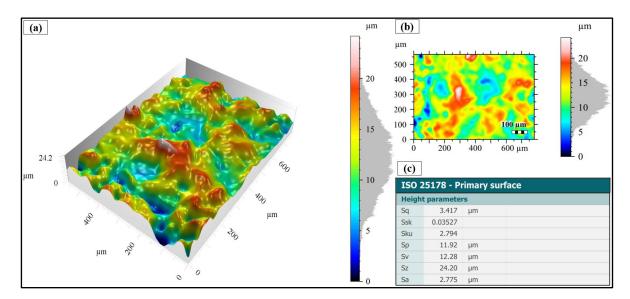


Figure 5. (a) Three-dimensional surface topography, (b) 2D roughness map, and (c) areal surface roughness values of conventionally shot-peened Ti6Al4V.

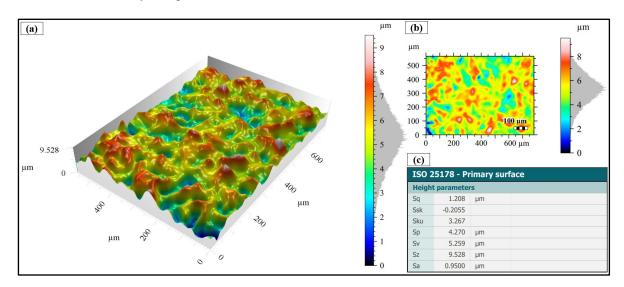


Figure 6. (a) Three-dimensional surface topography, (b) 2D roughness map, and (c) areal surface roughness values of fine shot-peened Ti6Al4V.

3.2. Surface Topography After Shot Peening

Surface topography plays a critical role in governing the mechanical and biological performance of titanium-based biomaterials. The results presented in Figures 5–7 reveal the profound influence of shot peening parameters on the three-dimensional surface features of Ti6Al4V alloy. Conventional shot peening, characterized by larger shot sizes and higher kinetic energy, produced an irregular surface profile with pronounced peaks (Sp) and valleys (Sv), as indicated by increased areal roughness values—maximum height (Sz) ≈ 24.20 µm and arithmetic mean height (Sa) ≈ 2.78 µm. In contrast, fine shot peening generated a much smoother surface ($Sz \approx 11.20 \,\mu\text{m}$, $Sa \approx 1.69 \,\mu\text{m}$), while still inducing beneficial topographic features necessary for mechanical interlocking and cellular interaction [44–47]. These findings agree with the previously discussed SEM-based morphology results (Figures 3 and 4), where larger and deeper impact craters were observed on the conventionally shot-peened surface, whereas the fine shot-peened surface exhibited more uniform and shallower surface features. These morphological observations support the interpretation that shot size plays a dominant role in determining local deformation mechanisms and surface reconstruction.

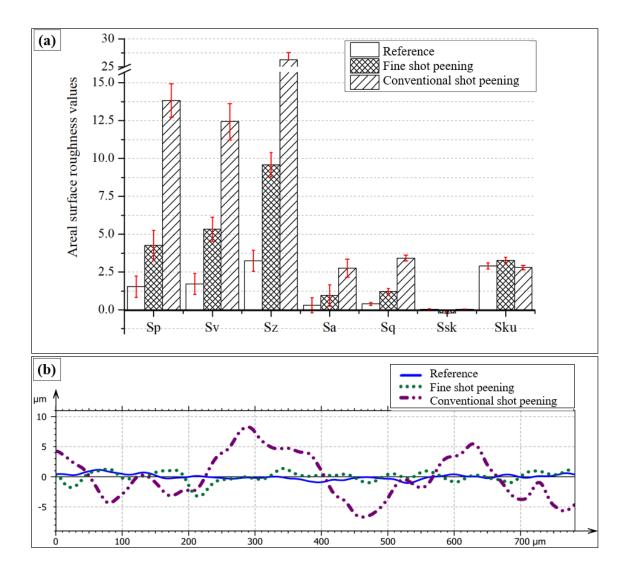


Figure 7. (a) Areal surface roughness results and (b) roughness profiles of conventionally and fine shot-peened Ti6Al4V.

The data extracted from ISO 25178 parameters (Figures 6c) and comparative bar plots (Figure 7a) highlight a clear correlation between shot size and roughness amplitude. *Sq* (root mean square height) increased with shot size, confirming higher roughness amplitude for conventional shot peening. Both shot-peened surfaces showed slightly negative *Ssk* (skewness), indicating valley-dominated morphologies favorable for fluid retention and potential cell adhesion. *Sku* (kurtosis) values >3 reflected sharper peak–valley features, enhancing potential mechanical interlocking, which are critical for evaluating functional surfaces in biomedical implants [48]. In addition, roughness profiles (Figure 7b) demonstrate the amplitude modulation and wavelength distribution across the treated surfaces, with fine shot peening yielding a more periodic and less jagged profile than the conventional counterpart.

From a fundamental standpoint, the observed differences in roughness and topography arise from the interplay between shot diameter, kinetic energy per impact, and impact frequency. Larger shots used in conventional peening impart higher momentum and localized energy, leading to deeper plastic deformation zones, irregular asperity formation, and increased surface roughness. Fine shot peening, on the other hand, involves smaller media with lower energy per impact but a higher number of impacts per unit area. This results in more homogeneous plastic flow, reduced microcutting, and smoother surface reconstruction due to cumulative overlapping deformation events [49,50]. Such differences in surface characteristics directly impact the implant's performance in vivo. Excessively rough surfaces may enhance mechanical anchorage but at the same time risk promoting

bacterial colonization and debris entrapment [51]. Conversely, ultra-smooth surfaces may reduce biological fixation. Therefore, the moderately rough and homogenously peened surfaces achieved via fine shot peening represent an optimized compromise, ensuring both structural integration and, potentially, a reduced infection risk.

The present findings align with the literature on shot peening–induced surface texturing of Ti6Al4V alloys, where controlled roughness plays a pivotal role in modulating osteoblastic activity, corrosion behavior, and long-term implant performance. These morphological and topographical improvements serve as a mechanistic foundation for understanding the antibacterial responses discussed below.

3.3. Wettability After Shot Peening

Surface wettability plays a pivotal role in the biological performance of metallic implants by influencing protein adsorption, initial cell attachment, and bacterial adhesion. The results of the contact angle measurements presented in Figure 8 reveal a notable change in wettability following both fine and conventional shot peening treatments compared to the reference condition. The reference sample, which possessed an ultrafine and mirror-polished surface, exhibited a low contact angle (~15°), confirming its hydrophilic nature. This behavior is consistent with its smooth topography and minimal surface roughness, as quantified in Figures 5-7. After shot peening, a clear transition toward hydrophobic behavior was observed. Specifically, fine shot peening increased the contact angle to ~90°, while conventional shot peening further elevated it to >100°, indicating strong water repellency. These wettability shifts are closely linked to the morphological and topographical alterations induced by the peening treatments. As shown in Figures 4 and 6, the generation of microcraters and overlapping deformation zones increased the surface roughness and introduced hierarchical surface features. These surface modifications reduce the real contact area and alter the solid-liquid interaction, resulting in an apparent increase in contact angle [52-54]. Such pronounced changes in wettability are expected to have direct implications for the biological response of the alloy surface. In particular, the transition from a hydrophilic to a hydrophobic state may influence subsequent protein adsorption, cell-material interactions, and bacterial adhesion, as will be discussed in the following sections.

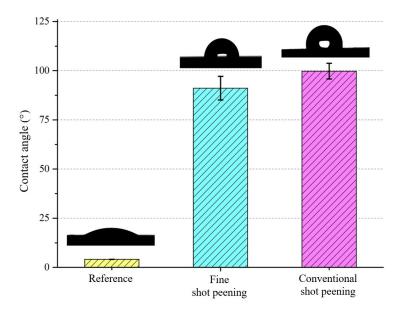


Figure 8. Contact angle measurements.

Fine shot peening applied in the present study appears to provide an optimized surface condition by tuning wettability and surface texture simultaneously. The finer and more uniformly distributed dimples generated by fine shot peening produced a moderately rough but

topographically ordered surface, supporting a transition to near-hydrophobic conditions while potentially maintaining favorable biological responses. In contrast, the irregular and sharper asperities caused by conventional shot peening likely reduce droplet spreading and enhance water repellency. From a functional perspective, the altered wettability is expected to influence subsequent biological interactions. Moderately hydrophobic surfaces can inhibit bacterial adhesion and delay biofilm formation, while still supporting protein anchorage and cell adhesion when combined with bioactive chemistry [44].

3.4. Antibacterial Activity After Shot Peening

Bacterial colony formation on Ti6Al4V was notably reduced compared to the control agar plate without material (Figure 9 and 10), confirming the alloy's intrinsic antibacterial behavior [55]. Shot peening increased surface roughness, which in turn promoted bacterial growth relative to the very smooth reference surface. Nevertheless, both shot-peened surfaces still exhibited markedly lower bacterial proliferation compared to the control, with fine shot peening outperforming the conventional process. Reduced *E. coli* proliferation was observed on smoother, fine shot-peened surfaces (Figure 10), whereas *S. aureus* showed higher proliferation on fine shot-peened surfaces (Figure 11).

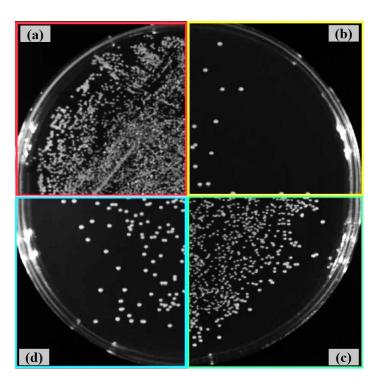


Figure 9. Antibacterial performance of samples against *S. aureus* after 6 hours of incubation. Representative images of bacterial colony formation on (a) control agar plate (no material), (b) untreated Ti6Al4V reference sample, (c) fine shot-peened Ti6Al4V surface, and (d) conventionally shot-peened Ti6Al4V surface.

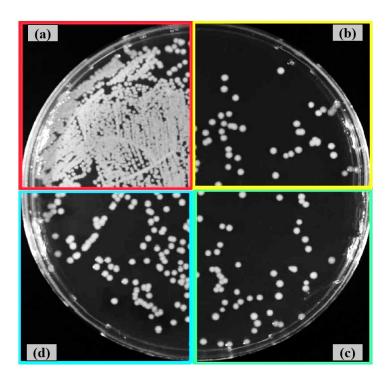


Figure 10. Antibacterial performance of samples against *E. Coli* after 6 hours of incubation. Representative images of bacterial colony formation on (a) control agar plate (no material), (b) untreated Ti6Al4V reference sample, (c) fine shot-peened Ti6Al4V surface, and (d) conventional shot-peened Ti6Al4V surface.

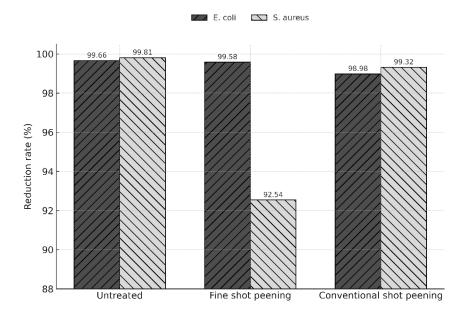


Figure 11. Reduction rates (%) of *E. coli* and *S. aureus* after 6 h incubation on Ti6Al4V surfaces: untreated, fine shot-peened, and conventionally shot-peened.

Consistent with earlier reports, Ti6Al4V demonstrates intrinsic antibacterial behavior [55]. Our findings confirm that antibacterial property is largely retained after shot peening i.e., not negatively affected by the applied surface modification methods (Figure 9 and 10). Among the peening techniques employed, conventionally shot-peened samples exhibited a notably higher surface roughness compared to fine shot-peened ones. It is well established that surface topography and roughness critically affect bacterial adhesion and proliferation. Surfaces with higher roughness may serve as a scaffold for bacterial colonization, especially when the average roughness (Sa) exceeds 0.8 μ m, facilitating bacterial attachment by providing protective niches [48]. In our study, both treatment methods yielded $Sa > 0.8 \mu$ m, while conventionally shot-peened surfaces exhibited the higher

roughness value ($Sa > 2.775 \mu m$). Additionally, the spacing of the surface grooves plays an important role in bacterial colonization. When the groove spacing is smaller than the bacterial cell size, adhesion becomes less favourable. In our case, the spacing formed by coarse shots ($Sz \approx 24.20 \mu m$) was much larger than that of fine shots ($Sz \approx 9.528 \mu m$), indicating higher potential bacterial adhesion on conventionally shot-peened surfaces. Priyanka et al. [48] reported greater bacterial adhesion on rougher TiN-Ag coated surfaces, which supports this claim.

Nishitani et al. [42] demonstrated that at lower surface roughness via fine particle bombardment, antibacterial activity improved, and particularly against both *E. coli* and *S. aureus*. Our results agree with these findings, as reduced *E. coli* proliferation was observed on the smoother, fine shot-peened surfaces (Figure 10). Moreover, the ~200 nm convex features formed during fine peening may have interacted with the *E. coli* cell membrane, causing membrane damage. However, to verify this, a further live/dead assay needs to be conducted. Interestingly, we observed a higher proliferation of *S. aureus* on fine shot-peened surfaces ($Sz \approx 9.528 \mu m$), as seen in Figure 9. This result aligns with prior studies by Cunha et al. [56] and Truong et al. [57], which reported *S. aureus* adhesion to microgrooves (10–20 μm) created by femtosecond laser treatment on titanium.

The wettability of the surface is determined by its roughness, topography, and chemical composition. Untreated Ti6Al4V surfaces are typically hydrophilic (Figure 8), and both peening methods increased hydrophobicity. Trapped air within the hydrophobic interface can act as a barrier, preventing bacterial attachment and motility, thereby enhancing antibacterial resistance [58]. Bright et al. [59] also observed that S. aureus preferred proliferation on hydrophilic surfaces. Thus, the increased proliferation of S. aureus on the less hydrophobic, fine shot-peened surfaces in our study (Figure 9) is consistent with the literature. Biofilm formation typically begins with the weak, reversible attachment of bacterial cells to the surface. Environmental factors such as surface roughness, hydrophobicity, and surface charge, as well as bacterial traits and surrounding pH or flow conditions, strongly influence adhesion and biofilm development. Once adhered, microorganisms produce extracellular polymeric substances (EPS), forming a protective matrix that increases resistance to antibiotics and environmental stressors [60]. Biofilms are therefore a major concern in the context of chronic polymicrobial infections and medical device-related complications [58]. Wu et al. [61] reported that micro- and nanoscale surface roughness notably affect bacterial adhesion and early biofilm development. The observed proliferation of S. aureus on rougher surfaces may indicate a higher potential for biofilm formation. Additionally, the increased growth of E. coli on more hydrophobic regions (Figure 10) could also suggest potential biofilm development. However, to confirm these trends, time-dependent antibacterial testing at various incubation durations (e.g., 12, 18, and 24 hours) is needed. Quantitative reduction rates for both bacterial strains after 6 hours of incubation further support these observations, confirming that both shot peening techniques were effective in reducing bacterial activity (Figure 11). It should be noted that there are strain-specific differences in reduction levels: While reduction efficiency remains same for E. coli in untreated and treated samples, this is only true for *S. aureus* for unpeened and conventionally peened sample where there was some reduction in efficiency for fine shot-peened sample.

4. Conclusions

In this study, a comparative investigation was conducted to evaluate the effects of conventional and fine shot peening techniques on the surface morphology, roughness, wettability, and antibacterial activity of biomedical-grade Ti6Al4V alloy. Using a custom-designed automated shot peening system, surface modification was achieved through either coarse or fine stainless-steel media to explore their impact on biofunctional surface design. Critical findings of the study include:

• Surface characteristics were strongly influenced by shot size and energy. Conventional shot peening (700–1000 μ m) induced rougher, irregular surfaces with higher peak-valley features ($Sa \approx 2.78 \mu$ m), while fine shot peening (100–200 μ m) produced more homogeneous textures with smoother topographies ($Sa \approx 1.69 \mu$ m), minimizing potential surface damage.

- Wettability shifted from hydrophilic to hydrophobic after peening, with fine shot peening
 achieving a contact angle of ~91° and conventional peening exceeding 99°. These changes are
 attributed to the formation of micro-dimples and hierarchical textures, which reduce solid—
 liquid interaction and may favor antibacterial behavior.
- Both shot peening techniques largely preserved the intrinsic antibacterial activity of the Ti6Al4V alloy, with some strain-specific variation. The smoother fine-peened surfaces may offer an improved balance between reduced bacterial adhesion and topographical suitability for biomedical integration.

While these findings clearly demonstrate the potential of mechanical peening as an effective and scalable technique for biofunctional surface engineering, the study has certain limitations: Live/dead bacterial assays, protein adsorption studies, and biofilm formation analyses were not conducted and should be addressed in future work. In addition, long-term durability and corrosion resistance of the modified surfaces under physiological conditions remain to be evaluated. Future studies may also explore hybrid approaches combining shot peening with antibacterial coating or chemical functionalization to further enhance both antibacterial efficacy and osseointegration potential.

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