Chapter 3
Laser Spallation of Biofilms and Cells on Sandblasted Ti-6Al-4V

Sahar Afshari and Martha E. Grady

Abstract Cell and bacterial adhesion at implant surfaces influence implant integration and infection outcomes. Surface roughening treatments such as sandblasting are widely used to enhance osseointegration of load-bearing implants, yet cell and biofilm adhesion are typically evaluated separately. This study utilizes the laser spallation technique to measure the adhesion strength of bacterial biofilms (*Staphylococcus aureus*) and mammalian cells (MG-63 osteoblast-like cells) on sandblasted Ti-6Al-4V substrates. Substrates are characterized using stylus profilometry and scanning electron microscopy to determine surface roughness and morphological features prior to culture and testing. Cells and biofilms are cultured separately on the substrates, and a range of laser fluences is applied to induce controlled interfacial stress. Failure data are analyzed using Weibull cumulative distribution functions to determine the 50% failure adhesion strength for each biological interface. The goal of this study is to directly compare mammalian cell and bacterial biofilm adhesion on the same surface in order to identify surface conditions that promote osteoblast attachment while reducing bacterial colonization for improved orthopedic implant design.

Keywords Laser spallation · biomedical implants · Ti6Al4V · adhesion · biofilms · cells

Introduction

Titanium and its alloys are widely used in load-bearing implants because of their favorable biocompatibility, corrosion resistance, and mechanical properties [1]. In particular, Ti-6Al-4V (Ti64), a common material in hip and knee replacements, dental implants, and fracture fixation devices [2], is selected here as a representative orthopedic implant material.

Surface topography influences biological interactions at the implant interface. Microscale surface roughening can improve osseointegration through increased bone–implant contact area and mechanical interlocking [3]. While no single roughness value is considered optimal, several studies have shown that titanium surfaces with moderate roughness (∼1–2 μm) exhibited enhanced bone–implant contact and integration [4]. However, direct comparison across studies remains challenging due to variations in roughness characterization techniques and reporting standards [4, 5]. Sandblasting, one of the most widely used surface treatments for clinical orthopedic implants [6], is therefore used in this study to generate controlled surface roughness on Ti64.

After implantation, device surfaces are prone to bacterial colonization and biofilm formation, which can ultimately lead to implant-associated infections and failure. Understanding cell adhesion in relation to biofilm formation is therefore critical for implant integration and long-term performance. Most prior studies evaluate mammalian cell adhesion or bacterial adhesion separately, which makes direct comparison difficult. In this study, the laser spallation technique is used to apply a controlled stress wave and quantify adhesion strength for both mammalian cells and bacterial biofilms. Ti64 surfaces with different sandblasted roughness levels are examined, and cell and biofilm adhesion strengths are measured across each surface condition. This study aims to identify Ti64 surface properties that preferentially promote MG-63 osteoblast-like cell adhesion over *Staphylococcus aureus* (*S. aureus*), a common pathogen in orthopedic implant infections.

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Materials and Methods

Ti64 sheet purchased from American Metal Xchange^{TM} Inc. was cut into 1-in $\times$ 1-in squares. The samples were then sandblasted using different particle mesh sizes and pressures to produce surfaces with varying roughness. Surface roughness (Ra) was measured on three samples per condition, with three measurements taken over a 4 mm scan length using a Bruker Dektak XT surface profiler in accordance with ISO 4288. The applied sandblasting parameters and resulting average Ra values are reported in **Table 1**. Scanning electron microscopy (SEM) was used to further characterize surface topography, and images are shown in **Figure 1c**. Images were acquired using an FEI Quanta SEM machine with an accelerating voltage of 5 kV under 300$\times$ magnification. One side of each Ti64 sample was coated with a 9 μm thick layer of aqueous sodium silicate using a spincoater. The substrates were adhered to the bottom of a petri dish containing a 13/16-inch hole, using a biocompatible NOA86H adhesive and cured under UV.

Staphylococcus aureus (ATCC 6538) was cultured by inoculating ~1 μL of frozen stock into 5 mL of Todd Hewitt Yeast (THY) broth and incubating at 37 °C for 24 h. Substrates were disinfected with 70% ethanol followed by UV exposure for 30 min. The bacterial suspension was diluted in THY to an optical density of 0.70 $\pm$ 0.05, measured using a GE Ultrospec 8000 spectrophotometer. Each substrate assembly was placed in a sterile Petri dish, sealed with parafilm, and inoculated with 1 mL of the adjusted bacterial suspension combined with 3 mL of THY with 75 mM glucose. Samples were incubated at 37 °C under 5% CO2 for 24 h. After incubation, media was removed and substrates were gently rinsed with phosphate-buffered saline to remove nonadherent bacteria.

MG-63 cells (ATCC CRL-1427) were cultured in a cell culture flask with Eagle's Minimum Essential Medium (EMEM, ATCC 30-2003), 10% fetal bovine serum (FBS, ATCC 30-2020), 1% penicillin streptomycin solution (ATCC 30-2300) until confluent. Cells were then detached using trypsin, counted with an automated cell counter, and seeded at a density of 225k cells per substrate assembly in fresh EMEM. Cells were incubated at 37 °C under 5% CO2 for 48 h to allow formation of a confluent cell layer.

In laser spallation, a high-energy pulsed Nd:YAG laser is used to generate a stress wave in the substrate. As this wave travels through the substrate and into the film, it reflects from the free surface as a tensile pulse; when this tensile stress exceeds the interfacial strength, detachment occurs [7]. However, the non-reflective nature of biomaterial films prevents direct measurement of interfacial adhesion strength, and calibration experiments are required, as described by Boyd *et al.* [8, 9]. The experimental setup of the laser spallation technique employed to measure biofilm and cell adhesion is illustrated in **Figure 1a**. An Nd:YAG laser pulse is controlled using an attenuator and focused to a desired spot size with a focusing lens. The focused laser pulse is directed upward toward the substrate assembly using a 45° mirror. A camera is used to capture biomaterial ejections during testing.

Table 1 Sandblasting parameters (pressure and particle mesh size) and resulting surface roughness (Ra) and roughness category for Ti64 substrates.

Sample	Roughness category	Sandblasting pressure	Particle mesh size	Surface roughness, Ra
1	Smooth	80 psi	400 Alumina + 200 Glass (1:1)	0.40 $\pm$ 0.03 μm
2	Moderately rough	50 psi	18-50 Alumina	1.47 $\pm$ 0.16 μm
3	Rough	80 psi	18-50 Alumina	2.57 $\pm$ 0.29 μm

Results and Discussion

A range of laser fluences (mJ/mm^2), from low to high, is applied to the substrates, and failure statistics are recorded. **Figure 1b** shows MG-63 cells cultured on sandblasted Ti64, illustrating regions of failure and no failure following laser loading. At lower laser fluence, where the induced interfacial stress is insufficient to detach the cells, the biological films remain adhered to the surface. At higher laser fluence, the cells are ejected from the substrate, exposing the underlying surface within the loaded region; these events are recorded as adhesion failure. **Figure 1d** exhibits the preliminary results for the adhesion testing of MG-63 cells on the as-received wrought Ti64 and sandblasted rough Ti64. Experiments are repeated at least ten times at each fluence level. Failure data are analyzed using a Weibull cumulative distribution function (CDF) to determine the median (50%) failure laser fluence. **Figure 1d** demonstrates that sandblasted rough Ti64 (Ra = 2.57 $\pm$ 0.29 μm)

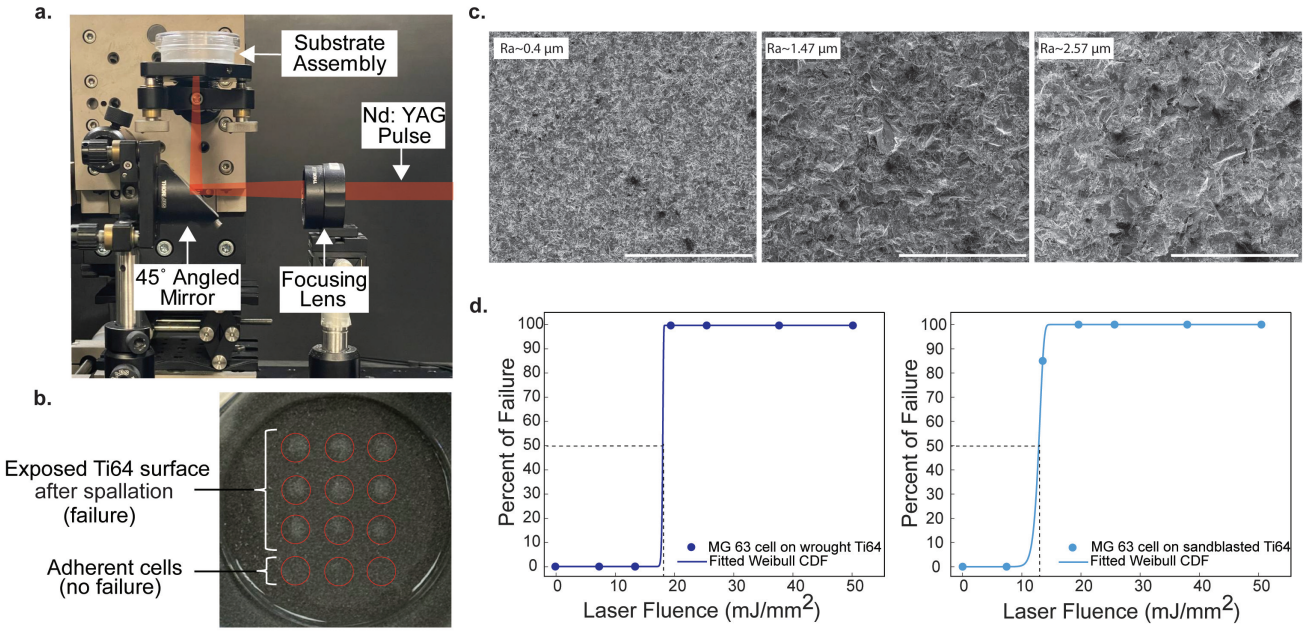

Fig. 1 (a) Photograph of the laser spallation setup for biological adhesion measurements. (b) MG-63 cells on Ti64 subjected to varying laser fluence, showing failed and intact regions. (c) SEM images of three sandblasted Ti64 surfaces (FEI Quanta, 5 kV, 300×; scale bar = 200 µm). (d) Weibull failure statistics for MG-63 cells on wrought and sandblasted Ti64; the dashed line denotes 50% failure. The 50% failure fluence is 18.21 and 12.85 mJ/mm^2 for wrought and sandblasted Ti64, respectively, indicating reduced cell adhesion on rougher surfaces.

exhibits a lower 50% failure laser fluence (12.85 mJ/mm^2) than as-received wrought Ti64 (18.21 mJ/mm^2), indicating a decrease in MG-63 cell adhesion with increased surface roughness.

Next Steps

As next steps, calibration experiments will be conducted on Ti64 substrates without biological films. These experiments will be used to calibrate the laser-induced stress wave propagating within the substrate and to determine the interfacial stress corresponding to the applied laser fluence. Additional experiments will investigate the effect of varying Ti64 surface roughness levels on cell and biofilm adhesion. A range of laser fluences will be applied to cells and biofilms cultured on sandblasted Ti64 substrates to obtain sufficient failure statistics under each condition (at least 10 repetitions per fluence level). The resulting failure data will be analyzed by fitting a Weibull CDF to determine the 50% failure interfacial adhesion strength for the biological films. This approach enables quantitative comparison of biological film adhesion strength across surface conditions and helps identify surface properties that better support osteointegration while limiting bacterial colonization in orthopedic implant applications.

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