Experimental study on the influence of hydrophilicity on bacterial adhesion in bioimplants

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Abstract: Titanium and its alloys are widely used as dental and orthopaedic implants because of their desirable mechanical properties and biocompatibility. However, implant failures due to bacterial infections occur in many cases unexpectedly. Formation of a biofilm on the implant surfaces facilitates growth of colony of bacteria which in turn reduces the effectiveness of antibacterial drugs. Hydrophilicity of implant surfaces diminishes the chances of biofilm formation. Micro surface features are created on the Ti6Al4V implant surface using a pulsed Nd-YAG laser and its influence on water wettability (and thus on hydrophilicity) has been studied and compared with a polished surface. Strains of Staphylococcus aureus have been inoculated on the test specimens and the bacterial adherence have been compared. The results show that micro-textured surfaces of Ti6Al4V have improved water wettability and thus are more hydrophilic than polished surface. This has reduced the formation of biofilm and bacterial growth.

1. Introduction

Ti6Al4V (Grade 5 Titanium alloy) is widely used for bioimplants due to its excellent properties such as low toxicity, corrosion resistance, high specific strength, MRI compatibility and high biocompatibility [1]. In many cases implantation fails due to reasons such as poor osseointegration, lack of adoption of proper procedures in placement of implants and infections developed after placing the implants in human body. Patients suffer due to implant associated issues which increases chances of co morbid conditions. Huge financial burden is also placed on patients and society due to more frequent implant failures. Bacterial infections are identified as a major cause of implant failure and usually require replacement of infected implants or treatment with prolonged use of antibiotics [2]. There are further issues such as bacterial drug resistance which is emerging. Bacterial infections associated with implants are mainly attributed to the formation of a biofilm on the surface of implants. Biofilm consists of bacterial colonies, teichoic acids and extra cellular polymeric substances, which give immunity against antibiotics [3]. Staphylococcus aureus (S.aureus) is identified as the most common pathogen responsible to majority of the implant related infections. S.aureus is typically hydrophobic in nature due to the presence of highly negative charged teichoic and lipoteichoic acid as its cell wall constituents [4]. Hydrophobic cells prefer attachment to the hydrophobic surface [5]. So a hydrophilic surface can inhibit the adhesion and maturation of biofilm on the surface.

Recent research has indicated that physical and chemical surface modification techniques can improve wettability and bio-tribological performance of implant surfaces [6]. The micro/nano textures on the implant surfaces can enhance the wettability and bio-tribological properties [6]. Hydrophilic properties of micro/nano textures depend on the surface energy, surface roughness and the topography of the
Several surface modifications techniques are available in order to improve the surface properties such as surface coating, texturing patterns with variable geometries, creating micro/nano structures, combination of texturing and coating etc. Surface coating techniques has a disadvantage that the coating may disintegrate in the presence of biological fluids which will diminish their effectiveness. Among various surface topography modification techniques, laser surface texturing has emerged as one of the most popular methods for creating a super hydrophilic surface. Periodic structures have been fabricated using laser surface texturing which will enhance the hydrophilicity and the surface free energy of the material. Laser texturing is found to have significantly reduced the adhesion of bacteria and reduced the formation of biofilm. The possibility of using ultra short pulsed laser for surface texturing of grade 2 Titanium alloy surfaces and the subsequent formation of biofilm have also been investigated recently.

In this study, the effects of surface texturing on altering the hydrophilicity of Ti surface and its influence on enhancing the antibacterial properties of Ti based implant surface have been investigated. Micro dimples (having diameter and depth measuring few microns) are created on the implant surface using laser texturing and the hydrophilicity of surfaces is evaluated in comparison with a polished surface. *S. aureus* cultures have been introduced on the prepared specimen surfaces and the bacterial growth has been studied.

2. Experimental details

2.1 Selection of materials and surface texturing

Commercially procured Ti6Al4V (Grade 5) specimens have been used in the experimental study. The samples are made in the form of tiny blocks of flat surface area of 10mm x 10 mm and 3mm thickness using wire cut EDM method (figure 1). Surfaces were ground sequentially with a series of SiC papers from 120 to 2000 grits using a polishing machine. Three surfaces each of Ti6Al4V were further polished with lapping paste and were kept as polished surfaces for comparing the antibacterial performance with textured surface. The laser surface texturing was performed using a femto-second pulsed Nd-YAG laser system having a power of 200W. The wave length of the laser was 1064nm. The samples were irradiated with the laser beam using pre-selected processing parameters of laser power 40W, scanning speed of 25mm/s and a stand-off distance of 10mm.

![Figure 1. Titanium Grade5 specimens (a) Polished (b) Textured](image)

The laser-textured area on the specimen samples were 10 x 10 mm which is fully covered by the dimples having a depth of 2.5μm. The laser textured samples were ultrasonically cleaned in ethanol bath for 10 min, further rinsed in distilled water and air dried.

2.2 Evaluation of Surface Topography

The surface roughness and 3D topography of the polished and laser-textured samples were assessed using a laser confocal microscope (Olympus LEXT OLS4000). Arithmetic mean roughness ($R_a$) and maximum height of the profile ($R_z$) were measured and compared with that of the polished specimen.
Dimples of depth of 2.5\(\mu\)m and diameter of 10\(\mu\)m each cover the entire length of the specimen surface.

2.3 Wettability Analysis
The sessile drop method was used to measure the water contact angle of the polished and laser-textured samples using a video-based goniometer (First Ten Angstroms, FTA 200). The image capture and analysis were performed using the FTA 32 video software. Deionized water was used as the testing liquid, and the volume of each sessile drop was controlled at 5\(\mu\)l using a microlitre syringe. Droplet images were captured in the direction perpendicular to the laser track orientation at fixed time intervals, counting since the start of the droplet deposition to the cessation of droplet spreading for at least 60s. Average contact angle of eight measurements were taken at different locations for each sample at room temperature.

2.4 Bacteria strain
Specimens are inoculated with \textit{S. aureus} which is cultured in Muller Hinton Broth (MHB) overnight (24 hrs) at 37 °C on a gyrotatory incubator with shaking at 100 rpm. After incubation, sterile MHB was used to adjust the overnight culture to an optical density of 0.3 at 550 nm and diluted 1 in 50 with fresh sterile MHB. This provided a bacterial medium of approximately 1 x 10^6 Colony Forming Units (CFU/ml). 1 ml of culture was applied to each sample at an inoculum not exceeding 2.4 x 10^6 CFU/ml, as verified by viable count. The samples were incubated for 24 hrs at 37 °C on a gyrotatory incubator with shaking at 100 rpm. Three samples of each type of specimen surfaces (polished and textured) were tested to ensure the consistency of the results. The antibacterial performance is compared by measuring the area covered by dead (red colour) and live (green colour) cells.

3. Results and Discussions
3.1 Influence of surface topography
The surface topography images of polished and micro-dimpled specimen are shown in figure 2. The influence of surface roughness on microbial retention has been investigated in literature [12]. Bacterial adhesion to surface depends on several factors, namely the topography, wettability and surface free energy, but the surface topography seems to be the most influential factor [13]. The Ra values for the polished and laser-textured samples extracted from the 3D roughness profiles are indicated in Table 1.

![Figure 2. Surface topography of Ti6Al4V (a) Polished (b) Textured](image)

The Ra values for the polished samples are in the range of 0.041 to 0.45\(\mu\)m. The Ra values increase significantly after laser treatment, lying between 3.6 and 3.7\(\mu\)m. Bacteria commonly adhere to low roughness polished surfaces, with Ra in the nanometre range, but also adhere to surfaces that present topographic features larger than the bacteria size [14]. It is found that surface roughness has a major role on cell adhesion and proliferation. Micro pattern supports the cell orientation, wound healing and repairing of cell defects [15].
### Table 1 Surface roughness and contact angle values of specimens

| No. | Specimen          | Measured Values |
|-----|-------------------|-----------------|
|     |                   | $R_a$ (µm)      | Contact angle(°) |
| 1   | Ti6Al4V - Polished | 0.451           | 121.2             |
| 2   | Ti6Al4V - Textured | 3.715           | 45.6              |

#### 3.2 Influence of water wettability

The smaller value of contact angle of liquid drop is a direct measure of the hydrophilicity of the surface [16]. The values of contact angles measured from the polished and textured specimens are shown in figure 3.

![Figure 3. Water contact angle on Ti6Al4V specimen (a) Polished (b) Textured](image)

Bacteria prefer to adhere on rougher surfaces but the surface is protected by unfavourable environmental disturbances such as shear force [17]. Laser textured specimen offers greater surface roughness than polished. From the Table.1 it is clear that the contact angle is significantly decreased after laser texturing and the surface become more hydrophilic. The hydrophobic nature of $S. aureus$ cells is due to the presence of highly negatively charged and hydrophobic teichoic and lipoteichoic acid [18].

#### 3.3 Influence on Bacterial adherence

The growth of Bacteria on the specimen surfaces have been evaluated with the help of confocal fluorescence microscopy. The images obtained are as shown in figure 4. Bacterial luminescence on the polished (figure 4a) and laser textured (figure 4b) samples are shown for comparison. Green fluorescence indicates viable bacteria with intact cell membranes whilst red fluorescence indicates dead bacteria with damaged membranes. Hydrophobic interactions considered to be an important factor for initial attachment of bacteria.

The results show that bacterial adherence is greatly reduced in laser-textured specimens (figure 4b) than the polished specimens. Textured surfaces are found to be unfavourable for bacterial colonization. This is assumed to be due to the hydrophilic nature of the textured surface and increased surface roughness ($R_a$) value. This is evident from the wettability studies carried out and reported in section 3.2. Surface peaks act as a spacer between the bacterial cell and surface, decreasing the interaction energy of the bacteria. Ti6Al4V shows better antibacterial behaviour due to its inherent chemical composition [14]. In Ti6Al4V, surface treatments convert the amorphous Titanium oxide to crystalline layer which reduces the chances of bacterial adhesion [9]. Bacteria produce Extracellular Polymeric Substances considered as the major cell to cell connecting substance, which are liable for the biofilm maturation and colonization of dead cells on the textured surface [4].
Figure 4. Microscopic images of bacterial luminescence in Ti6Al4V (a) Polished (b) Textured

Bacteria surface interaction through electrostatic, gravitational, Van der Waals forces, hydrophobic interaction of bacteria to matching biomaterial. The wavy nature of the laser textured surface reduces these interactions, through reductions in electrostatic and gravitational forces. S.aureus is typically hydrophobic in nature so they prefer hydrophobic surface for colonization [6]. Here the surface is hydrophilic in nature, which is liable for accumulation of dead cells on the textured surface. Biofilm maturation is found to be inhibited by the presence of micro features.

4. Conclusions

The present work is focused on the study of antibacterial behavior of Ti6Al4V biomaterial surfaces under the influence of micro textures. The antibacterial properties of the polished and laser-textured samples are compared and analyzed. A comparative study of the performance of antibacterial performance of Ti6Al4V has been made by analyzing the bacterial adherence and visual identification of live/dead using the bacterial luminescence microscopic images. The surface roughness of Ti6Al4V increased significantly after laser texturing. Increased surface roughness decreases the area of contact between individual bacteria and textured surface. The water contact angle of textured specimen was greatly reduced by laser texturing and the surface became more hydrophilic. These effects significantly reduced the adhesion of S.aureus cells on laser-textured surfaces. Laser surface texturing is thus found to be a promising method for endowing dental and orthopaedic Titanium implants with antibacterial properties, reducing the risk of implant-associated infections.

5. References

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