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Full Length Article

Enhancing the antibacterial performance of orthopaedic implant materials by fibre laser surface engineering

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ABSTRACT

Implant failure caused by bacterial infection is extremely difficult to treat and usually requires the removal of the infected components. Despite the severe consequence of bacterial infection, research into bacterial infection of orthopaedic implants is still at an early stage compared to the effort on enhancing osseointegration, wear and corrosion resistance of implant materials. In this study, the effects of laser surface treatment on enhancing the antibacterial properties of commercially pure (CP) Ti (Grade 2), Ti6Al4V (Grade 5) and CoCrMo alloy implant materials were studied and compared for the first time. Laser surface treatment was performed by a continuous wave (CW) fibre laser with a near-infrared wavelength of 1064 nm in a nitrogen-containing environment. *Staphylococcus aureus*, commonly implicated in infection associated with orthopaedic implants, was used to investigate the antibacterial properties of the laser-treated surfaces. The surface roughness and topography of the laser-treated materials were analysed by a 2D roughness testing and by AFM. The surface morphologies before and after 24 h of bacterial cell culture were captured by SEM, and bacterial viability was determined using live/dead staining. Surface chemistry was analysed by XPS and surface wettability was measured using the sessile drop method. The findings of this study indicated that the laser-treated CP Ti and Ti6Al4V surfaces exhibited a noticeable reduction in bacterial adhesion and possessed a bactericidal effect. Such properties were attributable to the combined effects of reduced hydrophobicity, thicker and stable oxide films and presence of laser-induced nano-features. No similar antibacterial effect was observed in the laser-treated CoCrMo.

1. Introduction

As a consequence of the soaring number of trauma cases, e.g. from road accidents and sports injuries, and with an increasingly elderly population, there is a strong global demand for orthopaedic prostheses. A recent market research report indicated that the global orthopaedic implants market was valued at USD 4.3 billion in 2015 [1]. Although significant advancements have been made to improve the osseointegration and mechanical properties of orthopaedic implants in the past two decades, orthopaedic implants are still challenged by failures due to various reasons including aseptic loosening and bacterial infections which contribute to 30% and 16% respectively of total joint revision in the hip and knee [2]. Implant failure caused by bacterial infection is costlier, time consuming, and more difficult to diagnose than aseptic loosening and usually requires the removal of the infected components [3]. Despite the severe consequence associated with implant failure, research into bacterial infection of orthopaedic implants is still at an early stage compared to the research effort on enhancing osseointegration, and on wear and corrosion resistance.

Bacterial infection is initiated through bacterial adherence to the implant surfaces, followed by bacterial colonization and biofilm formation. A biofilm is a community of microorganisms protected by a self-produced extracellular polymeric substance (EPS) matrix. It has been estimated that 99% of bacteria can exist within a biofilm state [4]. Surfaces of different components of orthopaedic implants such as the femoral stem, head and acetabular cup are designed for different purposes. For example, the stem and acetabular cup (back cup), usually made of a titanium alloy (Ti6Al4V), are designed with a rough texture to encourage osseointegration, while the femoral head, usually made of a cobalt-chromium alloy (CoCrMo), is smooth with the aim of reducing the friction between intercalating compo-

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nents. Nevertheless, bacteria can adhere to both rough and smooth surfaces, and to different types of materials. Bacterial adherence and subsequent biofilm development can hamper the performance of the implants, for example by interfering with the process of osseointegration [5]. Furthermore, biofilms are extremely difficult to remove with conventional antimicrobial therapies (e.g. antibiotics) and act as a reservoir of bacteria that can lead to chronic and systemic infection [6]. Therefore, strategies to minimise the chances of initial bacterial adherence to the implant surfaces are crucial to prevent bacterial infection.

Bacterial adherence to a surface is dependent on several interrelated surface properties of materials, such as surface roughness, topography, chemistry and wettability. Bacteria prefer to adhere to a rough surface than a smooth surface [7,8], and to hydrophobic rather than hydrophilic surfaces [9,10], while nano-scale surface features are more effective in reducing bacterial adhesion than micro- and macro-scale features [11,12]. Material chemistry can also influence bacterial colonization of a material surface, for example certain metal ions, e.g. silver (Ag), carbon (C), zinc (Zn), copper (Cu), and some metal oxides/nitrides, e.g., titanium dioxide (TiO_2), zinc oxide (ZnO), tantalum nitride (TaN), titanium nitride (TiN), and zirconium nitride (ZrN) [9] all exhibit intrinsic antibacterial properties.

Strategies to reduce bacterial adherence can generally be classified into coating and non-coating methods. The basic concept of coating methods is to coat the entire implant with the aforementioned antibacterial materials. However, the drawback of using antibacterial materials is the possibility of cytotoxicity to the host cells and tissues. For example, copper is known to display cytotoxicity towards mesenchymal stem cells [2]. Non-coating methods directly modify the surface properties of implants to achieve antibacterial characteristics. These methods include reducing surface hydrophobicity [10], creating surface nano-features [12] and modifying surface chemistry [10,13,14].

Laser surface treatment is emerging as a promising non-coating method to negate bacterial adherence. The advantages of laser technology include high speed, cleanliness, high precision and repeatability, as well as flexibility to modify surfaces in selective areas [15]. Further, laser technology can be used along with three-dimensional (3D) printing technology. The laser-based 3D printing technique of selective laser sintering (SLS) has recently been applied to fabricate bone scaffolds with antibacterial properties [16,17].

Recent successful examples of using laser surface treatment techniques to fabricate antibacterial surfaces for metallic implant materials are reviewed as follows: Gallardo-Moreno et al. [10] used UV irradiation at a wavelength of 258 nm to treat Ti6Al4V alloy. Their results indicate that the physicochemical changes on the UV treated surface caused a reduction in the adhesion rate of *Staphylococcus aureus* and *Staphylococcus epidermidis* cells. Kawano et al. [18] used a UV laser with a wavelength of 365 nm to treat commercially pure (CP) Ti. Their study suggests that exposure of CP Ti to a UV laser can decrease the number of attached *Porphyromonas gingivalis* bacterial cells, this bacterium being an important cause of dental implant infections. They ascribed the antibacterial effect to the decrease of water contact angle and increase of the Anatase phase in the surface layer on treated Ti surface. Gillett et al. [19] employed an excimer laser with a wavelength of 248 nm to surface pattern polyethylene terephthalate (PET). They reported that the surface treatment created micro-scale pits in surface and significantly influenced the distribution and morphology of attached *Escherichia coli* cells. Cunha et al. [5] created nano-features on CP Ti surface using a femtosecond laser with a wavelength of 1030 nm. They found that the nano-topography of the laser-induced features reduced adhesion of *S. aureus* cells, and attributed the effect to the reduction of contact area in the interface between individual bacterium and the metal substrate.

However, each of the studies above concerned only one particular type of materials (i.e. there was no direct comparison across different materials), and the majority of them used laser radiation in the ultraviolet wavelength range (i.e. less than 400 nm). Studies using near-infrared laser (i.e. 700–1800 nm) for enhancement of antibacterial properties of implant materials are very limited. In the work reported here, laser surface treatment was performed on three commonly-used orthopaedic metallic materials, namely CP Ti (grade 2), Ti6Al4 V (grade 5) and CoCrMo using a fibre laser with a near-infrared wavelength of 1064 nm in a nitrogen shielding environment. It is known that TiN forms on the surface when Ti-materials react with high power near-infrared laser in the nitrogen environment. TiN is a highly wear-resistant and biocompatible material [15]. *S. aureus*, the most common organism responsible for orthopaedic surgical site infections [20], was selected as the target bacteria in the study. The objectives of this study are (1) to compare the antibacterial effect of different orthopaedic materials before and after laser-treatment with near-infrared radiation, and (2) to explain the difference in antibacterial performance between treated and untreated surfaces in terms of the surface roughness, topography, chemistry and wettability.

2. Experimental details

2.1. Materials

Three different medical grade metallic materials were used for the laser treatment experiments, namely commercially pure Ti (99.2% pure, Grade 2) and Ti6Al4 V (Grade 5), and CoCrMo alloy. They were sourced from Zapp Precision Metals GmbH (Schwerte, Germany). The Grade 2 and Grade 5 titanium materials are labeled as TiG2 and TiG5 hereafter. The samples were in the form of discs 30 mm in diameter and 5 mm in thickness. Before laser treatment, the sample surfaces were ground sequentially with a series of SiC papers from 120 to 400 grits following standard metallography procedures to remove pre-existing oxides and ensure surface homogeneity. The samples were then ultrasonically cleaned in ethanol bath for 10 min, rinsed in distilled water for another 10 min, and finally dried thoroughly in a cold air stream.

2.2. Laser treatment experiments in nitrogen environment

The laser treatment process was performed using an automated continuous wave (CW) 200W fiber laser system (MLS-4030). The laser system was integrated by Micro Lasersystems BV (Driel, The Netherlands) and the fibre laser was manufactured by SPI Lasers UK Ltd (Southampton, UK). The wavelength of the laser was 1064 nm. The samples were irradiated with the laser beam using pre-selected processing parameters of: laser power of 40 W, scanning speed of 25 mm/s (meandered scan with lateral movement of 100 μm in the x direction), stand-off distance of 1.5 mm (laser spot size was measured as 100 μm) and shielding with high purity N_2 at 5 bar [21]. The N_2 gas was delivered coaxially with the laser beam via a standard laser nozzle with outlet diameter of 2 mm. The laser-irradiated area on the disk samples was 18 mm × 18 mm and fully covered by laser tracks with overlapping ratio of about 50% in track width.

2.3. Surface morphology, roughness and topography analysis

The surface morphology of the untreated and laser-treated samples was captured using a scanning electron microscope, SEM (Model 6500F, JEOL, Japan). The surface roughness and topography of the untreated and laser-treated samples were assessed