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In vitro biocompatibility and bacterial adhesion of physico-chemically modified Ti6Al4V surface by means of UV irradiation

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Abstract

UV irradiation leads to a "spontaneous" wettability increase of the Ti6Al4V surface while preserving bulk properties of the alloy that are crucial for its performance as an orthopedic and dental implant. We hypothesized that UV treatment of Ti6Al4V may impair bacterial adhesion without compromising the good response of human bone-forming cells to this alloy. The in vitro biocompatibility of the Ti6Al4V surface, before and after UV irradiation, was analyzed by using human cells related to the osteoblastic phenotype. The adhesion processes of bacterial strains related to clinical orthopedic infections, i.e., *Staphylococcus aureus* and *Staphylococcus epidermidis*, were studied theoretically and in vitro, under dynamic and static conditions as well as in the presence or absence of shear forces. While human cell adhesion was not altered by UV irradiation of Ti6Al4V alloy, this treatment reduced not only initial bacterial adhesion rates but also the number of bacteria retained on the surface after the passage of two air–liquid interfaces on the previously adhered bacteria. This study proposes the use of UV treatment prior to implantation protocols as an easy, economic and effective way of reducing bacterial adhesion on the Ti6Al4V surface without compromising its excellent biocompatibility.

Keywords: Ti6Al4V; Ultraviolet; Biocompatibility; Bacterial adhesion

1. Introduction

Microbial infection is one of the most destructive complications related to orthopedic implants because antimicrobial therapy usually lacks efficacy at the point when the infection process is detected [1,2]. Controlled antibiotic release from the biomaterial and antibiotic loading on the biomaterial surface are strategies employed to overcome this problem, but there is concern about the increased microbial resistance to antibiotics that these procedures may induce. An alternative approach in the fight against bacterial adhesion focuses on the modification of physico-chemical surface properties of the biomaterial, such as hydrophobicity, surface tension and electrical surface potential, because they are crucial in the initial approach and further retention of bacterial cells to various surfaces [3–5]. On the other hand, adequate adhesion of osteoblasts and their progenitors to the implant surface ultimately influences their capacity to proliferate and perform their specific functions. Surface characteristics of materials, such as chemistry and surface energy, play an essential role in cell adhesion to biomaterials. Thus, any modification of the surface characteristics introduced in order to diminish

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adhesion of microorganisms to a biomaterial should not compromise bone-forming cell adhesion.

The Ti6Al4V alloy is widely used in orthopedic and dental applications due to its low density, excellent mechanical and anti-corrosive properties, and good biocompatibility [6,7]. The spontaneous passivation of this alloy forms a thin outer layer, predominantly composed of amorphous or poorly recrystallized TiO_2. We have recently shown that the presence of TiO_2 changes the physico-chemical surface properties of Ti6Al4V after UV irradiation [8]. Interestingly, titanium, TiO_2 surfaces and anodized titanium alloy with a thin film of anatase show anti-bacterial properties under UV treatment [9–11]. However, to date there is no information available on the affinity of human cells and bacteria for physico-chemically modified Ti6Al4V surfaces after UV treatment. We hypothesized that UV treatment of Ti6Al4V may impair bacterial adhesion without compromising the behavior of human bone-forming cells on this alloy. The present study reports on the in vitro biocompatibility of the Ti6Al4V surface after UV irradiation by evaluating the behavior of three types of human cells related to the osteoblastic phenotype, including the osteoblastic Saos-2 cell line, mesenchymal cells from bone marrow (hMSC) and primary osteoblasts (hOB). We address the in vitro initial adhesion behavior and further retention of three staphylococci strains directly involved in implant-related infections, i.e., *Staphylococcus aureus* ATCC29213, *Staphylococcus epidermidis* ATCC35984 (producer of an extracellular polysaccharide substance, EPS) and *S. epidermidis* HAM892 (mutant of *S. epidermidis* ATCC35984, non-producer of EPS) [12–14]. The behaviors of human cells and bacterial strains on the titanium alloy surface after irradiation are extensively discussed in relation to UV-induced physico-chemical changes on the surface.

2. Materials and methods

2.1. Ti6Al4V

Disks of Ti6Al4V were cut from bars of 25 or 20 mm in diameter kindly supplied by SURGIVAL S.A. (Valencia, Spain). A TIMETAL 6–4 ELI alloy was processed as a hot rolled annealed bar at 700 °C for 2 h, then air cooled. The disks were abraded on successively finer silicon carbide papers, mechanically polished with diamond paste, and finished with silica colloidal.

Prior to their use, the Ti6Al4V disks were carefully cleaned with distilled water at 60 °C, vigorously rubbed with a smooth cotton cloth, then rinsed repeatedly, first with distilled water and finally with distilled and deionised water (Milli-Q system); they were then immersed in a beaker with distilled and deionized water and sonicated for 10 min, rinsed again with distilled and deionized water, dried in an oven at 40 °C for 1 h and stored in a desiccator for no longer than 24 h. Samples used as controls were not subjected to further treatment. A second set of samples was exposed to an UV source for 15 h. This period was sufficient to guarantee a complete hydrophilization of the surface, as we have recently shown [8]. G15-T8 UV lamps were kindly provided by Philips (Philips Iberica, Spain). The lamps emitted predominantly at a wavelength of 257.7 nm and the lamp glass avoided the production of ozone, which is produced by wavelengths lower than 200 nm. The disks were positioned at 10 cm from the light source and centered, receiving an intensity of 2.6 mW cm^{-2}. The irradiation installation was inside an opaque wood chamber to prevent interference from the room or daylight, or damage to the users.

2.2. Cell culture

Human osteosarcoma Saos-2 cells (ECACC, Salisbury, Wiltshire, UK) were grown in DMEM medium supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS), 500 UI ml^{-1} of penicillin and 0.1 mg ml^{-1} of streptomycin. Human mesenchymal stem cells from bone marrow (hMSC) were purchased from Cambrex Bio Science (Verviers, Belgium) and maintained in growth medium (Cambrex). Primary bone cells (hOB) were obtained from human bone specimens aseptically collected during orthopedic knee surgery and were cultured as has been described previously [15]. Bone samples were obtained from four patients aged 72 ± 5 years old. Each bone sample was processed in a separated primary culture and experiments were performed using cultures obtained from independent patients. Patients enrolled in this research signed Informed Consent and all procedures using human tissue designated "surgical waste" were approved by the Human Research Committee of Hospital La Paz (Date of Approval: 02-14-2007). hOB were cultured in DMEM containing 15% (v/v) FBS, 500 UI ml^{-1} of penicillin and 0.1 mg ml^{-1} of streptomycin. Cells were maintained at 37 °C under 5% CO_2 and 95% air in a humidified incubator.

For cell culture, Ti6Al4V disks of 20 mm in diameter were used. Ti6Al4V samples were routinely sterilised under UV light in a laminar flow hood for 15 min on each side and stored until use. Immediately before use in cell culture experiments, parallel sets of samples were left untreated or treated with UV light as described above.

2.3. Cell spreading assays

Cells were seeded on Ti6Al4V samples treated or untreated with UV light in 12-well plates (1.5×10^5 Saos-2 cells/well, 6×10^4 hMSC/well and 6×10^4 hOB/well) as described above and cultured for 3 or 24 h. Attached cells were fixed with 4% formaldehyde in PBS and permeabilized with 0.1% Triton X-100 in PBS. The cells were then stained with PBS containing 4×10^{-7} M phalloidine-TRITC (Sigma) to visualize filamentous actin and with PBS containing 3×10^{-6} M 4,6-diamidino-2-phenylindole (DAPI, Sigma) for nuclear DNA. Cells were examined

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