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Manipulation of the osteoblast response to a Ti–6Al–4V titanium alloy using a high power diode laser

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Abstract

To improve the bone integration of titanium-based implants a high power diode laser (HPDL) was used to modify the material for improved osteoblast cell response. The surface properties of un-treated and HPDL treated samples were characterized. Contact angles for the un-treated and the HPDL modified titanium alloy (Ti–6Al–4V) were determined with selected biological liquids by the sessile drop technique. The analysis revealed that the wettability of the Ti–6Al–4V improved after HPDL laser treatment, indicating that better interaction with the biological liquids occurred. Moreover, an in vitro human fetal osteoblast cells (hFOB 1.19) evaluation revealed a more favourable cell response on the HPDL laser treated Ti–6Al–4V alloy than on either un-treated sample or a mechanically roughened sample. It was consequently determined that the HPDL provides more a controllable and effective technique to improve the biocompatibility of bio-metals.

Keywords: High power diode laser; Wettability; Titanium alloy; Osteoblast cell

1. Introduction

Titanium–aluminum–vanadium alloys (Ti–6Al–4V) have been widely used in the dental and orthopaedic fields as implants. The success of these implant is dependent on the intimate apposition between bone and implant. A major determinant of the bone-biomaterial interfacial response is the initial attachment, spreading and growth of osteoblasts on the implant surface. Improvements in these processes may lead to faster and more extensive implant integration and higher long-term stability [1,2].

Classically, to improve bone tissue integration on implant surfaces, various techniques have been used to increase the roughness of the implant surfaces [3–5]. Many in vivo studies have compared the efficiency of various surface treatments in mechanically and morphologically improving bone tissue integration of implants. Various results have been obtained, depending on the roughness amplitude but also on the

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method used to produce the surface roughness [3–7]. Moreover, some methods have been used to improve the wettability of the biomaterials and in turn enhance the cell adhesion. Radiofrequency glow discharge has been used to increase surface energy and to enhance cell binding [8] and [9]. The heat treatment of the titanium showed that the greater surface roughness and higher surface energy resulted in greater numbers of adhered osteoblasts and higher cell activity [10]. Recently, Hao and Lawrence found that the improved wettability of the zirconia-based bioceramic following CO_2 laser irradiation resulted favourable fibroblast and osteoblast cell response [11,12].

In this study, a high power diode laser (HPDL) was used to modify the surface properties of Ti–6Al–4V alloy with the aim to improve osteoblast cell response. An in vitro test evaluated the osteoblast cell proliferation on the un-treated, mechanically roughed and laser modified Ti–6Al–4V alloy, thereby evaluating the mechanisms active on the osteoblast cell proliferation.

2. Experimental procedures

The as-received Ti–6Al–4V ELI alloy (ground annealed) was in the form of round bar with a diameter of 28.5 mm (Carpenter Inc.) and was cut into disks about 3 mm thick with a diamond rimmed blade cutter. A control sample was mechanically roughened by grounding with a grounding paper (180 Grit SiC) for 3 min. The beams of a 1.5 kW high power diode laser (Laserline, GmbH) fired and traversed across Ti6AL4V alloy surface at speed of 800 mm/min, whilst O_2 process gas was used to assist the surface treatment.

Surface analysis of the samples after laser treatment was conducted using X-ray photoemission spectroscopy (XPS), with surface roughness measurements being obtained using a profilometer (surface tester SV-600). To investigate the wetting and surface energy characteristics of the titanium alloy, contact angle, θ, measurement were carried out using distilled water, glycerol, simulated body fluid (SBF) and SBF plus bovine serum albumin (BSA) by a sessile drop measure machine (Fist Ten Ångstroms Inc.). The SBF has an inorganic ion concentration close to that found in human blood plasma. Bovine serum albumin (BSA) (Sigma A-9306) was dissolved in SBF at pH 7.40 with a concentration of 4 mg/ml.

The human osteoblastic cell line hFOB 1.19 was obtained from the American Type Culture Collection (Manassas, USA) and cultured in a culture medium in a 37 °C, humidified, 5% CO_2/95% air incubator. The Ti–6Al–4V alloy samples were placed in a 24-well tissue culture polystyrene plate (Falcon, BP) under a sterile environment and sterilized in 70% ethanol for 24 h. The samples were rinsed by phosphate buffered saline (PBS) and then were seeded with cell suspension. To analyse the cell attachment, the specimens were seeded with 0.5 ml cell suspension of 1×10^5 cell/ml for 24 h cell culture, then dehydrated in a graded ethanol series, critical point dried with CO_2 and gold coated for scanning electron microscope (SEM) analysis. Cell proliferation on each specimen was measured by MTT assay. The osteoblast cells culture 7 days on each specimen were gently washed with phosphate-buffered saline and were measured by MTT assay using 3(4,5-dimethylthiazole-2-yl)2,5-diphenyl tetrazolium bromide (MTT, Sigma Inc.). The MTT solution was added to each specimen and the cells were incubated for 4 h at 37 °C, then medium was replaced with dimethylsulfoxide. Absorbance of the solution was measured by a plate reader (EL312, Bio-Tek Instrument, USA) at 490 nm.

3. Results

As is evident in Fig. 1, the laser treatments brought about the rougher surface compared with un-treated sample. The HPDL laser treated sample with power density of 1142 W/cm^2 displays the highest R_a value. The results of the XPS tests on the titanium alloy samples reveals that there is a sharp increase in surface oxygen content for the laser treated titanium alloy. The surface oxygen content is 12.2 mass% for un-treated sample, 36.3 mass% for the laser treated sample with 857 W/cm^2 power density and 18.14 mass% for the laser treated sample with 1142 W/cm^2 power density.

From Table 1 it can be seen that the laser irradiation of titanium surface resulted in significant drop in θ measured using the various test liquids. It has showed signs of improvements in wettability of titanium alloy samples following HPDL laser treatments.