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Bone cell adhesion on ion implanted titanium alloys

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Abstract

The authors have previously reported than ion implantation can have a significant effect on osseointegration of an implant, specially when the latter is introduced in areas of poorer bone density. These results indicate that this process is particularly suited for implant devices introduced in elderly patients or in those regions that have a poor quality of bone. The aim of this work is directed to study osteoblast adhesion on Ti alloy surfaces with different ion implantation treatments, so osseoconductive properties of several surfaces can be assessed.

Polished discs of Ti–6Al–4V and Ti CP GR1 titanium alloy have been prepared and ion implanted with different species and parameters (dose and energy). Afterwards, the samples have been sterilized by UV light, inoculated with 1.5×10^5 human bone cells and incubated during 4 h at 37 C and 5% CO_2 atmosphere. Then, once fixed and rinsed, image analysis has been used to quantify the number of cells attached to the Ti discs. On a second round of tests, cell proliferation tests have been conducted during 24, 48, 144 and 192 h, respectively. Furthermore, surface analysis techniques (e.g. AFM) have been applied to learn about the qualitative behavior, i.e. morphology, of the attached cells.

Cell attachment has shown to be highly sensitive to ion implantation parameters. Although some quantitative differences have been observed, the more significant differences were qualitative. AFM analysis has shown that the star-shaped bone cells attached spread more and occupied larger surfaces like in osseointegration prone surfaces, most probably due to extracellular matrix synthesized around them, while other surfaces showed mainly large and narrow shaped or round shaped bone cells often with great cellular nucleus in the middle of the cells and little extracellular matrix around. So, ion implanted surfaces that facilitate osseointegration have been identified, in terms of initial bone cell attachment quality, where although the number of attached cells were not necessarily always larger, they tended to occupy wider areas with healthier cells.

Keywords: Ion implantation; Titanium alloys; Osteoblast; Cell culture; Cell proliferation; Implant

1. Introduction

The tissue integration of a biomaterial is a key factor in determining how well the implant materials commonly used in bone surgery or reconstruction are incorporated into the human body. The biocompatibility of a biomaterial is highly related to the behavior of the cells in contact and in particular the cell adhesion to its surface.

The surface characteristics of a material, including its topography and physical and chemical properties at a micro and nano-scale, play an important role in osteoblast adhesion on biomaterials [1]. Therefore, the attachment, adhesion and spreading of osteoblasts form a first phase of the interactions between cells and the material and will affect the cells' capacity to proliferate and get established in contact with the surface and ultimately generate bone tissue around the implant.

In the case of orthopedic and dental implants, the formation of a strong mechanical interface is of paramount importance to guarantee a long functionality. This implies a good joint between the surface of the implant material and the bone tissue without any fibrous tissue interface. The target in many cases is to ensure a good mechanically bonded implant even in areas of poor bone quality and, additionally, to shorten patient treatment times, by achieving shorter integration times. This objective responds to the wish of maintaining a healthy quality of life in addition to aesthetical

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aspects in a population with increased life expectancy. As an example only in Europe, one million people underwent a dental implant procedure in the year 2000, only a fraction of the people who present edentulism problems. This low implant penetration rate in the population is mainly due to cost and the lengthy implant treatment time. The latter would be enhanced significantly by engineering the cellular attachment to the implants surface.

A complete understanding of osteoblast adhesion on materials is therefore essential to engineer and optimize the bone–biomaterial interface, especially in the case of materials that have been designed to be osteoinductive. Different surface engineering processes have been applied to biomaterials to alter their surface properties, aiming at enhancing attachment of the cells. These include modification of the surface roughness by mechanical blasting [2] and acid etching [3] or altering the surface chemistry through plasma based sterilizing treatments [4], forming oxide layers by processes such as electrochemical anodization [5], and finally surface modification and coating deposition [6,7].

Ion based surface modification processes, including direct ion beam and plasma source ion implantation, are also good candidates to influence osteoblast–material interactions, since these can tailor the topography, surface chemistry and surface energy of a biomaterial. Ion implantation and related beam processing methods are today widely recognized as commercial surface treatments for orthopedic implants and for example are related to the reduction of polyethylene wear debris in articulated bearing surfaces such as hip joints and knees [8]. A number of studies have been carried out on the application of ion implantation to influence cell attachment to surfaces. For example, ion implantation from gaseous precursors has shown to improve the bone integration of dental implants [9,10]. Implantation of Na ions into titanium have been carried out to form sodium titanate at the surface and subsequently induce the precipitation of hydroxyapatite from the body fluid, which in turn would enhance osseointegration [11]. Additionally, implantation of Ca ions has also been carried out successfully to facilitate the osseointegration of titanium alloys [12].

In the case of ion implantation there is an existing background of in vivo and in vitro tests carried out by the authors [9,10] that have shown good results concerning the osseointegration of the surface treated material. To optimise the treatment conditions concerning cellular attachment and to gain a clear understanding on the effects of the treatment on cell adhesion, the authors have carried out in the present work cell adhesion and proliferation tests.

2. Experimental procedure

2.1. Sample preparation and ion implantation procedure

Two sets of experiments were carried out, in the first one two ion implantation treatments, designated as Type A and Type B, were evaluated for cell attachment in contact with bone cells for a fixed period of time. Secondly, cell proliferation tests were conducted with the selected treatment from the earlier study (Type A), investigating cell adhesion as a function of time.

In the first experiment, 16 samples of mirror polished titanium disks were prepared for each of the two treatments and the control material. Table 1 summarises the different material and surface treatment combinations that were applied in the cell attachment and proliferation tests. Two titanium alloys were used, a Ti–6Al–4V and a commercial pure Ti grade Ti CP GR1. The samples were 15 mm diameter discs cut from bars in the case of Ti–6Al–4V and 15×15 mm square samples in the case of Ti CP GR1 with 1 mm in thickness, polished from one side to a surface finish of 0.01 μm Ra. Ion implantation was carried out on the polished side of discs in a Danfysik high-current implanter Model 1090. All titanium discs were ultrasonically degreased and cleaned prior to ion implantation treatments, which were performed using a Chordis ion source at doses from 0.5 to 5×10^{17} ions/cm^2 and energies in the range of 40 to 100 KeV, using gaseous precursors [13]. The treatments were performed at low temperature (<170 °C) and at a vacuum better than 4×10^{-4} Pa.

Once that the cell attachment tests were completed and evaluated, the second group of assays concerning the study of cell proliferation was performed on Ti–6Al–4V samples with the Type A ion implantation process. In this case, 12 samples of the control unimplanted titanium alloy and another 12 specimens of the ion implanted titanium alloy were evaluated (see Table 1).

2.2. Cell culture

Cell adhesion and proliferation tests were determined with hFOB 1.19 human bone cells (ATCC, CRL-11372). Before performing these assays, the cells were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-

Table 1
Material and surface treatment combinations used in the cell attachment and proliferation tests

Test type	No. of samples	Sample ref.	Material	Surface treatment
Cell attachment	16	A (A1 to A16)	Ti–6Al–4V alloy	Type A ion implantation
	16	B (B1 to B16)	Ti–6Al–4V alloy	Type B ion implantation
	16	C (C1 to C16)	Ti–6Al–4V alloy	Unimplanted (control)
	16	D (D1 to D16)	Ti CP GR1	Type B ion implantation
	16	E (E1 to E16)	Ti CP GR1	Unimplanted (control)
Cell proliferation	12	Control Ti	Ti–6Al–4V alloy	Unimplanted (control)
	12	Treated Ti	Ti–6Al–4V alloy	Type A ion implantation