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Effects of calcium ion implantation on human bone cell interaction with titanium

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Abstract

The use of calcium ion (Ca) implantation of titanium (Ti), previously reported to encourage osseointegration in vivo, has been investigated using an in vitro model in order to understand the basic mechanisms involved in the response of target cells to such surfaces. Polished Ti discs were implanted with high, medium and low (1×10^{17}, 1×10^{16}, 1×10^{15} ions cm^{-2}) doses of Ca ions at 40 keV. The effects of different levels of Ca implantation on morphology, attachment and spreading of MG-63 cells seeded on the surface of control (non-implanted) Ti and Ca–Ti discs were assessed. Further, to understand cell–material interactions at a molecular level, the expression of $\beta 1$ and $\alpha 5\beta 1$ integrins and the formation of vinculin-positive focal adhesion plaques were examined. In addition, the effects of pre-immersion of the Ca (high)–Ti in tissue culture medium on cell attachment were measured and correlated with specific chemical changes at the Ti surface. Our findings suggest that Ca implantation can affect the adhesion of MG-63 cells both qualitatively and quantitatively. However, this effect appears to depend on the level at which Ca ions are implanted. Results showed that although cell adhesion on Ca (high)–Ti was initially reduced, it nevertheless was not only restored but substantially increased with progressing culture times. In addition, a significantly enhanced cell spreading, formation of focal adhesion plaques and expression of integrins were measured on this particular surface. In contrast, no marked differences were observed in cell behaviour on Ca–Ti (low and medium). Pre-immersion studies indicated that the decrease in cell attachment to Ca (high)–Ti at early time periods may be linked to the presence of Ca- and P-rich particles on the surface. The absence of these particles at 24 h was consistent with a significant increase in cell attachment.

Keywords: Titanium; Calcium-implantation; Cell adhesion; MG-63 cells; Integrin; Vinculin

1. Introduction

As events leading to the integration of an orthopaedic or dental implant into bone take place largely at the tissue–implant interface, it is essential that implant materials have optimum surface compatibility with the surrounding bone tissue. Surface characteristics such as surface energy, roughness, topography and chemistry play an important part in mediating the interactions between the implant and the host tissue [1,2]. Thus, certain changes in surface chemistry have been reported to influence the attachment of bone cells, their spreading characteristics, signalling cascades and maturation in vitro [3].

Several procedures have been developed to modify the surface chemistry of implant materials, including ion-implantation [1]. Main advantages of this method include speed, homogeneity and reproducibility of doping, exact dosage control and high dopant purity [4]. Although adequate biocompatibility and suitable physico-chemical characteristics of titanium (Ti) have made it the most frequently used metallic material for bone implants [5], numerous surface modifications have

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been carried out in order to improve its clinical performance and longevity. For example, implantation with nitrogen ions has been shown to improve resistance to corrosion and wear [4]. Biologically active calcium (Ca) ions have also been implanted into the surface of Ti in order to enhance biocompatibility, resulting in enhanced growth of bone tissue in vivo [5,6]. In contrast, implantation of Ca ions showed no beneficial effects on the viability or the functional activity of bone cells, despite increased corrosion resistance [7]. Such contradictory findings highlight the need to clarify the underlying mechanisms involved in cellular responses to ion-implantation, particularly with Ca.

We have previously investigated the effects of implantation of three elements with similar atomic masses but different chemical characteristics. Potassium (K) and argon (Ar) had no significant effect on the response of human alveolar bone and MG-63 osteosarcoma cells. In marked contrast, the growth of both types of bone cells was substantially enhanced on Ti implanted with relatively high levels of Ca ions. However, the initial adhesion of the cells to this particular surface was severely reduced compared with cell attachment to the K, Ar and non-implanted Ti surfaces [8,9]. Since this Ca-induced decrease in bone cell binding could adversely affect host responses to the Ti implant, the present study was undertaken to determine the effects of different levels of ion implanted Ca on a number of aspects of short-term bone–material interactions, including bone cell attachment, morphology, the expression of integrins which mediate cell–substrate adhesion [10] and the formation of vinculin-positive focal adhesion plaques which characterise the physical association of cells with surfaces [11]. In addition, since tissue culture conditions can change the surface chemistry of implant materials and thereby influence cell responses [12], the effects of pre-immersion of the Ca-implanted Ti on cell attachment were also measured and correlated with specific chemical changes at the Ti surface.

2. Materials and methods

2.1. Preparation of titanium discs

Commercially pure Ti discs (grade 1, 8×1 mm) (Goodfellow Cambridge, UK) were polished on one face to a mirror finish using a Rotopol 11 polisher (Struers, Glasgow, UK) and 1200–2400 silicon carbide grit followed by a 0.1 μm silica suspension containing 5% H_2O_2 (4:1 v/v). They were then sequentially ultrasonicated in toluene, acetone and deionized water, air-dried and stored in a desiccator. Ion-implantation was carried out (Department of Electrical and Electronic Engineering, Imperial College London, UK), at an implantation energy of 40 keV, with high, medium and low doses of $^{40}Ca^+$ ions, i.e. 1×10^{17}, 1×10^{16} and 1×10^{15} ions cm^{-2}, respectively. Following implantation, the samples were removed from the mounting plate using acetone and washed in deionized water. All discs were stored at room temperature (RT) in a desiccator and sterilized by ultraviolet light prior to cell culture.

2.2. Cell culture

MG-63 cells, derived from an osteogenic sarcoma of a 14-year old male [13], were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 2 mM L-glutamine (GIBCO, Invitrogen, Paisley, UK). In order to measure bone cell responses, a suspension of the cells was seeded onto the surfaces of the Ca-implanted and non-implanted Ti (control) discs in 24-well tissue culture plates (Becton-Dickinson Labware, Oxford, UK).

2.3. Scanning electron microscopy (SEM)

The MG-63 cells were seeded (1×10^3 cells/disc) on the test surfaces and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for 4 h. The discs were then removed, washed with phosphate-buffered saline (PBS) and fixed with 3% glutaraldehyde in 0.1 M cacodylic acid buffer (pH 7.4) overnight. After fixation, the discs were sequentially dehydrated in ethanol (20%, 50%, 70%, 90% and 100%) for 10 min each, immersed for 1.5 min in hexamethyl disilazane solution (HMDS) (TAAB Laboratories, Aldermaston, UK), a critical point drying fluid, and air-dried for 1 h at RT. A thin layer of gold/palladium was sputter-coated onto the discs using a Polaron E5000 (Emitech, Ashford, UK), which were then mounted on metal stubs and visualised using a Cambridge 90B SEM (LEO Electron Microscopy, Cambridge, UK) at an acceleration voltage of 15 kV.

SEM micrographs were captured digitally and the average area and shape factor [(area/perimeter^2) $\times 4\pi$] were measured on five representative fields of the SEM micrographs of the cells using Image-Pro Plus 4.01 analysis software (Media Cybernetics, Wokingham, UK).

2.4. Cell adhesion assay

To measure cell adhesion quantitatively, actively dividing MG-63 cells were first tagged by radiolabeling for 18 h with 1 μCi ml^{-1} of [^{3}H] thymidine (TdR) (44 C $mmol^{-1}$) (Amersham Biosciences, St. Giles, UK), a radioactive precursor of DNA. One sample was used to count the number of cells and a replicate sample was used to measure the amount of isotope incorporated into the DNA by liquid scintillation spectroscopy