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Osteoblastic cell behaviour on different titanium implant surfaces

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Abstract

The osseointegration of oral implants is related to the early interactions between osteoblastic cells and titanium surfaces. The behaviour of osteoblastic MC3T3-E1 cells was compared on four different titanium surfaces: mirror-polished (Smooth-Ti), alumina grit-blasted (Alumina–Ti) or biphasic calcium phosphate ceramic grit-blasted (BCP–Ti) and a commercially available implant surface (SLA). Scanning electron microscopy and profilometry showed distinct microtopographies. The BCP–Ti group had higher average surface roughness ($R_a = 2.5$ μm) than the other grit-blasted groups. Hydrophilicity and surfaces energies were determined on the different substrates by dynamic contact angle measurements. The most hydrophilic surface was the Alumina–Ti discs, while SLA was the most hydrophobic. The titanium surfaces were all oxidized as TiO_2 and polluted by carbon contaminants, as determined by X-ray photoelectron spectroscopy. Alumina–Ti samples also exhibited aluminium peaks as a result of the blasting. The BCP–Ti discs contained traces of calcium and phosphorus. MC3T3-E1 cells attached, spread and proliferated on the substrates. For both the SLA and BCP–Ti groups, the entire surface was covered with a layer of osteoblastic cells after 2 days. At high magnification, the cells exhibited cytoplasmic extensions and filopodia. Compared with plastic, cell viability was similar with the Smooth–Ti, slightly lower with the Alumina–Ti and superior with the SLA and BCP–Ti groups. Alkaline phosphatase activity increased with the culture time whatever the substrate. This study shows that BCP-blasting produces rough titanium implants without surface contaminants.

Keywords: Cell–biomaterial interaction; Osteoblasts; Surface roughness; Titanium; Dental implant

1. Introduction

The early osseointegration of titanium oral implants is an important factor for their clinical success [1,2]. The biological fixation of an implant to bone is influenced by numerous factors, including surface chemistry and surface topography [3–5]. These surface properties are crucial during the bone healing phase in the peri-implant region. After implantation, implant surfaces are in contact with body fluids and interact with a number of proteins and different cell types. The challenge in the engineering of implant surfaces is to attract, above all, osteoblasts that produce a bone extracellular matrix, which will ensure a high bone-implant contact. Cell adhesion is one of the initial stages for subsequent proliferation and differentiation of osteoblastic cells producing bone tissue. It has been shown that osteoblastic cell adhesion, growth and differentiation are related to surface energy and roughness [6,7]. Although many *in vitro* experiments have studied osteoblastic cell behaviour on titanium surfaces, the optimal surface properties for attachment, proliferation and differentiation of osteoblastic cells have not yet been clearly defined. Most oral implant surfaces are moderately roughened ($R_a = 1.0$–2.0 μm) [3]. Rough surfaces encourage the entrapment of fibrin protein, adhesion of osteogenic cells and mechanical stability of implants in host bone [8–10].

Various methods have been developed to create rough implant surfaces in order to improve the clinical performance of implants and to guarantee a stable mechanical

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bone-implant interface [11]. These roughened surfaces are usually obtained by alumina or titanium oxide grit-blasting followed by acid-etching (e.g. SLA from Straumann AG; TiOblast™ and OsseoSpeed™ from Astra Tech). However, contamination by conventional grit-blasting materials (e.g. particles of alumina or titanium oxide) is often observed on the surface of oral implants and these impurities might hamper the osseointegration process [12,13]. Another method for roughening titanium implants uses a biocompatible and resorbable blasting material, such as biphasic calcium phosphate (BCP) ceramic particles [14]. As calcium phosphate materials are soluble in acids, they can be easily removed from the implant surface after blasting [15,16]. Furthermore, manufacturers claim that acid-etching produces nanometre-sized topography favourable for the adsorption of proteins and the attachment of osteoblastic cells [17–20]. These claims are based on animal experiments with torque force and bone contact measurements. As these treatments produce surfaces with random topography and various chemical compositions, the optimal surface properties that result in rapid osteoblastic cell adhesion, proliferation and differentiation are not yet established [21].

The aim of this study was to compare osteoblastic cell behaviour on various titanium implant surfaces. Four groups were investigated: mirror-polished (Smooth-Ti), alumina-blasted and acid-etched (Alumina–Ti), SLA (sand-blasted, large-grit, acid-etched; supplied by Straumann AG) and biphasic calcium phosphate grit-blasted and acid-etched (BCP–Ti) titanium. After analysing the properties of the implant surfaces, osteoblastic cell attachment, morphology, viability and differentiation were studied.

2. Materials and methods

2.1. Surface preparation and characterization

Titanium discs, measuring 15 mm in diameter and 1 mm in thickness, were manufactured out of commercially pure titanium bars (MPTP SARL, Arnage, France). These titanium discs perfectly fitted the bottom of the wells in 24-well cell culture plates. Smooth–Ti discs were obtained by polishing first with SiC papers of decreasing grain sizes (Struers #1200–4000; Struers SAS, Champigny sur Marne, France) and then with a diamond suspension (DP-Suspension 3 μm; Struers SAS). SLA-treated titanium discs were supplied by the Institut Straumann AG (Waldenburg, Switzerland). According to the manufacturer, the SLA discs were made using alumina-blasting with large-grit 0.25–0.50 mm in size and then acid-etched with a mixture of HCl/H_2SO_4. The Alumina–Ti discs were obtained by alumina grit-blasting (Al_2O_3 particles Cerami Jet 110 μm; GEMA, Horbourg, France) using a dental blasting cabinet (Sanduret 3 K; Reitel, Bad Essen, Germany) at an air pressure of 4 bars for 1 min. The BCP–Ti discs were obtained by blasting with a biphasic calcium ceramic powder (BCP; Biomatlante SARL, Vigneux de Bretagne, France). The BCP consisted of a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) in an HA/TCP ratio of 60/40 sintered at 1250 °C. The powder was ground and sieved between 100 and 200 μm. After grit-blasting, the Alumina–Ti and BCP–Ti discs were acid-etched using nitric acid at 5% in an ultrasonic bath (Fisher Bioblock Scientific) for 5 min. The discs were cleaned with 15 min sonication cycles in demineralized water, ethanol and acetone, and then air dried. The titanium discs were then packaged and autoclaved at 121 °C for 20 min.

The different surfaces were characterized using a mechanical profilometer (Dektak 8; Veeco, Dourdan, France). Quantitative three-dimensional topographical analysis was obtained by the calculation of dimensional roughness parameters. The average roughness (R_a) and the roughness mean square (R_{ms}) of the surfaces were measured. The surface morphology was examined by scanning electron microscopy (SEM; LEO 1450VP, Germany). The contact angles and surface energy were determined by dynamic contact angle measurements (GBX Instruments, Valence, France). Contact angles were measured in triplicate using pure water, diiodomethane and formamide. The surface energy was calculated using the Owens–Wendt equation [22]. The chemical composition of the surfaces was analysed by X-ray photoelectron spectroscopy (XPS; Leybold LHS 12), by using an Mg source operated at 12 kV and 10 mA (120 W) and a pressure of 5×10^{-8} mbar. The area of the surfaces analysed was of 3 mm × 5 mm. XPS analyses (SpecLab data system) were performed at the University of Nantes–CNRS.

2.2. Cell culture

A newborn mouse calvaria-derived cell line (MC3T3-E1, subclone 4; ATCC, USA) was used. The MC3T3-E1 cells exhibit an osteoblastic phenotype, as evidenced by the expression of alkaline phosphatase (ALP) activity [23], the synthesis of extracellular matrix (ECM) components such as osteocalcin and type-1 collagen [24], and their ability to mineralize *in vitro*. MC3T3-E1 were cultured in alpha-MEM medium (In-Vitrogen Corporation, Paisley, UK) supplemented with 10% of fetal calf serum (Dominique Dutcher, Brumath, France), 1% penicillin/streptomycin (In-Vitrogen Corporation) and 1% L-glutamine (In-Vitrogen Corporation) at 37 °C in a humidified atmosphere of 5% CO_2 in air. The complete medium was replaced every 2–3 days and confluent cells were subcultured through trypsinization (trypsin/EDTA; In-Vitrogen Corporation). After counting, cells were seeded and cultured on plastic, Smooth-Ti, Alumina–Ti, SLA and BCP–Ti using 24-well culture plates. The culture medium was refreshed every 2 days. Each experiment was performed in quadruple ($n = 4$) for each group and repeated twice.

2.3. Cell morphology

Cells were seeded on to the titanium discs in 24-well plates at a final density of 10,000 cells cm^{-2}. After 2 days,

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