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Osteoblastic cell behaviour on modified titanium surfaces

Magdalena Lukaszewska-Kuska[a], Przemysław Wirstlein[b], Radomir Majchrowski[c], Barbara Dorocka-Bobkowska[a,*]

[a] Department of Gerostomatology and Oral Pathology, Poznan University of Medical Sciences, Bukowska 70, 60-812 Poznan, Poland
[b] Division of Reproduction, Department of Gynecology and Obstetrics, Poznan University of Medical Sciences, Polna 33, 60-535 Poznan, Poland
[c] Division of Metrology and Measurement Systems, Institute of Mechanical Technology, Poznan University of Technology, Jana Pawła II 24, 60-965 Poznan, Poland

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ABSTRACT

Introduction: The surfaces of endoosseous dental implants have been subjected to numerous modifications in order to create a surface which can provide rapid bone healing and fast implant loading. Each modification has involved changes to the chemical composition and topography of the surfaces which have resulted in various biological reactions to the implanted material.

Aim: The aim of this study was to evaluate the surface topography and chemistry of various modified titanium surfaces: (1) machined surface (MA), (2) alumina-blasted (Al2O3), (3) alumina-blasted and acid-etched (Al2O3 DE), (4) hydroxyapatite/tricalcium phosphate grit-blasted (HA/TCP) and (5) hydroxyapatite/tricalcium phosphate grit-blasted and acid-etched (HA/TCP DE) and to analyse the effects of surface roughness, and chemical composition on human osteoblast vitality, differentiation, morphology and orientation.

Materials and methods: The modified surfaces were subjected to topographic analysis using Scanning Electron Microscopy (SEM), optical profilometry, roughness analysis and chemical composition evaluation using Energy Dispersion Spectroscopy (EDS) analysis. The biological effects of the titanium modifications was analysed using human osteoblasts cell culture where the cell morphology, vitality (MTS assay) and differentiation (ALP activity) was analysed.

Results: The machined surfaces were classified as anisotropic, smooth and composed of titanium and oxygen.

The blasted surface samples along with the blasted and etched samples were found to be isotropic and rough. The grit-blasting procedure resulted in the incorporation of components from the blasting material. In the case of the blasted and etched samples, etching decreased the surface development as indicated by the Sdr and also reduced the amount of chemical compounds incorporated into the surfaces during the blasting procedure.

The attached NHOst cells, proliferated the surfaces. With regard to the MA samples, the cells spread close to the titanium surface, with expanded cytoplasmic extensions and lamelipodia and were oriented in line with the groves left after machining. On the rough substrates, cells were less dispersed and exhibited numerous cytoplasmic extensions, filopodia and interconnections, they were not oriented with respect to the surfaces features. The cell viability of all samples except for Al2O3 decreased after the first day of culture. For all Al2O3, Al2O3 DE and HA samples the viability increased with culture time after an initial reduction.

At the end of the culture period the ALP activity was slightly greater on Al2O3 and HA samples compared to the control with the HA DE sample having the same activity as the control. The Al2O3, HA and HA DE ALP samples showed comparable activity and were statistically different from MA and Al2O3 DE samples.

Conclusions: In this study, variously treated titanium surfaces were correlated with osteoblastic cell viability, morphology and differentiation in comparison with the plastic and smooth titanium. All examined surfaces were found to be biocompatible. Favourable cell reactions were observed for Al2O3 and HA blasted surfaces. The surface roughness patterns influenced the growth orientation while the surface topography influenced osteoblast morphology. Further animal studies are necessary to compare the in-vivo effect on osseointegration of these modified titanium surfaces.

* Corresponding author.
E-mail addresses: abys@wp.pl (P. Wirstlein), radomir.majchrowski@gmail.com (R. Majchrowski), b.dorocka@gmail.com (B. Dorocka-Bobkowska).

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1. Introduction

Titanium and its alloys are the best metallic materials for biomedical implants. This is due to their biocompatibility, atoxicity, high mechanical resistance, corrosion resistance and low modulus of elasticity (Niinomi, 2003; Boehlert et al., 2005)

The titanium endoosseous implant surface facilitates bone reaction and implant osseointegration. Such surfaces influence bone healing and have been subjected to numerous modifications in order to create a surface which can provide rapid bone healing and permit fast implant loading. The modifications have involved changes to the chemical composition and topography of the surface leading to various bone reactions. (Lincks et al., 1998)

The aim of this study was to evaluate the effects of modified titanium surface properties on human osteoblasts. Five groups of modified titanium specimens were investigated: (1) machined surface (MA), (2) alumina-blasted (Al2O3), (3) alumina-blasted and acid-etched (Al2O3 DE), (4) hydroxyapatite/tricalcium phosphate grit-blasted (HA/TCP) and (5) hydroxyapatite/tricalcium phosphate grit-blasted and acid-etched (HA/TCP DE). The effects of surface roughness, organisation and chemical composition on human osteoblasts vitality, differentiation, morphology and orientation was analysed.

2. Materials and methods

2.1. Sample preparation and characterisation

Titanium discs 8 mm diameter and 1 mm thickness were used. The discs were machined from commercially pure titanium class 4b rods (G&S Titanium Inc, USA). Five different titanium surface modification were produced: machined surface (MA), alumina-blasted (Al2O3), alumina-blasted and acid-etched (Al2O3 DE), hydroxyapatite/tricalcium phosphate grit-blasted (HA/TCP) and hydroxyapatite/tricalcium phosphate grit-blasted and acid-etched (HA/TCP DE). The machined surface was obtained by cutting discs from rods on a lathe. The Al2O3 surface was produced by alumina grit blasting using a large grit size (53–75 um). The Al2O3 DE surface was obtained by alumina grit-blasting as above followed by acid-etching with a mixture of 36% HCl/96% H_2SO_4 (1:6) for 10 min. The HA/TCP surface was prepared by blasting with a mixture of hydroxyapatite and tricalcium phosphate HA/TCP powder. The grit consisted of 70 +/− 5% HA and 30 +/− 5% TCP with a grain size < 300 μm. The HA/TCP DE surface was obtained by HA/TCP grit blasting and acid-etched with HCl/H_2SO_4 as above.

After surface preparation all discs were subjected to ultrasonic washing in a surfactant for 15 min at 55 °C, followed by 2-propanol for 15 min at 22 °C, a disinfectant for 15 min at 22 °C, and finally twice washed in distilled water for 15 min at 55 °C. The implants were then double packed and sterilized by radiation from an electron accelerator with a radiation dose of 25 kGy.

Scanning electron microscopy analysis (Zeiss EVO 25, Carl Zeiss, Germany) with 6000x magnification was performed to study the surface morphology.

The presence of aluminium, phosphorus and calcium deposited during the titanium surface treatment along with carbon contaminants was analysed by energy-dispersive spectroscopy (Quantax AXS, Bruker, Germany) as described previously (Lukaszewska-Kuska et al., 2017).

An optical profilometer (WYKO NT1100, Veeco Instruments, USA) was used for surface roughness analysis. The measured area was 0.9 × 1.2 mm and a magnification of x20 was used in the VSI Mode using WYKO Vision software 3.0 for NT-1100. The surface roughness of the examined discs was measured at 5 random locations from which the Sa, Sdr and Str values were obtained.

Le Guehennec et al. (2008) developed a methodology for the comparison of osteoblastic cell behaviour on various titanium surfaces using cell morphology, viability, alkaline phosphate activity and total protein content.

2.2. Cell culture

The cell culture study was performed in the Laboratory of Tissue Cultures of the Department of Reproduction at the University of Medical Sciences in Poznan. The human osteoblast cell line NHOst (NHOst-Osteoblasts OGM, cryo amp, Lonza USA) was used for the study. The culture was grown in a medium (Osteoblasts Basal Medium, Lonza, USA) supplemented with fetal bovine serum, ascorbic acid and gentamicin/amphotericin-B (Osteobast Growth Medium SingleQuots Lonza, USA) and with Hydrocortisone-21-hemisuccinate and beta-glycerophosphate (Differentiation SingleQuots, LONZA USA) at the temperature of 37 °C in a 5% CO_2 atmosphere. The culture was carried out with daily changes of the medium. After confluence, the cells were trypsinized with 0.25% Trypsin/EDTA (Lonza, USA). The MA, Al2O3, Al2O3 DE, HA/TCP, HA/TCP DE samples and plastic – polyester coverslips (Thermanox, Thermo Scientific, Denmarc) used as a control were placed in 24-well culture plates (Multidish 24 #144530, Roskilde, Denmark). The cell suspensions containing 4×10^4 cells were seeded on all samples and cultured for 1, 3, 7 and 14 days. The medium was completely renewed every 24 h. Each experiment was performed 6 times for each group and repeated twice.

2.3. Cell morphology

After 3 days NHOst cultured on titanium discs and plastic were fixed with 2% glutaraldehyde in PBS for 24 h. The specimens were then dehydrated in graded alcohol (30, 50, 70 and 96% and in absolute alcohol) After dehydration, a critical point dryer was used to dry the samples, followed by sputter-coating with gold. SEM was used for the specimen surface study and the images were recorded at different magnifications. Areas were randomly chosen from each sample during SEM imaging.

2.4. Cell viability

The NHOst cells were cultured on the various titanium discs and on plastic for 14 days. Immediately prior to testing a solution of MTS (Promega, USA) (20 vol.) and PMS (Promega USA) (1 vol.) was prepared. This solution was added to the medium at a ratio of (1:10) and then incubated for a maximum of 60 min at 37° C in 5% CO_2. The resulting colored solution was transferred to a 96-well ELISA plate # 167008 TC Nunclon MicroWell (Roskilde, Denmark). The absorbance was measured at λ = 490 nm using a Dynex MRX spectrophotometer (Dynex Technologies, USA) and the results were expressed as relative MTS activity compared to the control conditions with cells cultured on plastic.

2.5. Total protein content

The NHOst cells were cultured onthe various titanium discs and on plastic for 14 days. Micro BCA™ Protein Assay Kit (Thermo Scientific, USA) was used to determine the protein concentration. Culture medium was removed and the cell layer was placed in 0,5 mL of double-distilled water and homogenized by triple freezing in liquid nitrogen and thawing at room temperature. Reaming cells were scraped and added to suspension. A series of bovine albumin (Sigma Aldrich, USA) dilutions were prepared. A BCA working reagent was prepared according to manufacturer's instructions. A 150 μL aliquot of every sample and of every dilution of bovine serum was transferred to a 96-well ELISA plate # 167008 TC Nunclon MicroWell (Roskilde, Denmark) then 150 μL of BCA working reagent was added to every well and the plate was incubated at 37 °C for 2 h. After cooling the absorbance of the solutions was examined at λ = 507 nm using a Dynex MRX spectrophotometer (Dynex Technologies, USA). Readings were made using the blind test – working solution mixed with double distilled water. A calibration curve was prepared from standard solutions of bovine serum dilutions to