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Effects of phosphoric acid treatment of titanium surfaces on surface properties, osteoblast response and removal of torque forces

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ABSTRACT

This study investigated the surface characteristics and biocompatibility of phosphate ion (P)-incorporated titanium (Ti) surfaces hydrothermally treated with various concentrations of phosphoric acid (H_3PO_4). The surface characteristics were evaluated by scanning electron microscopy, thin-film X-ray diffractometry, X-ray photoelectron spectroscopy, optical profilometry, contact angle and surface energy measurement and inductively coupled plasma mass spectroscopy (ICP-MS). MC3T3-E1 cell attachment, spreading, proliferation and osteoblastic gene expression on different surfaces were evaluated. The degree of bony integration was biomechanically evaluated by removal torque testing after 4 weeks of healing in rabbit tibiae. The H_3PO_4 treatment produced micro-rough Ti surfaces with crystalline P-incorporated Ti oxide layers. High concentration H_3PO_4 treatment (1% and 2%) produced significantly higher hydrophilic surfaces compared with low H_3PO_4 treatment (0.5%) and untreated surfaces ($P < 0.01$). ICP-MS analysis showed P ions were released from P-incorporated surfaces. Significant increased cell attachment ($P < 0.05$) and notably higher mRNA expressions of Runx2, alkaline phosphatase, osteopontin and osteocalcin were observed in cells grown on P-incorporated surfaces compared with cells on untreated machined surfaces. P-incorporated surfaces showed significantly higher removal torque forces compared with untreated machined implants ($P < 0.05$). Ti surfaces treated with 2% H_3PO_4 showed increasing tendencies in osteoblastic gene expression and removal torque forces compared with those treated with lower H_3PO_4 concentrations or untreated surfaces. These results demonstrate that H_3PO_4 treatment may improve the biocompatibility of Ti implants by enhancing osteoblast attachment, differentiation and biomechanical anchorage.

1. Introduction

Numerous studies have demonstrated that a titanium (Ti) oxide layer modified by various methods improves bone healing around Ti implants. A micro-porous crystalline Ti oxide layer or one incorporated with potentially bioactive ions accelerates implant bone healing by promoting osteoblast cell differentiation in vitro, and by increasing bone–implant contact and biomechanical anchorage in vivo [1–6].

Several studies have performed anodic oxidation treatment using phosphoric acid in order to produce biocompatible Ti surfaces for biomedical applications [5,7,8]. These, however, did not produce crystalline phosphate ion (P)-incorporated oxide layers. Recently, we have shown that hydrothermal treatment using phosphoric acid produces a crystalline P-incorporated oxide surface, which exhibited highly wettable and micro-rough surface features [9]. P-incorporated Ti surfaces significantly increased bone–implant contact percentages and removal torque forces in rabbittibiae compared with various commercial microstructured surfaces.

Surface properties, including micro-topography, chemistry and wettability, are important factors affecting the quality of bone healing by influencing the biological responses of bone-interfacing implants [1–6,9–14]. Thus, Ti implants with surface properties that combine optimal micro-roughness, superior wettability and potentially bioactive chemistry may be effective for achieving favorable implant bone healing. Micro-rough P-incorporated surfaces showed improved osseointegration in vivo [9], but it might be expected that P-incorporated oxide layers produced by various hydrothermal treatment conditions would have different surface properties, which may affect their osteoconductivity.

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Table 1
Primer sequences for polymerase chain reaction.

Target	Primer sequences
Runx2	Forward primer 5′–3′: CCAGAATGATGGTGTTGACG Reverse primer 5′–3′: GGTTGCAAGATCATGACTAGGG
ALP	Forward primer 5′–3′: CTTGACTGTGGTTACTGCTG Reverse primer 5′–3′: GAGCGTAATCTACCATGGAG
Osteopontin	Forward primer 5′–3′: TCAAGTCAGCTGGATGAACC Reverse primer 5′–3′: CTTGTCCTTGTGGCTGTGAA
Osteocalcin	Forward primer 5′–3′: TGCTTGTGACGAGGTATCAG Reverse primer 5′–3′: GTGACATCCATACTTGCAGG
GAPDH	Forward primer 5′–3′: GGCATTGCTCTCAATGACAA Reverse primer 5′–3′: TGTGAGGGAGATGCTCAGTG

GAPDH: glyceraldehydes-3-phosphate dehydrogenase.

Therefore, the aim of this study was to investigate the surface characteristics of Ti surfaces produced by varying the phosphoric acid concentrations for use in future biomedical applications. The surface in vitro osteoconductivity was evaluated by observing cell attachment, spreading, proliferation and osteoblastic gene expression using MC3T3-E1 pre-osteoblast cells, and in vivo implant integration was biomechanically evaluated by comparing removal torque forces in rabbit tibiae.

2. Materials and methods

2.1. Sample preparation

Disks made from commercially pure Ti (ASTM grade 3) rods, 14 mm in diameter and 2 mm thick, were used to characterize

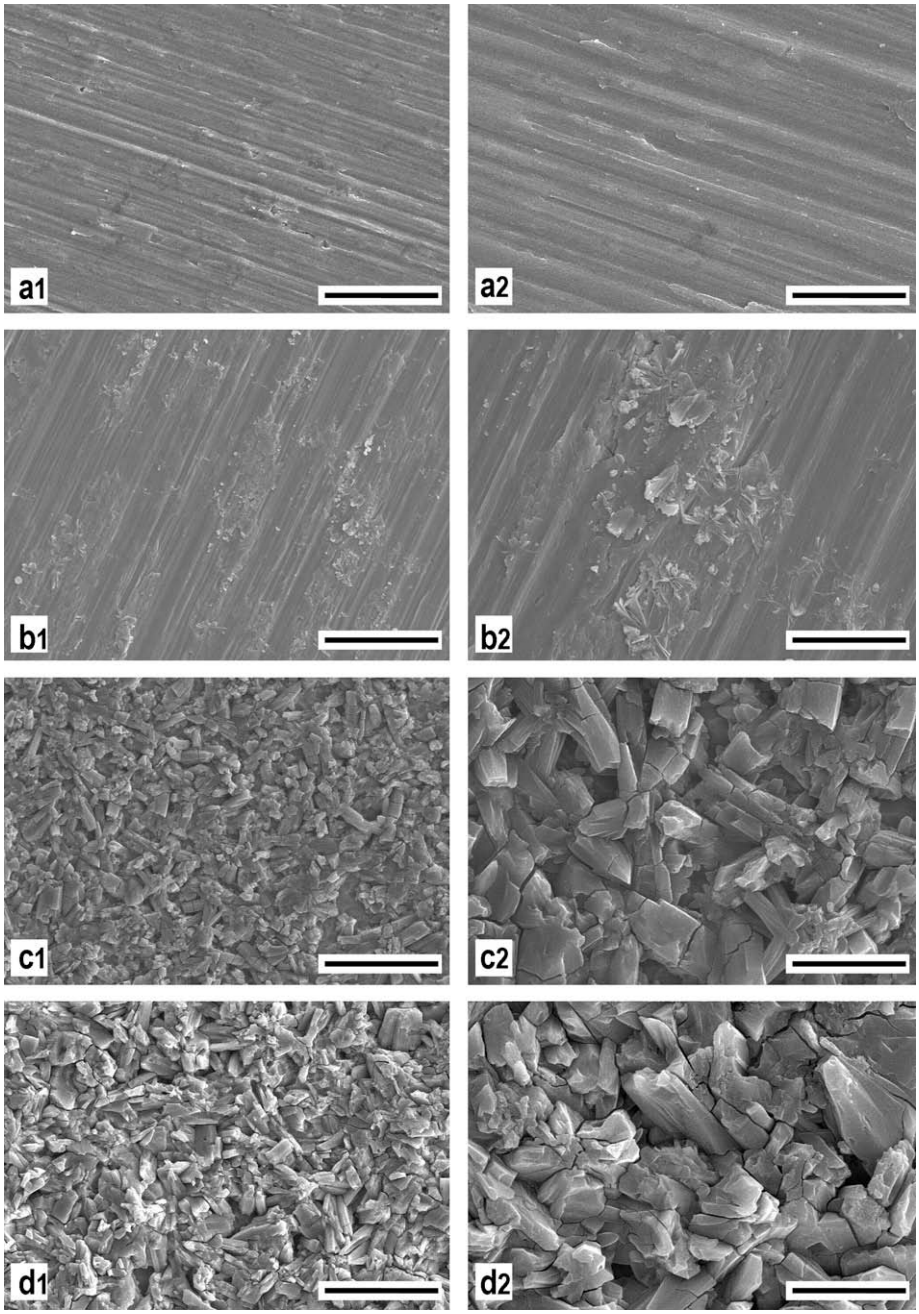

Fig. 1. Scanning electron microscope images of different samples. (a1 and a2) machined, (b1 and b2) TiP-1, (c1 and c2) TiP-2 and (d1 and d2) TiP-3 surfaces at magnifications of 1000× (a1–d1) and 3000× (a2–d2). SEM images show different surface morphologies of investigated samples. Scale bars = 30 μm (a1–d1) and 10 μm (a2–d2).

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