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# Osteoconductivity of hydrophilic microstructured titanium implants with phosphate ion chemistry

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## Abstract

This study investigated the surface characteristics and bone response of titanium implants produced by hydrothermal treatment using $H_3PO_4$, and compared them with those of implants produced by commercial surface treatment methods – machining, acid etching, grit blasting, grit blasting/acid etching or spark anodization. The surface characteristics were evaluated by scanning electron microscopy, thin-film X-ray diffractometry, X-ray photoelectron spectroscopy, contact angle measurement and stylus profilometry. The osteoconductivity of experimental implants was evaluated by removal torque testing and histomorphometric analysis after 6 weeks of implantation in rabbit tibiae. Hydrothermal treatment with $H_3PO_4$ and subsequent heat treatment produced a crystalline phosphate ion-incorporated oxide (titanium oxide phosphate hydrate, $Ti_2O(PO_4)_2(H_2O)_2$; TiP) surface approximately 5 μm in thickness, which had needle-like surface microstructures and superior wettability compared with the control surfaces. Significant increases in removal torque forces and bone-to-implant contact values were observed for TiP implants compared with those of the control implants ($p < 0.001$). After thorough cleaning of the implants removed during the removal torque testing, a considerable quantity of attached bone was observed on the surfaces of the TiP implants.


*Keywords:* Titanium implant; Wettability; Phosphate ion chemistry; Osseointegration; Histomorphometry

## 1. Introduction

Titanium (Ti) is widely used as an implant material in dentistry and orthopedics because of its biocompatibility and corrosion resistance. The long-term success rates of Ti implants have been well documented [1,2]. However, relatively high failure rates occur when the recipient bone is of poor quality [3,4]. Various means of improving the clinical performance of Ti implants in poor-quality bone and of shortening healing periods have been assessed. The surface properties of Ti implants (topography, chemistry and wettability) influence biological responses at the interface between the bone tissue and the implant and, consequently, their osseointegration [5–10]. Microrough surfaces produced by grit blasting and/or acid etching promote osteoblast differentiation and enhance bone apposition on Ti implants [6,9].

Microarc oxidation (spark anodization), a recently introduced surface treatment method, produces a porous surface oxide structure with a bioactive composition. Ti implants prepared by this method showed rapid and strong bone integration, indicating their potential biochemical bonding behavior [11,12]. Hydrothermal treatment is also an effective surface treatment method used to provide Ti implants with bioactive surface composition. Our recent studies have demonstrated that incorporation of calcium ions into a Ti oxide layer produced by a hydrothermal treatment improves the

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osseointegration of implants, stimulates osteoblast differentiation in vitro, and enhances bone apposition and removal torque in vivo [8,13,14]. In addition to calcium ions, phosphate ions are an important component of bioactive hydroxyapatite. However, there are few studies on the effects of incorporation of phosphate ions into Ti implants. Several in vitro studies have demonstrated that coating Ti surfaces with phosphorus or phosphate using the self-assembly monolayer technique or ion implantation accelerated apatite deposition on their surfaces [15–17]. The bioactivity of such surfaces may promote bone healing. Sul [11] reported increased bone-implant contact with phosphorus ion-incorporated Ti implants prepared by microarc oxidation compared to machined implants in rabbit tibiae. To our knowledge, few studies have reported the effects of a crystalline phosphate ion-incorporated Ti oxide layer with microrough surface topography on bone healing around Ti implants. As surface topography and composition both affect cell responses to Ti implants, a surface in which microrough surface topography is combined with phosphate ion chemistry may enhance osseointegration.

Therefore, we investigated whether hydrothermal treatment using $H_3PO_4$ would produce a suitable surface layer combined with a phosphate ion-incorporated oxide structure possibly favorable for enhancing bone healing around Ti implants. Furthermore, we compared the osteoconductivity of the resultant surface and compared it with that of Ti implants with various surface structures produced by commercial surface treatment methods – machining, acid etching, grit blasting, grit blasting/acid etching and spark anodization – by removal torque testing and histomorphometric analysis in rabbit tibiae.

## 2. Materials and methods

### 2.1. Sample preparation

Disks made from commercially pure Ti (ASTM grade 3) rods, 12 mm in diameter and 2 mm thick, were used to characterize the surface properties. To prepare Ti disks with a surface structure similar to that of the machined surfaces of commercial implants, Ti disks were wet-abraded to 1200 grit SiC abrasive paper and cleaned successively in acetone, alcohol and deionized water (machined surface). To produce the phosphate ion-incorporated Ti oxide surface (TiP surface), the disks were then treated hydrothermally in 2 wt.% $H_3PO_4$ solution at 180 °C for 6 h in a Teflon-lined reactor, followed by heat treatment at 400 °C for 12 h to eliminate incorporated hydrogen [18]. Five different microstructured Ti surfaces were used as controls: (i) machined surface; (ii) acid-etched with $H_2SO_4$/HCl (etched surface); (iii) grit-blasted with resorbable blast media (RBM; 100 μm sized-hydroxyapatite particles) followed by cleaning in nitric acid (RBM surface); (iv) grit-blasted with RBM and acid-etched with $H_2SO_4$/HCl (BE surface); and (v) spark anodized at up to 340 V in an electrolyte mixture containing 0.02 M α-glycerophosphate disodium salt and 0.2 M calcium acetate (anodized surface). For the animal study, screw-type implants ($n = 126$) with an external diameter of 3.3 mm and a length of 5.3 mm were prepared from commercially pure Ti (ASTM grade 3) rods. The screw implants were turned and then further treated by the same methods used to produce the Ti disks. Before the animal experiments, all implants were sterilized by γ-irradiation.

### 2.2. Surface characterization

The surface morphologies of the Ti disks and screw implants were observed by scanning electron microscopy (SEM; S-4300, Hitachi, Tokyo, Japan). The crystalline structure and chemical composition of the $TiO_2$ layer were evaluated by thin-film X-ray diffractometry (XRD; X'Pert-APD, Philips, Almelo, Netherlands) and X-ray photoelectron spectroscopy (XPS; Quantera SXM, ULVAC-PHI, Tokyo, Japan). Surface roughness measurements of the Ti disks were made with stylus profilometry (Form Talysurf Series 2, Taylor Hobson, London, UK); three samples from each group were measured, and two measurements were performed on each sample to evaluate the average surface roughness ($R_a$) values. The thickness of the phosphate ion-incorporated Ti oxide layer was evaluated by SEM measurement using cross-sectioned samples embedded in resin blocks to protect the surface layer. Surface wettability was determined by measuring the contact angles with one drop (0.5 μl) of deionized water using an automatic contact angle meter (Phoenix 300, Surface Electro Optics, Seoul, Korea); six samples from each group were measured, and two measurements were performed on each sample to evaluate the average contact angle.

### 2.3. Animals and surgical procedure

Twenty-one adult male New Zealand White rabbits weighing 3.5–4 kg were used in this study. This experiment was approved by the Institutional Animal Care and Use Committee of Kyungpook National University Hospital, Daegu, Korea. Korean national regulations (equivalent to NIH guidelines; NIH Publication No. 85-23 Rev. 1985) for the care and use of laboratory animals were observed. General anesthesia was induced by intramuscular injection of a combination of 1.3 ml of ketamine (100 mg $ml^{-1}$; Ketara, Yuhan, Seoul, Korea) and 0.2 ml of xylazine (7 mg $kg^{-1}$ body wt.; Rompun, Bayer Korea, Seoul, Korea). The medial surfaces of the proximal tibiae were used as the surgical sites. The surgical areas were shaved and the skin was washed with a mixture of iodine and 70% ethanol before surgical draping. Local anesthesia was induced with 1 ml of 2% lidocaine (1:100,000 epinephrine; Yuhan) to control bleeding and to provide additional local anesthesia. The surgical sites were exposed with an incision through the skin, fascia and periosteum at the medial surface of the proximal tibiae using a sterile surgical technique. Numerous animal studies have used the rabbit tibial model to evaluate the osteoconductivity of implants,