Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 27 (2006) 926–936
Biomaterials
www.elsevier.com/locate/biomaterials

Fluoride modification effects on osteoblast behavior and bone formation at TiO_2 grit-blasted c.p. titanium endosseous implants

Lyndon F. Cooper[a,*], Yongsheng Zhou[b], Jun Takebe[a], Juanli Guo[c], Armin Abron[d], Anders Holmén[e], Jan Eirik Ellingsen[f]

[a]*Bone Biology and Implant Therapy Laboratory, Department of Prosthodontics, University of North Carolina at Chapel Hill, 404 Brauer Hall, CB #7450, Chapel Hill, NC 27511, USA*
[b]*Peking University, School and Hospital of Stomatology, Beijing, China*
[c]*Oral Biology, Universtiy of North Carolina at Chapel Hill, Chapel Hill, NC, USA*
[d]*The Dental Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*
[e]*Astra Tech AB, Molndal, Sweden*
[f]*Oslo University, Oslo, Norway*

Received 8 June 2005; accepted 1 July 2005
Available online 19 August 2005

Abstract

Increasing bone formation at endosseous titanium implants may be achieved by modification of topographically enhanced surfaces. The aim of this study was to determine the effect of fluoride ion modification of TiO_2 grit -blasted, c.p. titanium implants on osteoblastic differentiation and interfacial bone formation by parallel in vitro and in vivo investigations. Human mesenchymal stem cells (Osiris Therapeutics, Inc.) were cultured on TiO_2 grit -blasted c.p.titanium disks with and without fluoride ion modification. Cell adhesion, proliferation, and osteoblastic gene expression was measured by scanning electron microscopy, tritiated-thymidine uptake into insoluble DNA, and reverse transcription polymerase chain reaction detection of mRNAs encoding collagen 1, osteopontin, bone sialoprotein, osteocalcin and BMP-2. After 24 h, there were no differences in cell adhesion among the surfaces tested. Fluoride-treated surfaces supported greater proliferation and increased bone sialoprotein and BMP-2 expression. Additionally, 12 TiO_2 grit-blasted and 12 fluoride ion modified implants were placed randomly into medial and distal osteotomies prepared in the tibia of 300 g Sprague Dawley rats. After 21 days, the tibiae were harvested and 100 μm ground sections were examined by backscatter scanning electron microscopy. The bone-to-implant contact formed at TiO_2 grit-blasted and fluoride-treated versus TiO_2 grit -blasted surfaces was 55.45% versus 34.21% ($p<0.027$), respectively. Fluoride ion modification of the TiO_2 grit -blasted surface enhanced osteoblastic differentiation in vitro and interfacial bone formation in vivo. This parallel in vitro and in vivo investigation demonstrates that fluoride ion modification enhanced osteoblastic differentiation and interfacial bone formation. The mechanism(s) by which fluoride ion modification of c.p.titanium enhanced osteoblastic differentiation and osseointegration merit careful investigation.

Keywords: Osseointegration; Surface modification; Cell culture; Titanium; Endosseous implant; Stem cell

1. Introduction

The current, expanding use of endosseous dental implants is based upon the acceptance of well-defined success criteria and a significant body of clinical data that has proven the concept of osseointegration to support dental prostheses [1,2]. Osseointegration is defined at histological and clinical levels [3]. Neither definition provides an absolute value for the amount of bone that is necessary to support a functioning implant prosthesis. Nonetheless, it is largely assumed that optimization of bone volume supporting individual

*Corresponding author. Tel.: +1 919 966 4579; fax: +1 919 966 3821.
E-mail address: lyndon_cooper@dentistry.unc.edu (L.F. Cooper).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.07.009

implants by treatment planning, technique and bone augmentation procedures is a key to proper clinical care [4]. Further efforts to ensure osseointegrated implant success are illustrated by attempts to increase the bone formed at endosseous implants, irrespective of host bone volume. Efforts since the late 1980s also focused on the osteoconductive nature of hydroxyapatite coatings [5]. Most efforts have recently come through the topographic alteration of the implant surface of c.p. titanium implants per se [6,7]. In fact, a majority of implant manufacturers provide some surface topographic alternative to the original-machined c.p.titanium implant. Available data from implant systems using a grit-blasted c.p. titanium surface with 10 years of clinical experience [8–10] and data concerning more recent blasted and etched [11] or dual-etched c.p.titanium surfaces [12] indicate that modification of the machined c.p.titanium surface does not negatively influence the clinical success of c.p.titanium dental implants.

Pre-clinical information concerning grit-blasted, blasted and etched, and dual-etched c.p.titanium implants indicate that the extent of bone formation at these topographically modified surfaces is greater than at machined c.p. titanium implants [13–15]. Numerous studies from several independent laboratories have confirmed this advantage. Associated with the increased bone formed at the modified c.p.titanium implant is the enhanced biomechanical interlocking of the implant with bone when measured using a common reverse torque method [16–18]. Beyond these qualitative conclusions, a temporal argument that bone formation may be modestly accelerated at topographically modified c.p.titanium implants is further suggested. Evidence from animal models and recently from clinical biopsies indicates that the bone-to-implant contact formed at a given time is greater at topographically modified c.p.titanium implants [19,20]. Although a longitudinal analysis has not been presented, the current data is interpreted to reflect more rapid bone formation at the topographically modified surface. This may be the most reasonable interpretation, since high (>85%) bone-to-implant contact areas have been reported from retrieval studies concerning human dental implants. Thus, topographically modified c.p. titanium implants may offer a temporal advantage in formation of the implant–bone interface. Given the current strong interest in rapid and immediate loading of dental implant prostheses, the role of implant surface topography is elevated in significance.

Both the temporal limits and volumetric extent of bone formation at endosseous implants are issues that are directly related to cellular or physiological limitations of the host. Yet, changes in implant surface chemistry and topography have increased bone formed at implants in a limited time period. Existing data indicates that c.p.titanium surfaces, irrespective of surface topography, consistently supported less bone formation than at hydroxyapatite-coated c.p.titanium implants [13]. Thus, beyond surface topography, surface chemistry may provide important and possibly synergistic cues for bone formation at dental implants. In fact, recent investigation implicates surface chemistry changes in many surface topographic modifications [21].

The significance of surface chemistry is illustrated by the specific cellular responses to different Ti alloys [22], to different grades of c.p.titanium [23], and to different bulk metals [24,25]. Some of the newer approaches to changing the reactivity of the c.p.titanium endosseous implant surface include direct chemical modification of titanium surfaces by treatment in simulated body fluid [26], covalent attachment of biological molecules [27], changes in the surface ion content [28], glow discharge [29] or alkali treatment [30]; to wholesale chemical modification of the titanium [31], or biological grafting with adhesive polymers [32] that can serve to biologically activate the implant surface; as well as alteration of surface hydrophobicity [33]. These different studies indicate that culture cell responses are affected by both marked as well as modest changes in the surface chemistry of the c.p.titanium substrate chemistry.

If two assumptions are made concerning implant surface topography; namely (1) that surface topography contributes important environmental cues for enhanced bone formation and (2) that surface topography has a key role in determining the subsequent biomechanical behavior of the implant bone interface, then the chemical modification of a topographically enhanced c.p.titanium implant could provide surface-directed signals to further positively influence bone formation. The aim of the present study was to determine the effect of fluoride ion modificaton of TiO_2 grit-blasted c.p.titanium implants on osteogenesis as measured in a cell culture assays and the degree of interfacial bone formation following endosseous implant placement in vivo. Fluoride ion treatment of TiO_2 grit-blasted titanium substrates enhances adherent osteoblastic differentiation of human mesenchymal stem cells and significantly increases the bone-to-implant contact at TiO_2 grit-blasted c.p.titanium implants in the rat tibia model.

2. Materials and methods

2.1. Titanium disk preparation

Five- and 8-mm titanium disks were machined and TiO_2 grit-blasted (25 and 75 μm) and treated with hydrofluoric acid under different conditions resulting in reproducible surfaces of varying fluoride ion content for testing (Table 1). The implants were treated and cleaned in pure trichloretylene acid, 20 ml (Prolabo, Fontenay, France) for 15 min, washed in ethanol