Evaluation of a predictive model for implant surface topography effects on early osseointegration in the rat tibia model

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Statement of problem. Alterations in commercially pure titanium (cp Ti) implant surface topography can be made to increase bone formation or the interfacial shear strength of bone at the functioning implant. It is not known whether these 2 goals are congruent or mutually exclusive.

Purpose. The aim of this study was to determine the effect of implant surface topography parameters of calculated biomechanical significance on the process of bone formation in a rat tibia model of osseointegration.

Material and methods. Implants (cp Ti grade IV) were machined and subsequently treated by grit blasting or grit blasting and 6.4 mol/L HCl. Measurements of surface roughness were made by atomic force microscopic analysis of similarly treated titanium disks. Cleaned and sterilized implants (12 machined, 12 with nonideal pit morphology, 12 with ideal pit morphology) were placed into the tibiae of 400-g male Wistar rats by using a series of drills, irrigation, and a self-tapping procedure. After 3 weeks, tibiae were harvested and processed and embedded in methyl methacrylate resin. Polished sections were examined by backscatter electron microscopy, and the percentage implant surface contacting bone was measured with the Scionics PC image analysis program.

Results. The implants possessing a proposed ideal pit morphology supported significantly greater bone formation at the implant surface (54% ± 7% bone-to-implant contact [$P<.003$]) than the nonideal pit morphology (40% ± 15%) or machined surfaces (34% ± 6%).

Conclusion. Implant surfaces with a proposed ideal pit morphology (which possess a calculated biomechanical significance) enhanced bone formation at early periods after placement in the rat tibia model. (J Prosthet Dent 2001;85:40-6.)

CLINICAL IMPLICATIONS

There exists a congruence of surface topography influences on the biologic determinants of bone formation and on the biomechanical determinants of implant function. Additional steps to increase the percentage of bone contact at implants in low-density (type IV) bone may take advantage of both topographic effects.

The formation of bone at commercially pure titanium (cp Ti) implants is a prerequisite process for successful osseointegration.[1] Some investigations have indicated that osteogenesis occurs at cp Ti in the absence of inflammation shortly after surgical placement in bone.[2,3] Current therapeutic goals, which include more rapid healing of implants and more extensive osteogenesis at implants in bone of low density, can be met using biologic and alloplastic approaches to increasing osteogenesis, osteoconduction, or osteoinduction.[4]

The role of the implant surface in the determination of osseointegration has been recognized for many years. In the 1990s, several investigators pursued detailed investigations of the effect of implant surface topography on the extent of bone formation. Buser et al[5] demonstrated a broad spectrum of possible responses to different surfaces using titanium cylinders in a minipig model. The result indicated that titanium could be modified such that the bone-to-implant contact could rival that of plasma-sprayed hydroxyapatite (a standard for osteoconductive behavior). In subsequent studies, several different approaches to

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enhancing the surface roughness of cp Ti were shown to increase bone-to-implant contact.[6-8] In a series of publications, Wennerberg et al[9-12] demonstrated in the rabbit model that grit blasting with different sizes of TiO_2 or Al_2O_3 particles altered the cp Ti topography and resulted in a similar enhancement of bone formation at the implant. These studies also demonstrated that specific surface modifications increased the biomechanical interlock of the implant with bone when measured with a torque device. Explanations for increased bone-to-implant contact vary, and proof of one or another surface-variable–specific mechanism remains elusive.

Consideration of precisely how the bone-to-implant interface biomechanically interlocks with bone was the subject of a theoretical analysis presented by Hansson and Norton.[13] In this study, the interfacial shear strength of bone at the implant was modeled in terms of the interlocking of formed bone within surface irregularities. Alteration of cp Ti surfaces achieved by grit blasting, acid etching, or combinations thereof results in the formation of pits in which formed bone eventually resides. Hansson and Norton's thesis used finite elemental analysis to successfully define the ideal pit morphology, pit dimension, and pit density (which intuitively is linked to pit dimension) for providing maximal biomechanical interlocking of formed bone with the implant. Hansson and Norton calculated the effectiveness of different shapes, sizes, and densities of pits to resist shear fracture of ingrown bone. A hemispheric pit approximately 1 to 5 µm in diameter and 1 to 5 µm in depth (not the same as average surface roughness) represented the ideal topographic element to resist shear force at the bone-implant interface. This dimension is represented among the spectrum of implant surface topographies available for clinical use. In fact, one available surface is based on this theoretical ideal, and its manufacture is directed toward establishing an ideal pit density and pit morphology. Several studies have shown the successful osseointegration of this altered cp Ti surface; they indicate that greater bone-to-implant contact is achieved and that biomechanical interlocking of bone occurs after healing.[14,15]

Surface topography effects may directly mediate changes in cell behavior.[16] What is the relationship between surfaces designed for ideal biomechanical interlocking with formed bone and the degree of osteogenesis that is possible? It was the aim of this study to compare the degree of bone formation that occurs at a surface that fulfills the Hansson and Norton theory (ideal pit density and pit morphology) with a nonideal surface (low pit density) and with a machined cp Ti implant. The rat tibia model of osseointegration was selected,[17-20] and the result of osseointegration was evaluated 3 weeks after implant placement.

MATERIAL AND METHODS

Implants were machined from cp Ti wire (Tico, Farmington Hills, Mich.) at the University of North Carolina Physics Machine Shop. Implants were manufactured as cp Ti screws 1.5 mm in diameter and 2.0 mm in length. Subsequently, implants were washed by ultrasonication in ethyl methyl ketone and then in 100% ethanol. Some implants were air-abraded with 50-µm–grit aluminum oxide abrasive at 10 psi for 30 seconds. Other implants were air-abraded with 50-µm–grit aluminum oxide abrasive at 20 psi for 60 seconds and, after ultrasonic cleaning in deionized distilled water, and subsequently etched in 6.4 mol/L HCl for 22 to 24 hours at room temperature. All implants were washed again by ultrasonication in distilled water and treated first in ethyl methyl ketone and then in 100% ethanol. Implants were sterilized from the 100% ethanol solution by treatment under ultraviolet light in a laminar flow hood.

To evaluate the surfaces of the implants, SEM analysis of 2 implants per group was performed with a JOEL6300 microscope (Joel USA, Inc, Peabody, Mass.). Images were recorded at ×20 and ×200 magnification. To gain more detailed information by using atomic force microscopy, titanium wire was also cut into disks that were prepared in parallel with the machined screw implants. The disks were subjected to atomic force microscopic analysis (AFM; Auto Probe CP, Park Scientific Instruments, Sunnyvale, Calif.) to determine the average roughness (R_a), root-mean-square roughness (R_{rms}), maximum peak-to-valley distance ($R_{p\text{-}v}$), surface area (SA), and surface volume (V) of the titanium specimens.

The anatomic force microscope uses a noncontacting stylus to image the surface of the implant and create a digitized image from which numerous surface parameters can be calculated by using the following relationship:

$$R_a = \sum_{n=1}^{N} \frac{\left|Z_n - \overline{Z}\right|}{N}$$

where N is the number of z (height) coordinates for each scan (N = 65,536 for each scan). The maximum peak-to-valley distance is given by:

$$R_{p\text{-}v} = z_{max} - z_{min}$$

where z_{max} is the highest z coordinate and z_{min} is the lowest z coordinate. For each preparation, three 50 × 50 µm AFM scans were made in air with a Si_3N_4 tip in contact mode.

Wistar rats (350 g, Charles River Labs, Boston, Mass.) were housed for 7 days before initiation of the experiment. All animals were cared for at the Dental

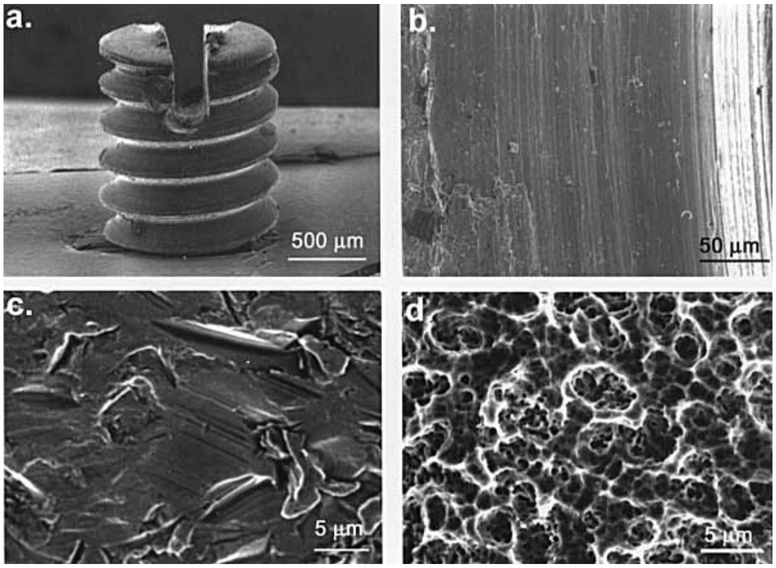

Fig. 1. SEM evaluation of prepared implants. **a,** Overview of implant morphology. Screw provided for self-tapping and assured primary stability. **b,** Machined implant surface represented by grooves arrayed in direction of machining. **c,** Nonideal surface bearing pits approximately 3 µm in diameter and 1.5-2 µm in depth that clearly were not densely arrayed on implant surface. **d,** "Ideal" surface bearing pits approximately 3 µm in diameter and 2 to 3 µm in depth that were maximally arrayed on implant surface.

Research Center vivarium (UNC Animal Protocol No. 94.434.0-C) in accordance with the rules and regulations of the Animal Welfare Act. Rats were anesthetized using pentobarbital sodium (Nembutal, 50 mg/mL, intramuscular). The tibiae were disinfected with Betadine and shaved, and a full-thickness incision was made on the dorsal aspect of the tibia. Implant sites were prepared by sequential drilling under cooled sterile saline irrigation using 0.4-, 0.5-, 1.0-, and 1.4-mm surgical stainless steel twist drills. The implants were threaded into the prepared sites, and intramedullary exposure of the implant with primary unicortical fixation was confirmed.[14] The sites were sutured closed. Recovery was demonstrated by mobilization of the hind limbs. At 21 days, animals were euthanized, and tibiae were obtained. For each of the 3 surfaces tested, 1 implant was placed 7 mm distal to the articular surface of each hind limb of a rat. Twelve implants of each type (total 36) were placed into 18 rats.

Tibiae were fixed in phosphate-buffered 3.7% paraformaldehyde for 24 hours. Fixed bones containing implants were dehydrated with a graded series of ethanol and embedded in methyl methacrylate resin. The embedded tibiae were sectioned through the implants with an Isomet tissue-grinding machine and a diamond disk (Buehler, Lake Bluff, Ill.). Resulting block sections were serially polished with diamond polishing pastes (Buehler) to 1 µm. Of the 12 sectioned implants in each group, 1 or 2 sections were not acceptable for further processing. The polished sections were gold sputter coated and imaged in the Joel SEM.

The extent of bone-to-implant contact was determined by linear measurement of only direct contact visualized on digitally captured backscatter SEM images made of the polished section. The sum of linear contact measures of bone at each implant was calculated, and the percentage of implant contacting bone was determined as follows:

$$100\% - \frac{\text{measured implant surface} - \text{sum linear contacts}}{100}$$

Each implant section (2 per implant) was measured twice, and the average value and standard deviation for each implant type were calculated. The Mann-Whitney *U* test was used for pairwise comparisons among the implant types.

RESULTS

The 3 surface preparations resulted in topographically and morphologically unique implant surfaces (Fig. 1). Note that the dimension of the pits created by blasting alone (Fig. 1, *c*) was similar to the dimension of those pits present on the surface that were subsequently treated by etching (Fig. 1, *d*). SEM evaluation of different aspects of several implants prepared by blasting indicated a similarity of pit density among all sites evaluated. The general distribution of pit diameter was qualitatively determined to be consistent and

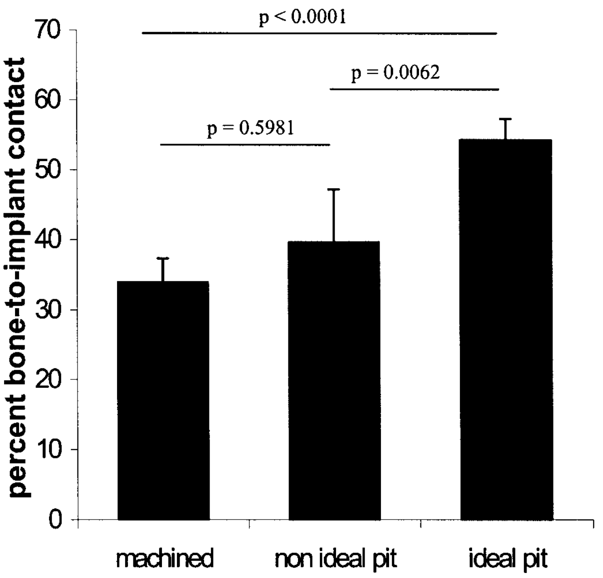

Fig. 2. Bone-to-implant contact at machined, nonideal pitted, and ideal pitted implant surfaces. Histogram indicates average bone-to-implant contact.

Table I. The following values were determined by anatomic force microscopy and are reported as the average (± SEM) of 3 separate scans (50 × 50 µm) of 3 individual disks

Surface	$R_{p\text{-}v}$	Surface area	R_a	V
Machined	1.48 µm (0.47)	2599 µm^2 (21.9)	0.142 µm (0.026)	2344.8 µm^3 (524.1)
Nonideal pit	4.406 µm (0.371)	3073.8 µm^2 (57.9)	0.582 µm (0.032)	6024.4 µm^3 (709.9)
Ideal pit	5.258 µm (0.554)	3528.6 µm^2 (277.3)	0.775 µm (0.058)	6216.4 µm^3 (509.6)

$R_{p\text{-}v}$ = Maximum peak-to-valley distance; R_a = average roughness; V = surface volume.

uniform along the implant and among implants. The titanium disks prepared in parallel were morphologically similar to their screw counterparts when viewed by SEM. AFM analysis of the disks provided measures of average surface roughness for comparison among the samples (Table I). Although the incompletely blasted surface represents a distinct topography from the blasted and etched surface, the average surface roughness (R_a) values and the average peak-to-valley distance ($R_{p\text{-}v}$) values were similar. These measures indicate that the magnitude of deviation from a mean surface plane or the average absolute magnitude of a surface element (depth of a pit) were not appreciably different for the 2 surfaces.

Surgical treatment and anesthesia resulted in the loss of 2 rats in the first 24 hours after implant placement. Subsequently, all rats tolerated implant placement well. On gross dissection, all implants were present in bone without apparent infection or inflammation and were immobile.

The direct bone-to-implant contact measured at machined implants was 33.9% ± 6.1% (Fig. 2). For implants with a topography represented by pits of low density (nonideal), the bone-to-implant contact was 39.7% ± 14.8%. This was not different from the machined surface (P=.5981). At implants with topography composed of densely arrayed pits (ideal), 54.3% ± 6.8% direct bone-to-implant contact was measured. This differed significantly from both the nonideal pit surface (P=.0062) and the machined surface (P=.0001). SEM images revealed sites of formed bone randomly distributed across the machined implant (Fig. 3). For the implants with nonideal pit density (grit blasted), bone formation was similar in extent of bone-to-implant contact. However, a bridging osteogenesis occurring from 1 contact site to another (Fig. 4) was observed in the grit-blasted surface implants (8 of 10). This newly formed bone was not included in measures of bone-to-implant contact or calculations of bone-to-implant contact. Direct bone-to-implant contact was seen against the majority of the implant surfaces with ideal pit density (Fig. 5).

DISCUSSION

This study confirms many previous reports that alterations in cp Ti implant topography alter the

Fig. 3. Representative backscatter SEM of bone formed at machined implant (original magnification, ×200).

Fig. 5. Representative backscatter SEM of bone formed at implant with ideal pit topography (original magnification, ×200). Note confluence of bone formation against implant surface.

Fig. 4. Representative backscatter SEM of bone formed at implant with nonideal pit topography (original magnification, ×200). Note bridging osteogenesis that occurred at distance from surface (not included in calculations).

degree of bone formed at the implant surface. A "rougher" implant surface is associated with increased bone-to-implant contact. This is particularly true at early time points.[6,7,14] In this study, 2 surfaces with very similar roughness parameters (nonideal vs ideal) showed differing abilities to support bone formation. Two surfaces with very different roughness (R_a and $R_{p\text{-}v}$) parameters (nonideal vs machined) did not show differing abilities to support bone formation. These results indicate the gross inadequacy of defining an implant in the general terms of "rough" or "smooth."

Unlike other studies of implant topography that used large animal models, this study used the rat tibia model of osseointegration. The rat model has been used in other studies of osseointegration, and similar degrees of osseointegration have been reported.[17-20] This model offers physiologically shorter bone healing periods and the ability to use a large number of animals, and it can be readily manipulated to invoke physiologic changes in the animal by surgical or pharmacologic intervention. Although there may be important concerns regarding the similarity of this model to large animal models in the context of bone remodeling events, a restricted interest in bone formation at early periods limits these concerns.

The rat tibia model allowed for unicortical fixation of implants, with a large portion of each implant exposed to the medullary space of the tibia. In this manner, the model revealed trabecular or woven bone formation at the implant in this space. Unlike larger animal models in which a relatively large portion of the implant may oppose cortical bone at placement, this model can efficiently display surface effects on osteogenesis. The measured effect of surface topography on the extent of bone-implant contact formed early after implant placement may be relevant to a clinical situation in which limited cortical bone is available for primary stability.

The main goal of this project was to determine whether a surface that conformed to a predicted topography for optimal biomechanical interlock with bone would enhance bone formation. The 2 surfaces created with pits of similar roughness parameters (Table I), but differing density showed marked differences in bone formation. Parallel biomechanical testing remains to be performed in this rat model. Surface chemical differences between the grit-blasted and subsequently etched surfaces existed; aluminum residue from the blasting procedure was effectively removed by the etching procedure. Direct comparison of aluminum-oxide–blasted and titanium-oxide–blasted implants indicated no differences in bone formation at these surfaces of comparable topography.[10-12] When machined implants were compared with aluminum-oxide–blasted, titanium-oxide–blasted, or SLA-treated implants, all investigations reported increased biomechanical interlocking of the implant in formed bone.[6,9-12]

It is possible to modify cp Ti to create distinct topographies that can be characterized to varying degrees. It is unfortunate that there exists no singular, accepted method of describing the surface topography of an implant. However, the Hansson and Norton hypothesis[13] argues that it is possible to describe surface topography in terms of topographic elements of biomechanical significance. These elements are described as pits of varying dimension and presence in a spectrum of densities along the surface. The function of each pit is to retain bone of defined shear strength and to resist shear forces at the interface. This description is the result of a mathematical modeling approach to understanding the effect of surface topography on the interfacial shear strength of bone at an implant surface. This study indicates that topographic features conforming to a predicted ideal topography also impart biologic effects (bone formation) at the altered titanium surface.

There exist several potential mechanisms by which the implant surface may have biologic effects that promote bone formation.[4,16] We speculate that there exists an ideal topography for supporting the biomechanical interaction of implants; further engineering efforts may result in the convergence of that topography with the ideal biologic surface for osteogenesis, osteoconduction, and osteoinduction.

CONCLUSIONS

In this rat tibia model, the bone-to-implant contact at a cp Ti implant surface that approximated an ideal biomechanical surface topography (predicted by the Hansson and Norton hypothesis) was significantly greater than: (a) the contact formed at a surface composed of similar topographic elements that did not conform to the theoretical ideal, and (b) the contact formed at a machined cp Ti implant. Additional effort is required to define the convergence of the ideal biomechanical and biologic properties of an endosseous implant surface.

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Noteworthy Abstracts of the Current Literature

Survey of dentists' knowledge and opinions about oral pharyngeal cancer

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Purpose. The purposes of this study were to determine general practitioners' knowledge of oral cancer risks and available diagnostic procedures for detection of oral cancer, to correlate the dentists' background characteristics and knowledge about oral cancer, and to describe the dentists' interest in continuing education (CE) courses about oral cancer.

Material and methods. A pretested, 34-item questionnaire was mailed to 7000 randomly selected general dentists in the United States. Of those returned, 3200 questionnaires were usable. Eighty-six percent of the dentists who returned usable questionnaires were men, 68% were solo practitioners, 12% were partners in a practice, and 14% were employees or independent contractors. Fifty percent of the 3200 respondents graduated from dental school between 1980 and 1995. Analyses of the answers were completed by using unweighted data. The extent of correct responses to the indicated items on oral cancer risk and diagnostic procedures was determined. Next, the overall set of selected background and practice characteristics was assessed in terms of the respondents' likelihood of achieving a high score on the following 3 indexes: oral cancer risk, diagnostic procedures, and a combination of risk and diagnostic procedures. The relationship between the practitioners' levels of oral cancer knowledge and their opinion on whether their knowledge of oral cancer was current was explored. Finally, a determination was made regarding the dentists' interest in oral cancer CE courses. Bivariate and logistic analytical techniques were used to evaluate the data (α=.01).

Results. On average, the respondents correctly answered 8 of 14 questions on oral cancer risks and 6 of 9 questions on oral cancer diagnostic procedures. Female practitioners were found to be 1.3 to 1.6 times more likely than male dentists to receive a high score on each of the 2 indexes; however, these findings were explained by other factors, particularly graduation year. Dental employees or independent contractors were found to be more likely than those in solo or partnership practices to receive high scores on the index of oral cancer risk and diagnostic procedures combined. Dentists who had not participated in an oral cancer CE course were 2 times less likely to receive a high score on the risk and diagnostic procedures indexes than were dentists who had participated in a CE course within a year of completing the survey. Eight-two percent of the respondents expressed interest in future oral cancer CE courses.

Conclusion. The results of this study suggest that general practitioners are not as knowledgeable as they could be about preventing oral cancer and early detection of oral cancer; they do, however, seem to recognize these deficiencies. High interest in oral cancer CE courses exists among general dentists. 15 References. —*DL Dixon*