HIGHLIGHTED TOPIC | Biomechanics and Mechanotransduction in Cells and Tissues

Modulation of bone ingrowth of rabbit femur titanium implants by in vivo axial micromechanical loading

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Submitted 28 September 2004; accepted in final form 22 December 2004

Clark, Paul A., Anthony Rodriguez, D. Rick Sumner, Mohammad A. Hussain, and Jeremy J. Mao. Modulation of bone ingrowth of rabbit femur titanium implants by in vivo axial micromechanical loading. J Appl Physiol 98: 1922–1929, 2005. First published January 7, 2005; doi:10.1152/japplphysiol.01080.2004.—Titanium implants commonly used in orthopedics and dentistry integrate into host bone by a complex and coordinated process. Despite increasingly well illustrated molecular healing processes, mechanical modulation of implant bone ingrowth is poorly understood. The objective of the present study was to determine whether micromechanical forces applied axially to titanium implants modulate bone ingrowth surrounding intraosseous titanium implants. We hypothesized that small doses of micromechanical forces delivered daily to the bone-implant interface enhance implant bone ingrowth. Small titanium implants were placed transcortically in the lateral aspect of the proximal femur in 15 New Zealand White rabbits under general anesthesia and allowed to integrate with the surrounding bone for 6 wk. Micromechanical forces at 200 mN and 1 Hz were delivered axially to the right femur implants for 10 min/day over 12 consecutive days, whereas the left femur implants served as controls. The average bone volume 1 mm from mechanically loaded implants (n = 15) was 73 ± 12%, which was significantly greater than the average bone volume (52 ± 21%) of the contralateral controls (n = 15) (P < 0.01). The average number of osteoblast-like cells per endocortical bone surface was 55 ± 8 cells/mm² for mechanically loaded implants, which was significantly greater than the contralateral controls (35 ± 6 cells/mm²) (P < 0.01). Dynamic histomorphometry showed a significant increase in mineral apposition rate and bone-formation rate of mechanically stressed implants (3.8 ± 1.2 μm/day and 2.4 ± 1.0 μm²·mm⁻²·day⁻¹, respectively) than contralateral controls (2.2 ± 0.92 μm/day and 1.2 ± 0.60 μm²·mm⁻²·day⁻¹, respectively; P < 0.01). Collectively, these data suggest that micromechanical forces delivered axially to intraosseous titanium implants may have anabolic effects on implant bone ingrowth.

Orthopedics; mechanobiology; mechanotransduction; bone histomorphometry

Metallic implants are used in orthopedic medicine and dentistry to replace defective skeletal structures such as bone and teeth. Because of its high strength and acceptable biocompatibility (36, 48), titanium has become the universal material for intraosseous implants and has demonstrated a remarkable capacity for integration with host bone (1, 30). Titanium integrates into host bone via two processes, bone ingrowth in which bone forms within the irregular surfaces of the titanium (30) followed by osseointegration, defined as “direct contact between living bone and an implant on the light microscope level” (6). The time required for implant integration in humans varies from an average of 3 mo for orthopaedic implants (31) and an average of 4–6 wk for dental implants (1). In animal models, it has been described to take a shorter time, ~4–6 wk (8, 18, 62). It is desirable to accelerate the bone ingrowth process of orthopedic and dental implants for the clinical benefit of recovery of skeletal and dental function.

Implant bone ingrowth is a complex process that begins with surgical trauma and inflammatory responses (35, 46, 30). The process of bone healing has been shown to follow similar patterns in both orthopedic and dental applications (14). Immediately after surgical trauma and implant insertion, the bone-implant interface fills with blood clots. Proteins from blood and tissue fluid then adsorb onto the implant surface, including bone cell adhesion molecules such as bone sialoprotein, osteopontin, and osteocalcin (4). Osteoblasts attach to the implant surface in a process mediated by cell adhesion molecules and begin the bone apposition process (7, 30, 35, 46).

Despite our increasingly improved understanding of molecular mediation of the implant bone ingrowth process, little is known about how mechanical forces modulate implant bone ingrowth. This is of surprise for two reasons. First, orthopedic and dental implants are both load-bearing structures and have substantially different material properties from surrounding bone (36, 48). Second, cyclic mechanical forces have repeatedly been shown to modulate the development and regeneration of bone in both appendicular and craniofacial skeletal lineages (11, 13, 33, 38, 49, 51, 53, 65, 66). In vivo cyclic loading has been shown to induce anabolic bone growth in both appendicular and craniofacial bones (13, 33, 52). In vivo, biochemical and biomechanical factors likely coregulate implant bone apposition and resorption (63). However, common intuition suggests that intraosseous implants should not be subjected to mechanical forces during the initial healing phase. This intuition is supported by several experimental studies showing micromotion caused by “excessive” mechanical forces applied too early in the osseointegration process leading to fibrous encapsulation and subsequent implant loosening (7, 9, 45, 59). It has been suggested that early micromotion leads to differentiation of cells at the bone-implant interface into fibroblasts that produce a fibrous capsule around the implant instead of the desired bone cells (61). The amount of micro-

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motion tolerated by healing tissue seems to be controlled by a strain threshold of ~100 µm for a bioinert surface (7). However, little is known about whether axial micromechanical forces applied to implants on completion of initial healing would have a metabolic effect on implant bone ingrowth.

The present study was designed to determine whether micromechanical forces applied axially to titanium implants modulate implant bone ingrowth processes. After a 6-wk healing period, micromechanical forces at 200 mN and 1 Hz were delivered axially to transcutaneously placed titanium implants in the right proximal femur for 10 min/day over 12 consecutive days, whereas the left femoral implants served as controls. The mechanically loaded implants showed significantly greater bone volume, endocortical osteoblast-like cells per bone surface, mineral apposition rate (MAR), and bone-formation rate (BFR/BS) than the contralateral control femur implants. These data appear to suggest that micromechanical forces delivered axially via intraosseous titanium implants may have anabolic effects on implant bone ingrowth.

MATERIALS AND METHODS

Animal model and titanium implants. A total of fifteen 9-wk-old male New Zealand White rabbits were used. Titanium implants were custom-made from commercially pure titanium. The implants had a machined-smooth surface with a screw-type geometric design (5 × 4.1 mm: length × diameter). The threaded area of the implant consisted of a core of 3.5-mm diameter with threads of 0.3-mm height and pitch 0.6 threads/mm. The implants were unicortical in design and contained a 3-mm abutment that remained nonsubmerged when implanted (Fig. 1, A and B). All experiments on animals were performed with the approval of the institutional animal care committee.

Surface roughness of titanium implants. Surface roughness of the titanium implants was quantitatively measured on the macrosopic scale using a noncontact optical profilometer (Wyko NT 3300 Surface Optical Profilometer) in white-light, vertical-scanning interferometry mode, which allowed a maximum height of 1.5 mm, resolution of 3 nm, and scans at an ~1-mm² area. Implants were cleaned using ultrasonication in chloroform to remove organic debris and rinsed with double-distilled, nonionized water, followed by air dry. The cleaned implants were mounted and stabilized for optical profilometry and scanned lengthwise along the surface of the implant in an area between the threads (Fig. 2A) that represented the area of the titanium implant that would make intimate contact with the host bone in vivo.

Surface roughness of the titanium implants was also analyzed using atomic force microscopy (AFM). Titanium implant samples were sonicated in chloroform for 30 min and rinsed with double-distilled, nonionized water. Water droplets were wicked off the sides of the implant and air dried. The samples were immediately mounted on a metal stub and loaded into the piezoscanner of multimode AFM with a NanoScope IIIa controller for imaging (Digital Instruments, Santa Barbara, CA). A J-type scanner (maximum scan size of ~100 × 100 µm²) was used to image a relatively large area. Standard silicon-nitride tips (Nanoprobe SPM Tips, Digital Instruments) with a spring constant of 0.58 N/m were used. Imaging was conducted at room temperature in ambient air within a custom-made chamber using contact mode operation. An area immediately adjacent to the threaded area of the titanium implant was used for scanning (Fig. 2A). The AFM probe was unable to access the identical area as used for optical profilometry (Fig. 2A), but because of the same machining techniques the two areas were assumed to have the same roughness. Both the “height” image and “deflection” image were obtained. The height image revealed detailed topographical features by tracking the surface with the probe and adjusting the tip-sample separation (defined as the z-axis) to sustain a constant imaging force using a feedback loop. The deflection signal (when feedback loop is still operational) or the error signal is caused by the gradient of the feature being traversed by the tip. Thus the deflection mode image enhances high spatial frequency information and improves sharp edges of the surface features. The color tables used on these images primarily indicate the rate of change of height along the fast-scan direction. All the presented images were flattened and plane-fitted to remove background slopes. The images were recorded at 1-Hz frequency of scanning. Scan rotation was 0° for all imaging, with the scan direction parallel to the long axis of the cantilever. Each image took ~8.5 min to complete at the scan rate of 1 Hz.

Quantitative measurements of the local root mean square (RMS) surface roughness were determined using height data from two scan sizes: 30 × 30 and 15 × 15 µm². The definition of RMS roughness (Rq) is a function of height fluctuations in a given area. Rq was determined by computing each image height data of 30 × 30 µm² size obtained from five random locations on the sample surface. This computation was done with a roughness analysis program included in the Digital Instruments software. Furthermore, a 15 × 15 µm² size scan was made in the same five locations to obtain a magnified view of surface features. All RMS values were calculated from height images, whereas corresponding deflection images were obtained for detailed topological feature presentation illustrating surface roughness.
Surgical implantation. General anesthesia was induced via intramuscular injection of 45 mg/kg ketamine (Ketaset, Abbott Laboratories) and 5 mg/kg xylazine (X-ject SA, Phoenix Scientific, St. Joseph, MO). Lidocaine/epinephrine (Abbott Laboratories, Libertyville, IL) was administered by intramuscular injection into the femoral muscle mass. After skin incision from the lateral side, separation of muscle and reflection of the soft tissue in the proximal femur region, the metaphysis region of the femur was marked with a round bur. Using a twist drill and torque control unit, a pilot hole was drilled, and the diameter of the pilot hole was sequentially increased using drill bits of 2.2, 2.8, and 3.5 mm at speeds of no greater than 800 rpm. The titanium implants were placed transversely and transctortically into the right proximal femur using a torque control unit. The abutment remained nonsubmerged. After replacement of the deflected soft tissue, the skin incision was closed. An identical titanium implant was placed in the corresponding proximal area of the left (contralateral) femur under the same conditions. The implants were allowed to heal for 6 wk, corresponding to the fringe of osseointegration for animal models (8, 18, 62). The rabbits were monitored daily for weight gain and cage behavior.

Application of micromechanical forces. Six weeks after in vivo implant placement, general anesthesia was induced as described above. The implant in the proximal femur was exposed as described above. A stainless-steel adaptor was attached to the abutment of the implant. The wound was sutured as close around the transcutaneous adaptor as possible.

The right-femur implant was immediately subjected to axial microcompressive forces using a servohydraulic system (858 Mini Bionix II, MTS Systems, Eden Prairie, MN) at 200 mN and 1 Hz (Fig. 1D), resulting in 600 cycles/day, for 10 min/day over 12 consecutive days (Fig. 1A). The force delivered was sinusoidal and, because limitations of the servohydraulic system prevented the regime from reaching a value of zero force, cycled between 200 and 50 mN (Fig. 1D). A low magnitude of force and an axial direction were desired to avoid potential micromotion leading to implant failure (7) and also because low-magnitude cyclic forces have been shown to stimulate bone growth in long-bone models (52). The frequency of 1 Hz was chosen because it has commonly been used in many mechanobiological studies (51, 65) and because of its approximation of the frequency of normal gait (27) and chewing (7, 27, 44, 71). The resulting halves were cut along the transverse axis of the proximal bone specimens, one-half of the specimen was decalciﬁed in 10% EDTA for 30 days (Fig. 1A), and therefore the bone response were equal for each half (7, 27, 44, 71). The resulting halves were cut along the transverse axis of the implant at the endocortical surface. Serial sections were then cut in both directions, further into the bone marrow and externally toward the cortex of the bone. Because the serial sections obtained gave similar results, the results of histological and histomorphometrical measurements were pooled (Fig. 1C). Once trimmed, the bone-implant samples were stored in 4% formalin. Implants were manually removed from all bone specimens with a pair of forceps. In the majority of cases, no bone loss was visually observed by separating the implant from bone, although considerable forces were needed to separate the implant from bone.

Data analysis and statistics. For each of control and mechanically loaded implant-bone specimens, one-half of the specimen was decalcified, embedded in parafﬁn, cut into 5-μm sections with microtome, and stained with various histological dyes including hematoxylin and eosin and safranin O/fast green. Bone histomorphometric parameters were quantiﬁed using computerized image analysis (ImagePro Plus, Media Cybernetics, Silver Spring, MD) (43). Bone ingrowth was quantiﬁed by static histomorphometry of bone immediately adjacent to implants. Bone density 1 mm from the implant-bone interface (BV/TV) was selected so that bone density near the implant would be quantiﬁed. To determine cellular response to mechanical loading, the number of osteoblast-like cells per bone surface (NOB/BS) were measured. BV/TV and NOB/BS were measured on hematoxylin and eosin-stained sections (42, 43). Safranin O stains for glycosaminoglycans in the extracellular matrix produced by cartilaginous and ﬁbrovascular tissue was used to detect whether any chondrogenic cells might be present in both control and mechanically loaded samples, since cartilage and ﬁbrocartilage is known to be present transiently during bone healing.

The other half of each specimen was used for undecalcified preparation, embedded in methyl-methacrylate, sectioned at 12-μm thickness with Leica Polycut (SM2500E, Leica), and used for quantiﬁcation of dynamic bone histomorphometry by ﬂuorescent microscopy (43, 54, 65). MAR was deﬁned as the distance between double labels divided by the number of days elapsed between the injection of acrylic leg mold was used to stabilize the right leg to ensure that accurate and reproducible forces were delivered to the implant. The left femur implant continued to serve as control. Calcein blue (20 mg/kg; Sigma) was injected intraperitoneally on day 5 after commencement of cyclic loading, whereas alizarin red (20 mg/kg; Sigma) was injected intraperitoneally on day 9.

Tissue harvest and processing. On day 12 after commencement of cyclic loading on the right femur implant, the rabbit was euthanized to obtain bone-implant specimens in both the left and right femurs, each with an approximate size of 1 ml using a surgical saw. The bone block was trimmed into two equal halves along the long implant axis with a diamond saw, with the proximal half to be used for ﬂuorescent microscopy and the distal half to be used for bone histomorphometry. Because the forces applied were axial, it was assumed that the force and therefore the bone response were equal for each half (7, 27, 44, 71). The resulting halves were cut along the transverse axis of the implant at the endocortical surface. Serial sections were then cut in both directions, further into the bone marrow and externally toward the cortex of the bone. Because the serial sections obtained gave similar results, the results of histological and histomorphometrical measurements were pooled (Fig. 1C). Once trimmed, the bone-implant samples were stored in 4% formalin. Implants were manually removed from all bone specimens with a pair of forceps. In the majority of cases, no bone loss was visually observed by separating the implant from bone, although considerable forces were needed to separate the implant from bone.
the two fluorescent labels on day 5 and day 9 after the commencement of cyclic loading. Bone-forming surface (BS/BS) was defined as the amount of double-labeled bone-forming surface divided by the total surface. BS/BS was used in the place of double-labeled surface because the second label was not present in some of the areas analyzed (65). A minimum of three areas for each section were analyzed, and all data were pooled for each rabbit so that the total number of samples remained the same for all parameters. BFR/BS was defined as $\text{MAR}/\text{H11003 BS}/\text{BS}$ (43, 65).

Following establishment of normal data distribution, paired sample Student’s $t$-tests were performed on all quantitative data between experimentally loaded and control samples using SPSS (Chicago, IL). Significance was attained with $P$ values of $\leq 0.05$.

RESULTS

Surface roughness of titanium implants. Surface roughness of titanium implants in the areas between two threads was measured as $R_q$ by optical profilometry. Macroscopically, the $R_q$ value of the threaded bone-contacting area of the titanium implants was 0.92 $\mu$m. With the use of AFM, the flat surface of the titanium implants was characterized. Using deflection images, detailed topological maps of the implant surface were obtained for both $30 \times 30 \mu$m$^2$ (Fig. 2B) and $15 \times 15 \mu$m$^2$ (Fig. 2C) scans. In $30 \times 30 \mu$m$^2$ scans, the $R_q$ value was measured as $215 \pm 24.8$ nm ($n = 5$). For $15 \times 15 \mu$m$^2$ scan sizes, the average $R_q$ was $197 \pm 34.7$ nm ($n = 5$).

Macroscopic examination of implant-bone interface. Each of the mechanically loaded and control implants, even after being cut into two halves, remained attached to the surrounding bone. On physical separation of the implant half from bone by using a pair of forceps, virtually no bone was lost. No loose implant was detected.

Histology and static histomorphometry of bone-implant interface. Representative microscopic sections of bone-implant interface are shown in Fig. 3. There was marked bone density in the implant surface that had received brief doses of mechanical forces for 10 min/day over 12 consecutive days under cyclic axial compressive forces at 200 mN and 1 Hz, resulting in 600 cycles/day. The bone adjacent to the implant seemed to be denser and also protruded further into the medullary cavity in the majority of samples (Fig. 3B) compared with the control implant (without mechanical loading) (Fig. 3A). This contrast in bone density between mechanical loading and the control was confirmed by static histomorphometry. BV/TV of mechanically loaded samples (73 $\pm$ 12%) was significantly higher than
controls without mechanical loading (52 ± 21%) (P < 0.01) (Fig. 4A). There were abundant osteoblast-like cells on the endocortical bone surface of mechanically loaded samples (Fig. 3D). By contrast, unloaded control samples had sparse osteoblast-like cells or bone-lining cells on the endocortical surface (Fig. 3C). Computerized quantification confirmed microscopic observations, showing that the NOb/BS in mechanically loaded samples (55 ± 8 osteoblasts/mm) was significantly higher than unloaded controls (35 ± 6 osteoblasts/mm) (P < 0.01) (Fig. 4B). Of tremendous interest was the appearance of chondrocyte-like cells in the bone matrix distant (>1 mm) from the bone-implant interface in some, but not all, mechanically loaded samples; these chondrocyte-like cells demonstrated strong positive reaction to safranin-O staining, indicating their synthesis of cell-associated matrices rich in cartilage glycosaminoglycans such as chondroitin sulfate (Fig. 3F). In contrast, there is a lack of positive safranin-O staining in any of the control bone-implant specimens as exemplified in Fig. 3E.

**Dynamic histomorphometry of bone-implant interface.** Bone apposition fronts were most noticeable in representative lacunae near the bone-implant interface with representative photomicrographic images shown in Fig. 5. Remarkable distances between double fluorescent labels (calcein and alizarin) in mechanically loaded samples were marked (Fig. 5B), compared with the narrow distances between double fluorescent labels in unloaded controls (Fig. 5A). Dynamic histomorphometry was used to quantify the distances between two fluorescent labels. The average MAR of mechanically loaded samples at 3.8 ± 1.2 μm/day was significantly greater than the average MAR of unloaded controls at 2.2 ± 0.92 μm/day (P < 0.01) (Fig. 4C). BSf/BS in dynamically loaded samples was 60 ± 12% compared with unloaded controls (53 ± 10%) but failed to reach statistical significance. The average BFR/BS, taken as the product of MAR and BSf/BS, was significantly higher for mechanically loaded samples at 2.4 ± 1.0 μm³·μm⁻²·day⁻¹ than the unloaded controls at 1.2 ± 0.60 μm³·μm⁻²·day⁻¹ (P < 0.01) (Fig. 4D).

**DISCUSSION**

The present data offer a glimpse of mechanical modulation of implant bone ingrowth after the initial healing period. Except for one parameter under study (BSf/BS), computerized static and dynamic bone histomorphometry measures demonstrate significantly greater bone volume, NOb/BS, MAR, and BFR/BS in loaded femur implants than corresponding values of the contralateral control femur implants. These original data are of interest for several reasons. First, the magnitude of mechanical forces likely is of considerable importance for anabolic bone responses (19, 20, 21, 34). However, force magnitude alone may not be sufficient to explain stress-induced anabolic bone responses because the present approach utilized cyclic forces that oscillated at 1 Hz instead of static forces. Force frequency or cyclic oscillation in force magnitude in both the appendicular and craniofacial skeletal lineages has been shown to induce more significant anabolic responses than static forces of the same peak magnitude and duration (11, 13, 38, 51, 61, 66). The direction of the presently applied mechanical forces was axial and likely accountable for potential equal transmission of mechanical stresses to the entire bone-implant interface as well as cortical bone (7, 27, 44, 71). The outcome of implant bone ingrowth is likely to differ if nonaxial forces are applied (47). Although not addressed in this study, there...
could have been differences in bone growth between the proximal and distal sides of the femur adjacent to the titanium implant. However, the axial forces probably led to equal force transmission and presumably equal stimulus to bone ingrowth throughout the entire interface (7, 27, 44, 71), and disparities between the proximal and distal sections would be most evident in nonaxial loading, such as if shear stresses or moments were introduced (47, 50, 57). Second, the timing of force application likely matters. The outcome of bone ingrowth may differ if forces are applied immediately after surgical implant placement. Third, the surface roughness as measured by optical profilometry and AFM of currently used titanium implants is similar to that of machined-smooth titanium implants (32). Measurements made by optical profilometry and AFM varied, but this difference was probably due to the scan sizes of the individual methods, ~1 mm² for optical profilometry and 30 × 30 and 15 × 15 μm² for AFM. Surface roughness has been shown to play an important role in cellular response to titanium implants (5, 37, 56, 58) and ultimately bone ingrowth (5, 46) in both dentistry and orthopedics (46, 30), with increasing surface roughness correlating to increased cell proliferation (37) and bony ingrowth (17, 25, 64, 70). Titanium implants with a smooth surface, such as the implants used in the present study, have adequate cell adhesion (37, 55) and bony ingrowth (1, 24), but the process occurs slower than for roughened surfaces (23, 60). In some cases, it has been shown that increased surface roughness can have a negative effect on cellular development (69) and bone ingrowth, especially in the loaded case if shear stresses develop (47). Strain and stress concentrations may also develop in the microgrooves of a surface-roughened implant on loading, as is the case for macroscopic screw threads (67). Nevertheless, further studies must be undertaken to determine whether micromechanical loading and surface roughness have synergistic or antagonistic effects. Fourth, although increased endocortical osteoblast density corroborates with the increased bone volume, BFR/BS, and MAR, the present study was not designed to address the mechanisms for the apparently coordinated increases in bone formation parameters. One may speculate, however, that short daily doses of axial mechanical forces have upregulated osteogenesis-related genes responsible for the proliferation, differentiation, and matrix synthesis of endocortical bone, leading to increased volume of bone apposition. This conjecture cannot be ascertained until additional mechanistic experiments are performed. Finally, the surprising appearance of safranin-O-positive, chondrocyte-like cells in bone adjacent to implant-bone interface is remarkable. It is probable that menenchymal progenitor cells resident in bone marrow have been induced to differentiate into chondrocyte-like cells by the presently applied mechanical forces (2, 3); under the same notion, these menenchymal progenitor cells would have differentiated toward the osteogenic lineage in the absence of exogenous mechanical forces. The absence of safranin-O-positive, chondrocyte-like cells in the unloaded contralateral femur appears to signify that marrow-resident precursor cells differentiate and form new bone by the process of endochondral ossification, serving as additional evidence of mechanically induced differentiation of marrow-resident progenitor cells (2, 3, 10).

The cells responsible for the presently observed increases in bone volume and bone density are likely osteoblastic cells in the endocortical surfaces adjacent to surgically placed titanium implants. These cells line the lacunae and display the cuboidal morphology of typical osteoblasts that actively deposit bone matrix. Mechanical stimulation, such as in the present study, likely has activated the proliferation of the endocortical osteoblastic cells, as evidenced by significantly increased NObs/BS in the endocortical surfaces of mechanically loaded samples over unloaded controls. Because the titanium implants penetrate the cortical bone into bone marrow of the medullary cavity, the biomechanical forces are also likely to have stimulated marrow-resident osteoprogenitor cells to differentiate into osteoblasts that in turn begin producing osteoid. Osteoblastic cell proliferation and differentiation may be a direct response of mechanical forces by increasing mitosis (15, 29). On the other hand, significantly more bone density surrounding mechanically loaded implants over unloaded controls suggests increased matrix synthesis by osteoblastic cells, as evidenced by significantly more MAR and BFR/BS than unloaded controls. Thus mechanical forces appear to have stimulated the proliferation of osteoblasts as well as their activation to produce more and denser peri-implant bone.

Two issues central to bone healing and bone ingrowth following the placement of intraosseous titanium implants are 1) whether mechanical forces can be applied to enhance bone ingrowth and 2) the specific timing of when this functional loading can be applied. Exogenous forces applied during the initial bone ingrowth period of titanium implants have long been associated with fibrous encapsulation and implant failure. This association has required long osseointegration periods for orthopaedic implants (~3 mo (31)) and even longer for intraosseous dental implants (~4–6 mo (1)). Although the present study is not designed to provide a complete answer to either of the aforementioned questions, the outcome of the present study appears to indicate that micromechanical forces applied along the axial orientation of the titanium implant may enhance bone ingrowth. This conclusion is aligned with mechanically induced anabolic responses in both the appendicular and craniofacial skeletal lineages by cyclic forces (11, 13, 38, 51, 61, 66). Recent interest in shortening the amount of initial bone healing time on implant placement has evoked the question of whether mechanical forces may indeed facilitate bone healing of intraosseous titanium implants (22, 26, 61). For the animal model used in the present study, the application of mechanical forces after 6 wk of bone ingrowth is likely on the fringe of complete primary ingrowth, with many healing processes still strongly intact (8, 18, 62). Visual evaluation of the freshly harvested implants in the present study shows good bone anchorage and a lack of fibrous capsule surrounding mechanically loaded samples. Early application of mechanical forces may also be advantageous, as demonstrated by the current increases in BV/TV and NObs/BS. However, the present data should not be viewed as experimental evidence in advocating immediate loading of intraosseous implants, given that loading has been applied only after 6 wk of healing. Commencement of mechanical loading of titanium implants at shorter time periods less than the presently used 6 wk needs to be demonstrated before a definitive conclusion is made regarding presumably widespread success of early loading. The timing of the loading regime is also important in that it was probably applied during a late-healing phase of bone ingrowth, during which the peri-implant bone is still actively modeling. The response to micromechanical loading may be different if
applied during the remodeling process in which the immature woven bone is replaced by well-organized bone tissue. To determine whether the tissue response due to micromechanical loading observed in the present study is a transient or permanent adaptation to mechanical loading requires observations at time points throughout the modeling as well as remodeling processes (40).

The presently identified increases in BV/TV may need to be supplemented with mechanical testing such as pull-out tests (7) to determine whether greater bone density indeed leads to higher strength of the bone-implant interface. The present study also used only a single applied force magnitude and single frequency in eliciting the osteogenic response. Peak magnitude and frequency as well as many other parameters have been found to play a large part in stimulating osteogenesis of skeletal structures (38, 52). Thus additional studies must be performed to determine whether these parameters lead to different amounts of bone ingrowth surrounding intraosseous implants. In the present work, differences in BSf/BS between mechanically loaded and control samples failed to reach statistical significance probably for several reasons. As shown by Robling et al. (49), at high levels of BSf/BS, mechanical loading does not significantly increase BSf/BS in long bones. Also shown in long bone-growth systems, large bending forces and strains were needed to significantly accelerate bone growth, whereas the small axial forces used in the present study may not have been large enough to increase BSf/BS (49, 65). During the studied period, bone healing likely is still underway. Accordingly, a similar study may be performed after longer durations of implant healing (40). Within the constraints of the present approaches, the clear evidence of significantly increased osteoblastic proliferation and bone volume and density surrounding mechanically stimulated intraosseous titanium implants suggests that axial micromechanical forces may indeed induce anabolic responses of bone ingrowth.

ACKNOWLEDGMENTS

We thank both anonymous referees whose valuable comments helped improve the quality of the manuscript. A. Lopez, T. Joshi, and S. Han are acknowledged for capable processing of histological specimens. A. Clark and R. Vyas are thanked for general technical assistance. D. dadi and R. Vyas are thanked for general technical assistance.

GRANTS

This research was supported by a Biomedical Engineering Research Grant from the Whitaker Foundation and National Institutes of Health Grants DE-13964, DE-15391, and EB-02332.

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