イヌ大腿骨に埋植した改良型Grit-blastインプラントの力学的・組織学的評価

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<th>誌名</th>
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<td>p. 1191-1198</td>
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<td>発行年月</td>
<td>2008年11月</td>
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農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
Mechanical and Histological Evaluation of Improved Grit-Blast Implant in Dogs: Pilot Study

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(Received 23 October 2007/Accepted 7 July 2008)

ABSTRACT. Orthopedic fixation of irreversibly damaged joints entails the use of artificial joints. Porous coated prostheses require no bone cement, but coating to the implant is susceptible to avulsion from the surface. The purpose of this pilot study was to test an improved non-coated grit blast titanium implant having a direct surface roughness of 33.4 μm with a wide contrast of 251.6 μm between the valleys and peaks, i.e., about ten times rougher than conventional grit blast, and designed with bumpy, acutely articulated depressions. Fifty-six implants (28 grit blast, 28 smooth) were tested in the femora of 7 healthy beagles. Four roughened grit-blast implants were implanted in the left femur and four smooth implants (controls) in the right femur. The 33.4-μm grit-blasted roughening dramatically enhanced the histological effectiveness, and the mechanical effectiveness improved as a consequence. Bone-to-implant ongrowth attained high apposition and integrity at weeks 12 (76.38%) and 16 (80.35%). Shear strength increased with time and continued to be acceptable (14.5 MPa) at the end of the study. Bone matrix was particularly abundant and thick at the end of week 16, indicating progressive mineralization and maturation of remodeled bone matrix and compatibility of the roughened implant. In conclusion, the new grit blast device shows promise as a potentially useful prosthetic implant. Grit blast roughening would facilitate use of the bare implant without coatings or cement, would likely minimize the cost of joint fixation, and would allow relatively easy implantation.

KEY WORDS: arthroplasty, artificial joint, femur, noncement fixation, roughened grit blast.

The knee or hip joint damaged irreversibly by disease or injury often requires orthopedic fixation with an implant. Metallic endoprostheses today require no cement; however, avertion infections and other complications associated with cemented implants. Noncemented implants, however, are subject to failure if the bone does not bind sufficiently to the implant surface. Shear strength and long life of an implant depend on bone ongrowth at the bone-to-implant interface [15, 17, 22]. No consensus has been reached, however, as to the most suitable surface design or processing required to promote bone interlocking upon the implant.

Grit blast implants are designed with irregular surfaces created directly on the solid titanium base itself. To create a porous surface, additional processing may involve the coating of an implant with beads or mesh and the use of plasma spray, sintering, or diffusion bonding. But, the fastness of such coatings is markedly reduced by fatigue, often ending in avulsion of the surface coating from the metallic substrate [16, 31]. Uncoated grit blast implants, on the other hand, are notably weak in shear strength between the bone and implant [10, 20, 30]. Attempts to increase the roughness, or porosity, of the grit blast implants, have demonstrated that hydroxyapatite or A-W glass ceramic accelerated the vascularization and ossification within a short period after implantation [21, 30]. However, these coatings were found to lack integrity to the implant substrate, and histomorphometric examination disclosed that the hydroxyapatite layers cracked and delaminated at the implant-to-coating interface [8, 16, 23, 30].

In a previous report from our laboratory, bone-to-implant osseointegration was vastly improved by roughening of the implant surface with a regular pattern of small grooves created by laser processing [29]. Compared to the low cost of irregular grit blast, however, the cost of such detailed laser processing can be exorbitant and virtually out of the question in veterinary medicine. The need for a practical and reliable implant that is less expensive than laser processing has led us to reconsider grit blast titanium implants with special reference to increasing the surface roughness.

In a prestigious study of increased surface roughness created on titanium dowel pins implanted in the tibiae of sheep, Svehla et al. [30] reported that a roughened surface created by a thick coating of hydroxyapatite (50 μm) upon the grit blast implant achieved significant increases in interfacial bone ongrowth and shear strength as early as postoperative week 4. In that study, however, so impressive was the early biochemical effect of hydroxyapatite that the effect of the 50-μm surface roughening remained unanswered. Thick coating of those grit blast implants with hydroxyapatite increased fixation at all the time points tested (4-, 8-, and 12-week intervals), and the authors concluded that this phenomenon could not be explained solely by the surface roughness variations [30]. The possible contributive role played by the augmented roughness, however, was not ruled out; the mystery remains as to the extent to which the improved surface might have contributed to the favorable

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overall results achieved. Unlike most coatings, hydroxyapatite is the main component of bone and teeth and, therefore, has the advantage of being a bioactive model that promotes osteogenesis at the cellular level [7]. Although uncoated conventional grit blast implants show little ongrowth in the early weeks after implantation, progressive osseointegration on grit blasted titanium implants has been reported as a function of time [13, 18, 19, 22]. This peculiarity raises the question as to whether bone ongrowth might be accelerated if the roughness, i.e., the peak-to-valley contrast, was to be increased on the surface of the grit blast implant itself rather than by coating.

Much understanding has been gained from a wide range of experimental work addressing the surface geomorphology of implants tested in the femur, tibia, ulna, and mandible of animals [3, 6-17, 19, 21, 23-25, 27-30, 33, 34] as well as from orthopedic and oral surgery in human clinical studies [1, 4, 5, 18, 20, 26], and from bioengineering studies in vitro [32] and biological examination of cell attraction to roughened surfaces in vitro [2]. Despite an ongoing proliferation of reports, however, controversy remains on how to design the implant surface most conducive to osseointegration and apposition of remodeled bone matrix to the implant. Taken together, studies to date suggest that greater contrast between the peaks and valleys pitted into the surface of titanium implants prepared by grit blasting might accelerate bone-to-implant interlocking as well as the shear strength. Vercaigne et al. [34] noted that increased mechanical interlocking plays a role in the bone reaction when the roughness is greater than 10 μm.

The objective of the present pilot study was to re-evaluate grit blast implants for possible use in arthroplasty involving the femur, with special reference to surface roughening of the implant. Heretofore, in conventional grit blasted implants, the average surface roughness (Ra) has ranged from 5.7 μm to 6.2 μm with peaks (Ry) of 38.4-43.5 μm [10, 30]. In the present work, we tested an improved grit blast titanium implant prepared with an average surface roughness of 33.4 μm and a wide contrast of 251.6 μm between the valleys and peaks, i.e., about ten times rougher than usual, designed with an especially bumpy surface of acutely articulated concavities, or craters. The bone-to-implant ongrowth and shear strength of the implants were examined at 4-week intervals for 16 weeks in the femora of healthy beagles.

MATERIALS AND METHODS

Study design: A total of 56 implants (28 roughened grit blast, 28 smooth) were studied in the left and right femora of active dogs. In 7 healthy laboratory beagles, 4 years old and weighing 9.3 to 14.5 kg each, the two types of metallic implant were compared for the effect of the implant surface on bone-to-implant ongrowth and shear strength. Four roughened grit blast implants were surgically implanted into the left femur, and four smooth implants (controls) into the right femur of each dog. The study protocol was approved in advance by the Institutional Review Board for Animal Experiments at Rakuno Gakuen University, and animal care followed the university Guidelines for the Care and Use of Laboratory Animals. After the experiments, all animals recovered uneventfully and were returned to normal activity.

Implants: All implants were slim cylindrical rods, or dowel pins, fashioned of titanium alloy (Ti-6Al-4V) and provided by Nakashima Propeller Company (Okayama, Japan). The grit blast implant was 13 mm long with a diameter of 4.4 mm, and the smooth implant (as control), not subjected to grit blasting, was 13 mm long with a diameter of 4.5 mm. The grit blast implants had an average roughness of 33.4 μm, with peaks of 251.6 μm (Fig. 1). The surface was bumpy, though not abrasive, and the resulting depressions varied in depth and breadth. The smooth implants had an average roughness of 0.143 μm, with peaks of 1.589 μm (Fig. 1).

Surgical procedure: Each dog was anesthetized, and surgically the left femur was exposed. From the lateral aspect, four 4.5-mm crypt-like holes were drilled into the cortical bone of the femoral shaft 1.5 cm apart and not penetrating the medial aspect. One grit blast implant was seated in position in each hole and tapped gently with an orthopedic hammer, with half the length of the implant resting inside the femoral shaft and the remaining half left protruding from the femur into the surrounding lateral tissue (Fig. 2). The surgical wound was closed, and four smooth pins were inserted in the same manner into the shaft of the right femur (Fig. 2). After surgery, each dog was administered cefazolin sodium (25 mg/kg body weight, Cefamezin; Fujisawa Pharmaceutical Co., Osaka, Japan) as a prophylactic antibiotic twice daily for seven days, and analgesics (Meloxicam 0.1 mg/kg/bw once daily, Metacam, Boehringer Ingelheim Co., Hyogo, Japan; and buprenorphin hydrochloride 0.01 mg/kg/bw twice daily, Lepetan, Otsuka Pharmaceutical Co.,
Push-out test

Histological examination

Push-out test

Push-out test

Push-out test

Smooth

Grit blast

Fig. 2. A representative X-ray film ventrodorsal view showing the implants in the femora of a beagle dog soon after surgery. Smooth implants are in the right femur, and roughened grit blast implants in the left femur. Bilaterally, the second-most proximal implant was examined histologically, and the others were subjected to a push-out test of shear strength.

Tokyo, Japan) for seven days. No perioperative complications were encountered, and the dogs were walking 3 hr after surgery.

Implant removal: All implants were removed from one dog at the end of week 4, two dogs at week 8, 2 dogs at week 12, and 2 dogs at week 16. With the dog under general anesthesia, the femur was exposed and transverse specimens of bone, 15 mm long with the implant in situ, were removed with a sagittal saw and bone chisel.

Push-out test: To evaluate fixation of the implants in the femur, we subjected the implant specimens to a push-out test [6, 14, 21] with Instron Universal Testing Instrument, model 1130 (Instron Co., Canton, ND, U.S.A.) within two hours of implant removal. The implant specimens used from grit-blast implant and control implant respectively at week 4 (n=1), week 8 (n=2), week 12 (n=2) and week 16 (n=2). In this test, the fresh implants were pushed out from the surrounding bone at a crosshead speed of 0.5 mm/min, until the applied load peaked and started to decrease. Shear strength was calculated according to 2 formulas [15, 31]: shear strength (MPa)=load at failure/contact area (mm²); and contact area (mm²)=π × implant diameter × average cortical thickness (density).

Histological examination: On specimens from the implant in the second-most proximal site, bone ongrowth was examined by light microscopy (TMS-F, Nikon Co., Tokyo, Japan), according to the method of Tsukeoka [33]. Each specimen was fixed in 10% neutral buffered formalin, dehydrated with ethanol, and embedded in polymethylmethacrylate. Then, the specimen was sliced into 0.5 mm thickness with a microcutting machine, model BS3000 (EXACT Co., Norderstedt, Germany), then ground into 100-μm sections with a microgrinding machine, model MG400CS (EXACT Co., Norderstedt, Germany). The sections were stained with toluidine blue for examination by light microscopy. Toluidine blue enables distinction between osteoid (which stains blue) and bone matrix (which stains purple to gray). The rates (%) of bone matrix-to-implant contact at post-implantation weeks 4 (n=1), 8 (n=2), 12 (n=2), and 16 (n=2) were calculated with Photoshop Element 2.0 software (Adobe Systems Incorporated, San Jose, CA, U.S.A.) on the Macintosh personal computer system.

RESULTS

Implantation check: Intraoperatively, the distal-most implant felt a little unstable to the touch, and the cortical compact bone was noticeably thin and sparse. X-ray films taken a few hours after implantation, however, showed all implants to be apparently in place (Fig. 2). One smooth implant was loose at week 8 and one grit blast implant at week 12. At week 0, grit-blast implant was able to be pulled out easily by the hand, however smooth implant was not possible to pull it out by the hand.

Push-out test: The roughened grit blast implants showed high shear strength as compared with the smooth column throughout the observation period (Fig. 3). Shear strength of the grit-blast implant showed a minimal change of about 5 MPa through week 12, but increased to 11.8 ± 2.62 (mean ± SD) MPa at 16 weeks. In the smooth implants, maximum shear strength was 3.66 ± 0.59 MPa, observed at week 4. The implant occupying the distal site of each femur was excluded from analysis, because the amount of cortical compact bone at that site turned out to be insufficient for hosting and anchoring the implants.

Histological examination: In the roughened grit blast group, bone matrix increased from 59.63% of the implant circumference at week 4 to 80.35% at week 16 (Table 1). Conversely, in the smooth implant group, bone matrix showed a negligible through week 16.

Osteoid was observed in the narrow gap between the surface of the smooth implant and host hole that was drilled into the bone (Fig. 4A). Osteoid was seen in intervals of apposition around the grit-blast implant, and bone matrix formed between the apposition sites as well as around the osteoid itself (Fig. 4B). An inverse relationship was observed between osteoid and bone matrix around the roughened grit blast implants, i.e., with proliferation of bone matrix, the amount of osteoid receded, and this phenomenon was particularly evident at week 16 (Fig. 4). In contrast, however, osteoid around the smooth device showed minimal change except between week 4 (91.57%) and week 8 (60.7%), with 69.09% observed at week 12, and 68.26% at week 16 (Fig. 4). Moreover, greater amounts of osteoid were observed around the smooth device than around the grit blast device at all observational periods in all animals. Taken together with the bone matrix values, these results indicate that the osteoid matured into bone matrix around...
the roughened grit blast device but not around the smooth device (Fig. 4).

Consistently, bone matrix was more abundant around the roughened grit blast implant than around the smooth implant from our initial observation at week 4 to the final observation at week 16 (Fig. 4). At week 16, the percent of implant circumference to which bone matrix was attached to the grit blast device was almost the same as that at week 12 (Table 1), but at week 16 the bone matrix was dramatically thicker, and the gap was filled between the implant and implant hole (Fig. 4H). The marked thickening of bone matrix during this period may account for the improved shear strength observed at week 16 (Fig. 3).

DISCUSSION

This pilot study has re-evaluated a grit blast titanium implant with special reference to deep roughening of the implant surface. The results show that 33.4-μm grit-blasted roughening dramatically enhances histological effectiveness of the implant and that the histological benefits, in turn, improve the mechanical effectiveness. Three findings in particular raise the possibility of using the low-cost grit blast devices in joint fixation. First, bone-to-implant ongrowth attained a high level of integrity, as evidenced by the dramatic apposition of remodeled bone matrix to the roughened implant surface at weeks 12 and 16. Second, the shear strength, although not dramatic, increased with time and continued to be acceptable at the end of the study period, week 16, suggesting that shear strength of the roughened grit blast device improves with time. Third, the bone matrix was particularly thick and abundant at the end of week 16, indicating progressive maturation and mineralization of remodeled bone matrix and compatibility of the implant.

In conventional fine-surface 60-grit, 36-grit and 30-grit blast, the highest bone apposition reported has been 30 to 35 percent of the implant circumference at week 12 [12, 13]. In stark contrast, the 76.38 percent apposition to the circumference of our roughened grit blast implant at 12 weeks translates to approximately twice that of conventional grit blast surfaces, and the attraction of almost a complete ring of new bone to the circumference by week 16 is unprecedented in grit blast implants. This phenomenon underscores the importance of surface roughening and leads us to propose that maximum roughening may be the prerequisite heretofore lacking in implants designed with conventional fine-grit blasted surfaces. With the sole exception of an 18-μm roughness achieved by risky acid etching of the surface and tested in rabbit femur, no other implant has been reported to attract a complete ring of new bone surrounding the implant [11].

Among conventional grit blast implants reported to date, none have achieved shear strength much above that of smooth implants. Those fine-grit blast implants reported heretofore, however, had an average surface roughness of only 5.7 to 6.2 μm Ra [10, 30] as compared to the present 33.4-μm roughness (Ra), and their maximum peak had a

Table 1. Bone-to-implant ongrowth shown by percent of the implant circumference covered by bone matrix

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<tr>
<td>Grit Blast</td>
<td>59.63</td>
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(%)
Fig. 4. Micrographs of undecalcified section of the implant circumference at weeks 4, 8, 12 and 16. (A) Smooth implant, week 4; (B) Roughened grit blast implant, week 4; (C) Smooth implant, week 8; (D) Roughened grit blast implant, week 8. At week 8, the roughened grit blast implant shows bone ongrowth to the surface. (E) Smooth implant, week 12; (F) Roughened grit blast implant, week 12; (G) Smooth implant, week 16; (H) Roughened grit blast implant, week 16. Purple staining indicates bone matrix (black arrowheads) and blue staining indicates osteoid (white arrowheads). Osteoid appears on both the smooth device and roughened grit blast device. Bone matrix is nil on the smooth implant. As bone matrix increased on the surface of the roughened grit blast implant, osteoid decreased, indicating that the osteoid matured into bone matrix around the roughened grit blast device. Scale bar=1 mm. Toluidine blue staining.

narrow variation from 38.4 to 43.5 μm Ry [10, 30] as compared to the deep peak-to-valley contrasts in the present study. In sheep femora, a conventional grit blast implant having a roughness of 6.2 ± 0.9 μm Ra and a maximum peak of 43.5 ± 5.3 μm Ry was reported to result in complete failure in push-out tests [10]. Conversely, in our study, the surface roughening of the implant contributed vastly to favorable bone ongrowth to the implant, and this high rate of
ongrowth, in turn, contributed noticeably to improved shear strength in relation to time at week 16 in the dog examined. The increase in bone matrix from week 12 to week 16, in particular, may account at least in part for the increase in shear strength observed at week 16.

Clemow et al. [6], working with surface roughening by use of porous coatings on titanium cylindrical cores implanted in the femoral medullary canal of dogs, found consistently that the shear properties of the interface were proportional to the extent of bone ingrowth. Tsukeoka et al. [33] reported similar findings in relation to time, i.e., (i) that sufficient attachment strength could be achieved between bone and implant through interlocking of bone grown in titanium fiber mesh, and (ii) that the mechanical interlocking was a major contributor to the adhesive strength between bone and implant after long-term implantation. Clinical studies have reported, however, that, in actual prostheses, surface coatings are not necessary on grit blast roughened surfaces [1, 20]. In a 5- to 8-year clinical study of femoral prostheses slightly roughened by grit blast and used in human joint fixation, Blaha et al. [1] reported results comparable to those achieved with porous-coated implants and concluded that surface roughening by grit blast achieves satisfactory noncemented results without any addition of porous coatings. Similarly, Miyakawa et al. [20] noted that, in hip prostheses, an entirely grit blasted straight tapered stem facilitates solid biological fixation without porous or hydroxyapatite coatings.

In our study, the encouragingly high rate of osseointegration and apposition around the bare grit blast implant at week 16 was a strong sign of the implant's compatibility and potential use as a long-term prosthetic partner in hip or knee arthroplasty. The term osseointegration implies an interface with remodelled, viable bone in direct contact with the implant surface, with no interposed fibrous tissue membrane [18]. The proliferation and thickening of bone matrix seen between weeks 12 and 16 may be attributed to the grit blast roughening of the titanium implant surface as a function of time.

Without a porous coating or mesh, as the remodeled bone matrix oriented itself into the irregular depressions on the roughened grit blast titanium surface, the bone matrix began maturing and interlocking with the new implant in the femoral environment. Finally, the shear strength was noticeably improved at week 16, in keeping with the principle established by predecessors in the field, i.e., that the greater the amount of bone ongrowth, the greater the mechanical properties which can be expected in the long term [6, 33]. This principle has been informd repeatedly by experiments involving large and medium sized animals such as sheep [10, 30], goat [34], Siberian husky [28], large mongrel dogs [8, 17], turkey [25], and miniature pigs [3], in addition to smaller animals such as the beagle [21, 29, 33] and rabbit [11–14, 16]. In the light of our present results, which we interpret as the combined functions of surface roughening and time, further study is necessary to test the shear strength of the 33.4-μm grit-blasted titanium implant over longer durations than our 16-week period and in more dogs.

True to documented characteristics of grit blast titanium materials, both the osseointegration and shear strength got off to a somewhat slow start in the present study, as shown through the first 8 weeks. With time, however, far more favorable results were evident at weeks 12 and 16. The behavior of hydroxyapatite coating is just the opposite, offering early ongrowth but often delaminating and losing integrity with the passing of time. Two decades before the present level of grit blast surface roughening came into being, Linder et al. [18] proposed a 3-way combination for use in prostheses for total knee replacement: (i) a titanium alloy implant with the surface roughened by grit blast, (ii) hydroxyapatite coating of the roughened grit blast titanium alloy substrate, and (iii) a different type of coating added between the hydroxyapatite and the metallic implant as an effort to avert failure at the coating-to-implant interface. Aiming for early stability in the artificial knee, Linder et al. [18] advocated securing the hydroxyapatite-coated implant with a screw if necessary. In the present work, 20 years after Linder's proposal, if we had secured our uncoated implant with a screw, perhaps the shear strength would have increased as a result of minimizing the micromotion. Additional study is warranted, with and without a screw.

In spite of the overall good results of this pilot study, some implant loosening was observed. At week 0, grit-blast implant was able to be pulled out easily by the hand, however smooth implant was not possible to pull it out by the hand. In other words, grit-blast implant was experimented on unfavorable conditions compared with control implant. This tempts us to suspect that a larger implant diameter might have allowed a more solid anchoring of the grit blast implant in the hole into which the device was seeded in the femur and that the larger diameter might have improved both the osseointegration and shear strength. However, a roughened surface itself is known to increase the total surface area [34]. Therefore, the gain in surface area might be expected to compensate for the small diameter of the implant. For secure fixation in the host bone, rather than increasing the implant diameter, particularly in such a small femur as that of the beagle, we propose a slight reduction in the diameter of the implant hole in which the device is to reside in the host cortical bone. As it is vitally important to make sure of a tight fit at implantation, perhaps a firmer seeding of the implant might have been facilitated if all the implant holes in the left femurs of our study had been drilled to a 4.4 mm diameter, exactly matching the diameter of the grit blast implant itself. Cameron et al. [4] as well as Sandborn et al. [26], found that for titanium implants, large gaps (> 0.5 mm) delay the gap filling and reduce the bone quality at the interface. The gap in our study was a mere 0.1 mm, and with time the gap was filled with remodeled bone. While concuring with the notion that the implant hole ought to be prepared to match the implant diameter, Vercaigne et al. cautioned that minimal damage of bone tissue during surgery and during press-fit conditions is also a prerequisite for titanium surfaces to obtain bony attachment.
In clinical cases of total hip arthroplasty in 33 patients followed up for 11 to 14 years, Miyakawa et al. concluded that secure stability at implantation is essential for overcoming interfacial shear forces between implant and host bone [20]. Accordingly, in a 24-month clinical study of 25 implants in the human tibia, Linder et al. attributed lack of osseointegration to inadequate primary contact with bone [18]. “Primary contact” and “initial stability” are part of what happens at the time of implantation, thus putting great demand on both the surgical technique and the prosthetic design [18].

Given that implants are vulnerable to early loosening during routine activity of the recipient, the question arises as to whether the unused portion of the present implants was problematic. It is not known whether the half of the implant that remained protruding from the lateral aspect of the femur and into the surrounding tissues could have conducted micromotion to the embedded portion when the dog moved while in lateral recumbency. In orthopedic studies involving experimental animals with a relatively small femur, such as the femur of a beagle, perhaps an implant shorter than 13 mm would accomplish the work intended and at the same time curtail the possible risk of micromotion that may be associated with the protruding portion (in our case, ~7 mm) of “unused implant” that might loosen the embedded portion (~6 mm) when the animal moves, especially in the early postoperative period.

In conclusion, this pilot study sheds new light on grit blast surface roughening of titanium implants and adds impetus to accruing evidence in the arthroplasty outlook and valuable considerations for further research. The grit blast device tested here shows promise for grit blast roughening as a potentially useful technique in designing prosthetic implants for human and veterinary arthroplasty. The improved 33.4-μm grit blasted surface roughening promotes osseointegration and interlocking of remodeled bone matrix directly to the titanium implant surface and, consequently, a mediocre-to-good shear strength as a function of both time and surface roughening. These features would facilitate use of the bare implant without coatings and without bone cement, would likely minimize the cost of joint fixation, and would allow relatively simple implantation. Currently, further experiments are in progress in our laboratory to test a custom-designed artificial joint roughened by grit blast and used in a trial case of total joint replacement.

ACKNOWLEDGMENTS. We are grateful to Nakashima Propeller Company (Okayama, Japan) for supplying the implants. We are indebted to Nell L. Kennedy (PhD), Professor of Biomedical English, School of Veterinary Medicine, Rakuno Gakuen University, for valuable discussions and help with the manuscript.

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