Bone Response to Titanium Alloy Implants Placed in Diabetic Rats

Michael McCracken, DDS, PhD[1]/Jack E. Lemons, PhD[2]/Firoz Rahemtulla, PhD[2]/
Charles W. Prince, PhD[3]/Dale Feldman, PhD[4]

Although dental implants continue to provide consistent and predictable treatment options for most patients, some people with uncontrolled systemic disease may be denied implant treatment. Diabetes is one such disease. According to the U.S. Centers for Disease Control and Prevention, diabetes is a leading cause of blindness, kidney failure, and amputations of the lower extremities. These complications result from microvascular disturbances associated with diabetes. The effect of diabetes on the healing of titanium implants has not been well established. In this study of 32 rats, diabetes was induced in 16 animals by injection of streptozotocin (65 mg/kg); the remaining 16 animals served as controls. Titanium alloy implants were placed in the tibiae of all 32 rats using standard surgical techniques. Implants healed for 14 days. Blood samples were obtained for serum glucose, osteocalcin, and alkaline phosphatase analyses. Implants were retrieved and processed for histomorphometric analyses. Three quantities were measured using light microscopy, video capture, and computer analysis: percent osseointegration (ie, linear bone interface), associated bone volume percent, and contact frequency. Diabetic animals demonstrated significantly less osseointegration than controls. However, bone volume percent in diabetic animals was about 4 times greater than controls. Biochemical analyses were mixed; diabetic animals demonstrated increased serum osteocalcin levels compared to controls but decreased alkaline phosphatase. Based on the results of this study, it was concluded that the bone response associated with titanium alloy implants in the tibiae of diabetic rats is uniquely different from controls. (Int J Oral Maxillofac Implants 2000;15:345–354)

Key words: *alkaline phosphatase, bone, bone histomorphometry, diabetes, implants, osseointegration, osteocalcin, titanium alloy*

Diabetes describes a disease generally related to abnormal uptake of blood sugar by cells. The disease affects almost 16 million Americans. Classically, the disease is defined as a "disorder of carbohydrate metabolism. . . with specific microvascular complications and accelerated atherogenesis. Functionally, it has been termed a complex metabolic derangement, characterized by either relative or absolute insulin deficiency."[1p637]

The literature documents a variety of systemic and metabolic changes associated with diabetes. Notable among these are the effects of diabetes on bone. In the laboratory, after diabetes is induced with an agent such as streptozotocin (STZ), bone formation decreases, as do the relative dimensions of the osteoid matrix.[2,3] Some authors conclude that this decreased bone formation occurs because bone resorption remains constant while bone production declines.[3–6] Proteoglycans are also affected in diabetic rats, with lower glycosaminoglycans concentrations in cartilage and aberrations in macromolecular side chains.[7] The relative concentrations of bone calcium and phosphorous remain the same as in controls, but bone volume decreases.[8,9]

[1]Assistant Professor, Department of Biomaterials, University of Alabama School of Dentistry, Birmingham, Alabama.
[2]Professor, Department of Biomaterials, University of Alabama School of Dentistry, Birmingham, Alabama.
[3]Professor, Nutrition Sciences Department, University of Alabama, School of Health Related Professions, Birmingham, Alabama.
[4]Associate Professor, Department of Biomedical Engineering, University of Alabama, School of Engineering, Birmingham, Alabama.

Reprint requests: Dr Michael McCracken, Department of Biomaterials, SDB 49, University of Alabama School of Dentistry, 1530 3rd Avenue South, Birmingham, AL 35294- 0007. Fax: 205-975-6108. E-mail: mikemc@uab.edu

Some researchers have attempted to modulate or reverse the effects of diabetes on bone production in STZ-treated rats. Sasaki et al,[2] for example, found abnormal osteoblast structure and function in diabetic rats, characterized by a 91% decrease in the osteoid zone and lower bone formation rates. These authors concluded that administration of tetracycline can restore osteoblast and osteoclast structure and function, and that bone and matrix production in tetracycline-treated animals more closely resembles controls.[2,10,11] Another substance, xylitol, appears to prevent bone loss associated with diabetes.[12]

The effects of diabetes on dental implant integration remain unclear. Although diabetes generally causes osteopenia, the presence of a biomaterial may alter the local tissue response, even if generalized osteopenia exists. Iyama et al[13] reported decreased bone associated with hydroxyapatite (HA) implants placed in diabetic rats. These findings are supported by Takeshita et al,[14] who reported a 50% reduction of bone volume in diabetic rats associated with HA implants. However, el Deeb et al[15,16] reported an increase in bone associated with HA implants in diabetic rats, whether placed in soft tissue adjacent to bone or in cranial subperiosteal locations.

Although it seems certain that induced diabetes affects bone metabolism generally, specific effects around implanted biomaterials require additional study. The purpose of this paper was to assess bone response to titanium alloy implants placed in the tibiae of diabetic rats.

MATERIALS AND METHODS

Animal Preparation

Animals were maintained in a AAALAC-accredited (American Association for Accreditation of Laboratory Animal Care) animal care and use program in accordance with the standards of the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Thirty-two male Sprague-Dawley rats weighing 350 to 375 g (4 months old) were divided into 2 groups of 16 each. All rats were weighed to the nearest gram. Food and water were provided throughout the experiment ad libitum. The room was maintained at 23°C, with 12-hour light-dark cycles.

One group was given a parenteral injection of streptozotocin (Sigma-Aldrich, St. Louis, MO), at a dose of 65 mg/kg diluted in phosphate-buffered saline, 5 days before surgery. On the day prior to implant surgery, urine samples were collected. Urine glucose was tested using urinalysis strips (Bayer Corporation, Elkhart, IN) to confirm the onset of diabetic symptoms. Five days after STZ injection, implants were placed. Implant sites healed for 14 days.

The rats were anesthetized using volatile gas (enflurane) and shaved, scrubbed, and draped to provide a surgical field. A 1.5-cm incision was made on the medial-proximal surface of the tibia above the tibial protuberance. Tissue was reflected to expose the flat portion of the tibia below the joint. Using a slow-speed surgical handpiece (Nobel Biocare, Yorba Linda, CA) with a No. 4 round bur and copious warm saline irrigation, a pilot hole was drilled in the tibia 8 mm proximal to the tibial protuberance. A 1.3-mm-diameter surgical implant drill bit was used to create an oblique-transverse osteotomy, traveling through the medullary canal and the opposite cortical shaft. Rather than drilling perpendicular to the bone, an oblique path of implant placement was used to optimize the implant surface area in the canal for each specimen (Fig 1). A No. 6 round bur was used to increase the size of the hole in the medial aspect of the tibia. The osteotomy was irrigated with 20 mL of warm saline.

Titanium alloy (Ti-6Al-4V) screws measuring 1.5 × 8 mm (Walter Lorenz Surgical Inc, Jacksonville, FL) were placed by hand to engage the opposite cortical shaft but did not engage the medial cortical shaft, which had been enlarged with the no. 6 round bur (Fig 2). Primary closure was achieved for each animal by approximating the muscle layers with sutures and closing the skin with surgical staples.

Approximately 200 μL of blood was obtained from each animal using a tail snip. Tail hemostasis was achieved with cautery. Blood samples were left at room temperature for 1 hour and spun; serum was then extracted and frozen. Most follow-up observations of the animals were within normal limits. However, 2 diabetic rats died during the course of the experiment as a result of their condition.

Histomorphometric Analysis

After healing for 14 days, the rats were euthanized with carbon dioxide inhalation. At this time, blood samples were again collected and spun for serum collection. Tibiae were removed, cleaned of soft tissue, and fixed in phosphate-buffered paraformaldehyde for 12 hours. Specimens were dehydrated with progressive alcohols under vacuum over 14 days, cleared with xylene, and infiltrated and embedded with Technovit 7200 (Exakt Technologies Inc, Oklahoma City, OK). Samples were prepared for light microscopy by cutting and grinding techniques (as described by Donath and Breuner[17]) using the Exakt system (Exakt Technologies Inc). The final sample thickness was less than 60 μm; slides that were

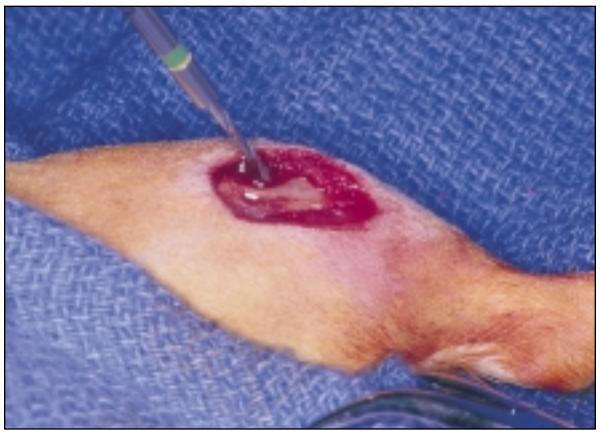

Fig 1 A surgical drill was introduced into the canal at an oblique angle to maximize the implant surface area exposed for osseointegration. The implant was stabilized by the opposite cortical plate. Copious saline irrigation was used during drilling to flush out the osteotomy.

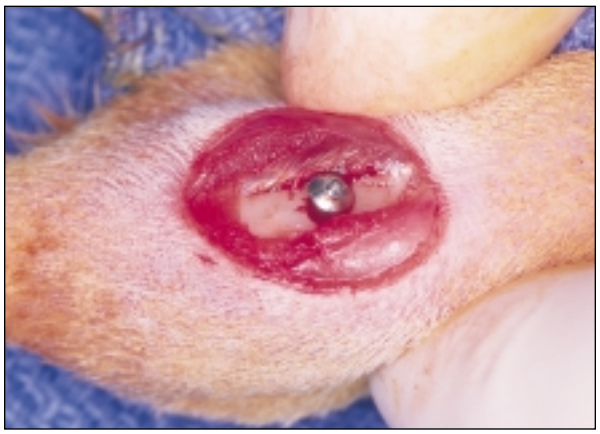

Fig 2 The implants were placed by hand into tibiae. The implants did not engage the proximal cortical plate, as the osteotomy was enlarged in this area, but they did engage the opposite cortical plate.

polished thinner yielded unpredictable retention of titanium implants in the section, which was necessary for measurement of osseointegration. Slides were stained with toluidine blue.

Samples were examined for histomorphometric analysis using an imaging system and microcomputer analysis, consisting of a Nikon light microscope (Nikon Corp, Tokyo, Japan), a Sony 3-chip CCD videocamera (Sony Corporation of America, New York, NY), a microcomputer, and a video capture board (Scion Corporation, Frederick, MD). Images were analyzed using NIH Image software (National Institutes of Health, Bethesda, MD).

Three different quantities were determined for each sample: percent osseointegration, bone volume percent around the implant, and frequency of bone contact along the implant surface. Percent osseointegration and bone volume were measured in 5 zones around the implants (Fig 3). Percent osseointegration (linear bone interface) was defined as the length of bone contacting the implant, divided by the length of the implant surface. Bone contact was defined as no visible gap at the light microscopic level; for this study, this represents any bone within 10 μm of the implant surface. Bone volume percent was defined as the area occupied by bone within 1.5 mm of the implant surface, divided by the total area of that region. Bone was identified by thresholding and digitizing techniques to minimize operator error. Total count was the frequency of bone nodes (areas of calcification) that were touching the implant. This is an integer for each implant and measures the frequency of contact, rather than volume or length of bone.

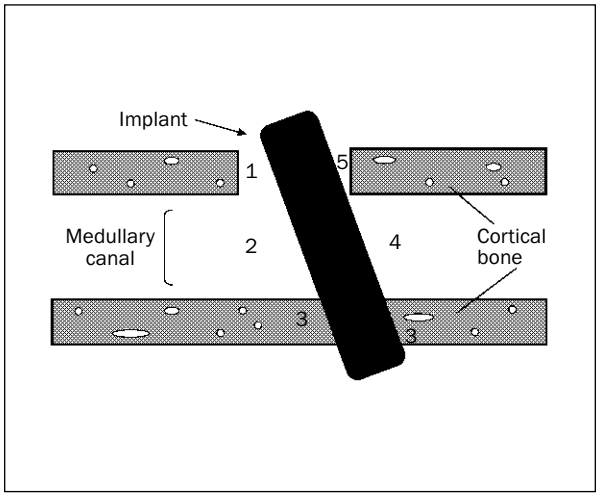

Fig 3 Schematic of implant placed in rat tibia. Zone 1 is the proximal portion of the medial cortical plate. Zone 2 is the proximal medullary canal. Zone 3 is the opposite cortical plate (proximal and distal aspects). Zone 4 is the distal medullary canal. Zone 5 is the distal portion of the cortical plate.

Blood samples taken at the time of surgery and sacrifice were analyzed for serum glucose, osteocalcin, and alkaline phosphatase. Serum glucose was measured using colormetric serum glucose analysis (Sigma-Aldrich). Osteocalcin was measured using an enzyme-linked immunosorbent assay technique specific for rat osteocalcin (Biomedical Technologies Inc, Stoughton, MA). Alkaline phosphatase was measured using p-Nitrophenyl phosphate colormetric determination (Sigma-Aldrich). All tests were performed per manufacturers' instructions.

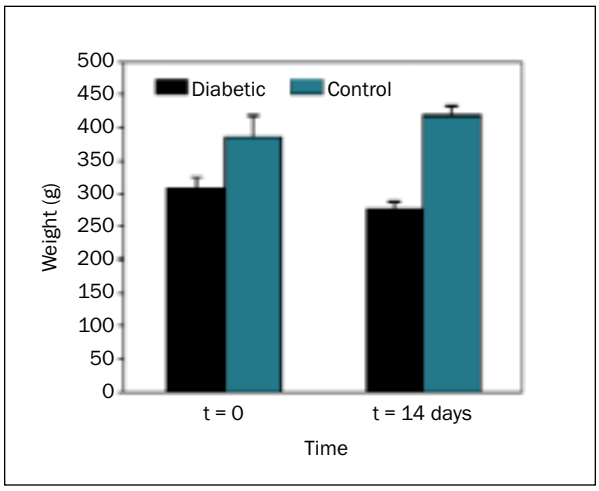

Fig 4 Weight of animals over time (± SE). Controls weighed significantly more than diabetic animals at both times measured ($P < .0001$).

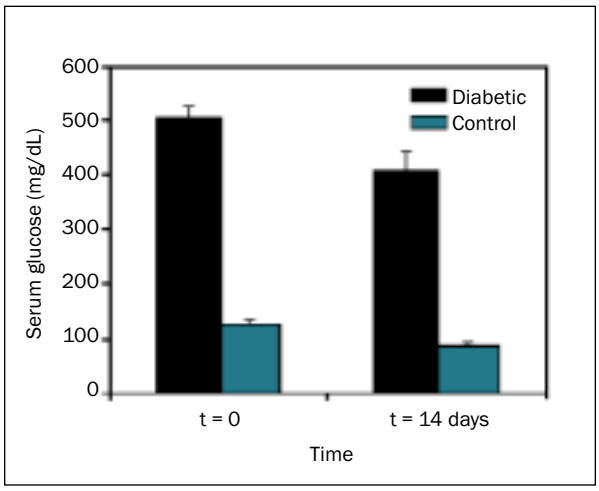

Fig 5 Blood glucose levels measured in diabetic and control rats over time (± SE). At both times, glucose levels in diabetic rats were significantly greater than in controls ($P < .0001$). Also, glucose levels in diabetic rats dropped significantly over time ($P < .01$).

Data were analyzed using StatView software (SAS Institute, Cary, NC). Comparisons of different measurements between groups were analyzed using multivariate analysis of variance (MANOVA). Since this test indicated a significant difference between groups, particular areas of analysis were examined with Fisher's PLSD post hoc analysis. Results were considered significant at the $\alpha < 0.05$ level.

RESULTS

Symptoms in the diabetic rats included weight loss, polyuria, polyphagia, and polydipsia. Diabetic animals weighed significantly less than controls (Fig 4). During the course of the experiment, control animals gained weight, while diabetic animals continued to lose weight. Diabetic animals showed significantly greater serum glucose levels than controls ($P < .00001$). Serum glucose levels are shown in Fig 5.

Histomorphometric Analysis

Diabetic animals developed total osseointegration of 16.2%, significantly less than controls, which had 24.5% integration ($P < .01$) (Fig 6). Measurements from each zone along the implant and associated standard errors are shown in Fig 6. Bone volume associated with implants placed in diabetic rats was dramatically greater than in controls. Total bone volume in diabetic rats was 25%, compared to 6.2% in controls, a significant difference ($P < .0001$) (Fig 7). Diabetic animals also demonstrated significantly greater amounts of bone volume in the neck region, where bone volume measured 48%, compared to 18% for controls. In Zone 2, diabetics demonstrated a bone volume of 32%, while controls produced only 4%, which was significantly different ($P < .0001$). Interestingly, bone volume in Zone 4 in diabetic rats was not significantly different from bone volume in controls.

Osteocalcin measurements for diabetic animals were lower than in controls, as measured on the day of surgery and 14 days after surgery (Fig 8). At the time of surgery, controls had an average of 81 ng/mL of serum before surgery, while diabetic rats had 26 ng/mL. After healing for 14 days, both controls and diabetic animals showed a decrease in serum osteocalcin levels; controls dropped to 53 ng/mL, and diabetic samples dropped to 16 ng/mL (Fig 8). Serum alkaline phosphatase levels in the diabetic rats were significantly higher than in controls ($P < .001$). Serum alkaline phosphatase (sigma units/mL) for diabetics was 23.1 (± 0.2), while controls measured 3.4 (± 0.6).

Histologic Description

Specimens were examined using a light microscope at 40× and 100× magnification. The entire tibia was visible in most cases, permitting examination of both cortices, the implant screw, marrow spaces,

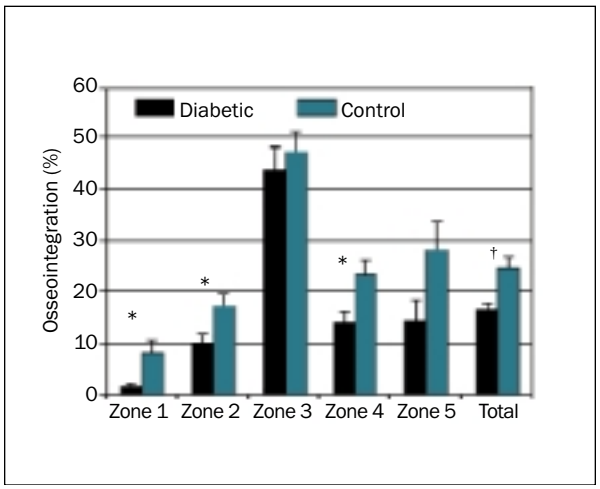

Fig 6 Osseointegration of implants in diabetic and control rats (± SE). Diabetic rats demonstrated significantly less osseointegration in Zone 1, Zone 2, and Zone 4 (*$P < .05$). Total diabetic osseointegration was also less than in controls (†$P < .01$).

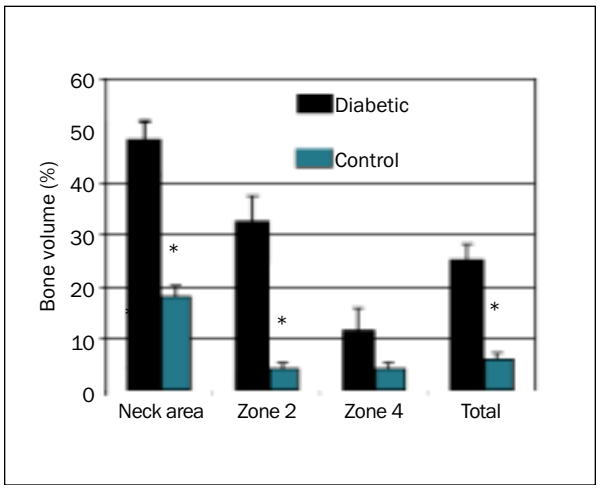

Fig 7 Bone volume associated with implants. Total bone volume associated with diabetic subjects was more than 4 times greater than in controls (*$P < .0001$). Diabetic animals demonstrated significantly more bone volume in the neck region, as well as in Zone 2 (*$P < .0001$). Bone volume in Zone 4 was not significantly different.

and the growth plates at the tibial joint. Toluidine blue stains chromatically, so that mature bone stained blue and new bone stained a darker violet.

Controls. When the implant was viewed from the epiphyseal plate, spicules of bone appeared in the marrow space (Fig 9). Typically, these spicules were within 400 µm of the implant surface in controls. This bone stained a dark blue and corresponded to the color of the newly formed bone near the epiphyseal growth plate. The bone was unorganized and woven in nature, and osteocytes were occasionally evident. The bone was similar in nature in the distal medullary canal adjacent to the implant. Within the thread spaces of the implant, new bone formed in slightly greater quantities than away from the implant.

Adjacent to the implant, some spicules of bone touched the implant so that no gap was present. This represents areas of osseointegration (Fig 10). The implant apparently acted as a substrate for bone growth, as more bone appeared to be present along the surface of the implant than in the medullary space directly adjacent to the implant. This bone along the surface of the implant appeared to be loosely organized woven bone, with osteocytes present.

At the neck of the implant, in Zone 1, the surgical margin of the osteotomy was defined by a straight line cut and light blue staining characteristic of mature bone (Fig 11). The cortical plates consisted of compact lamellar bone beneath a cortical shell. In the surgical margin between the cortical bone and the implant, new bone—staining dark blue—grew in the gap. Sometimes the bone approached the surface of the implant, but generally extended only part of the way from the cortical plate to the implant. In Zone 5, because of the geometric position of the implant and angulation, the cortical

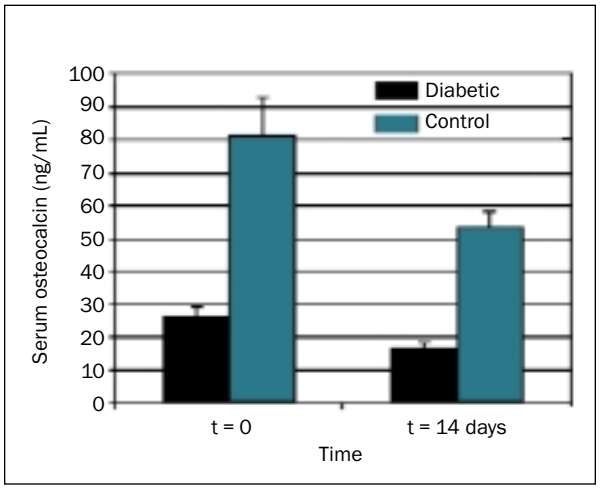

Fig 8 Serum osteocalcin levels in diabetic and control rats (± SE). Controls showed significantly more osteocalcin at both time intervals ($P < .001$). Higher levels of osteocalcin, a marker for bone turnover, indicate greater bone activity.

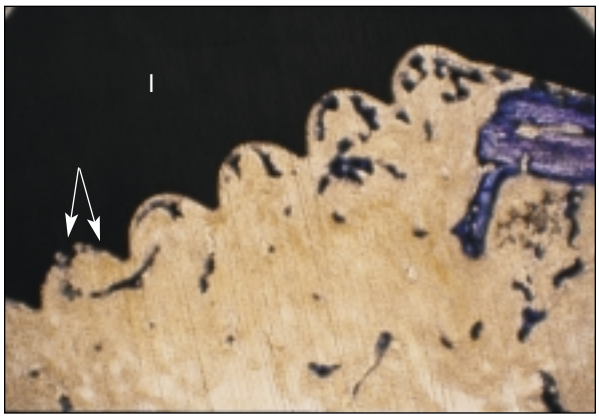

Fig 9 Bone response to a control implant. In this specimen, more bone is associated with the implant (I), yet the response remains modest. The areas of bone growth tend to be relatively large and few in number. Some areas of osseointegration can be identified (*arrows*), but the bone generally does not touch the implant. This photograph represents a strong bone response for a control implant (toluidine blue; original magnification ×40).

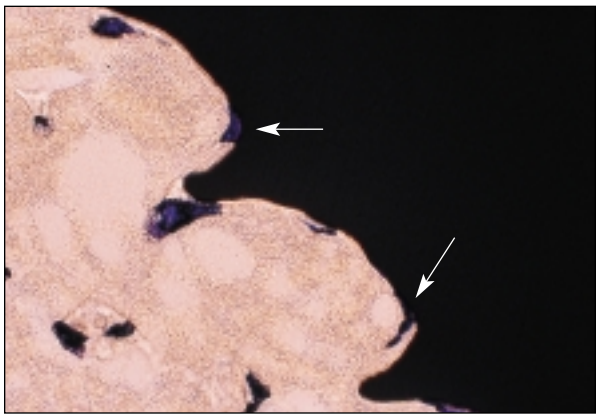

Fig 10 Osseointegration of a control implant. In this section, bone can be seen touching the implant in several areas (*arrows*). The volume of bone present adjacent to the implant, however, is not impressive (toluidine blue; original magnification ×100).

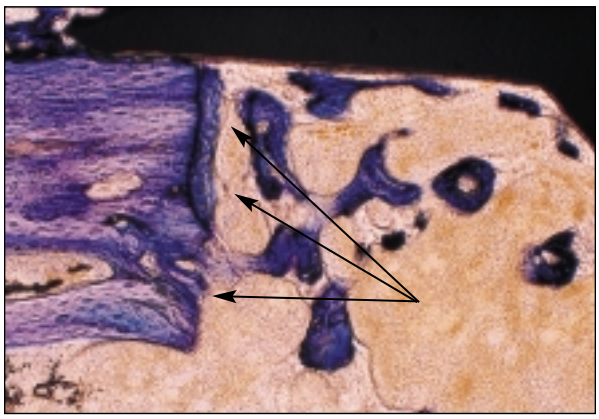

Fig 11 Neck of control implant in cortical plate. The bone response at the neck of the implant demonstrates some bone formation in the space created by the osteotomy. A line of demarcation created by the osteotomy is noticeable (*arrows*). New bone growth stains a slightly deeper blue than existing cortical bone (toluidine blue; original magnification ×40).

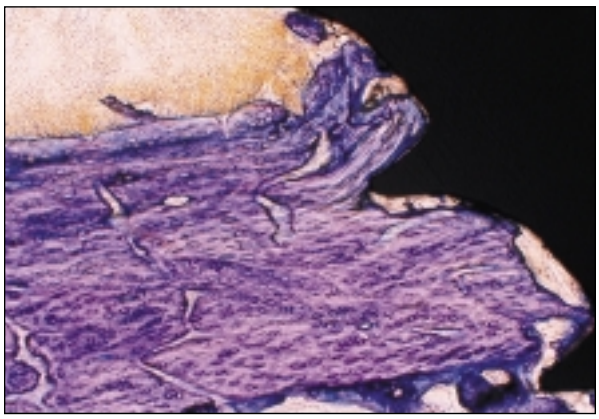

Fig 12 Control implant in opposite cortical plate. The implant was rigidly fixed in the opposite cortical plate. As such, osseointegration was consistently high in this region, Zone 3 (toluidine blue; original magnification ×40).

bone frequently approached the surface of the implant and demonstrated osseointegration. The surgical margin in this zone was readily apparent because of the different staining and morphometric characteristics.

In Zone 3, the implant was anchored in dense cortical bone, with primary fixation achieved at the time of surgery (Fig 12). New bone could be seen in gaps between the mature bone and the implant. In many places, the mature bone was lined with new bone (2 to 3 cells thick) and osseointegrated with the implant. The mature bone itself osseointegrated in places. Some remodeling and appositional growth in this region was evident from the location of new bone growth.

Outside the normal position of the periosteum, where the implant head was located or the implant tip extruded from the bone, evidence of callus formation existed. The callus seldom covered the implant completely but generally formed a characteristic dome shape on both sides of the implant. At this stage in the healing process, much of the callus had ossified in places, staining a color similar to areas of new bone growth in medullary canals.

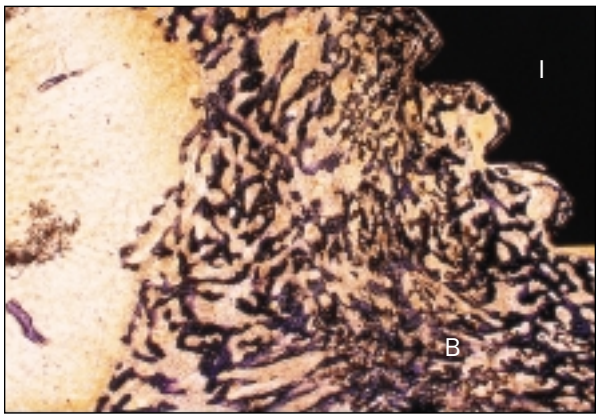

Fig 13 Diabetic bone response. A tremendous bone response (B) around implants (I) was consistently noted in specimens. The new bone displayed random architecture and stained deep blue. Osteocytes could be identified, but the bone was less organized than in control specimens (toluidine blue; original magnification ×40).

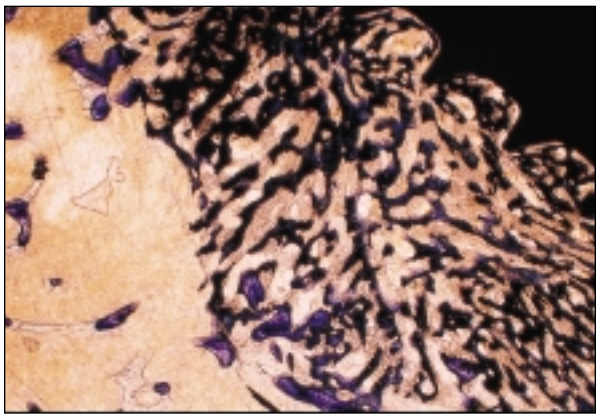

Fig 14 Diabetic bone response (2). A consistently aggressive bone growth was noted in these specimens (toluidine blue; original magnification ×40).

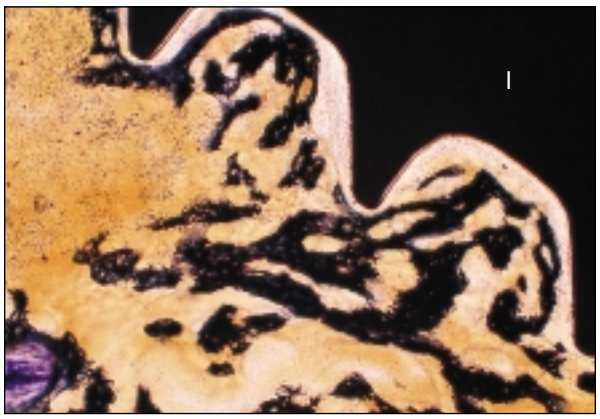

Fig 15 Osseointegration of implants in diabetic rats. Bone approached implants (I) in this sample population but generally did not osseointegrate (toluidine blue; original magnification ×100).

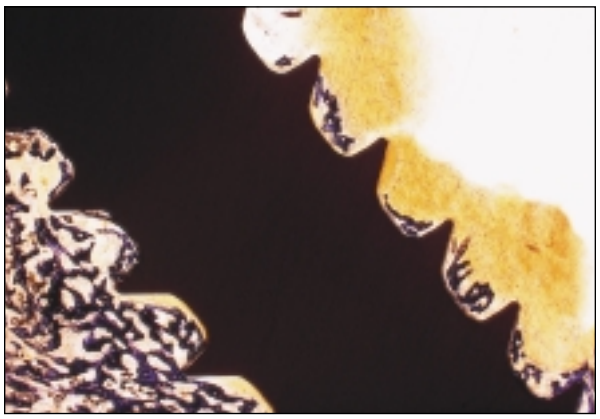

Fig 16 Proximal and distal bone response in diabetic animals. Greater bone volume occurred consistently in the proximal medullary canal than in the distal canal. In this figure, the proximal half of the section displays the typical bone response associated with this population. This response lacks symmetry, however, as the distal portion displays less bone volume. Also, an acellular region is noted in the middle of the canal (toluidine blue; original magnification ×40).

Diabetic Rats. The diabetic samples were immediately recognizable by massive and aggressive bone growth around the implants in the proximal medullary canal (Figs 13 and 14). This bone generally extended from the cortical plate at the neck of the implant and occupied a volume up to 1.5 mm from the implant surface. The bone was primarily woven in nature and loosely organized. It stained a deep blue-black. Osteocyte-like structures were present, but the bone lacked a familiar histologic structure. It somewhat resembled bone associated with an osteosarcoma or other malignant processes associated with bone.

In very few areas in the medullary canals did the bone touch the implant (Fig 15). Typically, the bone grew into the thread spaces but remained distinctly separate from the surface, outlining the implant but not touching it. This was a consistent feature among diabetic specimens.

The proximal medullary canal (Zone 2) appeared similar to controls in its cellular makeup. However, the distal canal (Zone 4) was markedly acellular, with only a small layer of cells lining the walls of the cortices (Fig 16). Also, the epiphyseal growth plate appeared consistently thinner than controls.

DISCUSSION

The diabetic-like symptoms induced by injection of STZ have been well established.[18–21] In this study, 4 days after injection of STZ, rats weighed 20% less than controls; at the end of the experiment, diabetic rats weighed 34% less than controls. While controls gained weight over the course of the experiment, diabetic animals continued to lose weight. These weight changes are typical for this dose of STZ.[18]

A blood glucose level in rats of approximately 500 mg/dL 4 days after STZ injection (65 mg/kg) agrees with other published reports.[3,6,18] However, the significant decrease in blood glucose levels after 14 days in this study suggests that the diabetic rats acclimated to the systemic effects of the STZ in some way. Controls also showed a slight decrease in mean glucose levels, but this difference was not significant and may be a result of normal diurnal changes in blood glucose.[18]

Induced diabetes offers an adverse systemic environment characterized by weight loss and high blood glucose levels. In such an environment, poor healing exists and has been associated with microvascular disturbances and improper cell function related to a high-glucose environment.[22] Typical changes noted in bone healing include diminished bone formation, lower bone turnover, lower mineral density in newly formed bone, and delayed fracture healing.[3,5,6,8,9,23] It is not surprising, therefore, that diabetic rats in this study demonstrated less osseointegration of titanium alloy implants.

In this study, controls osseointegrated more than diabetic animals in Zone 1, which was an area of cortical bone. Zone 1 was designed to be larger than the diameter of the implant so that the cortical bone would not be touching the implant after placement. In practice, it was found that the implant frequently contacted a region of the cortical bone because of the angulation of implant placement. However, the contact occurred in Zone 5, again as a result of surgical technique and angulation. In this way, Zone 1 and Zone 5 presented uniquely different healing environments. It may be for this reason that a significant difference in osseointegration existed in Zone 1, with controls showing significantly more osseointegration, while there was no difference between groups in Zone 5.

Zone 3 consisted of cortical bone with initial implant contact. In this area, the osteotomy was smaller than the implant, and initial stabilization of the implant occurred. This region exhibited the highest percent osseointegration in both groups. Conditions for healing are more favorable in the dense cortical bone, with an ample blood supply and bone cells present for proliferation. Often, osseointegration in cortical bone exceeds osseointegration in cancellous bone or medullary areas.[14,24] In this region of favorable healing, no difference was noted between groups.

In Zone 2 and Zone 4, osseointegration of controls exceeded that of diabetic animals by about 70%. The amount of osseointegration of controls in this region corresponds to values reported in the literature.[25,26]

To summarize the observations of osseointegration, where intimate contact between the implant and cortical bone occurred at the time of surgery, no significant difference was noted between diabetic and control groups. However, where no bone contact existed at the time of surgery, such as in the medullary canal, control specimens demonstrated significantly greater amounts of osseointegration. This supports the hypothesis that healing is impaired in some regions in uncontrolled diabetic animals. However, the data also suggest that osseointegration can occur when initial bone contact is present.

Analysis of bone volume surrounding the implants was surprising and striking. The total amount of bone surrounding implants in diabetic animals was approximately 4 times greater than controls and 8 times greater in Zone 2 (Fig 7). The results were highly significant ($P < .0001$). Published reports differ in their conclusions in this area, with some authors finding a decrease in bone volume adjacent to implants in diabetic animals and others reporting an increase.[13–16] The reason for this divergence is unclear, though it may be related to biomaterial effects or species-specific reactions. A simple regression analysis of the data in this study shows a correlation between high blood glucose levels and increased volume of bone associated with implants. Although the predictive value of this simple regression is small ($R^2 = 0.47$), the probability of relationship is significant ($P = .02$). This offers more evidence to suggest that blood glucose levels do affect the bone volume response to titanium alloy implants.

Interestingly, bone volume in the distal medullary canal (Zone 4) was not greater in diabetics than in controls. This suggests an anatomic variation in healing patterns in diabetic animals and may be a result of regional blood supply. The distal aspect of the osteotomy is further from the central blood supply and was partially disrupted by the surgery. The proximal blood supply remained intact after surgery. Also, occlusion of the canal by the implant itself may prevent new blood vessels from readily forming. The number of bone contact areas on the distal of the implant was lower than in controls, which may also suggest a decreased blood supply.

Serum osteocalcin levels in these diabetic rats were significantly lower than in controls. Osteocalcin serves as a marker for bone turnover and is typically depressed in diabetic rats because of a general decrease in bone cell activity.[4,27,28] In this study, serum osteocalcin levels were approximately 60% lower than in controls. Therefore, the increased bone production around the implants in diabetic rats is not reflected in serum osteocalcin levels compared to normal controls. The systemic decrease present in diabetic animals appears to overshadow any contribution that may be made by local bone activity around the implant. A more rigorous test might be to compare osteocalcin levels of diabetic animals with implants to those in diabetic animals without implants. If a difference is seen in this comparison, some correlation between serum osteocalcin levels and localized bone healing may be realized.

In contrast, serum alkaline phosphatase levels were much higher in diabetic rats than in controls. Alkaline phosphatase also serves as an indicator of bone formation since it is produced by cells that differentiate into osteoblasts.[29] It is commonly used as a measure of cytokine impact on osteoblasts.[27,30–35] One author reported a systemic increase in the levels of serum alkaline phosphatase in diabetic rats relative to controls,[5] which corresponded to increased serum calcium content. The authors offered no explanation for the increase in alkaline phosphatase. Another article also reported an increase in serum alkaline phosphatase about 10 times greater than in controls,[6] which correlates with the values presented in this study. However, the authors described a simultaneous decrease in bone alkaline phosphatase.[6] Some literature suggests that the increase in serum alkaline phosphatase seen in diabetic rats may result from an increased production of intestinal alkaline phosphatase.[36,37]

The depressed levels of osteocalcin and increased levels of alkaline phosphatase suggest abnormal bone activity in the diabetic animals compared to controls. Although it appears that the effects noted result from systemic actions, these findings might offer insight into the activity of bone cells at the implant interface. Specifically, the imbalance of these biochemical markers may help explain the imbalance seen between bone growth and poor osseointegration at the implant interface, especially when studied at the local rather than the systemic level.

CONCLUSIONS

The following conclusions are drawn from this study.

1. Rats with diabetic symptoms induced by STZ injection demonstrated less osseointegration of titanium alloy implants, particularly in the medullary canal area.
2. Compared to controls, diabetic rats showed a dramatic increase in bone volume associated with the implants.
3. Serum osteocalcin levels were decreased in these diabetic animals, while serum alkaline phosphatase levels were elevated.

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