Type 2 Diabetes Impairs Implant Osseointegration Capacity in Rats

Hideki Hasegawa, DDS, PhD[1]/Shogo Ozawa, DDS, PhD[2]/Kazuyoshi Hashimoto, DDS, PhD[3]/
Takuro Takeichi, DDS, PhD[4]/Takahiro Ogawa, DDS, PhD[5]

Purpose: *The effect of type 2 diabetes mellitus (adult-onset non–insulin-dependent), which is the most common form of diabetes in humans, on osseointegration capacity has not been addressed in an appropriate animal model. This study histologically and histomorphometrically examines bone healing around titanium implants in the type 2 diabetes rat model.* ***Materials and Methods:*** *Titanium implants with a chamber were placed into the femurs of normal male rats and genetically modified male rats with a close symptomatic resemblance to human type 2 diabetes, as characterized by late-onset hyperglycemia and obesity. Cross-sectional histology for the tissue grown into the implant chamber was examined.* ***Results:*** *Bone volume around implants was consistently (from weeks 4 to 8 postimplantation) smaller for the diabetes group than for the control group in the cortical area, while the bone volume in the marrow area was not affected by the diabetes. Bone-implant contact percentage was considerably lower for the diabetes group in both the cortical and marrow areas, with the week 4 bone-implant contact in the cortical area being 12% for the diabetes group and 61% for the control group. A 2-fold difference remained at week 8. Bone morphogenesis in the diabetic rats was characterized by fragmented bone tissues and extensive soft tissue intervention.* ***Conclusions:*** *Type 2 diabetes mellitus impaired osseointegration capacity disproportionally between the cortical bone and bone marrow areas. The reduction of the bone quantity in the cortical area and the bone-implant contact in both the cortical and marrow areas was remarkable.* INT J ORAL MAXILLOFAC IMPLANTS 2008;23:237–246

Key words: *blood glucose, bone histomorphometry, bone-titanium integration, endosseous titanium implant, risk factor*

It has been proven that titanium is a biocompatible material, and the use of endosseous titanium implants as an anchor has become a standard effective treatment modality to restore missing teeth and maxillofacial defects. However, the application of implants is still limited because of various risk factors, including host bone quality and quantity,[1] smoking,[2] age,[3] and systemic conditions.[4] Among the systemic conditions, diabetes mellitus is a major metabolic disease that significantly affects bone metabolic potential. There are 20.8 million children and adults in the United States with diabetes; they constitute about 7% of the population. The effect of diabetes on fracture healing has been well documented experimentally and clinically. Diabetes delays and impairs healing and remodeling in limb bone[5–9] as well as in the jaw and cranial bone.[10,11]

Diabetes mellitus, which is characterized by high levels of blood glucose, consists of type 1 (insulin-dependent) and type 2 (non–insulin-dependent) varieties. Type 1, previously called juvenile-onset diabetes, is induced by damage to the insulin-producing cells and is usually diagnosed in children and young adults, while type 2, previously called adult-onset diabetes, accounts for about 90% to 95% of all diag-

[1]Visiting Lecturer, Department of Fixed Prosthodontics, Aichi Gakuin University School of Dentistry, Nagoya, Japan.
[2]Associate Professor, Department of Maxillofacial Prosthodontics, Aichi Gakuin University School of Dentistry, Nagoya, Japan.
[3]Associate Professor, Department of Fixed Prosthodontics, Aichi Gakuin University School of Dentistry, Nagoya, Japan.
[4]Assistant Professor, Department of Fixed Prosthodontics, Aichi Gakuin University School of Dentistry, Nagoya, Japan.
[5]Associate Professor, The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, UCLA School of Dentistry, Los Angeles, California.

Correspondence to: Dr Takahiro Ogawa, The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, UCLA School of Dentistry, 10833 Le Conte Avenue (B3-081 CHS), Box 951668, Los Angeles, CA 90095-1668. Fax: +310 825 6345. E-mail: togawa@dentistry.ucla.edu

nosed cases of diabetes and is associated with older age, obesity, family history of diabetes, impaired glucose metabolism, and race/ethnicity. Although it is unclear whether diabetes mellitus is a critical determinant for unsuccessful implant therapy, clinical studies reported that type 2 diabetic patients showed higher implant failure rates than nondiabetic patients.[7,12,13] It seems agreed that certain oral hygiene procedures, such as the use of chlorhexidine rinses and premedication with antibiotics, and patients' motivation to employ them are important to obtaining successful outcomes in diabetic patients.[7,14]

The effect of diabetes on the osseointegration capacity of titanium implants has been studied using animal models. The percentage of bone-implant contact was decreased by over 50% in diabetic rats[15–17] and did not reach the level equivalent to the normal controls during the entire experimental period of up to 80 days postimplantation. The bone volume around implants was also reduced by approximately 50%.[15] Diabetes mellitus negatively influenced the mechanical retention of implants placed in the rabbit tibia.[18] These reports consistently suggested that diabetes mellitus impairs the osteogenic potential around implants; however, the diabetic condition in these studies was induced by intraperitoneal injection of streptozotocin, which irreversibly damages insulin-producing β-cells in the pancreas islet 10. Although this is a proven model for type 1 (insulin-dependent) diabetes, it does not represent the common diabetic status for which human dental implant therapy is considered. Streptozotocin-induced (type 1) diabetic rats show considerably lower weight (over 50% lower depending on the age of animals) than the untreated group,[16] while type 2 diabetes is normally associated with obesity. The effect of type 2 diabetes, which represents the majority of diabetes cases, on the process of osseointegration has been virtually uninvestigated with appropriate models.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats were developed and established as a genetically modified type 2 diabetes animal model symptomatically analogous to human type 2 diabetes.[19–22] Due to the recessive function of multiple genes, the OLETF rat features *(1)* late onset of hyperglycemia (after 18 weeks of age); *(2)* a chronic course of the disease; *(3)* progressive obesity; and *(4)* clinical onset of diabetes mellitus, mostly in the males.[23] This study histologically and histomorphometrically examined bone healing around titanium implants in this type 2 diabetes rat model. The hypothesis to be tested was that non–insulin-dependent diabetes mellitus delays the process of osseointegration or impairs host capacity of osseointegration.

MATERIALS AND METHODS

Titanium Implants and Surface Analysis

Experimental implants with a rectangular inner chamber (3.0 mm × 2.5 mm × 0.8 mm) were fabricated from commercially pure titanium by electrical discharge machining (Fig 1a). The chambered implant was originally designed by the University of Toronto.[24] It allows tissue to grow into the chamber, which makes it easier to analyze purely de novo tissue. Its usefulness for bone histomorphometry and molecular analysis was established in previous studies.[25,26] Surface morphology of the implants was examined by scanning electron microscopy (SEM; JSM-5900LV, Joel Ltd, Tokyo, Japan).

Animals

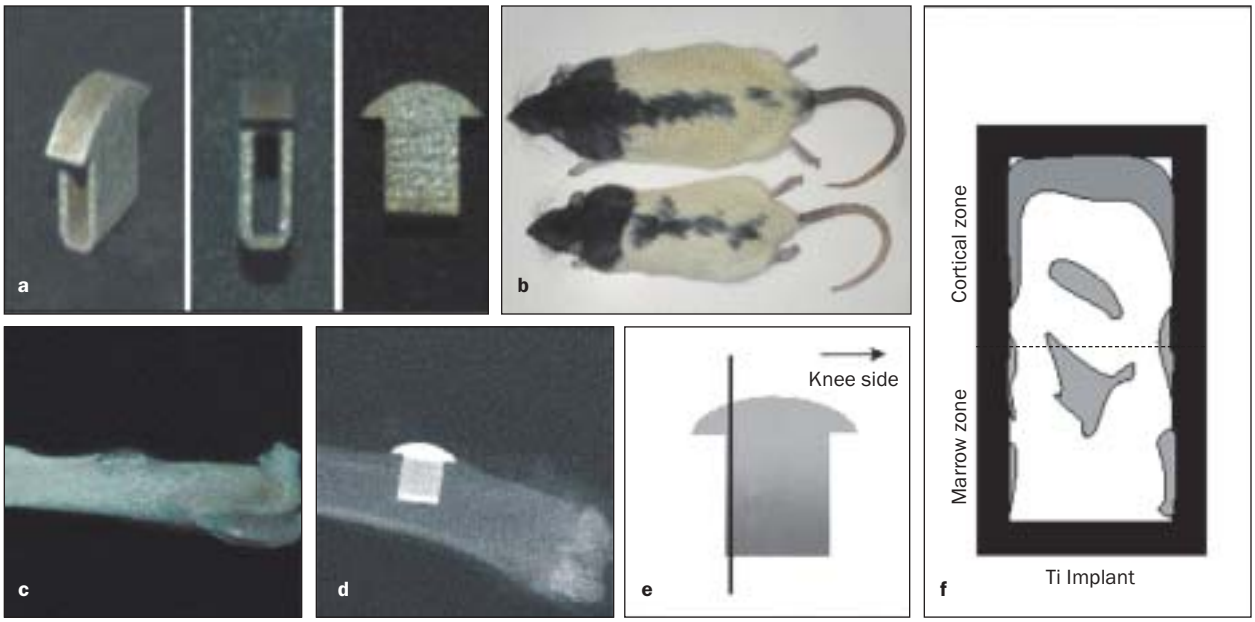
Thirty 38-week-old male rats, 15 OLETF rats, and 15 control animal (Long-Evans Tokushima Otsuka [LETO]) rats were purchased from Tokushima Research Institute (Otsuka Pharmaceutical, Yokushima, Japan; Fig 1b). The induction of high blood glucose and related complications of diabetes, such as progressive obesity, have been established in the type 2 diabetes rat model.[19–23]

Implant Surgery

The implants were cleaned with acetone and 70% ethanol and sterilized by autoclaving. Rats were anesthetized with an intramuscular injection of ketamine (7.5 mg/100 g body weight). After the leg was shaved and decontaminated with 10% povidone-iodine, the dorsal surface of the left femur was exposed. The initial pilot osteotomy was made by slow-speed drilling at a distance of 10 mm from the distal edge of the femur (Fig 1c). The osteotomy was expanded and finalized with a chisel (Aesculap, Center Valley, PA) with its width adjusted to the implant size. The implant was inserted until the implant umbrella structure reached the femur exterior, and stability was confirmed with a passive mechanical fit. Muscle and skin openings were closed separately. The lack of implant engagement into the cortical bone structure on the bottom side of the femur was confirmed by radiographic examination at the time of sacrifice (Fig 1d). This study protocol was approved by the Aichi-Gakuin University Animal Research Committee.

Histologic Preparation

Five rats from each group were sacrificed at 4 weeks postsurgery, 5 at 6 weeks postsurgery, and 5 at 8 weeks postsurgery. The bodies were then perfused through the abdominal aorta with a solution of 4% formaldehyde and 2% glutaraldehyde. Next, the femur was harvested and further fixed in 10%



Figs 1a to 1f Experimental titanium implants, surgical and histological procedures. *(a)* T-shaped implants with roof structure and inner chamber. *(b)* An OLETF rat (top) and a control (LETO) rat (bottom). Note that OLETF rat shows obesity. *(c)* The implant site. The flat surface of the distal femur was selected for implant placement. The implant is placed up to the roof structure level at 10 mm from the distal end. *(d)* A radiographic image of the implant placed in the femur at week 4 postimplantation. The complete insertion to the roof structure level and a lack of implant contact to the inferior side of the cortical bone was confirmed. *(e)* The embedded specimens were sawed perpendicular to the exposed implant roof structure at a site 0.5 mm from the proximal end of the implant, which produced cross sections parallel to the inner chamber opening. *(f)* A schematic representation of a histological section and zones for histomorphometry. Newly formed bone inside the chamber was analyzed in the upper half of the chamber (cortical zone) and lower half of the chamber (marrow zone).

buffered formalin for 2 weeks at 4°C. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in light-curing epoxy resin (Technovit 7200 VLC; Heraeus Kulzer, Wehrheim, Germany) without decalcification. The embedded specimens were sawed perpendicular to the exposed implant roof structure at a site 0.5 mm from the medial end of the implant (Fig 1e), which produced cross sections parallel to the inner chamber opening (Fig 1f). The specimens were ground to a thickness of 30 μm with a grinding system (Exakt Apparatebau, Norderstedt, Germany). The sections were stained with Goldner's trichrome stain and observed with a light microscope.

Bone Histomorphometry

A 20× magnification lens and 2× zoom on a computer display were used for computer-based histomorphometric measurements (Image Pro-plus; Media Cybernetics, Silver Spring, MD). To identify the details of the tissue structure, microscopic magnification up to 100× was used.

The following morphometric variables were analyzed:

- Percentage of total bone area = (bone area in the implant chamber)/(area of the chamber) × 100
- Percentage of bone area in cortical zone = (bone area in cortical zone)/(area of cortical zone) × 100
- Percentage of bone area in marrow zone = (bone area in bone marrow zone)/(area of bone marrow zone) × 100, where the cortical and marrow zones were defined as the upper and lower halves of the implant chamber, respectively (Fig 1f).
- Percentage of bone-implant contact in total (%) = (sum of the length of bone-implant contact)/(circumference of the inner chamber) × 100
- Percentage of bone-implant contact in the cortical zone
- Percentage of bone-implant contact in the marrow zone

Bone-implant contact was defined as anywhere that bone tissue was located within 10 μm of the implant surface without any intervening soft tissue.

Statistical Methods

A 2-way or 1-way analysis of variance (ANOVA) with $P < .05$ as the level of significance was applied to determine the effect of the healing time and diabetes on the histomorphometric variables. At each time point, the variables from the control and diabetes groups were compared using a Student *t* test.

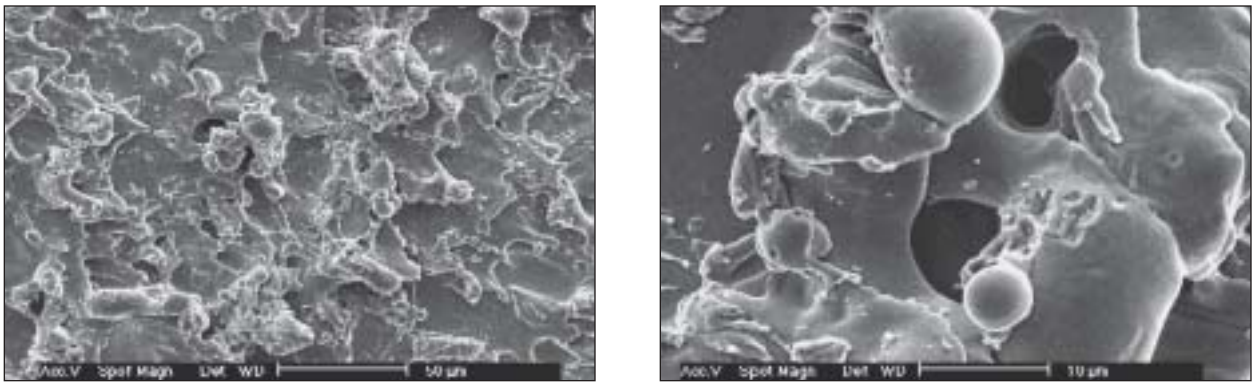


Fig 2 Surface morphology of titanium implants. *(a)* Low-magnification image and *(b)* high-magnification image.

RESULTS

Surface Topography of the Implant

SEM examination showed that the roughened surfaces of the implants consisted of structures irregular in shape but uniformly distributed (Fig 2). The irregular structures consisted of spheres, squama-like components, and pores ranging from 10 μm to 50 μm in size. High-magnification SEM revealed that the surfaces of the irregular structures were smooth at the micron scale (Fig 2).

Histologic Observation

At week 4, newly formed bone tissue was observed along the implant surface in both the control and diabetes groups (Fig 3). The middle of the chamber was filled with fatty bone marrow consisting of hematopoietic tissue. The appearance of the de novo bone tissue differed between the upper and lower halves of the implant chamber; the upper half of the implant chamber (cortical zone) was filled with more bone than the lower half (marrow zone). Also, the bone tissue in the upper half appeared thicker and more mature, indicating the growth of cortical tissue-stemmed bone into the area. The bone tissue in the cortical zone was thicker for the control group than for the diabetes group. The thickness ranged from 300 μm to 500 μm for the control group and from 100 μm to 300 μm for the diabetes group. In the control rats, an extensive area of bone tissue was in direct contact with the implant surface in the cortical zone, but there was only a limited area of bone-implant contact in the marrow zone. In the diabetes group, most of the bone tissue was accompanied by soft tissue at the implant interface, which prevented the establishment of direct bone-implant contact.

At week 6, the newly formed bone tissues appeared smaller in thickness than those observed at week 4 (Fig 3). A similar trend was found for the bone morphogenesis between the 2 groups; the bone tissue in the cortical zone was thicker for the control than for the diabetes group, while the bone tissue in the marrow zone showed an equivalent thickness between the control and diabetic rats. The bone tissue along the implant surface appeared continuous in the control group, while the bone tissue in the diabetes group appeared fragmented. Direct contact of bone to the implant was found more frequently in the control than in the diabetes group.

At week 8, the quantity of newly formed bone was further diminished. Instead, the thin bone tissue extensively encapsulated the implant surfaces in both the control and diabetes groups (Fig 3). Although the thickness of the bone was equivalent between the groups, ranging from 80 μm to 150 μm, the bone tissue in the diabetes group appeared less continuous in structure. An extensive area of bone was in direct contact with implant in the control group, while the bone in the diabetes group was largely intermixed with soft tissue at the implant interface.

Typical high-magnification images of the week 8 sections confirmed direct bone-implant contact in both the cortical and marrow zones for the control groups (Fig 4), while the intervention of the soft tissue was found extensively between the bone and implant in the diabetes group (Fig 4).

Histomorphometric Outcome

All 3 of the bone-area variables (total bone area, bone area in cortical zone, bone area in marrow zone) were significantly decreased with the healing time (2-way ANOVA, $P < .01$; Figs 5a to 5c). There were significant differences between the control and diabetes groups in total bone area and bone area in the cortical zone (2-way ANOVA, $P < .01$) consistently at weeks 4 through 8, with the diabetes group being 30% to 50% lower in area (*t* test, $P < .05$ or $P < .01$; Figs 5a and 5b). In contrast, no intergroup difference was found for the bone area in the marrow zone (Fig 5c).

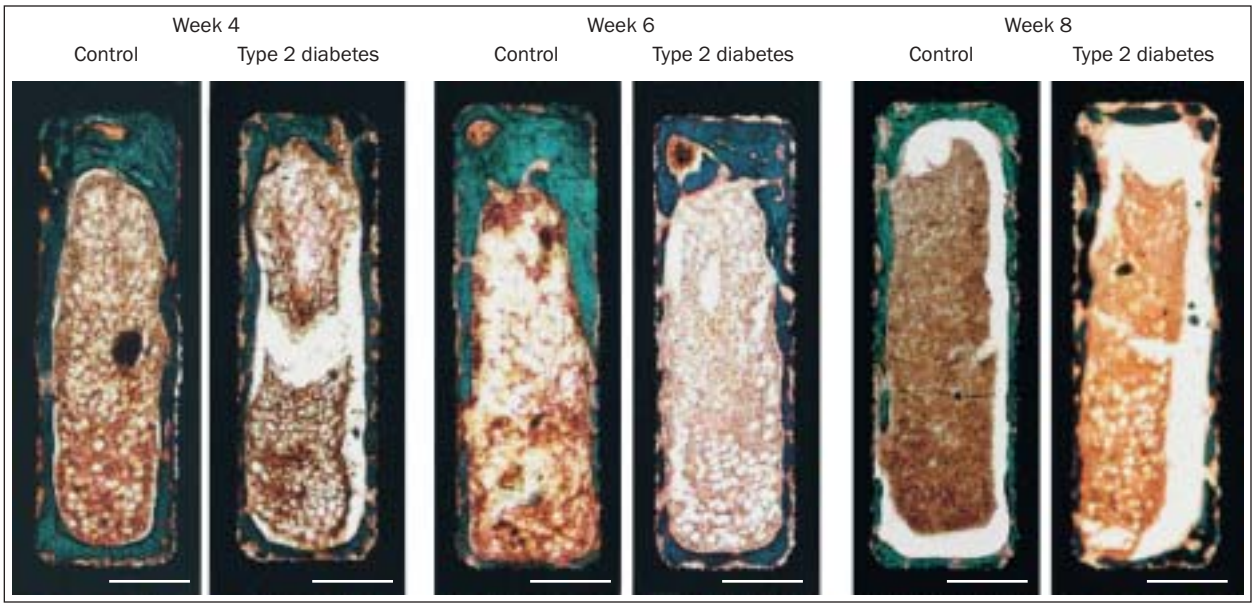


Fig 3 Histologic sections of the control and type 2 diabetes groups at 4, 6, and 8 weeks postimplantation. Overviews of the implant chamber. (Goldner's trichrome stain; bar = 400 μm).

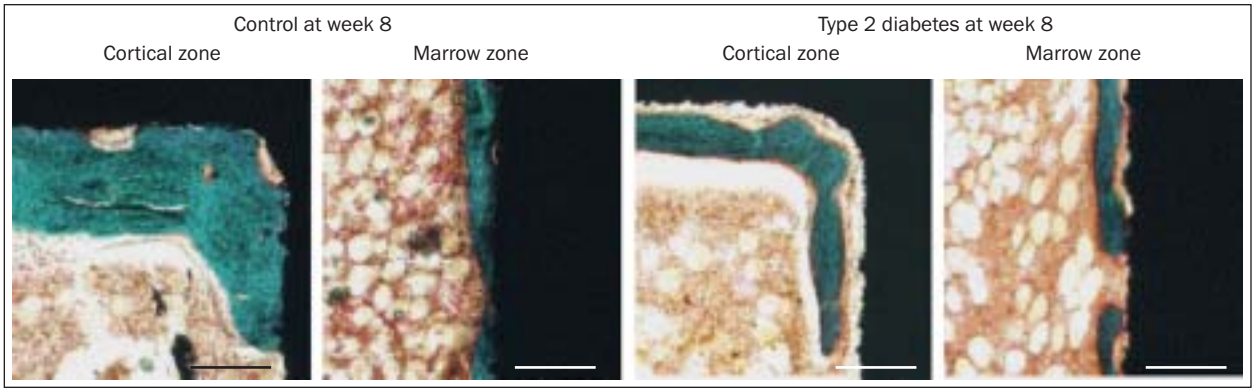
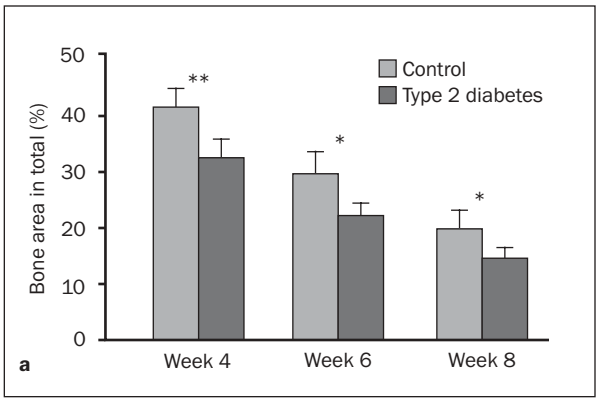


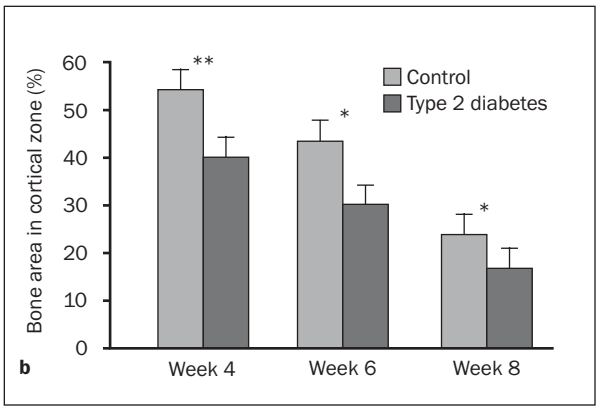
Fig 4 Histologic images of the control and type 2 diabetes groups at week 8 postimplantation. Close-up views of the newly formed bone adjacent to the implant surfaces are shown for both the cortical and marrow zones. (Goldner's trichrome stain; bar = 200 μm).

Bone-implant contact percentages showed a general increase with increased healing time (1-way ANOVA; $P < .05$; Figs 6a to 6c), which was a different trend than that found in the bone area variables (Fig 5). However, the bone-implant contact in the cortical zone was stable from weeks 4 to 8 in the control rats, without any time-dependent changes (Fig 6b). The bone-implant contact in the cortical zone was remarkably lower for the diabetes group than for the control group ($P < .01$; Fig 6b). Even though the difference tended to diminish in the later healing stages, it was approximately 5-fold at week 4 and 2-fold at week 8. Although bone-implant contact in the marrow zone showed an increase associated with healing time for both the control and diabetes groups, bone-implant contact was consistently lower for the diabetes group than for the control group by up to 60% (Fig 6c). As a result, the bone-implant contact in total was also significantly lower for the diabetes group throughout the healing time (Fig 6a).

DISCUSSION

To the authors' knowledge, this is the first report demonstrating that osseointegration capacity is significantly hindered in a type 2 diabetic rat model mimicking human adult-onset diabetes mellitus. This hindered osseointegration capacity was highlighted by the remarkably lower bone-implant contact percent-





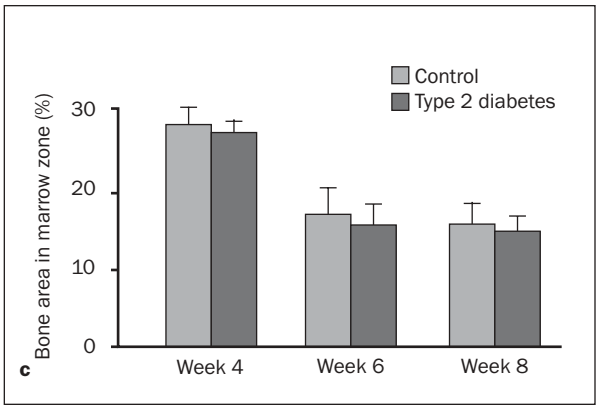
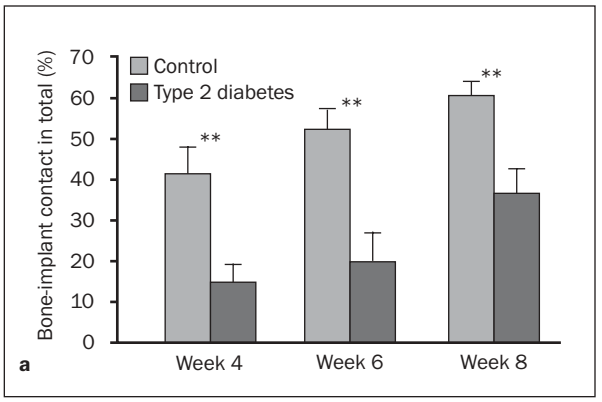
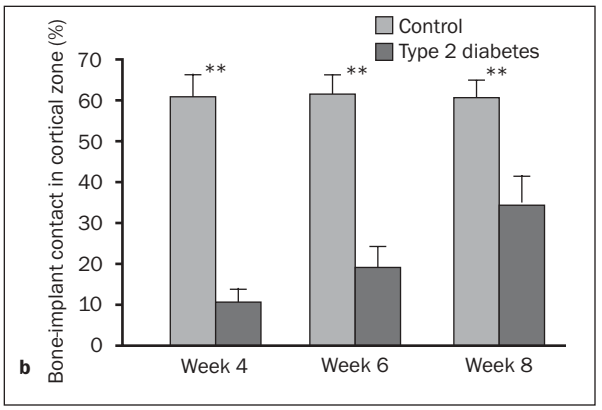


Fig 5 The average bone area with standard deviation (error bars) for *(a)* total bone area, *(b)* the cortical zone, and *(c)* the marrow zone. *Indicates significant difference ($P < .05$) between the control and type 2 diabetes groups (*t* test); **indicates significant difference ($P < .01$).





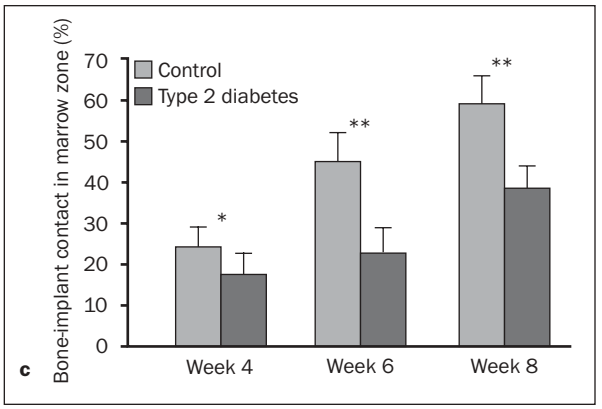


Fig 6 The average bone-implant contact percentage with standard deviation (error bars) for *(a)* total bone area, *(b)* the cortical zone, and *(c)* the marrow zone. *Indicates significant difference ($P < .05$) between the control and type 2 diabetes groups (*t* test); **indicates significant difference ($P < .01$).

age and diminished quantity of newly formed bone in the cortical area. Although bone quantity in the bone marrow area was not negatively affected by the diabetic status, the integrity of bone fragments along the implant surface and the direct bone-implant contact was apparently affected. The poor establishment of bone-implant contact was associated with the intervening soft tissue. The secure establishment of bone-implant contact and the concurrent elimination of soft tissue intervention in association with the accelerated differentiation of osteoblasts are well-documented benefits of a roughened implant surface.[26,27] Meanwhile, the integrity of the bone fragments may be dependent on the rate of osteoblastic proliferation. Therefore, it may be hypothesized that the type 2 diabetic host condition may impair both the osteoblastic proliferative and differentiation capabilities. This hypothesis needs to be investigated further.

The effect of diabetes on bone morphogenesis was disproportionate between the cortical and bone marrow areas; the diminished bone mass and bone-implant contact was more remarkable in the cortical area than in the bone marrow area. Bone regeneration processes in the cortical region, such as those associated with fracture repair, are primarily driven by the osteoprogenitor or stem cells originating in the periosteum.[28] The cells regenerate bone by a combination of intramembranous and endochondral ossification.[28] In contrast, bone generation in the bone marrow cavity is induced by bone marrow stromal stem cells.[29] As documented during the ablation healing in the femur, newly formed bone trabeculae replace blood clot, which will be eventually resorbed by osteoclasts and remodeled.[29] The types of bone morphogenesis by the 2 different cell populations could be clearly discriminated in the histologic images presented. Type 2 diabetes mellitus may affect the 2 different osteogenic processes differently, although it would be premature to make a firm conclusion.

The magnitude of the reduction of osseointegration capacity in the diabetes group was greater than expected. Mean bone-implant contact percentage in the diabetes group was only 12% in the week-4 cortical bone, while the mean for the control group was 61%. The present data fall within the reported range of histologic studies using experimentally induced type 1 diabetes rat models.[15,16] They reported that the bone-implant contact was reduced to 40% to 60% of the control group after 8 weeks. In the present study, the bone-implant contact percentage and bone area in the diabetes increased with healing time; however, they failed to reach the control values within the healing period tested, which is also in agreement with the previous reports.[15,16] A longer-term study is needed to determine whether the diabetes group ever "catches up" to the control group with respect to these histomorphometric variables over time.

The present study employed a very rough titanium surface created by electrical discharge machining. The surface, however, lacked the micro-scale roughness typically seen on acid-etched titanium surfaces and may be considered what is termed a smooth surface at the micron scale. There is a consensus that micron-scale roughness promotes osteoblastic activity, leading to faster and firmer osseointegration, compared to the relatively smooth machined surface.[30,31] In fact, rough surfaces are most in use in current dental implant therapy. The effect of type 2 diabetes on osseointegration capacity around such micro-roughened implants should be of great and immediate interest. Further, effects of the controlled type 2 diabetes, under a condition such as insulin control, also remain to be investigated in the animal model.

This study may provide reliable and valuable biological data on the effects of diabetes mellitus on implant osseointegration. The OLETF rat is an established model bearing close resemblance to type 2 diabetes mellitus in humans, including obesity and late-onset of hyperglycemia, with complications related to chronic diabetes.[19] Although multiple genetic loci have been identified, the cause of diabetes in the model is a combination of insulin resistance and impaired insulin secretion,[32] which is analogous to the etiology of human type 2 diabetes as opposed to type 1(insulin deficiency). In this model, general cellular and tissue sensitivity to insulin decreases with age; it is normal at 6 weeks but reduced by 40% at 12 weeks and by 80% after 18 weeks compared with age-matched control (LETO) rats.[33] Insulin secretion starts to be impaired at 40 weeks; lipotoxicity to islet β-cells may be involved.[34] In the present study, implants were placed in rats at the age of 38 weeks, which represents an overlapping stage of induced diabetes by progressively impaired insulin sensitivity and the subsequent advancement of chronic diabetes by the lessened insulin secretion.

Although the experimental factors, such as the age, race, genetic portrait, pathogenic mechanism, and living environment, are well controlled in animal models, their biological reaction often differs from that of humans. In fact, most of the previous experimental studies using animal models seem to demonstrate an impaired bone healing potential around implants in diabetic animals compared with nondiabetic controls, while the majority of clinical studies tend to indicate that diabetes does not have a critical adverse effect on the treatment outcome.[35] When interpreting and comparing the data from animal and human studies, it should be taken into account that *(1)* the diabetic condition is under control in most of the human studies; *(2)* the biological response sometimes differs among animal models and between animals and humans; and *(3)* in human studies, the research design and the inclusion criteria for patients are sometimes not well established in terms of the type and duration of diabetes and the levels and treatment of pathogenesis.[36] In fact, recent clinical studies have indicated a possible risk of implant failure in association with diabetes status.[7,12]

It should be noted that implants were placed in the femur in the present study. The femur provides an accessible site for implant placement in the rat model. However, it involves periosteal cells that show different biological potentials from the ones in the

jawbone. Periosteum-derived cells have been demonstrated to have the ability to differentiate into chondroblasts and osteoblasts and to form cartilage and bone both in vitro and in vivo.[37,38] Therefore, the periosteum plays an important role during growth and repair of bone through endochondral ossification.[38] Mandibular periosteal cells manifest only osteogenic capacity but not chondrogenic capacity.

Another important aspect of osseointegration is the biomechanical retention of implants, which may be the most clinically relevant parameter for assessment of the degree of osseointegration. In experimental diabetes models, fracture healing is delayed, and accordingly, the recovery of the biomechanical strength of bone is impaired.[39] Compromised osteogenic capability, including decreased or delayed mineralization and matrix formation, seems unavoidable.[8] Further, a study using diabetic rat femurs revealed that the diabetic state induces mechanical deterioration of bone, resulting in a 38% decrease in breaking strength of the femur.[40] Although a great contrast in bone morphogenesis between the type 2 diabetes and control rats was observed in the present study, how this affects the biomechanical fixation of implants remains unknown. In addition to long-term histologic study using the type 2 diabetes model, biomechanical evaluation of implant osseointegration will be essential.

Insight into the biological background and mechanisms of diabetes-associated impaired capacity of osseointegration can be gained from the literature. The association of reduced bone healing with inadequate insulin production has been demonstrated.[6,9] Extracellular matrix production, such as type X collagen, is reduced up to 70% in the endochondral ossification of diabetic animals.[41] Insulin directly stimulates osteoblastic extracellular matrix production and regulates the production of insulin growth factor-1 (IGF-1).[42] IGF-1, in turn, promotes bone formation by regulating osteoblastic proliferation and differentiation.[42–45] Therefore, the diminished production of insulin may have both directly and indirectly deteriorated the osseointegration capacity of the host site. In addition, although the pathways are unknown, experimental diabetes interferes with bone formation by failing to provide an adequate expression of key transcription factors to osteoblastic differentiation, such as Cbfa1/Runx-2.[46] Finally, sustained hyperglycemia increases the formation of advanced glycosylation end products (AGEs).[47,48] Osteoblasts express AGE receptors, which are known to reduce osteogenic potential.[47,48] Thus, an elevated AGE level caused by chronic hyperglycemia may have negatively affected the process of osseointegration. However, how these mechanisms result in the difference in osteogenic response around implants between the cortical and marrow areas is unknown. Also, the underlying mechanism responsible for the reduced bone-implant contact percentage, which is a critical factor for osseointegration, needs to be explored. Particularly, the effects of diabetes on the proliferative activity of osteoblasts, which is closely related to the bone volume, and on differentiation capacity, which is related to the speed of osteogenesis and eventually the direct bone-implant contact, need to be elucidated.

CONCLUSIONS

The present study examined for the first time the effect of diabetes on bone morphogenesis around titanium implants using a rat model with a close resemblance to type 2 diabetes which represents the majority of human adult-onset diabetes mellitus cases. The diabetic condition impaired osseointegration capacity disproportionally between the cortical and bone marrow areas. The percentage of bone-implant contact both in the cortical and marrow areas, and the bone volume in the cortical area, were significantly smaller for the diabetes group than for the control. Bone-implant contact in the cortical bone area was remarkably diminished at the early stage of healing (week 4); 12% for the diabetes group versus 61% for the control. These impaired osseointegrative capacities were not ameliorated to the levels of the control group within the observed healing period of 8 weeks.

ACKNOWLEDGMENTS

This study was supported by the "AGU High-Tech Research Center" Project for Private Universities matching fund subsidy from MEXT JAPAN 2003–2006. It was also supported by a High-Tech Research Grant from the Ministry of Education, Science, Culture and Sports, Japan.

REFERENCES

1. van Steenberghe D, Jacobs R, Desnyder M, Maffei G, Quirynen M. The relative impact of local and endogenous patient-related factors on implant failure up to the abutment stage. Clin Oral Implants Res 2002;13:617–622.
2. Kan JY, Rungcharassaeng K, Kim J, Lozada JL, Goodacre CJ. Factors affecting the survival of implants placed in grafted maxillary sinuses: A clinical report. J Prosthet Dent 2002;87:485–489.
3. Takeshita F, Murai K, Ayukawa Y, Suetsugu T. Effects of aging on titanium implants inserted into the tibiae of female rats using light microscopy, SEM, and image processing. J Biomed Mater Res 1997;34:1–8.

4. Ozawa S, Ogawa T, Iida K, et al. Ovariectomy hinders the early stage of bone-implant integration: Histomorphometric, biomechanical, and molecular analyses. Bone 2002;30:137–143.
5. Gandhi A, Dumas C, O'Connor J P, Parsons JR, Lin SS. The effects of local platelet rich plasma delivery on diabetic fracture healing. Bone 2006;38:540–546.
6. Gandhi A, Beam HA, O'Connor JP, Parsons JR, Lin SS. The effects of local insulin delivery on diabetic fracture healing. Bone 2005;37:482–490.
7. Morris HF, Ochi S, Winkler S. Implant survival in patients with type 2 diabetes: Placement to 36 months. Ann Periodontol 2000;5:157–165.
8. Follak N, Kloting I, Merk H. Influence of diabetic metabolic state on fracture healing in spontaneously diabetic rats. Diabetes Metab Res Rev 2005;21:288–296.
9. Follak N, Kloting L, Wolf E, Merk H. Delayed remodeling in the early period of fracture healing in spontaneously diabetic BB/OK rats depending on the diabetic metabolic state. Histol Histopathol 2004;19:473–486.
10. Ward DT, Yau SK, Mee AP, et al. Functional, molecular, and biochemical characterization of streptozotocin-induced diabetes. J Am Soc Nephrol 2001;12:779–790.
11. Santana RB, Trackman PC. Controlled release of fibroblast growth factor 2 stimulates bone healing in an animal model of diabetes mellitus. Int J Oral Maxillofac Implants 2006;21: 711–718.
12. Moy PK, Medina D, Shetty V, Aghaloo TL. Dental implant failure rates and associated risk factors. Int J Oral Maxillofac Implants 2005;20:569–577.
13. Olson JW, Shernoff AF, Tarlow JL, Colwell JA, Scheetz JP, Bingham SF. Dental endosseous implant assessments in a type 2 diabetic population: A prospective study. Int J Oral Maxillofac Implants 2000;15:811–818.
14. Abdulwassie H, Dhanrajani PJ. Diabetes mellitus and dental implants: A clinical study. Implant Dent 2002;11:83–86.
15. Takeshita F, Murai K, Iyama S, Ayukawa Y, Suetsugu T. Uncontrolled diabetes hinders bone formation around titanium implants in rat tibiae. A light and fluorescence microscopy, and image processing study. J Periodontol 1998;69:314–320.
16. Nevins ML, Karimbux NY, Weber HP, Giannobile WV, Fiorellini JP. Wound healing around endosseous implants in experimental diabetes. Int J Oral Maxillofac Implants 1998;13:620–629.
17. McCracken M, Lemons JE, Rahemtulla F, Prince CW, Feldman D. Bone response to titanium alloy implants placed in diabetic rats. Int J Oral Maxillofac Implants 2000;15:345–354.
18. Margonar R, Sakakura CE, Holzhausen M, Pepato MT, Alba RC, Marcantonio E. The influence of diabetes mellitus and insulin therapy on biomechanical retention around dental implants: A study in rabbits. Implant Dent 2003;12:333–339.
19. Iwase M, Uchizono Y, Tashiro K, Goto D, Iida M. Islet hyperperfusion during prediabetic phase in OLETF rats, a model of type 2 diabetes. Diabetes 2002;51:2530–2535.
20. Komaki K, Ohno Y, Aoki N. Gonadal hormones and gonadal function in type 2 diabetes model OLETF (Otsuka Long Evans Tokushima Fatty) rats. Endocr J 2005;52:345–351.
21. Jia D, Taguchi M, Otsuki M. Synthetic protease inhibitor camostat prevents and reverses dyslipidemia, insulin secretory defects, and histological abnormalities of the pancreas in genetically obese and diabetic rats. Metabolism 2005;54:619–627.
22. Yamamoto M, Jia DM, Fukumitsu KI, et al. Metabolic abnormalities in the genetically obese and diabetic Otsuka Long-Evans Tokushima Fatty rat can be prevented and reversed by alpha-glucosidase inhibitor. Metabolism 1999;48:347–354.
23. Kawano K, Hirashima T, Mori S, Natori T. OLETF (Otsuka Long-Evans Tokushima Fatty) rat: A new NIDDM rat strain. Diabetes Res Clin Pract 1994;24(suppl):S317–S320.
24. Dziedzic D. Effects of Implant Surface Topography on Osteoconduction [thesis]. Toronto: University of Toronto, 1995.
25. Ogawa T, Nishimura I. Genes differentially expressed in titanium implant healing. J Dent Res 2006;85:566–570.
26. Ogawa T, Nishimura I. Different bone integration profiles of turned and acid-etched implants associated with modulated expression of extracellular matrix genes. Int J Oral Maxillofac Implants 2003;18:200–210.
27. Takeuchi K, Saruwatari L, Nakamura HK, Yang JM, Ogawa T. Enhanced intrinsic biomechanical properties of osteoblastic mineralized tissue on roughened titanium surface. J Biomed Mater Res A 2005;72A:296–305.
28. Einhorn TA. The cell and molecular biology of fracture healing. Clin Orthop 1998(355 suppl):S7–S21.
29. Suva LJ, Seedor JG, Endo N, et al. Pattern of gene expression following rat tibial marrow ablation. J Bone Miner Res 1993;8: 379–388.
30. Cooper LF, Masuda T, Yliheikkila PK, Felton DA. Generalizations regarding the process and phenomenon of osseointegration. Part II. In vitro studies. Int J Oral Maxillofac Implants 1998;13: 163–174.
31. Masuda T, Yliheikkila PK, Felton DA, Cooper LF. Generalizations regarding the process and phenomenon of osseointegration. Part I. In vivo studies. Int J Oral Maxillofac Implants 1998;13: 17–29.
32. Ishida K, Mizuno A, Min Z, Sano T, Shima K. Which is the primary etiologic event in Otsuka Long-Evans Tokushima Fatty rats, a model of spontaneous non-insulin-dependent diabetes mellitus, insulin resistance, or impaired insulin secretion? Metabolism 1995;44:940–945.
33. Sato T, Asahi Y, Toide K, Nakayama N. Insulin resistance in skeletal muscle of the male Otsuka Long-Evans Tokushima Fatty rat, a new model of NIDDM. Diabetologia 1995;38: 1033–1041.
34. Man ZW, Zhu M, Noma Y, et al. Impaired beta-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. Diabetes 1997;46:1718–1724.
35. Mombelli A, Cionca N. Systemic diseases affecting osseointegration therapy. Clin Oral Implants Res 2006;17(suppl 2):97–103.
36. Kotsovilis S, Karoussis IK, Fourmousis I. A comprehensive and critical review of dental implant placement in diabetic animals and patients. Clin Oral Implants Res 2006;17:587–599.
37. De Bari C, Dell'Accio F, Vanlauwe J, et al. Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. Arthritis Rheum 2006;54:1209–1221.
38. Schantz JT, Hutmacher DW, Ng KW, Khor HL, Lim MT, Teoh SH. Evaluation of a tissue-engineered membrane-cell construct for guided bone regeneration. Int J Oral Maxillofac Implants 2002;17:161–174.
39. Funk JR, Hale JE, Carmines D, Gooch HL, Hurwitz SR. Biomechanical evaluation of early fracture healing in normal and diabetic rats. J Orthop Res 2000;18:126–132.
40. Reddy GK, Stehno-Bittel L, Hamade S, Enwemeka CS. The biomechanical integrity of bone in experimental diabetes. Diabetes Res Clin Pract 2001;54:1–8.
41. Topping RE, Bolander ME, Balian G. Type X collagen in fracture callus and the effects of experimental diabetes. Clin Orthop Relat Res 1994;(308):220–228.
42. Canalis E. Effect of insulinlike growth factor I on DNA and protein synthesis in cultured rat calvaria. J Clin Invest 1980;66:709–719.
43. Schmid C, Steiner T, Froesch ER. Insulin-like growth factor I supports differentiation of cultured osteoblast-like cells. FEBS Lett 1984;173:48–52.

44. Koch H, Jadlowiec JA, Campbell PG. Insulin-like growth factor-I induces early osteoblast gene expression in human mesenchymal stem cells. Stem Cells Dev 2005;14:621–631.
45. Thaller SR, Salzhauer MA, Rubinstein AJ, Thion A, Tesluk H. Effect of insulin-like growth factor type I on critical size calvarial bone defects in irradiated rats. J Craniofac Surg 1998;9: 138–141.
46. Lu H, Kraut D, Gerstenfeld LC, Graves DT. Diabetes interferes with the bone formation by affecting the expression of transcription factors that regulate osteoblast differentiation. Endocrinology 2003;144:346–352.
47. Santana RB, Xu L, Chase HB, Amar S, Graves DT, Trackman PC. A role for advanced glycation end products in diminished bone healing in type 1 diabetes. Diabetes 2003;52:1502–1510.
48. Cortizo AM, Lettieri MG, Barrio DA, Mercer N, Etcheverry SB, McCarthy AD. Advanced glycation end-products (AGEs) induce concerted changes in the osteoblastic expression of their receptor RAGE and in the activation of extracellular signal-regulated kinases (ERK). Mol Cell Biochem 2003;250(1–2):1–10.