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ARTICLE *in* IMPLANT DENTISTRY · JANUARY 2003

Impact Factor: 1.11 · DOI: 10.1097/01.ID.0000074440.04609.4F · Source: PubMed

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Bone Formation Around Titanium Implants in the Rat Tibia: Role of Insulin

José T. Siqueira, DDS, PhD,* Simone C. Cavalher-Machado, MSc,** Victor E. Arana-Chavez, DDS, PhD,*** and Paulina Sannomiya, PhD**

Background and Purpose: *Clinical and experimental studies show, with few exceptions, that type 1 diabetes mellitus is associated with a delay in bone repair around endosseous implants. The effect of insulin in bone repair/remodeling is not completely understood. The aim of this study was to investigate the course of histological and ultrastructural changes of the osseointegration process under the influence of insulin.* ***Materials and Methods:*** *Titanium implants were inserted into the tibiae of male Wistar rats. Animals were divided into three groups: 1) rats with alloxan-induced diabetes; 2) diabetic rats treated with isophane insulin (2 IU/day); and 3) matching controls. Histological and histomorphometric analysis of bone–implant sections were performed 10 and 21 days after implant placement.* ***Results:*** *Relative to control values, rats with alloxan-induced diabetes exhibited a 50% reduction in the area of formed bone (*$P < 0.001$*) and in the surface of contact between bone and implant (*$P < 0.01$*) 21 days after implant placement. There were no significant differences between groups 10 days after surgery. Values returned to normal levels in diabetic rats after insulin treatment. Presence of chondrocyte-like cells surrounded by a cartilaginouslike matrix in diabetic rats suggests a delay in the process of bone repair. Ultrastructural characteristics of bone–implant interface in diabetic rats treated with insulin resembled those observed in controls.* ***Conclusion****: The data presented suggest that bone repair around endosseous implants is regulated, at least in part, by insulin. The results imply that the control of the metabolic status of the diabetic patient is essential for a successful osseointegration. (Implant Dent 2003;12:242–251)*

Key Words: titanium implants, osseointegration, insulin

The replacement of teeth with dental implants continues to be a predictable option for most patients, providing that there is successful osseointegration during the healing period.[1] In the osseointegration process, the living bone tissue is induced to incorporate a synthetic anchorage element and the implants need to be accepted by, and integrated into, healthy, continuously remodeling bone tissue.[2] Diabetes mellitus (type 1 and type 2) is associated with poor wound healing and increased frequency and severity of oral infections, including periodontal diseases and dental caries.[3–5] Some studies support the use of dental implants in diabetic patients, provided that the patient's plasma glucose level is in the reference range.[6–8] Others indicate that despite a reasonable success, an increase in failure rate occurs during the first year after prosthetic loading.[9] Therefore, this study was undertaken to investigate the role of insulin in the process of osseointegration. The data to be presented include morphological and morphometric analyses of bone formation around titanium implants inserted transcortically into the tibiae of rats with alloxan-induced diabetes and the effect of insulin during the initial phases of osseointegration (at 10 and 21 days) using light microscopy and transmission electron microscopy.

*Supervisor of Orofacial Pain Team, Division of Dentistry, University of São Paulo Medical School, São Paulo, Brazil.
**Associate Professor, Research Division, Institute of Heart (Incor), University of São Paulo Medical School, São Paulo, Brazil.
***Associate Professor, Laboratory of Mineralized Tissue Biology, Department of Histology and Embryology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

ISSN 1056-6163/03/01203-242$3.00
Implant Dentistry
Volume 12 • Number 3

DOI: 10.1097/01.ID.0000074440.04609.4F

Materials and Methods

Animals

Male Wistar rats 3 months old and weighing 350 g at the beginning of the experiments were housed with a 12-hour light/dark cycle and allowed a standard pellet diet and tap water *ad libitum* throughout the observation period. University of São Paulo's guidelines for animal experiments were observed during the experiment.

Experimental Protocols

The experimental design included histological/histomorphometric analysis of whole bone–implant sections 10 and 21 days after implant placement. The animals were divided into three groups: controls (n = 17), diabetic rats (n = 18), and diabetic rats treated with insulin (n = 8). The experimental schedule is shown in Figure 1.

Induction of Diabetes Mellitus

Diabetes mellitus was induced by the intravenous injection of 42-mg/kg

alloxan monohydrate (Sigma Chemical Co., St. Louis, MO) dissolved in physiological saline. Control rats were injected with physiological saline alone. The presence of diabetes was verified by blood glucose concentrations > 200 mg/dL estimated with the aid of a blood glucose monitor (Advantage, Eli Lilly, São Paulo, Brazil). Blood samples were obtained from the cut tip of the tail of the animals.

Insulin Treatment

A group of diabetic rats received a single daily dose (2 IU) of isophane insulin (Eli Lilly, São Paulo, Brazil) by the subcutaneous route. Treatment started on the second or 10th day after alloxan injection and continued throughout the observation period.

Implants

Commercially pure titanium (grade II; INP System, São Paulo, Brazil) implants were designed for placement into the tibia of the animals. The implants were cylindrical (3.8 mm in length and 2.0 mm in diameter) with two V-shaped grooves and the apical one was screwed for better stabilization. The implant surface was roughened by sandblasting and acid treatment.

Surgical Procedures

The animals were anesthetized intraperitoneally with 400-mg/kg chlorohydrate (Sigma Chemical Co.) and the hind limbs were shaved and cleaned with an alcoholic solution of iodine. The implants were placed into both tibiae 1 cm distal from the knee joint. After skin incision, the muscles were displaced from the tibia to expose the periosteum. After careful dissection of the periosteum, a cortical hole was drilled with a slow-speed (800 rpm) dental handpiece with a sequential of two drills (1.0 and 1.9 mm) using copious physiological saline irrigation to minimize the temperature rise in the bone. The recipient site was carefully washed with saline solution and the implant was inserted initially by tapping and then by screwing at the inferior cortical level of the bone. The periosteum was reapproximated with cotton sutures and the muscles and skin were similarly closed. Penicillin (Wycillin, Wyeth Whitehall, São Paulo, Brazil; 25,000 IU) was given to the animals by the intramuscular route. Surgical procedures for implant placement were done 24 hours after alloxan injection.

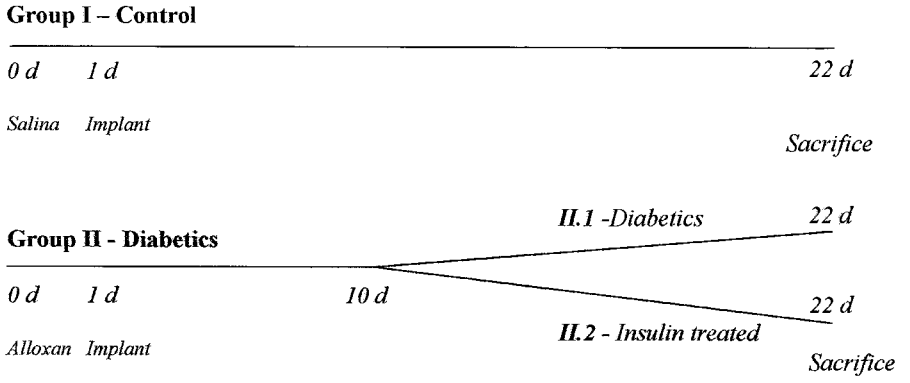


Fig. 1. Experimental schedule.

Light Microscopy

Under chlorohydrate anaesthesia (400 mg/kg), the bone was stripped of soft tissue and a block of bone surrounding the integrated implant was removed using a dental handpiece with a Carborundum separating disk. The samples were fixed in 4% paraformaldehyde solution in 0.5M sodium cacodylate buffer (pH 7.4) for 24 hours. The specimens were radiographed and then dehydrated through a graded series of ethanol solutions, air dried, and embedded in resin (Osteo-bed, Polysciences, Warrington, PA). The resin blocks were sectioned at a thickness of 15 μm parallel to the long axis of the implant using a diamond blade (Leica 1600, Leica Inc., Deerfield, IL) and stained with paragon.

Transmission Electron Microscopy

For electron microscopy analysis, the animals were anesthetized as described previously and submitted to an intracardiac perfusion with a solution of 2% glutaraldehyde and 2.5% paraformaldehyde in cacodylate buffer. The bone was stripped of soft tissue, and the bone–implant block was removed using a dental disk and immersed in the same perfusion solution overnight. After decalcification in 10% edetic acid for 8 weeks at 4°C, the specimens were rinsed in cacodylate buffer and postfixed in 1% osmium tetroxide for 2 hours, dehydrated through a graded series of acetone and embedded in Spurr resin (Electron Microscopy Sciences, Fort Washington, PA). The implants were removed by means of a fracture technique.[10] The pieces, freed from titanium implants, were re-embedded in Spurr resin; trimmed from semi thin sections (5 μm), and ultrathin sections were prepared with a ultramicrotome equipped with a diamond knife, then stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (Jeol 100 CX II, Tokyo, Japan).

Histomorphometric Analysis

For image processing, bone–implant sections were analyzed using a video camera (JVC, TK 1270, Victor Company of Japan LTD, Tokyo, Japan) incorporated to a triocular microscope (Carl Zeiss, West Germany) and a computer-based image analyser (Kontron Imaging System KS-300, Kontron Electronic GmbH, Munich, Germany). The V-shaped grooves on both sides of the sectioned implant were selected to measure growth of cortical bone inside the chambers and the contact of new bone with the surface of the implant. The schematic design of the implant section is illustrated in Figure 2. The total area of the groove (A) and the area of new cortical bone inside the groove (B) were measured to calculate the percentage of bone growth (% = B/A × 100 μm^2). Similarly, the total length of groove's metal surface (C) and the length of the surface border where the bone tissue directly contacted the implant (D) were measured to calculate

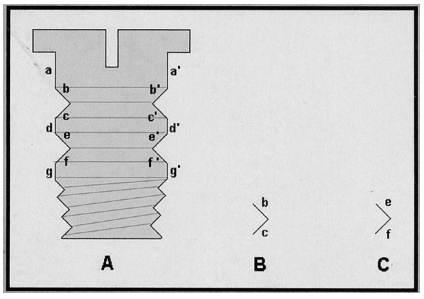


Fig. 2. Schematic design of the implant. Implant subdivisions are indicated as alphabetic symbols (A); first groove (B); second groove (C).

percent bone contact with the implant (% = D/C × 100 μm).

Statistical Analysis

Data are presented as means ± SEM. One-way analysis of variance was performed followed by the Tukey-Kramer multiple comparisons test. $P < 0.05$ was considered to be statistically significant.

Results

Characteristics of Study Groups

Relative to controls, diabetic rats exhibited a reduction in body weight gain and sharply elevated blood glucose levels during the experimental period (Table 1).

Light Microscopy of Bone–Implant Sections

At 21 days after implantation, bone–implant sections showed that in control animals, bone formation was evident around the implant, particularly at the level of cortical bone. However, diabetic rats showed less bone contact with the surface of the implant, suggesting a delay in the formation of new bone inside the cortical chamber of the implant. Growth of new bone was further increased in diabetic rats after insulin treatment (data not shown).

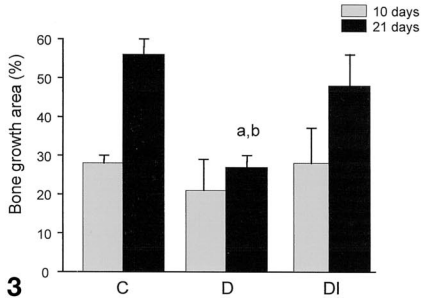
Histomorphometric Analysis

Results of histomorphometric analysis (Fig. 3) showed that the cortical locus of the implant served as a bone growth chamber as depicted by the area of new bone present within the chamber, 10 and 21 days after surgery of control animals. In contrast, the area of newly formed bone within the chamber of rats with alloxan-induced diabetes was reduced, particularly 21 days after surgery. Treatment of diabetic animals with insulin restored synthesis of new bone, and values attained matched those observed in the control group. Similar results were observed when the surface of contact between implant and bone was evaluated. There was less contact between the newly formed cortical bone and the surface of the implant in diabetic rats 21 days after surgery, and values returned to normal levels after insulin treatment (Fig. 4). Blood glucose levels (mean ± SEM) before and after insulin treatment were 395 ± 17 and 295 ± 24 mg/dL, respectively ($P < 0.05$). Differences in body weight between untreated and insulin-treated diabetic rats were not significant (−36 ± 10 vs. −26 ± 8 g, respectively).

Table 1. Characteristics of Study Groups

Animals	Body Weight Gain (g)	Blood Glucose (mg/dl)
Diabetic (11 d) n = 4	−25 ± 10*	365 ± 24**
Matching control n = 3	11 ± 8	123 ± 3
Diabetic (22 d) n = 17	−36 ± 10***	374 ± 18***
Matching control n = 14	36 ± 7	121 ± 7

Student's *t* test, unpaired: *p = 0.0518 (not quite significant); **p = 0.0003 (extremely significant); ***p < 0.0001 (extremely significant).



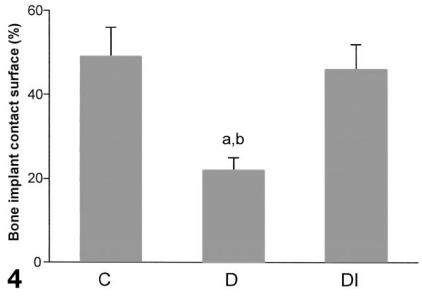


Fig. 3. Histomorphometric data for the percent of bone growth area inside the cortical chamber of the implant. C, control rats; D, diabetic rats; DI, diabetic rats treated with insulin (2 IU/day). Treatment started on the second or 10th day after alloxan injection and continued throughout the observation period, 10 and 21 days after implantation, respectively. Each bar represents the mean ± standard error of the mean of four to six animals in each group. [a]$P <$ 0.001 versus control group. [b]$P < 0.05$ versus insulin-treated diabetic rats.

Fig. 4. Histomorphometric data for the percent of bone–implant contact surface. C, control rats; D, diabetic rats; DI, diabetic rats treated with insulin (2 IU/day). Treatment started on the second or 10th day after alloxan injection and continued throughout the observation period, 10 and 21 days after implantation, respectively. Each bar represents the mean ± standard error of the mean of four to six animals in each group. [a]$P < 0.01$ versus control group. [b]$P < 0.05$ versus insulin-treated diabetic rats.

Transmission Electron Microscopy of the Bone–Implant Interface

Examination of the bone–implant interface in control rats 10 days after surgery revealed many areas in which flattened cells were covering the implant surface. Secretory osteoblasts showing well-developed synthetic organelles in contact with unmineralized bone matrix (osteoid) were identified forming bone toward the implant. In general, a region of connective tissue was present between the forming bone and the flattened cells adjacent to the implant. However, at this time point, some red blood cells and macrophages were identified in the connective tissue (Fig. 5 a and b). At 21 days after surgery, the new bone reached the implant. A layer of flattened cells, which resembled bone-lining cells, was always localized between the new bone and the implant. In addition, higher-magnification views at the interface revealed a thin electron-opaque line surrounding the implant (Fig. 5c).

The ultrastructural characteristics of diabetic rats 10 and 21 days after surgery were, in general, similar to those of control rats (Figs. 6 a–c). However, conspicuous chondrocyte-like cells inside the new bone were

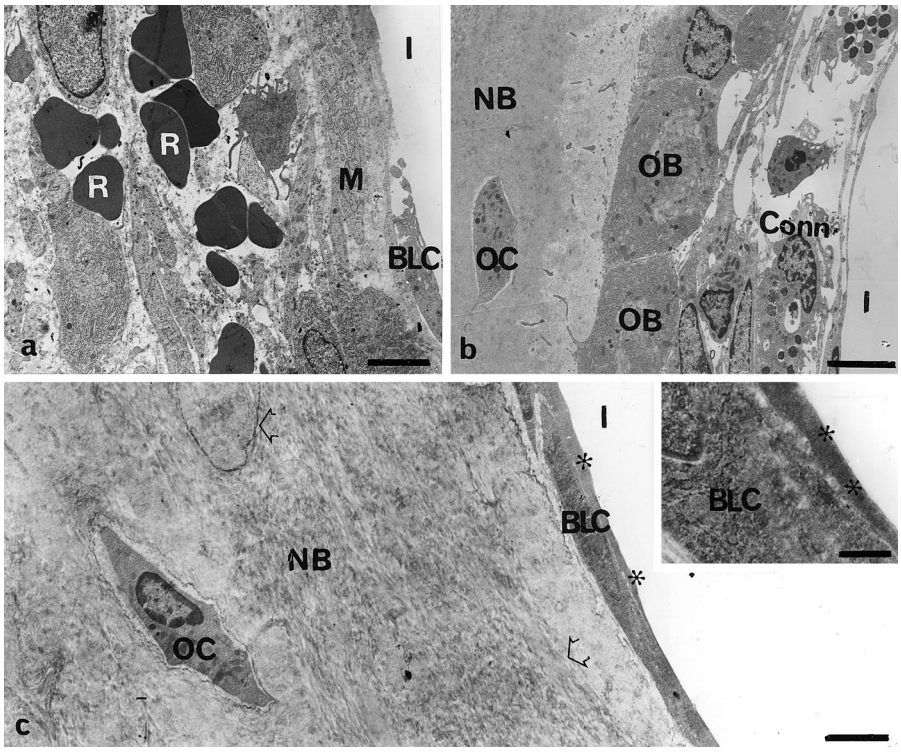


Fig. 5. Control group. Electron micrographs of the bone–implant interface where an implant space (I) is observed since the titanium implant was fractured out of the resin. **Ten days after surgery:** In **a**, a layer of organic matrix (M) and some cell processes are distinguished between a flattened cell (BLC) and the implant; some adjacent red blood cells (R) are also present. Scale bar = 3.0 μm. In **b**, an area of forming bone (NB) in which an osteocyte (OC) and typical secretory osteoblasts (OB) can be observed. Note that deposition of new bone occurs toward the implant (I). Some connective cells (Conn) are present between the forming bone and the implant. Scale bar = 4.0 μm. **Twenty-one days after surgery:** In **c,** a flattened cell resembling a bone-lining cell (BLC) is present between the new bone (NB), in which an osteocyte (OC) and cement lines (arrowheads) can be observed, and the implant space (I). The inset shows a higher-magnification view of the bone–implant interface. Observe that an afibrillar material (asterisk) can be distinguished between the implant and the flattened cell and the cell process. Scale bar = 3.0 μm; inset bar = 1 μm.

observed in regions adjacent to the implant in diabetic rats at day 21. In contrast to osteocytes, these cells presented numerous and short cytoplasmic processes and were surrounded by an extracellular matrix that was looser than that of the adjacent bone, showing a capsulelike appearance (Fig. 7).

Similar findings were observed in insulin-treated rats at 10 days after surgery (Fig. 8a). However, typical osteoclasts exhibiting a well-developed ruffled border were frequently seen in new bone facing the implant. Presence of typical cement lines was also conspicuous, and revealed that remodeling events took place in these specimens (Fig. 8b). Presence of active osteoclasts was more frequent in 21-day specimens. However, despite the resorbing stage of the new bone, a layer of bone-lining cells remained surrounding the implant (Fig. 9).

Discussion

These results suggest that insulin might regulate, at least in part, the process of bone repair/remodeling around endosseous implants. The suggestion is supported by the following observations: 1) the area of new bone within the cortical locus of the implant inserted into the tibiae of rats with alloxan-induced diabetes was significantly reduced, representing 50% of the values observed in the control group; 2) this was accompanied by a 50% reduction in the surface of contact between bone and implant; and 3) values returned to normal levels in diabetic rats after insulin treatment.

The schedule of insulin therapy was based in daily subcutaneous injections of 2 IU isophane insulin throughout the experimental period, starting on the second or 10th day after alloxan administration. The restorative effect of insulin on bone healing was clearly demonstrated 21 days after the placement of the implants into the tibiae of the animals. Both the area of new bone inside the cortical locus of the implant and bone-implant contact surface were of the same magnitude as those observed in the control group. Plasma glucose concentrations were sharply elevated in rats with alloxan-induced diabetes. Insulin treatment (2 IU/day) was not sufficient to maintain normal blood glucose levels in these animals, as indicated by the remaining hyperglycemia that, nevertheless, was of a lower magnitude than in untreated diabetic animals. Accordingly, the impairment in bone repair/remodeling around endosseous implants might be primarily linked to continuing insulin deficiency rather than to secondary hyperglycemia occurring in the diabetic rats. Insulin is an important hormone not only for glucose control but for skeletal growth.[11] Interestingly, diabetes induces a greater decrease in collagen than noncollagen protein production and a greater defect in collagen production than food restriction.[12,13] Nondiabetic semistarved rats with the same body weight as diabetic animals exhibit a similar delay in bone growth as diabetic rats, but did not have the same cellular defects as diabetics, including decreased bone formation, resorption, and plasma osteocalcin concentration.[14] Animals rendered diabetic by the injection of alloxan exhibited a significant loss of weight as compared with matching controls. Although body weight did not significantly change after insulin treatment in diabetic rats, there was a complete recovery in bone growth around endosseous implants in these animals. According to studies of food-restricted rats,[13,14] the abnormalities in bone repair/remodeling observed in animals with alloxan-induced diabetes cannot be explained simply by loss of body weight. The ability of insulin to restore bone formation around titanium implants indicates that the alter-

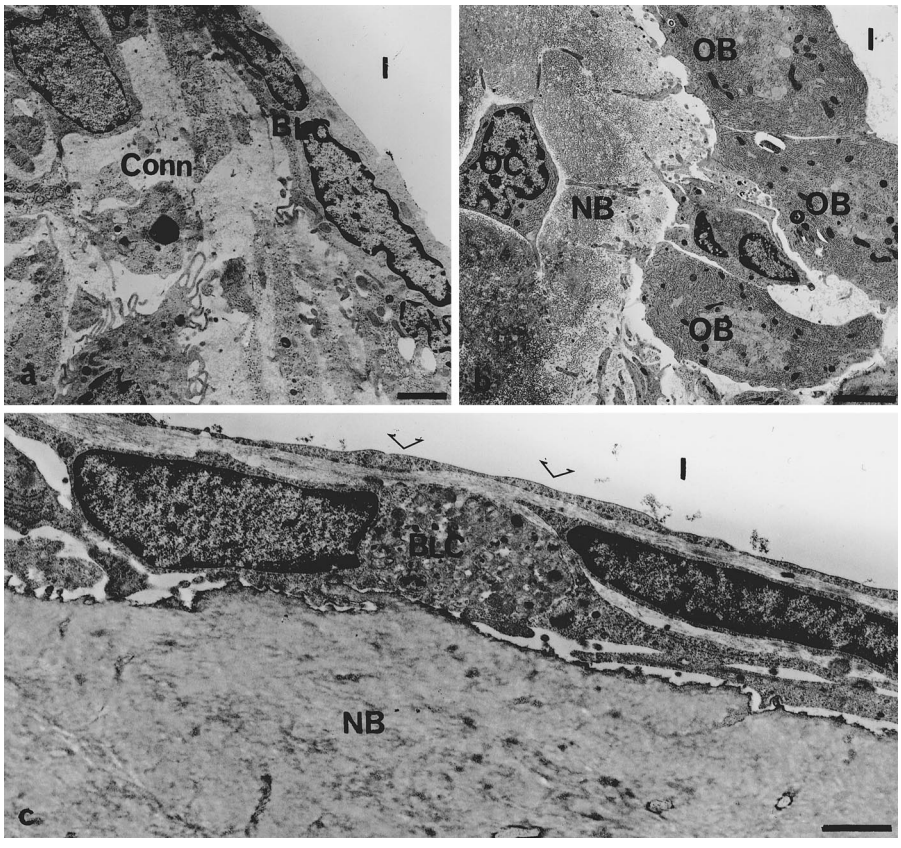


Fig. 6. Diabetic group. **Ten days after surgery:** Electron micrographs of the bone–implant interface showing, in **a**, a region of connective tissue (Conn) adjacent to the implant (I). Note the presence of a continuous layer of flattened cells (BLC) surrounding the implant. Scale bar = 2.0 μm. In **b**, an area of forming bone (NB), in which an osteocyte (OC) is observed, reached the implant. Note that secretory osteoblasts (OB) are in contact with the implant (I). Scale bar = 3.0 μm. **Twenty-one days after surgery:** In **c,** a higher-magnification of the bone–implant interface. The new bone (NB) is observed adjacent to the implant (I). However, a continuous layer of cell process–like structures (arrows) can be distinguished between the bone-lining cells (BLC) and the implant (I). Scale bar = 1.0 μm.

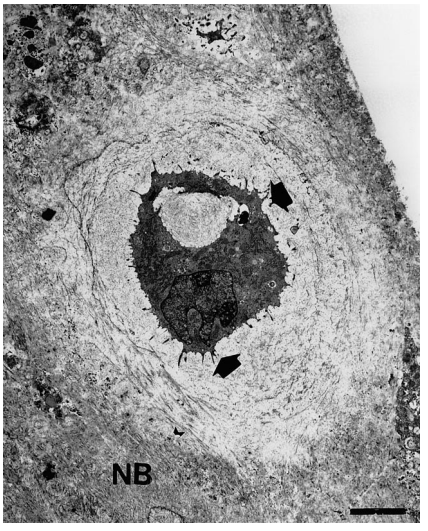


Fig. 7. Diabetic group 21 days after surgery: Electron micrograph of the bone–implant interface showing a chondrocytelike cell, which is characterized by many short processes (arrows) inside the new bone (NB). A loose fibrillar matrix, different from the dense adjacent bone matrix, can be seen surrounding the cell. Scale bar = 3.0 μm.

ation is a consequence of the diabetic state.

Studies carried out in the tibia of rats with streptozotocin-induced diabetes with hydroxyapatite[15,16] or titanium implants[17] show a delay in the repair around implants. By comparing the course of osseous healing around implants in normal nondiabetic and insulin-controlled diabetic rats, it has been shown that whereas insulin increases the formation of bone around implants inserted into the femora of rats with streptozotocin-induced diabetes, it does not increase bone-implant contact in these animals.[18] In this study it was demonstrated that both the total area of new bone formed, and the surface of contact between bone and implant, were normalized after treatment of diabetic rats with insulin. This suggests that insulin appears to be essential for bone repair/remodeling process during osseointegration.

Histological analysis of the bone–implant interface under transmission electron microscopy showed that the ultrastructural characteristics in diabetic and control animals were very similar 10 days after implant placement. Flattened cells covering the implant surface, osteoblasts with well-developed organelles surrounded by osteoid, were identified in bone–implant interfaces. At 21 days after surgery, the new bone reached the implant. In control animals, a layer of bone-lining cells, between the new bone and implant space, osteocytes, and cement lines were observed. In diabetic rats, a continuous layer of cell process–like structures was distinguished between the bone-lining cells and the implant. The presence of chondrocytelike cells, which were surrounded by a cartilaginouslike matrix in new bone, indicates that the pattern of bone formation may be altered in these animals. Ultrastructural characteristics of bone–implant interface in diabetic rats treated with insulin resembled those observed in control animals.

Bone is continually being remodeled through bone formation by osteoblasts and resorption by osteoclasts. Osteoblastic cells are known to express insulin receptors,[19] and physiological concentrations of insulin increase bone collagen production in osteoblasts[20] and chondrocytes[21] in culture. Furthermore, it has been shown that mature mononuclear and multinucleated osteoclastlike cells generated *in vitro* and primary neonatal rat and mouse osteoclasts express receptors for insulin.[22] In addition, when osteoclastlike cells are placed onto dentin slices, insulin induces a dose-dependent inhibition on pit formation.[22]

Insulin initiates cellular responses by binding to tyrosine kinase receptors, which regulate a variety of signal pathways, therefore controlling growth and development of bone. The insulin receptor substrates (IRSs) are

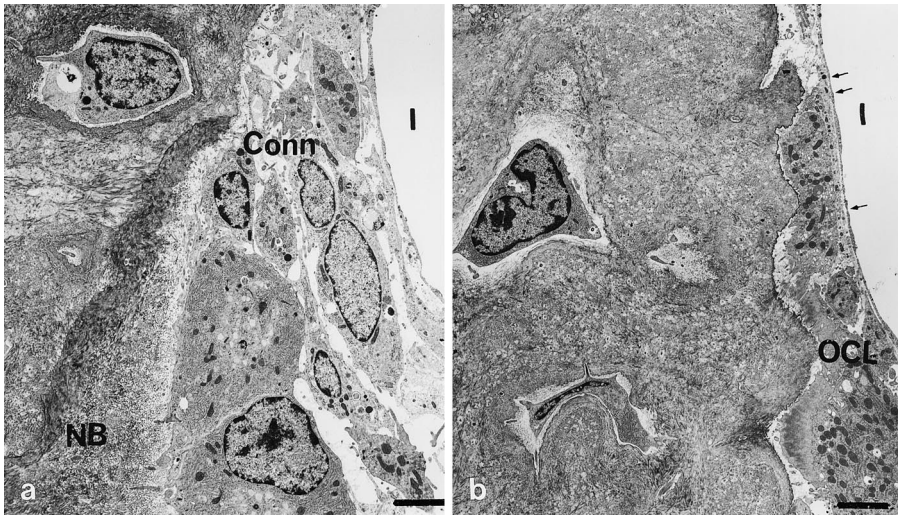


Fig. 8. Insulin-treated group 10 days after surgery. Electron micrographs of the bone–implant interface showing, in **a**, a region of connective tissue (Conn) near the implant (I). Note that a continuous layer of cell processes are surrounding the implant (I). Scale bar = 4.0 μm. In **b**, an area in which the new bone already reached the implant (I). Osteoclasts (OCL) are seen resorbing the new bone near the implant. However, the cell process layer (arrows) is still present surrounding the implant. Scale bar = 4.0 μm.

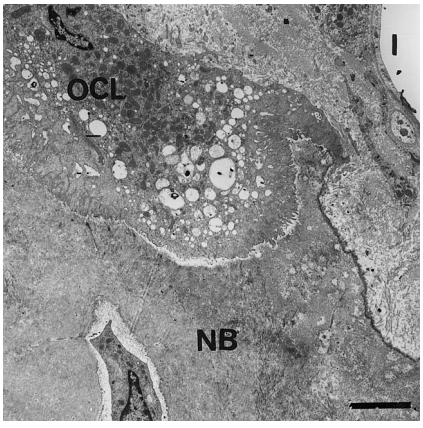


Fig. 9. Insulin-treated group 21 days after surgery. Electron micrograph of a region adjacent to the implant (I). An osteoclast (OCL) resorbing the new bone (NB) can be observed. Connective tissue remains between the osteoclast and the implant. Scale bar = 4.0 μm.

essential signal-transduction proteins that mediate the effects of insulin on cellular function.[23,24] IRS-1 expression is limited to osteoblastic cells, whereas IRS-2 is expressed in both osteoblastic and osteoclastic cells.[25] Recent observations on transgenic mice lacking the *IRS-1* gene (*IRS1*$^{-/-}$ mice)[25] or the *IRS-2* gene (*IRS2*$^{-/-}$ mice)[26] and wild-type mice showed that IRS-1 in osteoblasts is indispensable for maintaining bone turnover,[25] and that IRS-2 maintains predominance of bone formation over bone resorption. The integration of these two signals causes a potent bone anabolic activity by insulin and insulin-like growth factor-1.[26]

Specific alterations in bone formation and remodeling have been associated with type 1 diabetes mellitus, including altered mineral homeostasis[27,28] and reduced collagen production.[29] Bone turnover, as measured by percentages of osteoclasts, osteoblasts, and osteoid surface, and osteocalcin synthesis have been shown to be reduced in experimental models of diabetes.[30,31]

Conclusion

In this study, it was demonstrated that insulin restored bone formation around endosseous implants inserted into the tibiae of rats with alloxan-induced diabetes. Furthermore, ultrastructural characteristics of the bone–implant interface observed in diabetic animals under the influence of insulin resembled those of a normal osseointegration process. These results suggest that bone repair around endosseous implants is regulated by insulin, and imply that the control of the metabolic status of the diabetic patient is essential for successful osseointegration.

Acknowledgments

This work was supported in part by Fundação de Amparo à Pesquisa do Estado de São Paulo. The authors thank Sonja A. Bustamante, H.T. (ASCP), Gaspar Ferreira Lima, H.T. (TEM), and Irene Gouvea for their valuable technical assistance throughout the study.

Disclosure

The authors claim to have no financial interest in any company or product mentioned in this article.

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Reprint requests and correspondence to:
Paulina Sannomiya, PhD
Research Division
Institute of Heart (Incor)
University of São Paulo Medical School
Av. Dr. Enéas de Carvalho Aguiar, 44
05403–900 São Paulo, SP, Brazil
Phone/Fax: 55–11–30667178
E-Mail: expaulina@incor.usp.br

Abstract Translations [German, Spanish, Portuguese, Japanese]

AUTOR(EN): José T.T. Siqueira, DDS, PhD*, Simone C. Cavalher-Machado, MSc**, Victor E. Arana-Chavez, DDS, PhD***, Paulina Sannomiya, PhD**. **Leiter der Abteilung für Mund- und Gesichtsbeschwerden, Abteilung für Zahnheilkunde, zahnmedizinische Fakultät der Universität São Paulo, São Paulo, Brasilien. **A.O. Professorin, Forschungsabteilung, Institut für Herzleiden (Incor), LIM-11, medizinische Fakultät der Universität São Paulo, São Paulo, Brasilien. ***A.O. Professor, Labor für anorganische Gewebsbiologie, Abteilung für Histologie und Embryologie, Institut für biomedizinische Forschung, Universität von São Paulo, São Paulo, Brasilien. Schriftwerkehr:Paulina Sannomiya, PhD, Forschungsabteilung (Research Division), Institut für Herzleiden (Incor) (Institute of Heart (Incor)), medizinische Fakultät der Universität São Paulo (University of São Paulo Medical School), Av. Dr. Enéas de Carvalho Aguiar, 44 - 05403-900 São Paulo, SP, Brasilien. Telfon/Fax: 55-11-30667178. eMail: expaulina@incor.usp.br.*

Die Bildung von Knochengewebe um Titanimplantate herum - Untersuchung am Scheinbein der Ratte bezüglich des Einflusses von Insulin

ZUSAMMENFASSUNG: Hintergrund: Aus klinischen und experimentellen Studien ist mit wenigen Ausnahmen ableitbar, dass ein Zusammenhang zwischen der Erkrankung mit Typ 1 Diabetes und einer verzögerten Knochenneubildung um in das Knochengewebe eingesetzten Implantaten besteht. Es bestehen bislang keine gesicherten Erkenntnisse bezüglich der Auswirkung von Insulin auf den Prozess der Knochenwiederherstellung. Die vorliegende Studie zielte daher darauf ab, den Knochenintegrationsprozess anhand der histologischen und ultrastrukturellen Veränderungen unter dem Einfluss von Insulin zu beobachten und untersuchen. ***Methoden:*** In die Scheinbeine von männlichen Wistar-Ratten wurden Titanimplantate eingepflanzt. Es erfolgte eine Aufteilung der Versuchstiere in folgende drei Gruppen: 1) Ratten mit künstlich hervorgerufenem Alloxan-Diabetes, 2) diabetische Ratten, die mit NPH-Insulin in zwei Injektionen täglich behandelt wurden, und 3) Ratten als Mitglieder einer Prüfgruppe. 10 und 21 Tage nach Einsatz der Implantate wurden histologische und histomorphologische Messanalysen an den Übergangsstellen zwischen Knochengewebe und Implantat vorgenommen. ***Ergebnisse:*** In Abstimmung zu den Kontrollwerten wiesen die Ratten, bei denen ein Alloxan-Diabetes erzeugt wurde, 21 Tage nach Implantateinsatz einen 50 %igen Rückgang des Knochens im Bereich des neu gebildeten Knochengewebes ($p<0,001$) und an der Kontaktoberfläche zwischen Knochen und Implantat ($p<0,01$) auf. 10 Tage nach dem chirurgischen Eingriff konnten keine wesentlichen Unterschiede zwischen den Ergebnissen der einzelnen Versuchsgruppen festgestellt werden. Wurden die diabetischen Ratten mit Insulin behandelt, normalisierten sich die Werte. Die Ursache für die Verzögerung des Knochenheilungsprozesses liegt womöglich darin begründet, dass sich bei diabetischen Ratten chondrozytartige Zellen in einer kartilaginärähnlichen Umgebung finden. Bezüglich der Ergebnisse der Ultrastrukturanalyse waren die bei den mit Insulin behandelten diabetischen Ratten ermittelten Werte mit denen der Ratten der Kontrollgruppe gleichzusetzen. ***Schlussfolgerung:*** Die innerhalb der Studie ermittelten Daten lassen den Rückschluss zu, dass die Behandlung mit Insulin zumindest teilweise zur Normalisierung des Knochenwiederaufbaus bei in den Knochen eingelagerten Implantaten beiträgt. Daher kann die Überprüfung des Stoffwechselzustands bei einem an Diabetes erkrankten Patienten wesentlich zu einer erfolgreichen Implantation inklusive Knochenintegration der gesetzten Implantate beitragen. ***SCHLÜSSELWÖRTER:*** Titanimplantate, Knochenintegration, Insulin

AUTOR(ES): José T.T. Siqueira, DDS, PhD*, Simone C. Cavalher-Machado, MSc**, Victor E. Arana-Chavez, DDS, PhD***, Paulina Sannomiya, PhD**. **Supervisor del Equipo de Dolor Orofacial, División de Odontología, Facultad de Medicina de la Universidad de San Pablo, San Pablo, Brasil. **Profesor Asociado, División de Investigación, Instituto del Corazón (Incor), LIM-11, Facultad de Medicina de la Universidad de San Pablo, San Pablo, Brasil. ***Profesor Asociado, Laboratorio de Biología de Tejidos Mineralizados, Departamento de Histología y Embriología, Instituto de Ciencias Biomédicas, Universidad de San Pablo, San Pablo, Brasil. Correspondencia a: Paulina Sannomiya, PhD, Research Division, Institute of Heart (Incor), University of São Paulo Medical School, Av. Dr Eneas de Carvalho Aguiar, 44, 05403-900 Sao Paulo, SP, Brazil. Teléfono/Fax: 55-11-30667178. Correo electrónico: expaulina@incor.usp.br*

Formación del hueso alrededor de implantes de titanio en la tibia de ratas: el papel de la insulina

ABSTRACTO: ***Antecedentes***: Los estudios clínicos y experimentales demuestran, con algunas excepciones, que la diabetes mellitus tipo 1 está asociada con un retraso en la reparación del hueso alrededor de implantes endoóseos. El efecto de la insulina en la reparación o remodelación del hueso no se entiende completamente. El objetivo de este estudio es investigar el curso de los cambios histológicos y ultraestructurales del proceso de integración ósea bajo la influencia de la insulina. ***Métodos***: Se insertaron implantes de titanio en la tibia de ratas Wistar machos. Los animales se dividieron en tres grupos: 1) ratas con diabetes inducida por aloxán; 2) ratas con diabetes tratadas con insulina NPH (2 unidades internacionales por día); 3) controles. Se realizaron análisis histológicos e histomorfométricos de las secciones de hueso implantado a los 10 y 21 días luego de la colocación del implante. ***Resultados***: Con respecto a los valores de control, las ratas con diabetes inducida por aloxán exhibieron una reducción del 50% en el lugar del hueso formado ($p < 0,001$) y en la superficie de contacto entre el hueso y el implante ($p < 0,01$), 21 días después de la colocación del implante. No existieron diferencias significativas entre los grupos 10 días después de la cirugía. Los valores volvieron a los niveles normales después del tratamiento de las ratas diabéticas con insulina. La presencia de células similares a condrocitos rodeadas por una matriz tipo cartilaginosa en las ratas diabéticas sugiere un retraso en el proceso de reparación de hueso. Las características ultraestructurales del interfaz entre hueso e implante en ratas diabéticas tratadas con insulina se asemejan a las observadas en los grupos de control. ***Conclusión***: Los datos presentados sugieren que la reparación del hueso alrededor de implantes endoóseos es regulada, por lo

menos en parte, por la insulina. Los resultados sugieren que el control del estado metabólico del paciente diabético es esencial para lograr una integración ósea exitosa.
PALABRAS CLAVES: implantes de titanio, integración ósea, insulina

AUTOR(ES): José T. T. Siqueira, DDS, PhD*, Simone C. Cavalher-Machado, MSc**, Victor E. Arana-Chavez, DDS, PhD***, Paulina Sannomiya, PhD**. **Supervisor da Equipe de Dor Orofacial, Divisão de Odontologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil. **Professor Associado, Divisão de Pesquisa, Instituto do Coração (Incor), LIM-11, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil. ***Professor Associado, Laboratório de Biologia de Tecido Mineralizado, Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil. Correspondencia para: Paulina Sannomiya, PhD, Divisão de Pesquisa, Instituto do Coração (Incor), Faculdade de Medicina da USP, Av. Dr. Enéas de Carvalho Aguiar, 44 CEP: 05403-900 - São Paulo, Brasil. Telefone/Fax: 55-11-3066 7178. E-mail: expaulina@incor.usp.br*

Formaçγo Óssea ao Redor de Implantes de Titβnio em Tíbias de Ratos: Papel da Insulina

SUMÁRIO: **Antecedentes:** Estudos clínicos e experimentais demonstram, com raras exceções, que o tipo 1 de diabetes mellitus é associada com uma demora na reparação óssea ao redor de implantes endósseos. O efeito da insulina na reparação/remodelagem do osso não é completamente compreendido. O objetivo deste estudo foi investigar o curso de mudanças histológicas e ultra-estruturais do processo de ósteo-integração sob a influência da insulina. **Métodos:** Implantes de Titânio foram colocados na tíbia de ratos machos da raça Wistar. Os animais forma divididos em três grupos: 1) ratos diabéticos induzidos com aloxam; 2) ratos diabéticos tratados com insulina NPH (2UI/dia); 3) controles combinados. Análises histológicas e histomorfométricas das secões implantadas de osso foram realizadas 10 e 21 dias depois da colocação do implante. **Resultados:** Com relação aos valores de controle, os ratos diabéticos induzidos com aloxam exibiram uma redução de 50% da área de osso formado ($P<0.001$) e na superfície de contato entre osso e implante ($P<0.01$), 21 dias depois da colocação do implante. Não houveram diferenças significativas entre os grupos 10 dias depois da cirurgia. Os valores voltaram aos níveis normais depois do tratamento dos ratos diabéticos com insulina. A presença de células similares a condrócitos rodeadas por matrizes similares a cartilagens em ratos diabéticos sugere uma demora no processo de reparação do osso. As características ultra-estruturais da relação do implante ósseo em ratos diabéticos tratados com insulina assemelhou-se àquelas observadas nos controles. **Conclusão:** Os dados apresentados sugerem que a reparação óssea ao redor de implantes endósseos é regulada, pelo menos em parte, pela insulina. Os resultados implicam que o controle do estado metabólico de um paciente diabético é essencial para uma ósteo-integração bem sucedida.

PALAVRAS-CHAVE: implantes de titânio, ósteo-integração, insulina.

ラット顎骨へのチタン・インプラントの周辺部における骨形成；インシュリンが果す役割

著者：ジョセ・T・T・シケイラ、DDS、PhD*、シモーン・C・カバリァ・マシャドゥ、MSc**、ヴィクトール・E・アラナ-シャベス、DDS、PhD***、パウリーナ・サンノミヤ、PhD**

要約：

背景：タイプI糖尿病が骨内インプラントの骨修復の遅れに関連があることについては、これまでにも臨床研究と実験で示されてきた。しかし、骨修復・再形成へのインシュリンの影響についてはあまりよく知られていない。本研究の目的は、インシュリンの影響下における骨統合課程の組織学・微細構造学的な変化を知ることにあった。

方法：チタン・インプラントが雄ウィスター・ラットの顎骨に挿入された。ラットは1)アロキサン糖尿病群、2)NPHインシュリン（1日2 IU）処置群、3)対照実験群の3つのグループに別けられた。骨-インプラント断面の組織学・組織形態計測学分析が、インプラント設置の10日後と21日後に行われた。

結果：インプラント設置10日目には、各群の間に有意な差は認められなかった、手術21日目には、アロキサン糖尿病群において対照実験群と比べて骨形成（P<0.001）、骨とインプラントの接合部表面面積（P<0.01）ともに、50％少ないことがわかった。糖尿病ラットの分析値は、インシュリン治療を受けた後正常に戻ることがわかった。糖尿病ラットにおいて軟骨様基質に取り巻かれたchondrocite様の細胞が見られたことは、骨修復過程の遅れを示唆していた。インシュリン治療を受けた糖尿病ラットの骨-インプラント境界面の微細構造学的な性質は、対照実験群に近いことがわかった。

結論：骨内インプラント周辺部の骨修復は、少なくとも部分的にはインシュリンによって規制されていることがわかった、この結果から、糖尿病患者においては代謝水準の管理が骨統合の成功に描かせないものと推測する事ができる

キーワード：チタン・インプラント、骨統合、インシュリン

*サンパウロ大学メディカルスクール歯科学部口腔顔面疼痛チーム主任
(サンパウロ、ブラジル)
**サンパウロ大学メディカルスクールLIM-11心臓研究所（Incor）研究部准教授
(サンパウロ、ブラジル)
***サンパウロ大学バイオメディカル研究所組織・発生学部無機化組織生物研究所准教授
(サンパウロ、ブラジル)

問い合わせ先：Paulina Sannomiya, PhD, Research Division, Institute of Heart (Incar), University of São Paulo Medical School, Av. Dr. Enéas de Carvalho aguiar, 44 05403-900 São Paulo, SP, Brazil
電話／ファックス：55-11-30667178　Eメール：expaulina@incor.usp.br