Titanium Alloy Osseointegration in Cancellous and Cortical Bone of Ovariectomized Animals: Histomorphometric and Bone Hardness Measurements

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Purpose: *Histomorphometry and microhardness measurements were performed after Ti6Al4V implantation in cancellous and cortical bone of healthy and ovariectomized animals to determine characterization of the bone-biomaterial interface in osteopenic bone.* ***Materials and Methods:*** *Nine ovariectomized and 9 sham-aged rats were used. Four months later, nails were implanted in the distal femurs, and the animals were sacrificed after 8 weeks. Moreover, 3 ovariectomized and 3 sham-aged sheep were used. Twenty-four months later, screws were implanted in the tibial diaphyses and the animals were sacrificed after 12 weeks.* ***Results:*** *Histomorphometry showed the development of osteopenia in both trabecular and cortical bone, and revealed a significant decrease in the osseointegration rate in osteopenic versus sham-aged animals for both trabecular (Affinity Index: −18.6%,* $P < .001$*) and cortical bone (Affinity Index: −23.5%,* $P < .005$*; Bone Ingrowth: −9%,* $P < .05$*). At the interfaces of the sham-aged animals in both trabecular and cortical bone, a decrease of bone microhardness was observed in comparison with preexisting bone (trabecular: −9.8%,* $P < .0005$*; cortical: −19.3%,* $P < .0005$*). In case of osteopenia, this decrease was even more extensive (trabecular: −15.5%,* $P < .0005$*; cortical: −24.7%,* $P < .0005$*).* ***Discussion:*** *The present data suggest that bone formation around Ti6Al4V was not associated with complete bone maturation, even in healthy animals. In case of osteopenia, both bone formation and maturation were delayed.* ***Conclusion:*** *These results apparently demonstrate the utility of investigating biomaterials in osteopenic bone and the importance of careful evaluation of the healing rate and bone maturation degree around implanted biomaterials.* (Int J Oral Maxillofac Implants 2002;17:28–37)

Key words: *bone-biomaterial interface, implant, microhardness, osseointegration, osteoporosis, titanium alloy*

Osteoporosis is a health problem of clinical relevance that recognizes many different pathogenetic factors.[1,2] Structural modifications related to osteoporosis mainly affect cancellous bone, but cortical bone also is compromised with a reduction of cross-sectional area because of endosteal resorption and medullary expansion.[3,4] Osteoporotic patients develop pathologic fractures mainly affecting the hip, spine, and wrist, but the incidence of fractures also increases at many other sites,[5,6] necessitating the greater use of plates, pins, screws, and arthroprostheses in osteoporotic bones. The extent of oral bone loss in conditions that cause skeletal osteopenia is still unclear.[7] However, some experimental and clinical studies have shown jaw and mandible involvement in osteoporosis,[8–11] and they illustrate the role played by the systemic bone mass decrease in the development of tooth loss in postmenopausal women.[5,12,13] This suggests that the need for dental implants may be greater for osteoporotic patients.[14]

Clinicians recognize that the presence of osteoporosis could represent a risk factor for implanted

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biomaterials because of the poor or slow rate of osseointegration related to bone rarefaction, metabolic alterations, and possibly also to the presence of a local biochemical negative microenvironment.[2,13,15–18] It is known that not only bone histologic and structural modifications typical of the osteoporotic state are responsible for poor bone ingrowth, but also a decrease of some cytokins or growth factors which are useful for osseointegration processes,[19–21] associated with an increase in other damaging events,[22,23] have additional negative consequences on the bonding of bone to biomaterials.

Many experimental studies in orthopedics and dentistry conducted on biomaterial osseointegration have focused attention on the clinical situation of osteoporosis[24–33] because of the costs and severe effects on patients' physical and mental health.[34] In previous experimental studies in rats, the current authors observed a significant decrease in the osseointegration rate of some metallic and ceramic materials when implanted in osteopenic cancellous bone.[24–26] Histomorphometry was used to study the bone-biomaterial interface to yield quantifiable results.

The working hypothesis of the present study was that the osseointegration of Ti6Al4V can be delayed in trabecular and cortical osteopenic bone in comparison to healthy bone. Nails and screws made of Ti6Al4V were implanted in the cancellous and cortical bone of ovariectomized rats and sheep. First, the osteopenic state developed in animals was characterized, and then the bone-material interface was assessed. Histomorphometry was improved by microstructural data including microhardness measurements, thereby increasing the knowledge on bone maturity, mineralization rate, and mechanical resistance at the bone-biomaterial interface.

MATERIALS AND METHODS

Experimental Design

The specimens used in this project were obtained from a larger study currently in progress. The study was performed in compliance with the European and Italian Laws on animal experimentation and with the Animal Welfare Assurance No #A5424-01 of the National Institute of Health (NIH-Rockville, MD). The animal research protocol was approved by the responsible public authorities as requested by the Italian Law according to the EC rules (Law by Decree, 27 January, 1992, No. 116).

Intact rats and sheep served as control groups (sham-aged), while the ovariectomized animals comprised the experimental groups. Grade 5 Ti6Al4V (ASTM F-136), sterilized via gamma radiation (2.5 megarad), was implanted in animals. Osseointegration in trabecular bone was tested in rats where cylindrical nails, 2 mm in diameter and 5 mm in length, were used, while osseointegration in cortical bone was tested in sheep in which cylindrical tapered screws, 3.5 mm in outer diameter and 12 mm in length, were implanted (Fig 1).

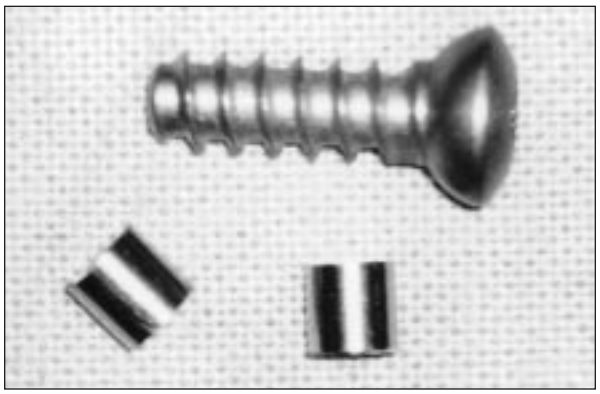

Fig 1 The Ti6Al4V nails and screws just before surgical implantation.

Bone implants were placed 4 and 24 months after ovariectomy in rats and sheep, respectively. The postovariectomy time intervals selected had already shown the development of an osteopenic state in previous tests by the current authors. Therefore, they proved to be useful for the present investigation in both animal species.[35,36] Rats were housed in metal cages for the entire study at 20°C ± 0.5°C and relative humidity of 55%; food (Mucedola Srl, Settimo Milanese, Milan, Italy, Ca 0.93%, P 0.64%) and tap water were allowed ad libitum. Sheep were maintained in a stall until postoperative day 20 (1°C and relative humidity of 55%; food (Mucedola Srl, Settimo Milanese, Milan, Italy, 0.85% Ca and 0.51% P) and tap water were allowed ad libitum. About 20 days after surgery, sheep were moved to external housing conditions.

All surgical procedures were performed in aseptic conditions and under general anesthesia. Animals were euthanized under general anesthesia with intravenous administration of Tanax (Hoechst, Frankfurt am Main, Germany) at selected experimental times.

Rats: Trabecular Bone Implants. Eighteen rats, weighing between 450 ± 30 g and aged 10 months at the beginning of the study were used; 9 animals were bilaterally ovariectomized through a lumbar approach and 9 nonoperated sham-aged rats were used as controls. General anesthesia was obtained

by subcutaneous injection of 87 mg/kg bw ketamine (Ketavet 100, Farmaceutici Gellini, SpA, Aprilia, Italy) and 13 mg/kg bw xylazine (Rompun Bayer AG, Leverkusen, Germany). Four months later, all 18 rats underwent exposure of the left distal femur through a lateral skin incision, and cylindrical nails were implanted. Antibiotics and analgesics were administered for 5 and 2 days, respectively (3 mg/100 g bw flumequine, Flumexil, Fatro, Ozzano Emilia Bologna, Italy; 0.01 ml/100 g bw ketoprofen, Orudis, Rhone-Poulenc Rorer SpA, Milan Italy). Eight weeks after implantation, the animals were pharmacologically euthanized and the femurs were harvested.

Sheep: Cortical Bone Implants. Six mongrel sheep, body weight 70 ± 5 kg, aged 5 years at the beginning of the study, were used. General anesthesia was obtained by premedication with 10 mg/kg bw ketamine, 0.3 mg/kg bw xylazine im and 0.0125 mg/kg bw atropine sulphate sc. It was induced with 6 mg/kg bw sodium thiopentone iv (2.5% solution, Pentothal, Hoechst AG, Germany), and maintained with O_2, N_2O and 1% to 2.5% halothane under assisted ventilation (Servo Ventilator 900 D, Siemens, Germany). Three sheep were submitted to bilateral ovariectomy while the remaining animals served as sham-aged control group. Twenty-four months later, a total of 18 screws were implanted in the left tibia of all sheep (3 per tibia). After incision of the skin and dissection of the fasciae, the left tibial diaphyses were exposed. A 2.7-mm-diameter drill was used to predrill 3 holes. They were tapped with a 3.5-mm device and 3 screws were then placed into the diaphyseal cortex of each bony segment, with the same final insertion torque of about 1.8 Nm. Antibiotics and analgesics were administered for 5 and 2 days, respectively (1 g/day cephalosporin, Totacef, Bristol Myers Squibb SpA, Italy; and 500 mg/day ketoprofen, Orudis, Rhone-Poulenc Rorer SpA, Milan, Italy). All the animals were euthanized after 12 weeks, and each implant site was isolated using a saw and harvested.

Histomorphometry

The bone specimens containing the implants were fixed in 4% buffered paraformaldehyde for 24 hours, dehydrated in graded series of alcohols until the absolute was reached (for 24 hours at each concentration), and then included in polymethylmethacrylate resin until solidification, which usually occurred after 7 days. All the processing was carried out at a temperature of 22°C ± 1°C and humidity rate of 48%. Blocks were sectioned along a plane perpendicular (nails in rats) or parallel (screws in sheep) to the long axis of the implants by using a Leica 1600 diamond saw microtome (Ernst Leitz, Wetzlar, Germany) and yielding undecalcified sections of 60 to 80 µm in thickness. The sections were ground and polished (Struers Dap-7, Strues Tech A/S, Rodovre/Copenhagen, Denmark) and stained with Fast Green. Histomorphometric analyses were performed on 3 consecutive sections using an optic microscope (Zeiss Axioskop, Carl Zeiss, Jena, Germany) connected to an image analyzer system (Kontron KS300 v.2, Kontron Elektronik, Munchen, Germany). The nomenclature approved by the American Society of Bone and Mineral Research (ASBMR) was used for cancellous and cortical bone calculations[37] and the following static measurements were calculated at 1.25x:

1. *In cancellous bone (bone bed around the implants):* trabecular bone volume (BV/TV, %), the whole spongy bone area, expressed as a percentage of the total tissue area in the sampling site and converted to a volume; trabecular thickness (Tb.Th, µm), trabecular separation (Tb.Sp, µm), trabecular number (Tb.N, /mm) and index of connectivity (number of nodes/number of trees, N.Nd/N.Tm) were calculated from BV/TV and the perimeter of trabeculae (BS/TV) according to Parfitt's formulas;[37]
2. *In cortical bone (mid-diaphysis):* cross-sectional area (Se.Ar, mm^2), area of bone and marrow cavity within the periosteal surface; medullary area (Me.Ar, mm^2), area delineated by the endocortical surface of the cross section; cortical bone area (Ct.Ar, mm^2), Se.Ar-Me.Ar; endocortical perimeter (Ec.Pm, mm), length of bone perimeter on the endocortical surface; and periosteal perimeter (Ps.Pm, mm), length of bone perimeter at the periosteal surface.

Affinity Index (the ratio of the length of the region in which bone is directly apposed to the implant without the presence of fibrous membrane divided by the total length of the bone-implant interface multiplied by 100) was calculated for nails implanted in rat trabecular bone. Affinity Index and Bone Ingrowth (amount of bone grown into the screw grooves, expressed as a percentage of the groove area) was calculated for screws implanted in sheep cortical bone.

Each measurement was made semiautomatically by an experienced blinded investigator.

Microhardness. After the histologic analysis, the resin-embedded blocks containing the remnant of the implanted material were ground and polished following the methodology described by Huja and associates,[38] and then used to measure the level of

bone hardness by means of an indentation test (Microhardness VMHT 30, Leica, Wien, Austria). Microhardness measurements were taken with a Vickers indenter (4-sided pyramid with square base and an apex angle between opposite sides of 136 ± 15 degrees) applied to the bone at a load of 0.05 kgf and dwell time of 5 seconds. The Vickers hardness degree (HV) was calculated by dividing the indentation force by the surface of the imprint (4 pyramid surfaces) observed at the microscope. The resulting formula was where F is the weight applied to the pyramid expressed in kg, β is half of the pyramid angle, and d is the average diagonal length of the imprint expressed in μm. The average value for each sample was calculated on 10 measurements for each examined area, eg, at the interface and at 1,000 μm from it. By allowing a minimum distance of about 3d between the imprints, their mutual influence was avoided, too.

Statistical Analysis

Statistical analysis was performed by using a SPSS version 7.5 software (SPSS/PC, Chicago, IL). Data were reported as mean ± SD at a significance level of $P < .05$. After having verified normal distribution and homogeneity of variances with Levene's test, Student t test was used to compare data between groups. Paired Student t test was used in case of dependent samples.

RESULTS

Rats: Trabecular Bone Implants

Percent variations in static histomorphometric parameters of the cancellous bone between experimental and control groups are reported in Fig 2. These data confirmed the development of a significant osteopenic state in the bone bed where Ti6Al4V was implanted at 4 months after ovariectomy.

Figures 3a and 3b show the histologic appearance of healthy and osteopenic trabecular bone implanted with Ti6Al4V. When Ti6Al4V is implanted in osteopenic bone, some areas without any apparent bone-to-implant contact can be observed.

Morphometric data also confirmed the histologic appearance: the Affinity Index decreased significantly ($P < .001$) in osteopenic versus healthy bone (experimental group: 54.63% ± 8.88%; control group: 67.08% ± 10.51%).

Bone microhardness results obtained around implants at the interface and at 1,000 μm from it are reported in Table 1. Percent decrease in microhardness data observed at the interface and compared to that obtained at a 1,000-μm distance revealed a significant difference ($P < .0005$) in experimental (–15.51% ± 5.05%) and control (–9.79% ± 9.95%) groups.

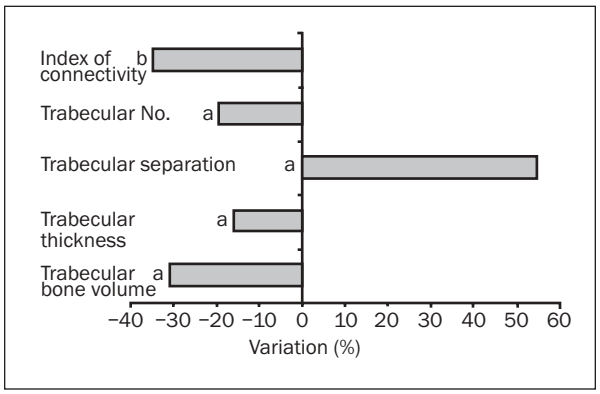

Fig 2 Percent variation in histomorphometric parameters of rat cancellous bone bed where Ti6Al4V was implanted between experimental and control groups. The significant decrease in trabecular bone volume, trabecular thickness, trabecular number, and the significant increase of trabecular separation in experimental group indicate the development of an osteopenic state in rats 4 months after ovariectomy (a = $P < .0005$; b = $P < .05$).

Sheep: Cortical Bone Implants

The percent variations in cortical static histomorphometric parameters measured in tibia and reported in Fig 4 demonstrated the development of osteopenia in cortical bone.

Figures 5a and 5b show less bone-to-implant contact in osteopenic cortical bone versus healthy bone.

Morphometric data are consistent with histologic observations: Significant decreases in the Affinity Index ($P < .005$: experimental group: 57.99% ± 6.17%; control group: 75.79% ± 12.17%) and Bone Ingrowth ($P < .05$: experimental group: 79.38% ± 6.12%; control group: 87.24% ± 8.57%) were observed in osteopenic cortical bone.

Figure 6 shows an image depicting microhardness rhomboidal imprints after an indentation test at the bone-biomaterial interface. The microhardness values measured at the Ti6Al4V-bone interface and at a 1,000-μm distance in both the experimental and control groups are shown in Table 2. Percent decreases in microhardness data observed at the interface and compared to those obtained at 1,000 μm, revealed a significant difference ($P < .001$) in experimental (–24.70% ± 2.95%) and control (–19.33% ± 1.61%) groups. A significant decrease

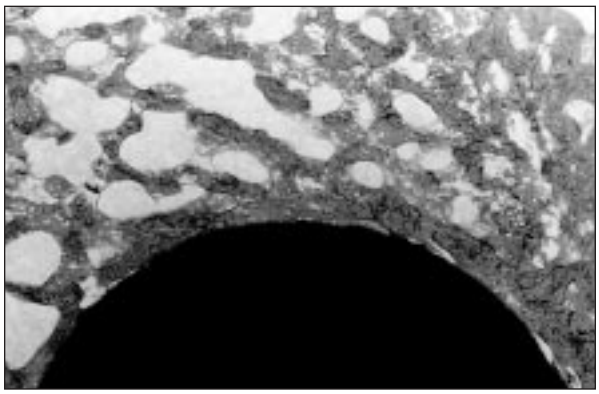

Fig 3a Rat trabecular normal bone implanted with a Ti6Al4V nail 8 weeks after surgery. Bone is directly apposed to the implant surface (Fast Green, 4×).

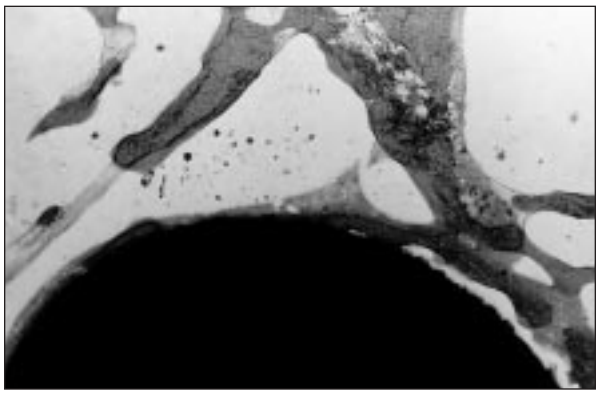

Fig 3b Rat trabecular osteopenic bone implanted with a Ti6Al4V nail 8 weeks after surgery. Some areas without bone-to-implant contact are visible. The rarefaction of bone trabeculae in the implanted bone bed caused by osteopenia is also observable (Fast Green, 4×).

Table 1 Bone Microhardness Around Ti6Al4V Nails Implanted in Rats Expressed in Vickers Degrees

	Control group		Experimental group	
	Interface	1000 µm	Interface	1000 µm
Ti6Al4V	60.15 ± 5.33[a]	66.87 ± 2.97	57.35 ± 4.08[a]	67.89 ± 2.95

Paired Student *t* test: [a]Comparison between interface and 1000-µm data within each group ($P < .0005$).
Average of 10 determinations; mean ± SD, n = 9.

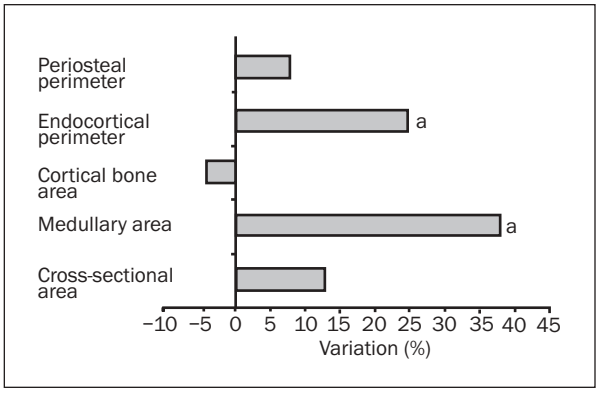

Fig 4 Percent variation in histomorphometric parameters of cortical bone bed where Ti6Al4V was implanted between experimental and control groups. The significant increase of endocortical perimeter and medullary area together with the increase of periosteal perimeter, cross-sectional area and the decrease of cortical area indicate the development of an osteopenic state in sheep 24 months after ovariectomy (a = $P < .05$).

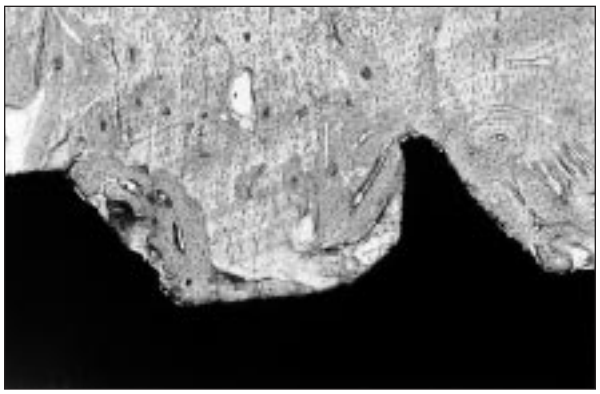

Fig 5a Sheep cortical normal bone implanted with a Ti6Al4V screw 12 weeks after surgery. Bone is visible in the screw threads directly apposed to the biomaterial surface (Fast Green, 4×).

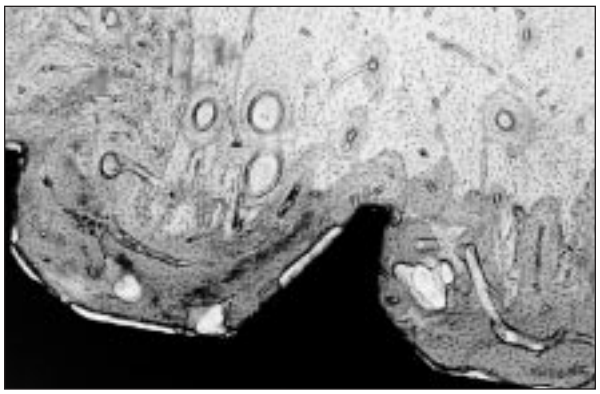

Fig 5b Sheep cortical osteopenic bone implanted with a Ti6Al4V screw 12 weeks after surgery. In some areas bone is not directly apposed to the screw surface (Fast Green, 4×).

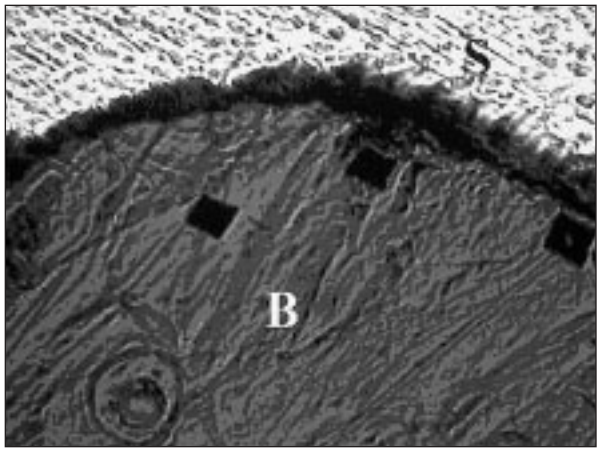

Fig 6 Microhardness rhomboidal imprints after indentation test at the bone-Ti6Al4V screw interface. S = screw; B = Bone; rhomboidal imprints after indentation test.

Table 2 Bone Microhardness Around Ti6Al4V Screws Implanted in Sheep Expressed in Vickers Degrees

	Control group		Experimental group	
	Interface	1000 µm	Interface	1000 µm
Ti6Al4V	63.97 ± 7.67[a]	79.30 ± 5.15	59.29 ± 9.71[a,b]	78.77 ± 5.77

Paired Student *t* test: [a]Comparison between interface and 1000-µm data within each group ($P < .0005$) (paired); [b]Comparison between experimental group and control group at interface ($P < .05$) (unpaired).
Average of 10 determinations; mean ± SD, n = 9.

in bone hardness ($P < .05$) was also observed at the interface of the experimental group when compared to the control group (–16.7%).

DISCUSSION

Longer life expectancy, at least in western countries, is associated with an increased interest in the behavior of devices implanted in pathologic bone. The common metabolic bone disorders affecting potentially implanted patients are different and include renal osteodystrophy, osteomalacia, and Paget disease. However, aged osteopenic and osteoporotic bone seems to represent a significant concern. Symptomatic osteoporosis, related to aging, and secondary osteoporosis, subsequent to estrogen deficiency and to many systemic disorders or therapies, affect a great part of the population, depending on age, race, gender, and lifestyle.[1,2,13,39]

In the present study, histomorphometry of the implanted bone bed in ovariectomized animals showed the development of osteopenia both in cancellous and in cortical bone. Ti6Al4V highlighted a significant osseointegration decrease, when implanted in trabecular and cortical osteopenic bone and compared to control animals. Microhardness measurements made distant to the implant, showed no difference in trabecular and cortical bone hardness between experimental and control animals. In fact, according to the definition of osteoporosis (reduction of bone mass without alterations in the composition of bone, leading to fractures), the microhardness value, which mainly depends on bone mineralization, is expected to be similar for healthy and osteoporotic bone. Such a hypothesis was confirmed by present results, where measurements were taken at 1,000 μm from the implant in preexisting bone.

In healthy and in osteopenic bone a significant decrease in bone hardness was observed at the Ti6Al4V-bone interface compared to the preexisting bone located distant to the implant. Such a result obtained for both groups—even in the absence of pathologic conditions—confirmed that bone around the material was still immature and not well mineralized at 8 and 12 weeks for trabecular and cortical bone, respectively. Histomorphometric investigation had, in fact, shown better osseointegration of Ti6Al4V in trabecular and cortical bone of control animals. However, the newly formed bone around the materials was less resistant to the indentation test as compared to the distant bone also found in th healthy bone of control animals. A significant decrease in bone hardness at the Ti6Al4V-bone interface was observed in osteopenic versus normal bone, but only in the cortical diaphyseal implants and not in the trabecular bone. These data, which exceed the hypothesis, require some pathophysiologic considerations that are based on clinical experience and, regretfully, on limited basic science evidence of the established osteoporosis effects on fracture healing.[40] Bone osseointegration could be considered as a reparative process similar to fracture healing.[41,42] In this regard, fractures of osteoporotic patients that usually occur in the trabecular bone are commonly observed in clinical practice and appear to heal with the formation of a normal callus and bone repair as in healthy subjects. On the contrary, when studying fracture healing in diaphyseal cortical bone, some authors have failed to find differences,[40] but most of them observed abnormalities in osteoporotic bone[43–46] both in the early and late phases of bone healing.

The present data seem to suggest that modeling and remodeling processes in cortical bone are differently affected by osteoporosis than in trabecular bone, but further histodynamic investigations concerning bone quality around implants are necessary.[47]

The rat was selected because the characterization of such a model for studies on osteoporosis is excellent.[48,49] Moreover, it is the only osteopenic animal that has been used for research on osseointegration, except for a few studies in which dogs and rabbits were employed.[30,31,50] However, placement of screws or implants in the diaphyseal cortical bone of this small-size animal for the evaluation of biomaterials cannot be recommended, according to the Standards of the International Organization for Standardization.[51] Moreover, the rat is reported to be a poor model for the study of the effects of ovariectomy in cortical bone because of the lack of Haversian systems.[52] Hence, the sheep was used for the study of cortical bone implants, and this animal seems to be a good model for postmenopausal osteoporosis, even though it is less standardized than the rat.[35, 53–55]

As far as the experimental times are concerned, the interval of 8 weeks after implantation was chosen for the rats because the main significant differences in biomaterial osseointegration between osteopenic and normal animals are first observed at that time and persist until week 24, according to the present authors' previous experiences.[24,32] In sheep, 12 weeks postsurgery represent the shorter experimental time for evaluating bone-implant osseointegration in cortical bone following ISO 10993-6 rules.[51] As far as the present authors know, this study is the first to report on bone implants and

osteopenia in diaphyseal cortical bone of long-term ovariectomized sheep.

Microhardness tests have also been used by other researchers to assess the bone-material interface.[38,56–59] They have emphasized that bone hardness measurements in the proximity of an implant can provide important information on many qualitative bone characteristics, such as calcification degree, arrangement and number of collagen fibers, ratio between collagen fibers and ground substance, mineral quantity per volume unit, and elastic modulus.[56,59] However, as far as the current authors know, the microstructural technique has never been used in studies investigating osseointegration in pathologic osteopenic bone. Most of these studies concentrated mainly on morphologic tests such as histomorphometry, although the importance of characterizing the newly formed bone around biomaterials in osteopenic animals is well recognized both in orthopedics and dentistry.

Moreover, microhardness measurements enable the study of bone hardness at different distances from the implant,[59] obviating the need for additional specimens since the same specimens can also be used for undecalcified bone histomorphometry.[56–58] Bone fixation and infiltration processing may always be considered and standardized to achieve reliable measurements and results.[38]

Finally, these data suggest that even in healthy animals, bone formation around Ti6Al4V was not associated with complete bone maturation; when an osteopenic state was present, both bone formation and maturation were delayed.

Present histomorphometric data are consistent with other results achieved on pure Ti and Ti6Al4V implants in osteopenic bone, which demonstrated the superiority of ceramic materials, especially in trabecular bone, although their osseointegration was affected by the presence of osteoporosis in some cases.[24,27–29,32]

The osteopenic state delayed bone formation around endosseous implants both in trabecular and cortical bone. Osseointegration of Ti6Al4V was obtained, but bone remodeling processes leading to maturation took longer both in trabecular and cortical bone in healthy bone.

SUMMARY

These results cannot be automatically transferred into clinical practice where implants are differently loaded and further studies focusing more on clinical experience are mandatory. The current findings would seem to suggest that biomaterials should be preclinically tested in pathologic bone and, in addition, the bone-to-implant interface may be characterized with both morphologic and microstructural investigations to acquire a better knowledge of the relation between bone ingrowth and bone status. In fact, although it is still unclear what size of implant-bone contact area or degree of bone maturation is required to ensure adequate anchorage for successful implantation of a load bearing device,[60] it may be useful to take into account the various different healing responses of osteopenic bone, thus providing clinicians with suitable macro- or micromorphology of implants, healing period, and loading modalities.[61]

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REFERENCES

1. Kleerekoper M, Avioli LV. Osteoporosis pathogenesis and therapy. In: Avioli LV, Krane SM (eds). Metabolic bone disease. San Diego: Academic Press, 1998:387–409.
2. Dao TTT, Andersen JD, Zarb GA. Is osteoporosis a risk factor for osseointegration of dental implants? Int J Oral Maxillofac Implants 1993;8:137–144.
3. Cheng S, Toivanen JA, Suominen H, Toivanen JT, Timonen J. Estimation of structural and geometrical properties of cortical bone by computerized tomography in 78-year-old women. J Bone Miner Res 1995;10:139–148.
4. Ruff CB, Hayes WC. Sex differences in age-related remodeling of the femur and tibia. J Orthop Res 1988;6:886–896.
5. Krall EA, Garcia RI, Dawson-Hughes B. Increased risk of tooth loss is related to bone loss at the whole body, hip, and spine. Calcif Tissue Int 1996;59:433–437.
6. Kannus P, Niemi S, Palvanen M, Parkkari J. Continuously increasing number and incidence of fall-induced, fracture-associated, spinal cord injuries in elderly persons. Arch Intern Med 2000;160:2145–2149.
7. Miller SC, Hunziker J, Mechman M, Wronsky TJ. Intermittent parathyroid hormone administration stimulus bone formation in the mandibles of aged ovariectomized rats. J Dent Res 2000;76:1471–1476.
8. Von Wowern N, Klausen B, Kollerup G. Osteoporosis: A risk factor in periodontal disease. J Periodontol 1994;65: 1134–1138.
9. Miller SC, Bowman BM, Miller MA, Bagi CM. Calcium absorption and osseous organ-, tissue-, and envelope-specific changes following ovariectomy in rats. Bone 1992;12: 439–446.

10. Nishimura I, Hosokawa R, Atwood DA. The knife-edge tendency in mandibular residual ridges in women. J Prosthet Dent 1992;67:820–826.
11. Bollen AM, Taguchi A, Hujoel PP, Hollender LG. Case-control study on self-reported osteoporotic fractures and mandibular cortical bone. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90:518–524.
12. Kribbs PJ. Comparison of mandibular bone in normal and osteoporotic women. J Prosthet Dent 1990;63:218–222.
13. Becker W, Hujoel PP, Becker BE, Willingham H. Osteoporosis and implant failure: An exploratory case-control study. J Periodontol 2000;71:625–631.
14. Fujimoto T, Niimi A, Nakai H, Ueda M. Osseointegrated implants in a patient with osteoporosis: A case report. Int J Oral Maxillofac Implants 1996;11:539–542.
15. Barrios C, Brostrom LA, Stark A, Walheim G. Healing complications after internal fixation of trochanteric hip fractures: The prognostic value of osteoporosis. J Orthop Trauma 1993;7:438–442.
16. Blomqvist JE, Alberius P, Isaksson S, Linde A, Hansson BG. Factors in implant osteointegration failure after bone grafting. An osteometric and endocrinologic matched analysis. Int J Oral Maxillofac Surg 1996;25:63–68.
17. Roberts WE, Simmons KE, Garetto LP, DeCastro RA. Bone physiology and metabolism in dental implantology: Risk factors for osteoporosis and other metabolic bone diseases. Implant Dent 1992;1:11–21.
18. Weber RL, Wiesen MJ, Iacono VJ, Baer PN. Osteoporosis: A risk factor for dental implants and in the prognosis of periodontal therapy. Periodontal Clin Investig 1997;19:5–8.
19. Celiker R, Arslan S. IGF-I levels were found to be lower in women with osteoporosis. Rheumatol Int 2000;19:205–208.
20. Marie PJ. Cellular and molecular alterations of osteoblasts in human disorders of bone formation. Histol Histopathol 1999;14:525–538.
21. Linkhart TA, Mohan S, Baylink KDJ. Growth factors for bone growth and repair: IGF, TGF beta and BMP. Bone 1996;19:1S–12S.
22. Jilka RL. Cytokines, bone remodeling, and estrogen deficiency: A 1998 update. Bone 1998;23:75–81.
23. Raltson SH. Role of cytokines in clinical disorders of bone metabolism. In: Gowen M (ed). Cytokine and Bone Metabolism. Boca Raton: CRC, 1992:362–367.
24. Fini M, Nicoli Aldini N, Gandolfi MG, et al. Biomaterials for orthopaedic surgery in osteoporotic bone: A comparative study in osteopenic rats. Int J Artif Organs 1997;20:291–297.
25. Fini M, Giavaresi G, Nicoli Aldini N, et al. The effect of osteopenia on the osteointegration of different biomaterials: Histomorphometric study in rats. J Mat Sci Mat Med 2000; 11:579–585.
26. Fini M, Giavaresi G, Torricelli P, et al. Biocompatibility and osseointegration in osteoporotic bone. A preliminary in vitro and in vivo study. J Bone Joint Surg Br 2001;83:139–143.
27. Hara T, Hayashi K, Nakashima Y, Kanemaru T, Iwamoto Y. The effect of hydroxyapatite coating on the bonding of bone to titanium implants in the femora of ovariectomised rats. J Bone Joint Surg Br 1999;81:705–709.
28. Yamazaki M, Shirota T, Tokugawa Y, et al. Bone reactions to titanium screw implants in ovariectomized animals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87: 411–418.
29. Pan J, Shirota T, Ohno K, Michi K. Effect of ovariectomy on bone remodeling adjacent to hydroxyapatite-coated implants in the tibia of mature rats. J Oral Maxillofac Surg 2000;58:877–882.
30. Mori H, Manabe M, Kurachi Y, Nagumo M. Osseointegration of dental implants in rabbit bone with low mineral density. J Oral Maxillofac Surg 1997;55:351–361.
31. Fujimoto T, Niimi A, Sawai T, Ueda M. Effects of steroid-induced osteoporosis on osseointegration of titanium implants. Int J Oral Maxillofac Implants 1998;13:183–189.
32. Hayashi K, Uenoyama K, Mashima T, Sugioka Y. Remodeling of bone around hydroxyapatite and titanium in experimental osteoporosis. Biomaterials 1994;15:11–16.
33. Takeshita F, Iyama S, Ayukawa Y, Kido MA, Murai K, Suetsugu T. The effects of diabetes on the interface between hydroxyapatite implants and bone rat tibia. J Periodontol 1997;68:180–185.
34. Oleksik A, Lips P, Dawson A, et al. Health-related quality of life in postmenopausal women with low BMD with or without prevalent vertebral fractures. J Bone Miner Res 2000;15: 1384–1392.
35. Giavaresi G, De Terlizzi F, Gnudi S, et al. Discriminant capacity of quantitative ultrasound versus dual-X-ray absorptiometry to determine cancellous bone loss in ovariectomized rats. Bone 2000;26:297–303.
36. Fini M, Pierini G, Giavaresi G, et al. The ovariectomised sheep as a model for testing biomaterials and prosthetic devices in osteopenic bone: a preliminary study on iliac crest biopsies. Int J Artif Organs 2000;23:275–281.
37. Parfitt AM, Drezner MK, Glorieux FH, et al. Bone histomorphometry: Standardization of nomenclature, symbols and units. J Bone Miner Res 1987;2:595–610.
38. Huja SS, Katona TR, Roberts WE. Microhardness testing of bone. In: An YH, Draughin RA (eds). Mechanical testing of bone and the bone-implant interface. Boca Raton: CRC, 1999:247–256.
39. Wiktorowicz ME, Goeree R, Papaioannou A, Adachi JD, Papadimitropoulos E. Economic implications of hip fracture: Health service use, institutional care and cost in Canada. Osteoporos Int 2001;12:271–278.
40. Wheeler DL, Eschbach EJ, Montfort MJ, Maleshwari P, McLoughlin SW. Mechanical strength of fracture callus in osteopenic bone at different phases of healing. J Orthop Trauma 2000;2:86–92.
41. Soballe K. Hydroxyapatite ceramic coating for bone implant fixation. Acta Orthop Scand 1993;255:1–57.
42. Glowachki J, Spector M. Tissue responses to bone-derived and synthetic materials. In: Davies JE (ed). The Bone Biomaterial Interface. Toronto: Toronto Press, 1991:265–274.
43. Walsh WR, Sherman P, Howlett CR, Sonnabend DH, Ehrlich MG. Fracture healing in a rat osteopenia model. Clin Orthop 1997;342:218–227.
44. Muschler GF, Lane JM. Orthopaedic surgery. In: Habal MB, Hari Reddi A (eds). Bone Grafts and Bone Substitutes. Philadelphia: WB Saunders, 1992:375–407.
45. Kubo T, Shiga T, Hashimoto J, et al. Osteoporosis influences the late period of fracture healing in a rat model by ovariectomy and low calcium diet. J Steroid Biochem Mol Biol 1999;68:197–202.
46. Namkung-Matthal H, Appleyard R, Jansen J, et al. Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. Bone 2001;28:80–86.
47. Chappard D, Aguado E, Hurè G, Grizon F, Basle MF. The early remodeling phases around titanium implants: A histomorphometric assessment of bone quality in a 3- and 6-month study in sheep. Int J Oral Maxillofac Implants 1999;14:189–196.
48. Yamazaki I, Yamaguchi H. Characteristics of an ovariectomized osteopenic rat model. J Bone Miner Res 1989;4: 13–22.

49. Frost HM, Jee WSS. On the rat model of human osteopenias and osteoporoses. Bone Miner 1992;18:227–236.
50. Martin RB, Paul HA, Bargar WL, Dannucci GA, Sharkey NA. Effects of estrogen deficiency on the growth of tissue into porous titanium implants. J Bone Joint Surg Am 1988; 70:540–547.
51. ISO 10993:1994, Biological evaluation of medical devices-Part 6: Tests for local effects after implantation.
52. Newman E, Turner AS, Wark JD. The potential of sheep for the study of osteopenia: Current status and comparison with other animal models. Bone 1995;4:277S–284S.
53. Bellino FL. Nonprimate animal models of menopause: Workshop report. Menopause 2000;7:14–24.
54. Thorndike EA, Turner AS. In search of an animal model for postmenopausal diseases. Front Biosci 1998;3:17–26.
55. Thompson DD, Simmons HA, Pirie CM, Ke HZ. FDA guidelines and models for osteoporosis. Bone 1995;17: 125–133.
56. Stea S, Visentin M, Savarino L, et al. Microhardness of bone at the interface with ceramic-coated metal implants. J Biomed Mater Res 1995;29:695–699.
57. Moroni A, Caja VL, Maltarello MC, et al. Biochemical, scanning electron microscopy, and microhardness analyses of the bone pin interface in hydroxyapatite coated vs uncoated pins. J Orthop Trauma 1997;11:154–161.
58. Savarino S, Stea S, Ciapetti G, et al. Microstructural investigation of bone cement interface. J Biomed Mater Res 1995; 29:701–705.
59. Huja SS, Katona TR, Moore BK, Roberts WE. Microhardness and anisotropy of the vital osseous interface and endosseous implants supporting bone. J Orthop Res 1998; 16:54–60.
60. Hämmerle CHF, Lang NP. Tissue integration of oral implants. In: Lang NP, Karring T (eds). Proceedings of the 1st European Workshop on Periodontology. London: Quintessence, 1994:297–316.
61. Buser D. Effects of various titanium surface configurations on osseointegration and clinical implant stability. In: Lang NP, Karring T, Lindhe LJ (eds). Proceedings of the 3rd European Workshop on Periodontology-Implant Dentistry. London: Quintessence, 1999:88–101.