Osteoporosis-like Bone Conditions Affect Osseointegration of Implants

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Purpose: *Usage of dental implants has become common for the treatment of edentulous patients, but concerns exist over the use of implants in patients where orofacial bone loss occurs. In the present study, the osseointegration of implants in rabbits under osteoporosis-like (OP-like) conditions simulating several clinically relevant conditions is reported.* ***Materials and Methods:*** *Forty rabbits were divided into 4 groups of 10. Three groups of animals received daily intramuscular injections of glucocorticoids (7.5 mg/kg) for 8 weeks to induce OP-like conditions either before, simultaneous to, or after implant placement.* ***Results:*** *The injections of glucocorticoids resulted in cortical thinning, irregular trabecular patterns, and impaired extracellular (ECM) matrix formation and mineralization. Although interfacial strength (8.5 ± 1.3 MPa for the control group; 9.3 ± 4.0 to 10.1 ± 4.0 MPa for the experimental groups) was apparently not affected in this limited sample cohort (n = 3 per group), statistically significant decreases (*$P < .05$*) in implant-bone contact were observed in animals with OP-like conditions (49% ± 10% for the control group; 24% ± 16% to 42% ± 16% for the experimental groups).* ***Discussion:*** *Histologic features characteristic of OP-like conditions were observed in each experimental group. ECM expression also appeared to be altered and compromised in all animals with OP-like conditions, which may affect long-term biomechanical stability of the implants.* ***Conclusion:*** *OP-like conditions affect the osseointegration characteristics of implants, but long-term biomechanical stability under forces of mastication is unknown as yet.* INT J ORAL MAXILLOFAC IMPLANTS 2004;19:687–694

Key words: *dental implants, glucocorticoids, osseointegration, osteoporotic conditions*

Since the pioneering work of Brånemark[1] and others,[2,3] the use of dental implants has become a standard and accepted treatment regimen for the edentulous patient. The utilization of dental implants has expanded, and implants are being placed in a variety of biochemically and biomechanically compromised bone states, such as atrophic mandibular and maxillary sites and in patients who have been diagnosed with oral manifestations of systemic diseases. Medical conditions such as diabetes, xerostomia, radiation treatment for cancer, and osteoporosis (OP) have been suggested as potential contraindications for the use of dental implants. OP-like conditions often manifest themselves in the geriatric female population, for which dental implant therapy has become common. OP has received attention in the dental implant field, as it is characterized by the loss of bone mass, structure, and function. OP is thought to be a result of altered bone remodeling capacity, ie, bone formation decreases while resorptive capacity remains relatively constant. In relation to dental implant use, the impaired regenerative capacity of bone may reduce bone healing around dental implants, thereby limiting their use.[4]

Patients who suffer from OP-like conditions do not possess the optimum bone conditions for placement of dental implants and the establishment of osseointegration (ie, type 1 or type 2 bone conditions), which is critical for the long-term success of dental implant treatment.[4] Therefore, dental implant use in patients with various bone disorders,

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including OP, has been controversial. Earlier reports have questioned the use of dental implants in clinical situations where decreased bone volume or regenerative capacity is observed or suspected.[5–10] More recently, it has been suggested that while OP may not necessarily contraindicate implant use, the formation and maintenance of an appropriate bone interface with implants may be affected by these conditions.[10,11]

The categorization of OP as a contraindication for implant placement presumes that a systemic diagnosis of OP affects the bone environment found in the oral cavity. There is debate as to whether the diagnosis of skeletal OP is in fact manifested in the oral cavity.[12,13] Nonetheless, a number of reports point to the possibility that OP or a reduction in bone mass or density could be problematic to the initiation and maintenance of osseointegration of dental implants.[5,8–10]

On the contrary, several clinical case reports indicate that OP may not be necessarily problematic for dental implant placement or maintenance.[10,14,15] For example, Friberg[14] reported a case in which a woman diagnosed with severe OP (type 4 bone) was able to maintain dental implant stability for 5 years after implantation. Minimal marginal bone loss and the absence of significant peri-implant radiolucency were noted. Other clinical studies suggest that the placement and osseointegration of implants in atrophic edentulous ridges may lead to load-related beneficial bone remodeling that can minimize or counteract physiologic bone loss.[4,8,16] It has been reported from several case studies that osseointegration of dental implants is possible in patients experiencing OP-like conditions; however, longer healing times are apparently required prior to prosthetic loading of the devices.[10,15] These clinical findings indicate that the chronologic sequence of implant placement and acquisition and maintenance of stability with the onset of an altered (perhaps weakened) bone state may be a significant factor in the success of implants in patients suffering from metabolic bone loss.

The objective of this work was to attempt to clarify the relationship between implant placement and the onset of an OP-like bone state as a determining factor in the prognosis of the establishment and maintenance of osseointegration. The hypothesis of this research was that administration of glucocorticoids in rabbits would produce OP-like conditions[17–20] that would affect the potential for osseointegration of dental implants. When used experimentally to induce altered bone states, corticosteroid treatments are known to deplete bone of the progenitor cell pool, which may play an important role in the ability of bone tissue to repair itself or become integrated with an implant.[21]

MATERIALS AND METHODS

Implant Preparation

Dental implant prototypes (2 mm in diameter; 4 mm in length) were prepared from titanium bar stock (Goodfellow Cambridge, Cambridge, United Kingdom). Internal threads were created at one end of each implant to facilitate placement with the aid of a holding tool with matching external threads. The implants were then prepared in a process similar to that used with commercially available implants.[22] Briefly, the implants were sandblasted with 50-μm alumina particles and ultrasonically cleaned in ion-free, organic-free water (Millipore-Q Plus Water Systems; Millipore Corporation, Bedford, MA). The implants were acid passivated (ASTM F86-76) and rinsed in the clean water prior to air drying in a vacuum desiccator. The implants were sterilized individually via dry heat autoclaving.

Animal Model

Adult male New Zealand white rabbits (2.7 to 3.6 kg, Harlan, Indianapolis, IN) were selected as the animal model, and the proximal tibiae of the animals served as the implant site. Ten animals were used in each of 4 groups (1 control and 3 experimental groups). To induce experimental OP-like conditions, the methodology of Aschraft and colleagues was used.[17] In the experimental groups, 7.5 mg/kg of cortisone (Cortisone Acetate; Sigma Chemical, St Louis, MO) was administered daily by intramuscular injection to induce OP-like effects on the rabbit skeleton. For the control group, physiologic saline was administered daily by injection beginning the day of surgery and for 4 weeks after surgery. To establish OP-like conditions prior to implantation and initiation of osseointegration, the animals in group 1 received daily injections from 2 weeks before implant surgery through 4 weeks postimplantation. To simulate the onset of an OP-like condition after osseointegration, the animals in group 2 received daily injections for 4 weeks beginning 4 weeks after surgical implantation. Finally, to model the initiation of OP-like conditions at the time of osseointegration, the rabbits in group 3 received daily injections from the day of implant surgery through 4 weeks postimplantation.

Implantation Procedure

The utilization of rabbits for this project was monitored and approved by the University of Iowa Animal Care and Use Committee and was performed in accordance with "The Guide for the Care and Use of Laboratory Animals," National Institutes of Health publication no. 85-23. The animals were

anesthetized using halothane (1.5% to 2.0%), an inhalant, and a mixture of ketamine (31.5 mg/kg), xylozine (7.3 mg/kg), and acepromazine (0.75 mg/kg), which was administered intramuscularly. The surgical site (the proximal lateral tibia) was prepared with a betadine scrub and surgical drapes. Either the right or left tibia was selected randomly for each surgery; only 1 leg of each rabbit was used. Following surgical exposure of the bony site, a pilot hole was drilled into the tibial cortex with a 1.0-mm-wide fissure bur (SS White Burs, Lakewood, NJ). The recipient bone bed was enlarged by a succession of increasingly wider drills until an interference fit was allowed for the 2-mm diameter implant using the placement tool. The implant was placed such that the superior aspect of the implant was flush with the bone cortex. The surgical site was then closed in layers using 4-0 Vicryl sutures (Ethicon, Somerville, NJ). The animals were allowed to recover from anesthesia and then were kept under standard laboratory conditions until the end of the postimplantation period. All animals were provided oral antibiotics in their drinking water for 7 days. The postoperative period was uneventful.

Implant Retrieval

Animals were euthanized by intramuscular injection of the ketamine mixture followed by an intravenous injection of Euthasol (0.22 mL/kg; Virbac/Delnarva Laboratories, Fort Worth, TX). After euthanasia, the tibial shaft was retrieved, together with the surrounding tissues. The specimens were placed into 3% glutaraldehyde solution for histologic fixation or were wrapped in wet towels and frozen for future mechanical property testing. Of the 10 implant sites per group, 5 sites were dedicated to undecalcified light microscopic evaluation and histomorphometric analysis, 3 sites were assigned to mechanical property testing, and 2 sites were used for immunohistochemical studies of the implant/tissue interface.

The processing and sectioning of undecalcified specimens was performed according to the general methods previously established by Krizan and associates.[23] Briefly, the tissue/implant blocks were fixed in 3% glutaraldehyde in sodium cacodylate buffer (Tousimis, Rockville, MD), dehydrated in a series of graded ethanol solutions, and embedded in modified Spurr Plastic Mixture (Tousimis). The implants were sectioned along their long axes into wafers about 200 to 300 μm thick using a low-speed saw (Isomet 1000, Buehler, Lake Bluff, IL) with a diamond wafering blade. The 3 sections from the center of the implant were used for morphometric analysis. The thick sections were then mounted onto acrylic plastic slides (Rohm and Haas, Philadelphia, PA) using Permabond 910 cement (Permabond, Englewood, CA) under constant pressure until cured. The sectioned wafers were hand ground and polished to approximately 25 μm using graded metallographic papers.

Microradiographs of the undecalcified sections were made using a Faxitron x-ray cabinet (Field Emissions, McMinnville, OR) (22-second exposure at 70 kV, 2.5 mA; working distance = 30 cm) on 4889 EM film (Eastman Kodak, Rochester, NY). The radiographs were projected and enlarged approximately 10 times their original size using an SAC GP-6 digitizer (Science Accessories, Southport, CT). Tracings were made to measure the perimeter of the implant and the amount of bone contact in the cortical and cancellous portions of the bone. Slides were stained with hematoxylin-eosin or Gomori's trichrome to examine the overall tissue architecture and cellular morphology.

Immunohistochemistry

Immunohistochemical techniques were utilized on 2 implant/tissue blocks per experimental group to determine the changes in the expression of extracellular matrix (ECM) proteins and their respective integrin receptors.[24] Briefly, after paraffin embedding and sectioning, the sections were deparaffinized, rehydrated, treated with hydrogen peroxide (H_2O_2) for 10 minutes, rinsed in phosphate buffer solution (PBS), treated with 0.5% Triton (Fischer Scientific, Fair Lawn, NJ), and rinsed again in PBS. The sections were blocked with 10% goat nonimmune serum, rinsed in PBS, and stained with primary antibody (monoclonal antibodies from the Hybridoma Facility at the University of Iowa). They were then rinsed, and biotinylated secondary antibody and 3-amino-9-ethylcarbazole chromagenic substrates (Zymed Labs, San Francisco, CA) were applied. Negative control samples were treated only with nonimmune serum or myeloma-conditioned media instead of primary antibody. The specific ECM protein studied was bone sialoprotein (BSP; antibody WVID1-9C5).

Mechanical Property Testing

The mechanical property testing of the specimens was performed according to the methods developed by Chang and coworkers.[25] Prior to testing, the frozen samples were thawed at room temperature. New bone growth that covered the implant head (the portion containing the internal thread) was carefully removed with a high-speed drill to eliminate interference during the mechanical testing. A holding rod was attached to the implant and aligned

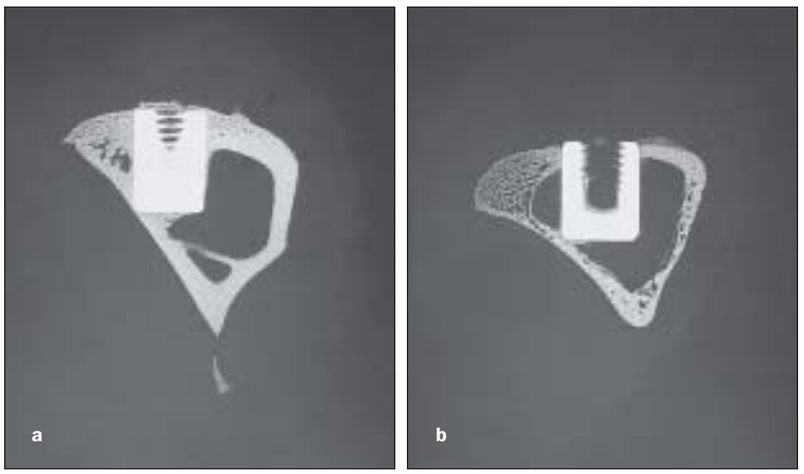

Fig 1 Microradiographs of plastic-embedded ground selections illustrating the implant-bone interface in *(a)* the control group and *(b)* group 1.

in position using a metal bushing around the rod. Acrylic resin was placed in the gap between the sample and the inside channel of the testing device to ensure even stress distribution during testing. The metal bushing was then removed to prevent contact between the holding rod and the implant device. This assembly was then attached to the Zwick 1445-60 universal testing machine (Zwick, Ulm, Germany) by 2 pull chains on both ends. The pull-out test was performed at a speed of 5 mm/min and a preload force of 1 N, with automatic shutdown at 35% maximum force. The load displacement curve and ultimate failure force were recorded. Interfacial attachment strength was derived from the maximum force divided by the surface area in contact with bone according to the previously measured implant diameters.

RESULTS

Histologic evaluation, including quantitative histomorphometric analysis of the bone contact at implant-tissue interfaces, indicated that administration of cortisone resulted in the formation of OP-like conditions in bone. The most dramatic alteration in host bone was the presence of decreased bone density in the cortical bone, which was not present in normal bone (control). Figure 1 contrasts the dramatic presence of porous areas of in a sample of cortical bone from group 1 compared to a sample from the control group. This observation is further demonstrated in the series of histologic sections shown in Figs 2a to 2d. Note that 4 weeks after implantation, an intimate bone-implant interface had formed in the control implant site (Fig 2a). There was no intervening layer of fibrous tissue present at this interface. For group 1 (Fig 2b), there was sporadic bone-implant contact; furthermore, there were large porous areas and highly stained cement lines, indicating OP-like bone conditions. For groups 2 and 3 (Figs 2c and 2d, respectively), the bone-implant interface appeared to be somewhat more extensive, but porous areas in the cortical bone were present, along with prominently stained cement lines.

The presence of OP-like conditions in the groups treated with cortisone was also demonstrated in the quantitative histomorphometric analysis of bone contact at the bone-implant interface. As indicated in Table 1, there was a significant reduction in the percentage of bone contact when OP-like conditions were present prior to the establishment of osseointegration (group 1, 24% ± 14% bone contact) compared to the control implant sites (49% ± 10% bone contact, $P < .05$). Administration of cortisone leading to OP-like conditions on a histologic basis resulted in an intermediate level of bone contact for groups 2 and 3 (31% ± 6% and 42% ± 16% bone contact, respectively). These percentages were not significantly different from the control group, although the qualitative histologic examinations of these implant sites indicated a disruption of the overall bony architecture at the bone-implant interface.

The immunohistochemical studies also revealed an altered bone response. Figs 3a to 3d illustrate the changes observed in staining for selected ECM proteins. BSP was abundantly stained adjacent to osteoblasts and within the bone matrix in control sections; however, there was little to no staining for BSP in the experimental groups (Figs 3b to 3d).

The biomechanical stability of the bone-implant interface was determined by measuring the interfacial attachment strength of 3 implant sites per group. The pull-out forces for the 4 animal groups are shown in Table 2. Although there were minor

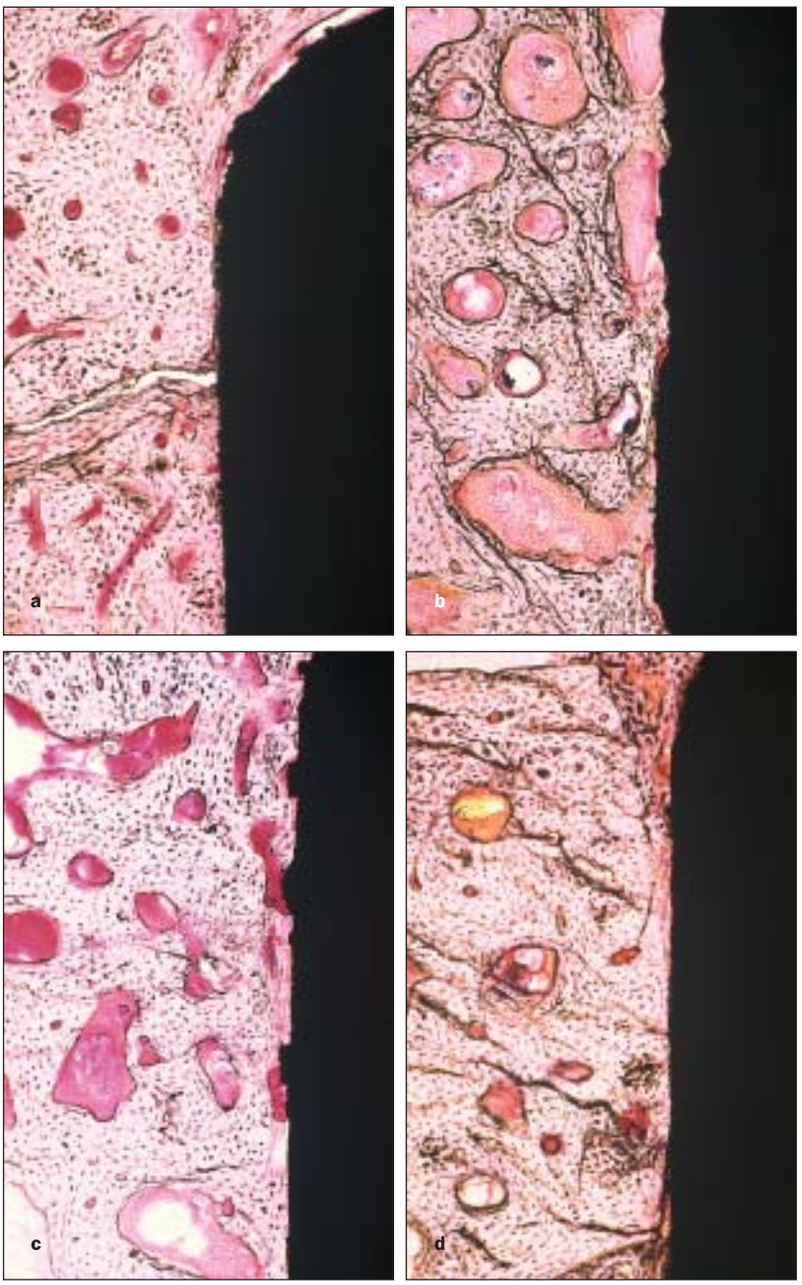

Fig 2 Light microscopic images illustrating the bone-implant interface in *(a)* the control group, *(b)* group 1, *(c)* group 2, and *(d)* group 3. Note the disruption of the interface, loss of cortical bone, and enhanced bone cement lines where OP-like conditions were present compared to the control group (hematoxylin-eosin; original magnification ×25).

individual differences in pull-out strength between the various groups, no significant differences ($P < .05$) were found.

DISCUSSION

The overall goal of this research was to understand the osseointegration of dental implants under OP-like conditions. The administration of cortisone was used to mimic OP-like conditions in the rabbit tibial implant model. Cortisone is known to inhibit osteoblast replication and differentiation; specifically, cortisone is known to inhibit gene expression for type I collagen, resulting in a disruption in ECM production and inhibition of intercellular signaling.[20,21] As osteoblastic activity leading to bone deposition is disrupted, osteoclastic activity remains unaffected, resulting in net bone resorption. In this study, the effectiveness of daily administration of cortisone was readily apparent from the histologic results shown in Figs 1 and 2. Cortical porosity and trabecular thinning were observed, along with reduced bone matrix and the formation of prominent

Table 1 Bone Contact (%) at the Implant Interface

Group	Bone contact (%)
Control	49 ± 10
Onset of OP-like conditions before implantation	24 ± 14 *
Onset of OP-like conditions after implantation	31 ± 6
Onset of OP-like conditions simultaneous with implantation	42 ± 16

Five implant sites per group; 3 slides per implant site.
*There were statistically significant differences at $P < .05$.

Table 2 Biomechanical Pull-out Force (MPa)

Group	Force (MPa)
Control	8.5 ± 1.3
Onset of OP-like conditions before implantation	10.1 ± 4.0
Onset of OP-like conditions after implantation	10.0 ± 3.5
Onset of OP-like conditions simultaneous with implantation	9.3 ± 2.9

Three implants per group were tested. No statistically significant differences were found.

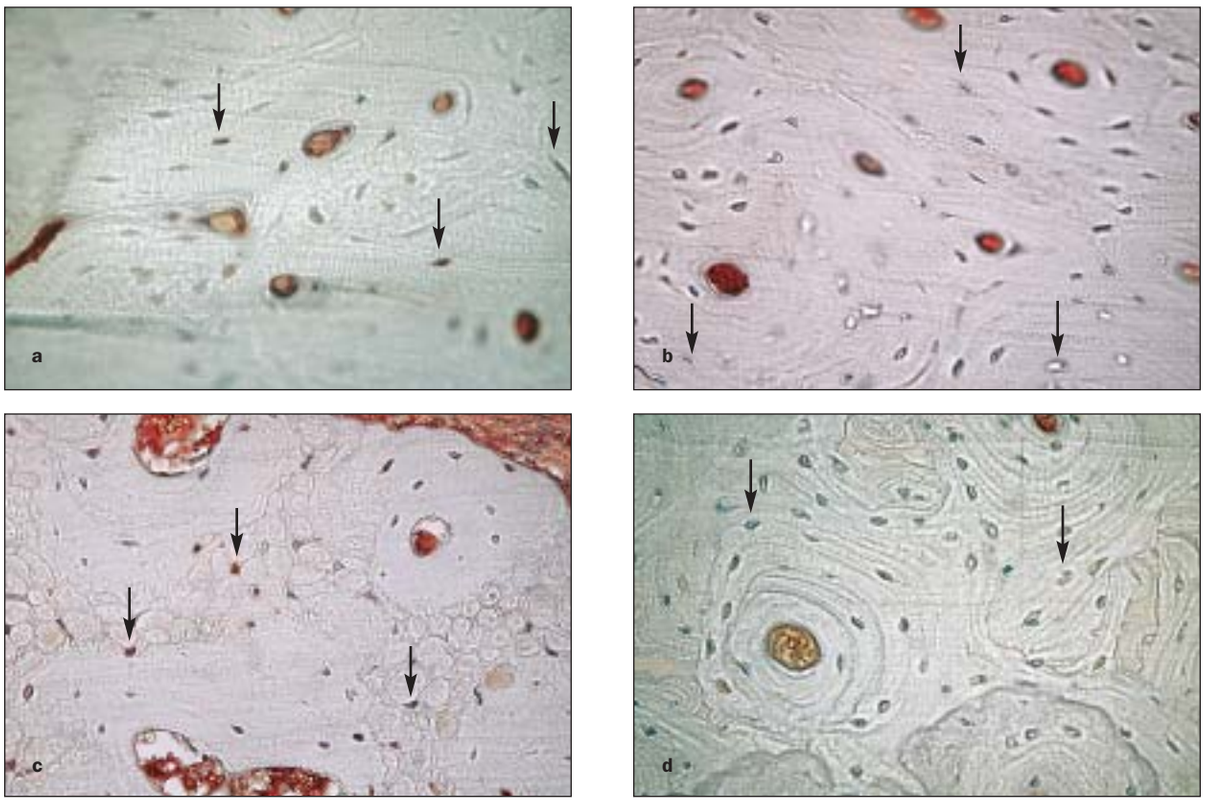

Fig 3 Light microscopic images of paraffin sections immunohistochemically stained for BSP illustrating the bone-implant interface in *(a)* the control group, *(b)* group 1, *(c)* group 2, and *(d)* group 3. Arrows indicate the loss of staining adjacent to and within osteoblasts where OP-like conditions were present compared to the control group (hematoxylin-eosin; original magnification ×40).

cement lines in each of the 3 groups in which cortisone was administered. The study was designed was set to mimic several chronologic possibilities regarding the establishment and maintenance of osseointegration and the onset of OP-like conditions. OP-like conditions were established prior to implantation (group 1), after implantation (group 2), and simultaneous with implantation (group 3) to model several clinically relevant possibilities.

It is clear from the histologic studies that administration of cortisone to each of the experimental groups stimulated OP-like conditions in the rabbit model.[17] All of the experimental groups experienced a disruption in the normal patterns of bone architecture characteristic of osseointegration (Figs 2a to 2d). The control group had the most well established bone-implant interface (Fig 2a); bone contact of approximately 50% was determined by histomorphometric analyses at 4 weeks after implantation. As shown in Fig 2a, dense cortical bone with well-established haversian systems was observed immediately adjacent to the bone-implant interfaces, which lacked an intervening fibrous tissue layer. In each experimental group, disruption of the adjacent cortical bone and a decrease in the percentage of bone contact were observed. In group 1 (Fig 2b) a statistically

significant decrease in bone contact ($P < .05$) was observed, along with a reduction in cortical density and increased bone cement line formation. A reduction in the percentage of bone contact (although not statistically significant) was also observed in group 2 (Fig 2c). Although the implants were placed 4 weeks prior to the start of cortisone administration, a substantial reduction in cortical density was observed with this group. Bone cement lines were observed in this group, but they were not the dominant histologic feature as in group 1. When oral implantation and OP-like conditions were introduced simultaneously (group 3), a slight reduction in the percentage of bone contact compared to the control group was found (42% ± 16% vs 49% ± 10%). Following 4 weeks of daily administration of cortisone, cortical porosity and prominent cement lines were noted.

When OP-like conditions were imposed by daily administration of cortisone, a bony architectural pattern involving decreased bone mineral density and reduced bone volume in the cortical and trabecular regions akin to OP-like conditions in humans was observed.[18–20] The results of this work clearly indicate a disruption in the normal formation and maintenance of bone deposition indicative of osseointegration. These results are similar to those of Lugero and associates[26]; in their study, a diminished level of bone deposition and overall bone volume was observed around hydroxyapatite-coated implants in an ovariectomized rabbit model. Similarly, Fini and colleagues[11] observed only slightly decreased levels of bone contact with implants but a significant loss of bone volume in the cortical regions adjacent to implants placed in the tibiae of ovariectomized rats.

In the present study, considering the prominent reduction in cortical density observed in each of the experimental groups where OP-like conditions resulted from administration of cortisone, one might have expected a more dramatic effect on the interfacial attachment strength (Table 2). Within the experimental limitations of this work, there was no statistical difference in interfacial strength between any of the experimental groups and the control group. The overall clinical ramifications of these results are unclear, and caution should be taken in the interpretation of these results. While one could argue that no alteration in biomechanical strength was observed, which might suggest a stable bone-implant interface, the histologic results clearly demonstrate the disruption of the bone architecture as a result of cortisone administration. Impaired osteoblast function associated with an OP-like state may be the result, in part, of a relative loss of function associated with the inability of the cells to interact with, synthesize, or secrete the appropriate ECM (eg, BSP). Some studies have indicated that impaired ECM synthesis may affect adhesion-mediated signaling cascades through focal adhesion kinase activity.[27] Altered osteoblast signaling may affect the ability of the cells to regulate ECM synthesis and assembly and contribute to the maintenance or loss of mineralized tissue.[28,29] The integrin receptors of osteoblasts bind to a variety of ECM ligands depending on the state of cell differentiation; thus the coordinated interaction of an integrin with a specific ECM, such as BSP, may be an important signal to the differentiating osteoblast. Adhesion-integrin mediated signaling could be altered in osteoporotic osteoblasts and thus impede the ability of osteoblasts to adhere to and interact with ECM, leading to loss of mineralization, a hallmark feature of OP-like conditions. The results presented here correlate with previous studies that suggest osseointegration is possible in OP-like bone, but because the regenerative capacity of bone is diminished, longer healing periods are necessary to obtain adequate osseointegration.[11,26]

While OP-like conditions were established in each experimental group, the overall results in terms of the percentage of bone contact and interfacial attachment strength were likely affected by the length of time allowed for the establishment of osseointegration and OP-like conditions and by the limited sample size (3 implant sites per group) available for the biomechanical testing. It would be helpful to conduct longer-term experiments; however, to do so may require the development of other models (eg, ovariectomized animals) to establish OP-like conditions. Long-term administration of cortisone is known to negatively compromise the immune system of small animals.[17] Therefore, this model may not prove useful for longer-term studies.

CONCLUSIONS

The administration of cortisone in the rabbit model proved effective in evoking an OP-like response in bone. The cortical porosity, trabecular thinning, and altered bone matrix were likely related, in part, to the diminished recruitment of osteoprogenitor cells, which led to impaired ECM formation and mineralization. The OP-like conditions resulted in the disruption of normal patterns of osseointegration in all 3 experimental groups. Significantly smaller percentages of bone-implant contact were noted in the experimental groups than in the control group. The hallmarks of OP (cortical porosity, trabecular thinning, altered matrix) were demonstrated histologically and

immunohistochemically. No significant effect on the interfacial strength of the implants was found. In conclusion, although the results of the present study suggest that implants may successfully osseointegrate in OP-like bone, future research in this area should continue to address the complicated clinical situation of dental implant use in metabolically altered bone states.

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