Histomorphometric and Fluorescence Microscopic Evaluation of Interfacial Bone Healing Around 3 Different Dental Implants Before and After Radiation Therapy

Michael Weinlaender, MD, DDS[1]/John Beumer III, DDS[2]/E. Barrie Kenney, DDS[3]/Vojislav Lekovic, DDS[4]/Ralph Holmes, MD[5]/Peter K. Moy, DDS[6]/Hanns Plenk Jr, MD[7]

Purpose: *Radiation therapy influence on bone healing around 3 types of endosseous dental implants in dogs was evaluated.* ***Materials and Methods:*** *Implants with 3 different surfaces (A = machined commercially pure titanium screws, B = commercially pure titanium plasma spray-coated cylinders, C = hydroxyapatite [HA] -ceramic coated cylinders) were first implanted unilaterally into the right posterior edentulous mandibles of 7 dogs as nonirradiated controls. After 12 weeks without functional loading and after sequential fluorochrome labeling these implants were retrieved by block dissection. In this same surgery, implants were placed on the contralateral side. Three weeks postimplantation the implant-containing hemimandibles were Cobalt 60 irradiated with the biologic equivalent of 5,000 cGy. Twelve weeks postimplantation and after labeling these irradiated implants were retrieved at sacrifice. On scanning electron, light, and fluorescence microscopic images of undecalcified longitudinal ground sections of the implants with surrounding tissues, percent bone-to-implant contact (% BIC), bone formation, and remodeling were histometrically and subjectively evaluated.* ***Results:*** *Woven bone formation started 1 week after implantation at the implant interfaces on both the nonirradiated and the irradiated sides. Average BICs (total/cortical/spongious bone bed) of 26%/49%/36% for surface A, 46%/48%/64% for surface B, and 81%/83%/78% for surface C were observed. In the irradiated hemimandibles average BICs (total/cortical/spongious bone bed) were reduced to 11%/9%/4% for surface A, 43%/46%/43% for surface B, and 63%/85%/76% for surface C, with increased resorption of peri-implant bone and retarded bone formation after irradiation.* ***Discussion:*** *Reductions of total % BIC in all irradiated implants, though not statistically significant, were significant ($P \leq .05$) on implant surfaces A and B in the spongious bone bed.* ***Conclusion:*** *Retarded bone formation on surfaces A and B in the spongious bone bed represented a more radiation-sensitive situation at the time of radiation onset compared to advanced bone formation and maturation at surface C.* Int J Oral Maxillofac Implants 2006;21:212–224

Key words: *bone-implant contact, histomorphometry, implant surfaces, radiation*

[1]Research Associate, Bone and Biomaterials Research, Institute for Histology and Embryology, Medical University of Vienna, Austria; and Private Practice, Vienna, Austria.
[2]Director, University of California at Los Angeles (UCLA) Maxillofacial Clinic, UCLA Dental School, Los Angeles, California.
[3]Tarrson Family Endowed Chair in Periodontics, and Director of the UCLA Periodontal Clinic, UCLA Dental School, Los Angeles, California.
[4]Professor and Dean, University of Belgrade Dental School, Belgrade, Serbia.
[5]Professor and Director, FACESplus and Center for Craniofacial Disorders, Children's Hospital and Health Center, San Diego, California.
[6]Director, UCLA Implant Center, UCLA Dental School, Los Angeles, California.
[7]Professor and Director, Bone and Biomaterials Research, Institute for Histology and Embryology, Medical University of Vienna, Austria.

Correspondence to: Dr Michael Weinlaender, Private Practice, Rotenturmstrasse 19, A-1010 Vienna, Austria.
Fax: +0043 1 535441024. E-mail: office@drweinlaender.at

A combination of radiotherapy and ablative surgery is often the treatment of choice for malignant tumors in the oral and maxillofacial regions. Reconstructive procedures involving dental implant placement in tissues that have suffered radiation injury carry the risk of producing soft-tissue and osteoradionecrosis.[1] Twenty-five years ago, the complication rate of oral reconstructive procedures involving necrotic tissues that had received more than 5,000 cGy was 81%.[2] Considering the low survival rate of these patients at that time, there was a reluctance to perform reconstructive surgical procedures on tissues that carried such a high risk of radiation complications. New radiation regimens and surgical techniques, in combination with hyperbaric oxygen treatment, have improved this situation.[3–5]

Endosseous implant-retained dental/maxillofacial prostheses can provide prosthodontists with a treat-

ment modality for achieving acceptable functional and esthetic rehabilitation of these patients.[6–8] It was the goal of this study to investigate the impact of radiation therapy on bone formation during the first 3 months of healing using 3 different dental implant surfaces—machined, plasma flame–sprayed titanium oxide, and hydroxyapatite (HA) -coated. The implants were placed in dogs 3 weeks before radiation therapy. Implant placement at the time of tumor resection but prior to radiation therapy was simulated. The rationale for placing implants at the time of surgical tumor eradication was to avoid secondary trauma by delayed implant surgery to tissues after radiation tissue injury.[9]

MATERIALS AND METHODS

Implants

Three types of implants were used:

- Surface A: Commercially pure titanium machined screw-type implants 10 mm in length and 3.75 mm in diameter (Biotes; Nobel Biocare, Göteborg, Sweden)
- Surface B: Commercially pure titanium plasma flame–sprayed cylinders 11 mm in length and 4 mm in diameter (IMZ; Interpore International, Irvine, CA)
- Surface C: Commercially pure titanium HA ceramic-coated cylinders 10 mm in length and 4 mm in diameter (Integral; Calcitek, Carlsbad, CA)

Surgical Procedures

The teeth of 7 healthy adult mongrel dogs (5 males, 2 females) with an average body weight of 16.2 kg were extracted bilaterally in the premolar areas of the mandible. The study protocol was approved by the UCLA Animal Protection Committee. After a 6-month postextraction healing period, the 3 different implant types were placed in the same distal-to-mesial order in the edentulous area of the right mandible. These implants served as nonirradiated controls.

All implants were placed by 1 surgeon using the specific techniques and instrumentation recommended for each system. Flaps were closed with resorbable sutures. The dogs received preoperatively atropine sulfate (0.04 mg/kg body weight) subcutaneously. All dogs were anesthetized with intravenous pentobarbital sodium (30 mg/kg body weight). They were intubated and received intraoperatively approximately 500 mL of lactated Ringer's solution intravenously. All dogs received 500 mg cefazolin sodium (Kefzol; Eli Lilly, Indianapolis, IN) intramuscularly immediately postsurgery and at 1 and 2 days thereafter. The dogs' postsurgical diet consisted of soft canned dog food and water ad libitum for 14 days, followed by conventional dry dog chow and water for the remainder of the observation period. All 7 dogs were housed individually for the duration of the study. Clinical evaluations of the dogs' mandibles were made throughout the duration of the study. Radiographs of the implants were made under general anesthesia 3 weeks postimplantation. Twelve weeks postimplantation all 7 dogs were anesthetized as previously described and, following the elevation of mucoperiosteal flaps, 3 block sections (approximately 1 × 1 cm), each containing one of the implants with surrounding tissues, were harvested for histologic evaluation using an oscillating saw under saline cooling. The resulting bone defects were filled with a porous xenograft (Interpore R200) to restore continuity of the mandibular bony architecture and to ensure soft tissue closure. The flaps were closed with resorbable sutures. After a healing period of 3 months, the same implant types and procedures were again used on the contralateral side of the dogs' mandibles. All 7 animals received the same pre- and postoperative treatment used for their earlier surgery. Three weeks postimplantation of this second set of implants, radiation therapy with a biologic equivalent of 5,000 cGy was performed. After a subsequent healing time of 12 weeks postimplantation, the animals were sacrificed, and the implants and surrounding tissues were harvested for histologic evaluation.

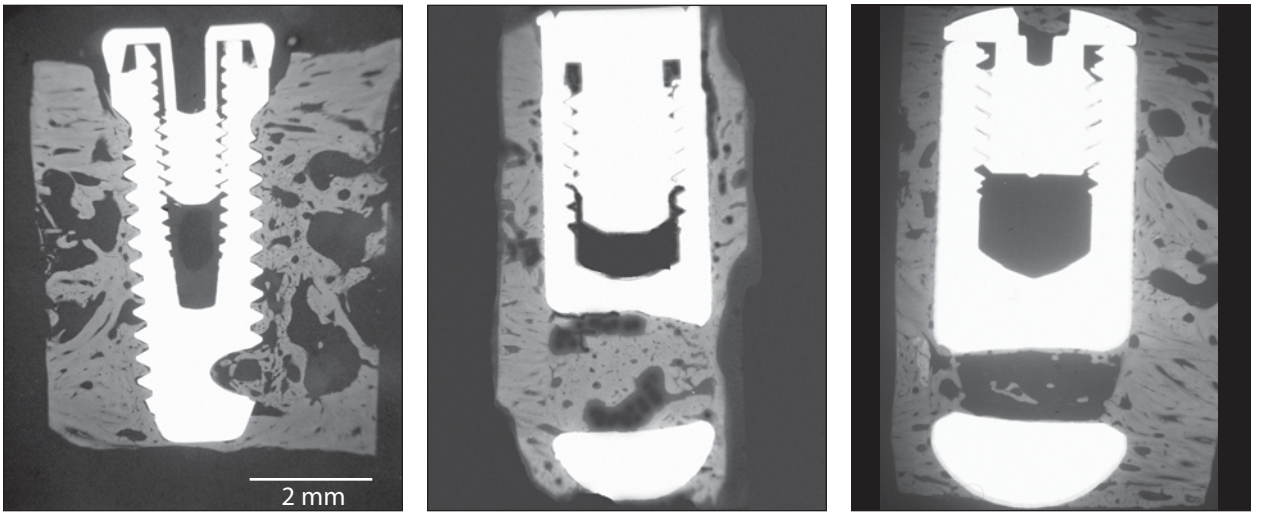
Polyfluorochrome Labeling

After each set of implantations, the following fluorochrome labels of new bone formation were administered subcutaneously to all dogs[10]:

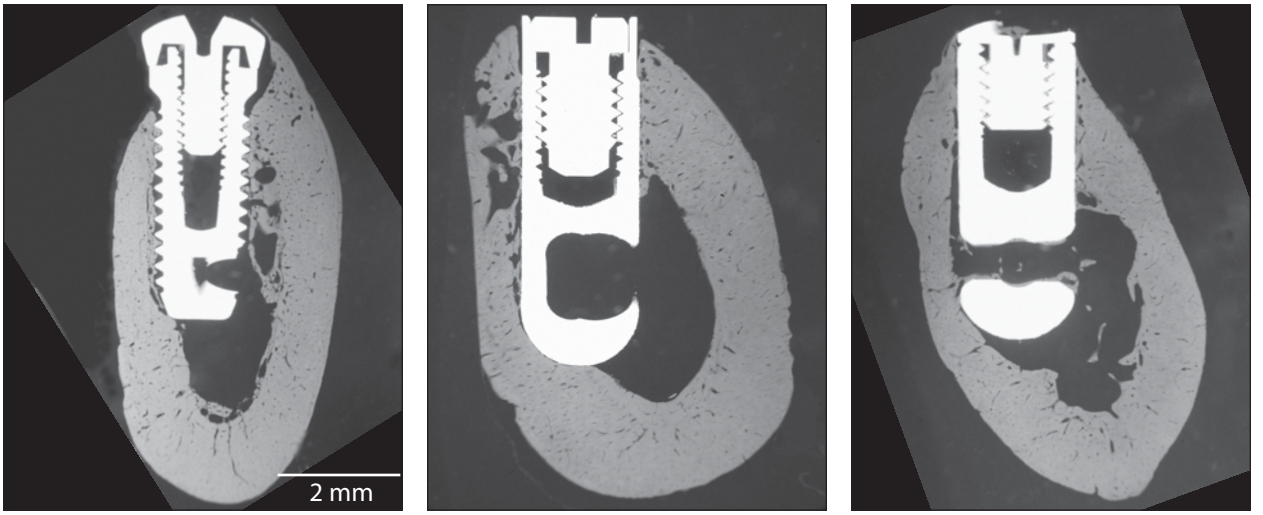
- Oxytetracyclin (Reverin Aventis, Frankfurt, Germany) 7 days postsurgery (25 mg/kg body weight)
- Alizarin Complexon (Merck, Darmstadt, Germany) 21 days postsurgery (30 mg/kg body weight)
- Calcein (Merck) 2 days before implant harvest (20 mg/kg body weight)

Radiation Therapy

Three weeks after implantation, the left hemimandibles, which contained the implants, were irradiated with the biologic equivalent to a total dose of 5,000 cGy, which is usually applied for radiotherapy in patients with head and neck cancer. The calculation of this equivalent dose/fractionation scheme was based on a study by Powers and associates[11] and resulted in a total dose of 2,600 cGy adminis-



Figs 1a to 1c Microradiographs of ground sections of nonirradiated block sections of *(a)* a machined titanium screw (surface A), *(b)* a titanium plasma flame–sprayed coated titanium cylinder (surface B), and *(c)* an HA-coated titanium cylinder (surface C) 12 weeks postimplantation.



Figs 2a to 2c Microradiographs of irradiated ground sections of mandibles with *(a)* a machined titanium screw (surface A), *(b)* a titanium plasma flame–sprayed coated titanium cylinder (surface B), and *(c)* an HA-coated titanium cylinder (surface C) 12 weeks postimplantation.

tered in 2 fractions per week over a 2-week period. Under general anesthesia, induced as already described, a field of 7 × 15 cm on the left mandible was irradiated for 6.04 minutes in a caudo-cranial direction at a source–skin distance of 80 cm using a AECL Theratron 80 Cobalt 60 machine (Theratronics International, Kanata, ON, Canada). This resulted in a dose of 650 cGy/fraction in a depth of 4 cm.

Histologic Procedures

The block sections from the right mandibles and the implant-containing specimen blocks from the left hemimandibles were immediately placed in Schaffer's fixative solution[10] (100 mL formalin [36%] neutralized over calcium carbonate and 200 mL ethanol [80%]; should have a pH of 7.2) and embedded in methylmethacrylate resin. After specimen radiography, a central longitudinal section of each implant was cut in either a mesiodistal direction (nonirradiated specimens) or a buccolingual direction (irradiated specimens). This section was then ground to a thickness of 50 to 70 μm and polished. The ground sections were surface-stained with a modified Paragon or a Giemsa stain. Corresponding microradiographs were also prepared (Figs 1 and 2) and were evaluated by transmitted light, polarized light, and fluorescence microscopy. For technical details see Plenk.[10]

The cut surfaces of the remaining specimen blocks (ie, the surfaces of the remaining implant

halves) were sputtered with a gold-palladium film (Ion Sputter Technics; Angstrom Sciences, Duquesne, PA) and evaluated by scanning electron microscopy (SEM, Cambridge S360; Leica/Cambridge, Cambridge, United Kingdom) in the backscattered electron mode (BSE).

Microscopic Evaluation and Bone Morphometry

For computerized histomorphometric evaluation, backscattered electron images at 40× magnification were taken of the remaining block specimens, and then 2 × 2-mm fields of view (4 on each side, 8 per implant) were digitized and further processed.[12] In addition to morphologic evaluation and photographic documentation, a subjective quantification was performed on the surface-stained and fluorochrome-labeled ground sections by an experienced observer (HP). Using an eyepiece grid at 40× magnification, encompassing approximately 1 millimeter of the implant surface in either cortical or intramedullary spongious bone bed, the following parameters in the peri-implant tissues were subjectively estimated in each field of view:

- Amount of pre-existing cortical or spongious bone
- Amount of newly formed bone, labeled with *(1)* oxytetracycline, *(2)* alizarin-complexon, and *(3)* calcein
- Presence of resorption lacunae and apparently resorbed old and/or new bone
- Amount of soft tissue interface

Each field of view was evaluated 3 times and given a rating from 0 to 4 on each of these criteria (0 = not detectable; 1 = up to 25%; 2 = up to 50%; 3 = up to 75%; 4 = up to 100%). Differentiation between pre-existing cortical and spongious (ie, intramedullary or cancellous) bone areas from these subjective quantifications was superimposed and correlated with the computerized histomorphometric data. Thus, quantitative results were obtained not only for total percentages of bone-to-implant surface contact (% BIC) but also for cortical and spongious % BIC for all irradiated and nonirradiated implants. Data are presented as pooled values per implant type with and without irradiation.

Statistics

Using paired *t* tests for implant-type–related samples in the same animal, the irradiated values were compared with the nonirradiated values. The required normal distribution assumption was tested with the Kolmogoroff-Smirnoff test, which did not show that the samples deviated from the normal distribution assumption. However, the test only showed low power, because the sample size was relatively small. Therefore, the corresponding nonparametric Wilcoxon signed rank test results have been used as the basis for the statistical significance calculations of all the data. The differences in results were regarded as statistically significant if the calculations resulted to an error probability of $P \leq .05$. For graphic presentation the data were illustrated by box plots. The box plot graphic contains the median, minimum, and maximum data values. The box itself contains the middle 50% of the data. The upper edge indicates the 75th percentile and the lower hinge the 25th percentile.[13] All the statistics were calculated using SPSS 11.02 (Chicago, IL).

RESULTS

Clinical and Radiologic Observations

All 7 dogs appeared to be healthy and showed no signs of impairment to their normal behavior during the full observation period. The control radiographs taken at 3 weeks postimplantation in both parts of the study showed all implants in situ and revealed no gross changes in the alveolar bone architecture.

Six weeks following the first implant placement surgery, however, 1 surface-A implant perforated the crestal alveolar mucosa; it was lost at week 8. At week 9 following placement of the irradiated implants, 2 implants, 1 with surface A and 1 with surface B, perforated through the oral mucosa and were subsequently lost. All other implants remained covered by normal nonulcerated keratinized mucosa until the end of the respective observation periods.

Histologic Observations

All implants were seated with their coronal part in the mandibular cortical layer. The middle and apical portions of the implants protruded into the medullary cavity of the mandible with more spongious bone and sometimes reached the mandibular canal. The apical parts of some implants contacted the neurovascular bundle at the lower part of the medullary cavity and therefore had no bone formation at these surfaces. If the implants contacted the buccal or lingual aspect of the mandibular cortical bone, additional cortical support was also seen at the apical parts of these implants. All other implants achieved most of their primary stability at the crestal cortical layer only. Additional to the aforementioned cortical bone fixation, varying amounts of peri-implant cancellous bony trabeculae in the area of the medullary cavity provided additional primary stability (Figs 1 and 2).

Nonirradiated Implants. *Periosteal Healing.* After 12 weeks' healing time the coronal portions of the nonirradiated implants were surrounded by various amounts of newly formed periosteal bone, which was mostly diffusely yellow (oxytetracycline) labeled. Consecutive lamellar compaction was demonstrated by bands of red (alizarin) and green (calcein) labeling. However, for most surface-A and surface-B implants, new periosteal bone was separated from the implant surface by connective tissue interposition reaching down about 1 mm to the neck of surface-A implants and to the beginning of the titanium plasma-sprayed surface of surface-B implants. This interposition of periosteal connective tissue was not seen with surface-C implants. Osteoconductive growth of bone along the HA-coated implant surface was visible even if the implant head was placed slightly supracrestal.

Endosteal Transcortical Healing. In the area of the crestal cortical layer 2 different bone reactions at the original bone bed could be seen, depending on the initial fit of the implant in the cortical bone bed. In the case of poorly fitting screw-type implants (surface A) or tilted cylinder-type implants (surfaces B and C), there was diffuse yellow-labeled woven bone formation starting from the prepared pre-existent bone as early as 1 week postimplantation, indicating the beginning of bridging of the tissue gap between bone and implant surfaces. If the implant had been tightly seated in the cortical bone bed, only alizarin red–labeled reversal lines were visible, indicating preceding postimplantation resorption of the bone bed, with peri-implant bone formation after 3 weeks. These 2 phenomena could be observed with all 3 implant types.

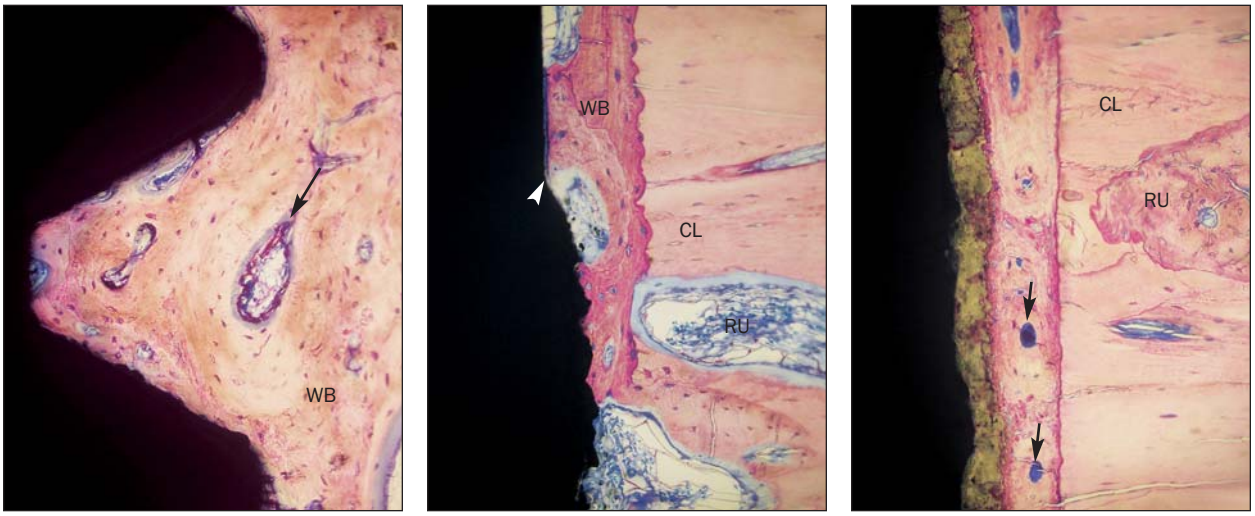
Bone formation at the A and B surfaces mostly originated from the prepared surfaces of the preexistent bone bed (ie, "implantopetal"; Figs 3a and 3b), but on surface C (Fig 3c) and occasionally on surface B simultaneous direct bone apposition was indicated by tetracycline labeling on both the implant surface (ie, "implantofugal") and the surrounding bone structures. Consecutive lamellar bone formation on this peri-implant woven bone was indicated by red and green bands of labeling around primary osteons. Polarized light microscopy revealed that these lamellar layers on the HA surfaces resembled so-called "gap healing" (Fig 3c) during stable osteosynthesis.[14]

Signs of resorption and remodeling of these new and pre-existing bone structures could also be seen at 12 weeks postimplantation.

Intramedullary Healing. The implants also elicited bone healing with new woven bone formation starting as early as 1 week postsurgery, as indicated by diffusely yellow tetracycline labeling of the cut bone trabeculae and on endosteal surfaces. The amount of peri-implant new bone formation in the medullary areas was dependent upon the quality of the available adjacent bony structures. Most of the bone consisted of mainly diffusely red-labeled irregular woven bone formations starting from pre-existing bone surfaces, which provided secondary stabilization to the implants. These contacts developed earlier and were more extensive on the HA-coated than on the titanium oxide surfaces. Whereas the titanium plasma-sprayed implants (surface B) occasionally demonstrated direct woven bone apposition, the surface of the osteoconductive HA coating had from the beginning a lamellar layer of bone covering parts of the implant surface, even in the absence of close peri-implant bony structures. This layer of lamellar bone had a thickness of about 30 to 40 μm (as spot measured in the backscattered electron mode) and showed consecutive layers of tetracycline-, alizarin-, and calcein-labeled bone tissue. Areas of the HA coating which were in close proximity to the endosteal surface of the cortical layer exhibited tetracycline-labeled osteoconductive woven bone formation originating from these surfaces. Red and green labels indicated consecutive lamellar bone. Resorption and remodeling of these new and pre-existent bone structures could also be seen. Most of the bone in the apical openings was diffusely red-labeled, indicating woven bone formation, at 3 weeks postimplantation, with bands of green at 12 weeks. If the entrance to the apical openings was in close proximity to neighboring bone structures, osteoconductive ingrowth of diffusely yellow-labeled woven bone, especially around the HA-coated implants, could be observed.

There were areas where either the coating was not applied (apical openings) or detachment of the HA coating was visible. Connective tissue with foreign body giant cells and macrophages incorporating particles of the HA coating was seen in these areas of detachment. Detached particles of the titanium plasma-sprayed coating of the surface B could also be seen occasionally, provoking a similar foreign body reaction.

Irradiated Implants. At the periosteal level implants of all groups exhibited similar bone formations labeled 1 week and 3 weeks postimplantation. Where cover screws were exposed before the end of the study, acute inflammatory cells with resorption of newly formed periosteal bone tissue could be observed (this occurred with 2 implants with surface A). Whereas the initial tetracycline- and alizarin-labeled bone formations in the cortical and medullary spongious bone areas around the irradiated set of implants were comparable to those



Figs 3a to 3c Details from stained ground sections of nonirradiated implants 12 weeks postimplantation. *(a)* Transcortical threads of a machined screw-type implant, filled with early woven bone (WB). Lamellar compaction with primary osteons can be observed *(arrow)*. *(b)* Transcortical portion of a plasma flame–sprayed implant. Newly formed WB filled the gap between the smooth collar/plasma-sprayed surface transition *(arrowhead)* and the cortical bone bed, marked by a cementing line (CL). Note the remodeling units (RU) with active bone formation. *(c)* Transcortical portion of an HA-coated implant. Perfect "gap healing" with primary osteons *(arrows)* and direct lamellar bone formation on CLs on cortical bone and the HA-coated implant surface. Note a more mature RU (Giemsa stain; original magnification ×140).

observed with the nonirradiated implants, there was a reduction of the amount of newly formed calcein green-labeled bone surfaces. Although these reductions did not reach statistical significance in any of the 3 different implant types, they were more distinct with surfaces A and B (Figs 4a to 5b). The lamellar bone formation at the HA-coated surface of nonirradiated implants was also present with the irradiated implants (Figs 6a and 6b). These bone formations were mostly labeled yellow and then red. The presence of connective tissue at the bone-implant interface with all 3 implant types was more extensive in the irradiated specimens than in the nonirradiated specimens, but these estimations again did not reach statistical significance. No general fibrous marrow response to radiation could be observed. In the areas of the apical openings, lamellar compaction was reduced compared with the nonirradiated specimens. Major vascular changes, both in vessel diameter and architecture, could not be observed in the bony bed of the irradiated implants.

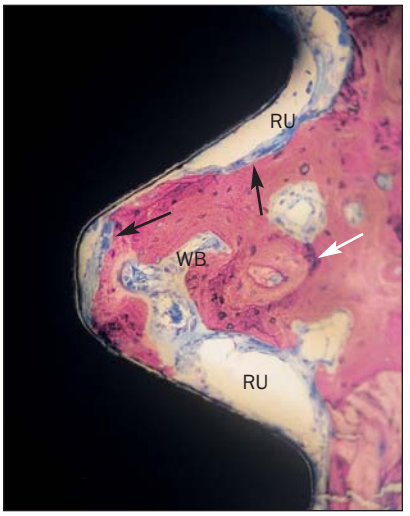
Bone Morphometry

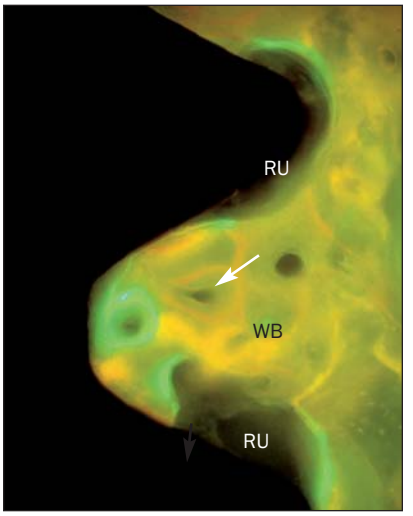
The reduction of total % BIC values in the irradiated group compared to the nonirradiated groups was not statistically significant ($P > .05$) (Table 1, Fig 7a). However, when irradiated cortical and spongious % BIC values were compared separately with nonirradiated values, they were significantly lower ($P \leq .05$) for surface A, as were the spongious % BIC values for surface B (Table 1; Figs 7b and 7c). The total % BIC values as well as the % BIC values for cortical and spongious bone alone generally demonstrated greater BIC with surface B than with surface A and with surface C than with surface B. This general pattern was followed in all of the investigated nonirradiated and irradiated groups. These differences in total BIC were statistically significant from surfaces B to C and from surfaces A to C with respect to the nonirradiated implants. In the irradiated implant group the difference from surface A to surface B also reached statistical significance. The comparison of cortical- and spongious-area % BIC values between the 3 implant types in the nonirradiated group versus the irradiated group again followed the general finding of increasing % BIC values from surface A to surface B and from surface B to surface C. These differences were also statistically significant ($P \leq .05$).

Subjective Estimations of Different Tissue Parameters

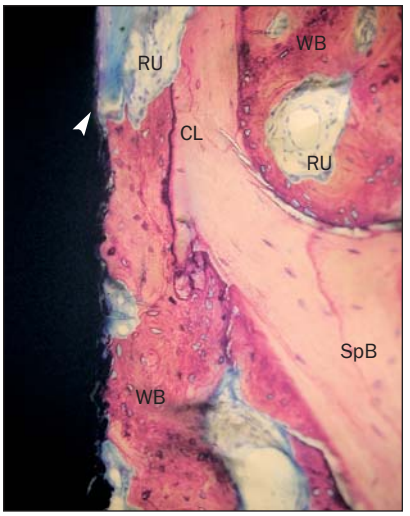
The data from the estimations in the fluorescence optical mode indicated that

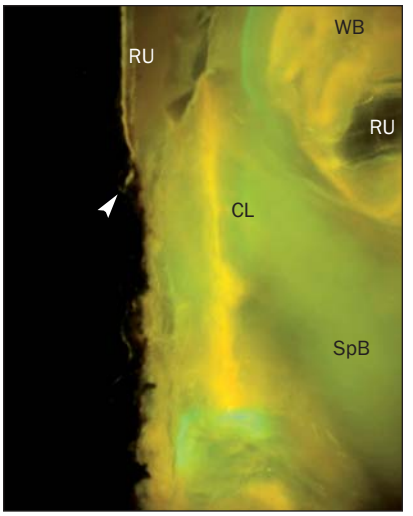
1. There was a gradual increase in pre-existent old bone from surface A to surfaces B and C in both the irradiated and nonirradiated groups. However, only surface C had significantly ($P \leq .05$) more pre-existent bone than surface A in both the irradiated and nonirradiated groups (Table 2, Fig 8a).



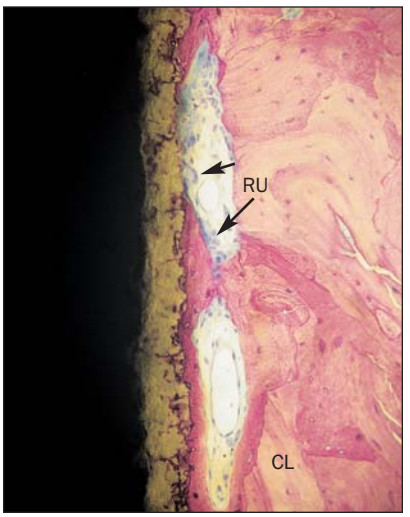


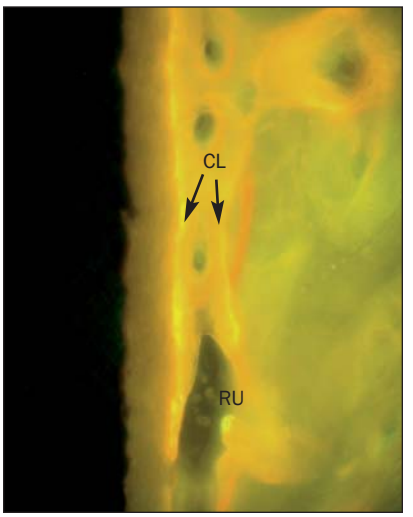
Figs 4a and 4b Details from *(a)* a Giemsa surface-stained ground section and *(b)* a corresponding fluorescence micrograph of a transcortical thread of a radiated implant with surface A 12 weeks postimplantation. The yellow oxytetracycline-labeled woven bone (WB) and red alizarin-labeled primary osteons *(white arrow)* were partly resorbed by active osteoclasts *(black arrows)* in remodeling units (RU). Some calcein green-labeled bone formation can be observed (magnification ×140).





Figs 5a and 5b Details from *(a)* a Giemsa surface-stained ground section and *(b)* a corresponding fluorescence micrograph of the transition from smooth collar to titanium plasma-coated surface *(arrowhead)* of a radiated implant with surface B 12 weeks postimplantation. Woven bone (WB), partly oxytetracycline yellow-labeled, filled the spaces between the implant and the old spongy bone (SpB) marked by a cementing line (CL). Note the resorption in remodeling units (RU).

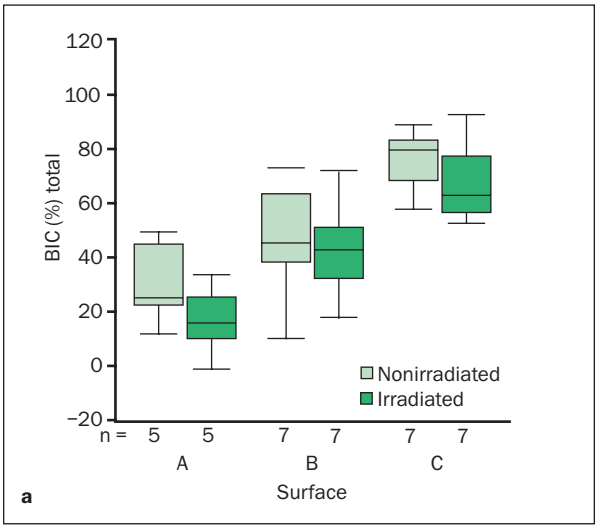


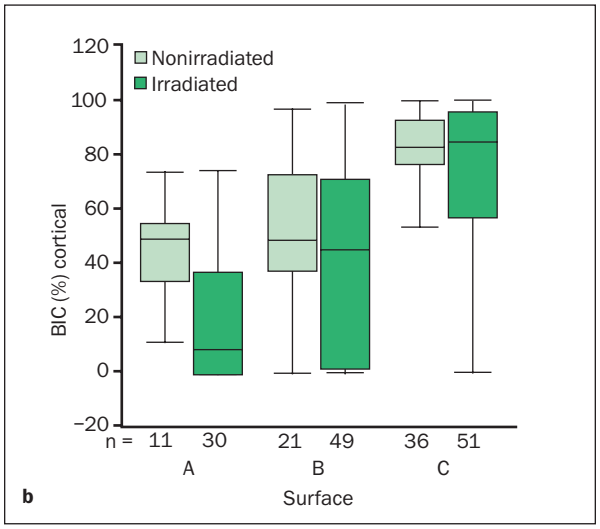


Figs 6a and 6b Details from *(a)* a Giemsa surface-stained ground section and *(b)* a corresponding fluorescence micrograph of a radiated implant with surface C implant 12 weeks postimplantation. Direct BIC on yellow oxytetracycline-labeled cementing lines (CL) and red alizarin labeling can be observed. Note the remodeling units (RU) with active osteoclastic resorption *(black arrows)* along the implant surface and the absence of calcein green-labeled bone formation.

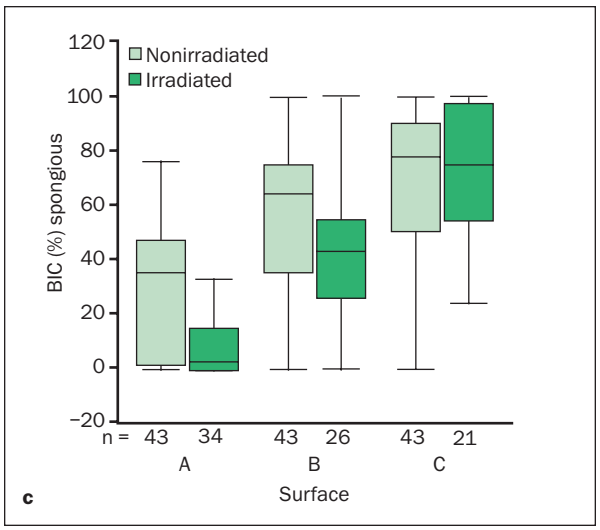
Table 1 Median % BIC

	Total		Cortical area		Spongious area	
Implant type	**Median**	***P* ≤ .05**	**Median**	***P* ≤ .05**	**Median**	***P* ≤ .05**
Surface A						
Nonirradiated	26.37 ± 37.17	A-C	49.12 ± 61.68		36.31 ± 75.94	
Irradiated	11.45 ± 34.15	A-B, A-C	9.09 ± 74.10	A-A	3.68 ± 70.89	A-A
Surface B						
Nonirradiated	45.64 ± 61.45	B-C	48.49 ± 97.25		64.40 ± 100.00	
Irradiated	43.15 ± 53.15	B-C	45.82 ± 99.13		43.20 ± 100.00	B-B
Surface C						
Nonirradiated	80.72 ± 54.71	A-C, B-C	83.15 ± 45.80		78.22 ± 100.00	
Irradiated	63.28 ± 39.95	A-C, B-C	84.62 ± 100.00		75.77 ± 75.62	





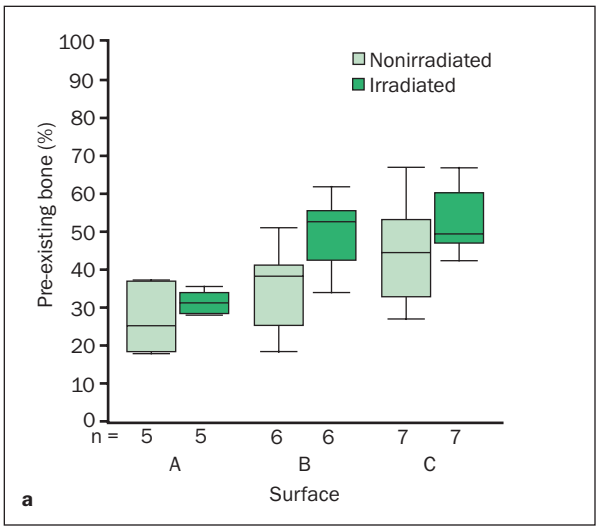
2. When the different implant types in the irradiated and nonirradiated groups were compared, it was found that the amount of labeled bone increased from surface A to surfaces B and C for all 3 labels. These differences reached statistical significance in some comparisons (Table 3). There were basically no differences between the nonirradiated and the irradiated surfaces with respect to tetracycline and alizarin labeling. The amount of calcein green labeling uptake was reduced in the irradiated implant group; however, this finding did not reach statistical significance ($P > .05$) (Table 3; Figs 9a to 9c).
3. Bone resorption values were reduced in the irradiated group versus the nonirradiated group for implant surfaces B and C. With surface C, there was significantly less bone resorption in the irradiated group compared to the nonirradiated group ($P \leq .05$). Resorption in the irradiated group with surface A was increased in comparison to the nonirradiated group (Table 2, Fig 8b).

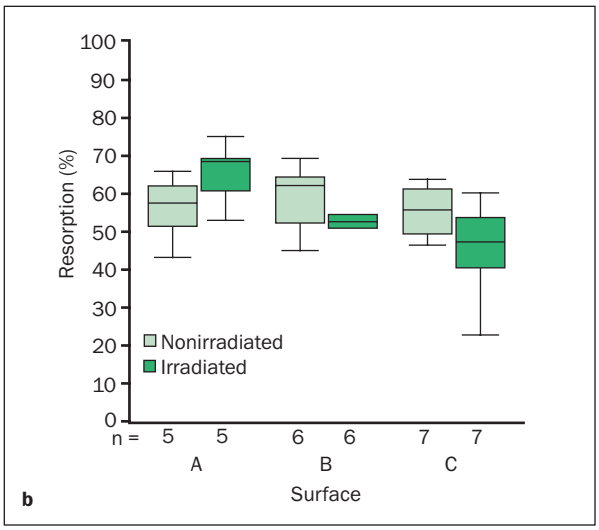


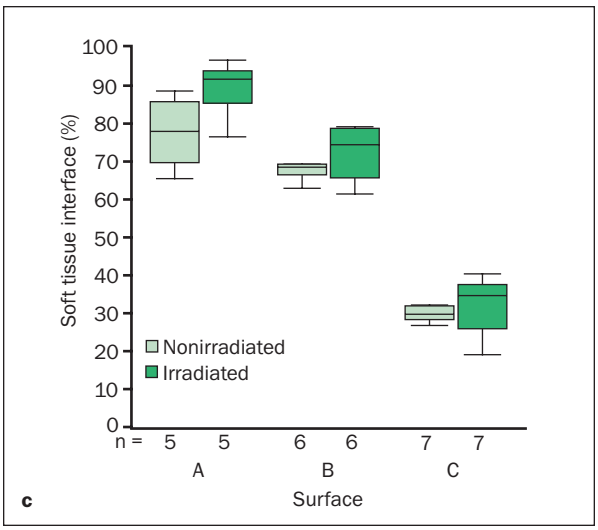
Figs 7a to 7c Box-and-whisker plots showing *(a)* % BIC over the total area, *(b)* % BIC in the cortical areas, and *(c)* % BIC in the spongious areas.

Table 2 Median % Pre-existing Bone Resorption and Soft Tissue

	Pre-existing bone		Resorption		Soft tissue	
Implant type	Median	$P \leq .05$	Median	$P \leq .05$	Median	$P \leq .05$
Surface A						
Nonirradiated	26.39 ± 19.41	A-C	58.33 ± 22.04		77.78 ± 23.18	
Irradiated	32.94 ± 23.44	A-C	64.84 ± 29.69		92.93 ± 20.21	
Surface B						
Nonirradiated	38.39 ± 32.72		62.57 ± 24.13		68.13 ± 17.12	
Irradiated	52.38 ± 37.50		51.79 ± 8.98		76.56 ± 17.57	A-B
Surface C						
Nonirradiated	44.44 ± 38.89	A-C	56.25 ± 16.46		30.00 ± 38.61	A-C, B-C
Irradiated	50.00 ± 24.02	A-C	48.08 ± 37.18	C-C	35.71 ± 20.38	A-C, B-C







Figs 8a to 8c Box-and-whisker plots showing percentages of *(a)* pre-existing bone, *(b)* resorption, and *(c)* soft tissue interface.

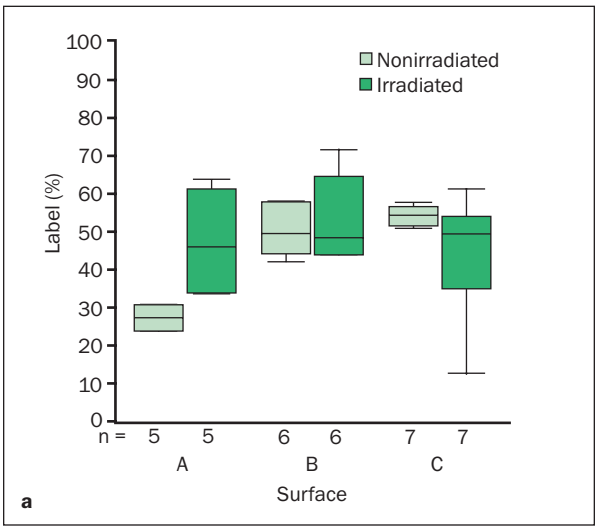
4. The amount of soft tissue interface was higher with surface A in comparison to surfaces B and C in both the nonirradiated and irradiated groups. In both the irradiated and nonirradiated groups, there was a significant reduction in soft tissue interface between surfaces A and C and between surfaces B and C. In the irradiated group, the difference between surfaces A and B was also statistically significant ($P \leq .05$) (Table 2, Fig 8c).

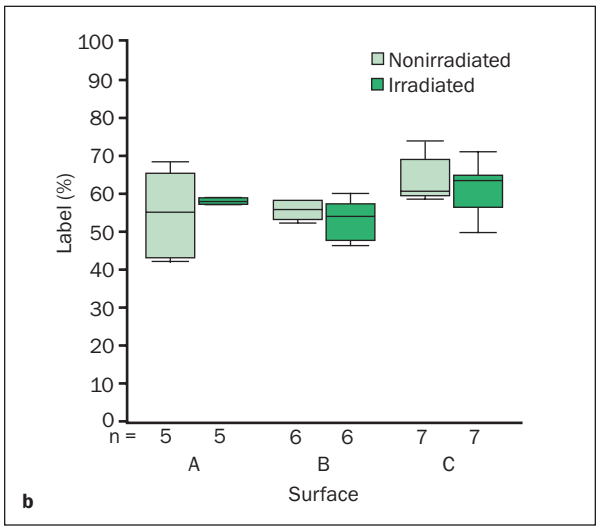
DISCUSSION

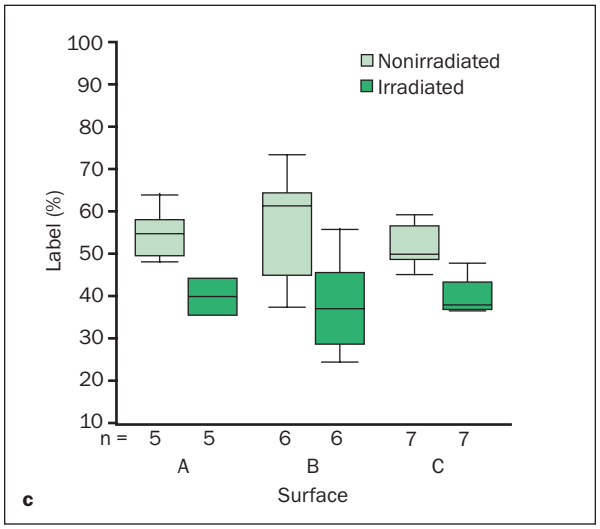
It is now well accepted that endosseous dental implant success or lack thereof is governed by several factors, such as implant design and surface, together with the amount and quality of the pre-existing bone.[15–24] The data for the present histomorphometric measurements in the nonirradiated group are in agreement with other reports regarding the

Table 3 Median % Labeled Bone

	Oxytetracycline		Alizarin red		Calcein green	
Implant type	Median	$P \leq .05$	Median	$P \leq .05$	Median	$P \leq .05$
Surface A						
Nonirradiated	27.78 ± 41.67	A-B	55.56 ± 26.11		55.56 ± 16.04	
Irradiated	41.00 ± 30.13		58.63 ± 25.00		41.41 ± 47.71	
Surface B						
Nonirradiated	49.89 ± 16.10	A-B, B-C	56.45 ± 13.29	B-C	61.88 ± 36.11	
Irradiated	45.59 ± 37.50		54.41 ± 29.28		36.90 ± 31.25	
Surface C						
Nonirradiated	55.00 ± 27.78	A-C, B-C	61.11 ± 14.06	B-C	50.00 ± 14.41	
Irradiated	50.00 ± 24.02		63.33 ± 21.43		38.46 ± 33.33	







Figs 9a to 9c Box-and-whisker plots showing the percentages of bone labeled with *(a)* oxytetracycline, *(b)* alizarin complexon, and *(c)* calcein green.

percentage of total BIC after a 3-month healing period.[25–29] The somewhat lower levels of total BICs with machined titanium oxide–surfaced implants are known to level out after longer observation periods, with the initially higher BIC with HA-coated implant surfaces.[28]

With respect to radiation influences on bone healing around implants, some researchers advocate a delay of at least 1 year between radiation therapy and surgical implant placement,[30] but there are also reports of endosseous implants and osteosynthesis materials which have been irradiated during the primary healing time.[31–33] Hum and Larsen[34] demonstrated a 19% reduction of BIC of a cylindric titanium plasma-sprayed implant in rabbit tibiae after 4050 cGy irradiation during the initial healing time. Brogniez and colleagues[35] compared titanium plasma-sprayed and HA-coated implants in dog mandibles. The experimental group, which received radiation therapy after implant placement, had significantly better results

than the one receiving the radiation treatment before implantation. However, numerous clinical studies evaluating the success rates of intraoral endosseous implants placed in previously irradiated bone beds with and without adjunct hyperbaric oxygen treatment have demonstrated success rates slightly to substantially less than in nonirradiated patients.[36–40]

The present histomorphometric results demonstrated differences, but not significant differences, in total implant BIC for the 3 implant surfaces when a biologic equivalent of 5,000 cGy was administered at 3 weeks postimplantation. In this animal model bone tissue around the experimental implants was allowed to heal undisturbed for 3 weeks before radiation therapy started. After this time period lamellar compaction of newly formed bone had occurred in nonirradiated implants and was most advanced with surface C. Histomorphometry showed highest reductions in BIC areas after irradiation with surface-A implants and to a lesser extent (mainly in the spongious bone bed) with surface-B implants. Surface-C implants showed practically no reduction in total or cortical % BIC, while in the spongious bone bed even more BIC was found in the irradiated group. These results for BIC in the cortical and spongious bone areas are similar to other studies showing that more mature lamellar bone seems more resistant to ionizing radiation than newly formed woven bone.[30,33,41,42]

The subjective estimations of different histologic parameters in the present study, though not yielding exact measurements like computer-assisted histomorphometric data, rendered additional information about new bone formation and remodeling dynamics at different time intervals before and after onset of radiation therapy and complemented the histomorphometric results. The statistically significant reduction of % BIC in cortical bone areas in surface-A implants in spongious bone beds with surfaces A and B has to be seen in context; there was a smaller amount of pre-existing bone because of implant position in the mandible, and bone formation activity was reduced at the machined titanium oxide surface of implant A compared to implants B and C. Whereas the new bone formation and lamellar compaction seen at surface-C implants was almost complete at radiation onset, the bone gap between the machined titanium oxide surface (A) and the preexistent cortical or spongious bone bed was still partly occupied by immature woven bone.

The greater amount of cells susceptible to irradiation may be responsible for the greater impairment of new bone formation at the cortical bone bed of surface-A implants as well as at the spongious bone bed with surfaces A and B. These findings are further supported by a statistically significant reduction in resorption in the irradiated implants with surface C and the reduction of calcein-labeled new bone formation. The estimates for the amount of soft-tissue interfaces around the different implant surfaces parallel the results from the histomorphometric evaluation. The statistically significant differences in total BIC for both irradiated and control implants confirmed previous findings that HA-coated implants were able to induce early bone formation and maturation than the 2 titanium oxide–surfaced implants. However, more BIC was seen in irradiated B implants than in irradiated A implants, pointing also to the superiority of rough surfaces versus machined surfaces. The absence of statistical significance for some of the data where clearly trends are visible (for example, calcein labeling; Fig 9c) may be explained by the low statistical power associated with the low sample size.

From data relating to the removal of teeth before radiation treatment, most of the clinical incidence of osteoradionecrosis developed when the time allowed for the tissue to recover before the beginning of radiation treatment was less than 2 weeks postextraction.[1] No cases of osteoradionecrosis developed if these tissues were allowed to recover for 21 days or more. Asikinen and coworkers[43] showed in an animal experimental model that implant survival is dose related.

Certainly no animal model truly reflects the human situation. Therefore, a tissue damage dose in such a model equal to a given human dose is not really equivalent because host tissue response, recovery, and late cellular loss is species specific.[44]

CONCLUSIONS

Endosseous implants placed in bone which had been irradiated with a biologic equivalent of 5,000 cGy 3 weeks after implant placement had, depending on implant surface, reduced total % BIC when compared to nonirradiated implants in the same animal. Mature lamellar bone around implants with surface C showed less radiation impact than newly formed woven bone, as expressed by significantly ($P \leq .05$) reduced BIC in the pre-existent cortical bone areas surrounding surface-A implants and in the spongious BIC of implants with surfaces A and B. Histologically, these conclusions are supported by evidence of impaired new bone formation after radiation onset for all 3 implant types, as indicated by reduced calcein labeling of bone surfaces and by a statistically significant reduction of resorption at irradiated C-surface implants at the end of the observation time of 12 weeks postimplantation. The effects of radiation also led to an increase of total implant–soft tissue contacts when

compared to nonirradiated implants with surfaces A and B. Importantly, the more mature the peri-implant bone was at irradiation onset, the less impact radiation had on peri-implant bone formation. The combination of slower bone formation at the machined titanium oxide surface of surface-A implants with a poorer pre-existent bone bed compared to implants with surfaces B and C created the most irradiation-sensitive combination and resulted in the greatest irradiation impact of all 3 implant surfaces.

ACKNOWLEDGMENTS

(1) Thanks for specimen preparation and histotechnics to Mrs B. Wallner and Mrs G. Schlotter, Bone and Biomaterials Research, Institute for Histology and Embryology, Medical University of Vienna. *(2)* The authors thank Prof DDr H. Toutenburg, Dipl Stat, and S. Scheid, Department for Biostatistics, University of Munich, Germany, for the statistical support. *(3)* The authors thank Friadent-Dentsply and Dr Neugebauer for their support.

REFERENCES

1. Marx RE, Johnson R. Studies in the radiobiology of osteoradionecrosis and their clinical significance. Oral Surg Oral Med Oral Pathol 1987;64:379–390.
2. Adamo AR, Szal RL. Timing, results and complications of mandibular reconstructive surgery. Report of 323 cases. J Oral Surg 1979;37:755–763.
3. Marx RE. Osteoradionecrosis of the jaws: Review and update. Hyperb Oxygen Rev 1984;41:283–288.
4. Marx RE, Johnson RP, Kline SN. Prevention of osteoradionecrosis: A randomized prospective clinical trial of hyperbaric oxygen versus penicillin. J Am Dent Assoc 1985;111:49–54.
5. Granström G, Jacobsson M, Tjellström A. Titanium implants in irradiated tissue. Benefits from hyperbaric oxygen. Int J Oral Maxillofac Implants 1992;7:15–25.
6. Roumanas E, Freymiller E, Chang T-L, Aghaloo T, Beumer J III. Implant retained prostheses for facial defects: An up to 14-year follow-up report on the survival rates of implants at UCLA. Int J Prosthodont 2002;4:325–332.
7. Jacobsson M, Tjellström A, Albrektsson T, Thomsen P, Turesson I. Integration of titanium implants in irradiated bone. Histological and clinical study. Ann Otol Rhinol Laryngol 1988;97:337–340.
8. Taylor TD, Worthington P. Osteointegrated implant rehabilitation of the previously irradiated mandible: Result of a limited trial at 3 to 7 years. J Prosthet Dent 1993;69:60–69.
9. Marx RE. Osteoradionecrosis: A new concept of its pathophysiology. J Oral Maxillofac Surg 1983;41:283–288.
10. Plenk H Jr. The microscopic evaluation of hard tissue implants. In: Williams DF (ed). Techniques of Biocompatibility Testing. Boca Raton, FL: CRC Press, 1986:35–81.
11. Powers BE, Gilette EL, McChesney SL, LeCouteur RA, Withrow SJ. Bone necrosis and tumor induction following experimental intraoperative irradiation. Int J Radiat Oncol Biol Phys 1989;17:559–567.
12. Holmes RE, Hagler HK, Coletta CA. Thick section histometry of porous hydroxyapatite implants using backscattered electron imaging. J Biomed Mater Res 1987;21:731–739.
13. Tukey J. Exploratory Data Analysis. Reading, MA: Addison-Wesley, 1977.
14. Schenk R, Willenegger H. Zum Bild der sogenannten Primärheilung der Knochenkompakta nach experimentellen Osteotomien beim Hund. Experentia 1963;19:593–606.
15. Albrektsson T. The response of bone to titanium implants. CRC Crit Rev Biocomp 1984;1:53–84.
16. Adell R, Lekholm U, Rockler, Brånemark P-I. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981;10:387–416.
17. Kasemo B, Lausmaa J. Biomaterial and implant surfaces: A surface science approach. Int J Oral Maxillofac Implants 1988;3:247–259.
18. Brånemark P-I, Breine U, Adell R, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg 1969;3:81–100.
19. Albrektsson T, Jacobsson M. Bone-metal interface in osseointegration. J Prosthet Dent 1987;57:597–607.
20. Schroeder A, Stich H, Straumann F, Sutter F. Ueber die Anlagerung von Osteozement an einen belasteten Implantatkoerper. Schweiz Mschr Zahnheilk 1978;88:1051–1058.
21. Schroeder A, van der Zypen E, Stich H, Sutter F. The reaction of bone connective tissue and epithelium to endosteal implants with sprayed titanium surfaces. J Maxillofac Surg 1981;9:15–25.
22. Sutter F, Raveh J, Schuerch H, Stich H. Titanplasma- beschichtetes Hohlschrauben- und Rekonstruktionsplattensystem (THRP) zur Ueberbrueckung von Kieferdefekten; Grundprinzipien und mechanische Eigenschaften. Chirurg 1984;55:741–748.
23. Kirsch A, Mentag PJ. The IMZ endosseous implant system: A complete oral rehabilitation treatment concept. J Oral Implantol 1986;12:576–589.
24. Babbush CA, Kirsch A, Mentag P, Hill B. Intramobile cylinder (IMZ) two-stage osteointegrated implant system with the intramobile element (IME): Part I. Its rationale and procedure for use. Int J Oral Maxillofac Implants 1987;2:203–216.
25. Gerner BT, Barth E, Albrektsson T, Ronningen H, Solheim LF, Wie H. Comparison of bone reactions to coated tricalcium phosphate and pure titanium dental implants in the canine iliac crest. Scand J Dent Res 1988;96:143–148.
26. Cook SD, Kay JF, Thomas KA, Jarcho M. Interface mechanics and histology of titanium and hydroxylapatite-coated titanium for dental implant applications. Int J Oral Maxillofac Implants 1987;2:15–22.
27. Weinlaender M, Kenney EB, Lekovic V, Beumer J III, Moy PK, Lewis S. Histomorphometry of bone apposition around three types of dental implants. Int J Oral Maxillofac Implants 1992;7:491–496.
28. Block MS, Kent JN, Kay JF. Evaluation of hydroxylapatite-coated dental implants in dogs. J Oral Maxillofac Surg 1987;45:601–607.
29. Gottlander M. On Hard Tissue Reactions to Hydroxyapatite-Coated Implants [thesis]. Göteborg, Sweden: Göteborg University, 1994.
30. Jacobsson M, Jönsson A, Albrektsson T, Turesson I. Short- and long-term effects of irradiation on bone regeneration. Plast Reconstr Surg 1985;76:841–850.
31. Ehrenfeld M, Cornelius CP, Hildebrandt H, Riediger D, Schwenzer N. Besteht aus onkologischer Sicht eine Indikation zur Sofortrekonstruktion beim Mundhöhlenkarzinom. Fortschr Kiefer Gesichtschir 1992;37:50–53.

32. Bergermann M, Dieckmann J, Machtens E. Präoperative Kurzzeitbestrahlung mit 3x6Gy, direkte anschließende radikale Tumorresektion und postoperative Aufsättigungsbestrahlung bis 60 Gy als effektives Therapiekonzept zur Behandlung von T2 Plattenepithel- karzinomen der Mundhöhle. Ergebnisse einer prospektiven randomisierten bizentrischen Studie. Fortschr Kiefer Gesichtschir 1992;37:17–20.
33. Schön R, Kohsuke O, Masato K, Kenichi M. Peri-implant tissue reaction in bone irradiated the fifth day after implantation in rabbits: Histologic and histomorphometric measurements. Int J Oral Maxillofac Implants 1996;11;228–238.
34. Hum S, Larsen P. The effect of radiation at the titanium-bone interface. In: Laney W, Tolman D (eds). Tissue Integration in Oral, Orthopedic and Maxillofacial Reconstruction. Chicago: Quintessence, 1990:234.
35. Brogniez V, D'Hoore W, Gregoire V, Munting E, Reychler H. Implants placed in an irradiated dog mandible: A morphometric analysis. Int J Oral Maxillofac Implants 2000;15:511–518.
36. Esser E, Wagner W. Dental implants following radical oral cancer surgery and adjuvant radiotherapy. Int J Oral Maxillofac Implants 1997;12:552–557.
37. Franzen L, Rosenquist JB, Rosenquist KI, Gustafsson I. Oral implant rehabilitation of patients with oral malignancies treated with radiotherapy and surgery without adjunctive hyperbaric oxygen. Int J Oral Maxillofac Implants 1995;10: 183–187.
38. Keller E, Tolman DE, Zuck SL, Eckert SE. Mandibular endosseous implants and autogenous bone grafting in irradiated tissue: A ten-year retrospective study. Int J Oral Maxillofac Implants 1997;12:800–813.
39. Nimi A, Ueda M, Keller EE, Worthington P. Experience with osseointegrated implants placed in irradiated tissues in Japan and the United States. Int J Oral Maxillofac Implants 1998;13:407–411.
40. Watzinger F, Ewers R, Henninger A, Sudasch G, Babka A, Woelf G. Endosteal implants in the irradiated lower jaw. Craniomaxillofac Surg 1996;24:237–244.
41. Jacobsson M, Kaläbo P, Tjellström A, Turesson I. Bone cell viability after irradiation. An enzyme histochemical study. Acta Oncol 1987;26:463–465.
42. Jacobsson M, Albrektsson T, Turesson I. Dynamics of irradiation injury to bone tissue. A vital microscopic investigation. Acta Radiol Oncol 1985;24:343–350.
43. Asikainen P, Klemetti E, Kotilainen R, et al. Osseointegration of dental implants in bone irradiated with 40, 50, or 60 Gy doses. An experimental study with beagle dogs. Clin Oral Implants Res 1998;9:20–25.
44. Marx RE. Discussion of histomorphometric examination of healing around hydroxyapatite implants in Co 60 irradiated bone. J Oral Maxillofac Surg 1994;52:167–172.