
A Histomorphometric Study of Bone Reactions to Titanium Implants in Irradiated Bone and the Effect of Hyperbaric Oxygen Treatment

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The present study was undertaken to histomorphometrically analyze early peri-implant bone tissue reactions that occur after radiotherapy and to determine whether hyperbaric oxygen therapy (HBO) affects bone tissue at the microscopic level by altering bone morphology. Twelve rabbits received a single dose (15 Gy) of cobalt⁶⁰ radiation to one hind leg and the other hind leg served as a control. Titanium screws were placed into the femur and tibia directly after irradiation. Six animals received HBO during the first 4 postoperative weeks. After 8 weeks of follow-up, bone specimens containing the screws were prepared for histomorphometry. Bone-metal contact and the amount of bone in the thread areas and in the mirror areas were measured in a blinded manner. Periosteal bone formation and bone remodeling decreased after irradiation; also after HBO treatment. Hyperbaric oxygen therapy improved bone formation in nonirradiated bone and to some extent also in the irradiated bone. Bone maturation was improved in the HBO animals after irradiation. It was concluded that irradiation reduces the capacity for osseointegration of titanium implants. Hyperbaric oxygen treatment may improve bone formation and especially has positive effects on bone maturation after irradiation.
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Key words: acute tissue reactions, histomorphometry, hyperbaric oxygen treatment, irradiation, osseointegrated implants

The osseointegration protocol is an established clinical technique by which titanium implants can be anchored in different bones of the body.¹ The concept has mainly been used to replace lost teeth with bone-anchored prostheses in the oral cavity,² but also to replace lost parts of the face

with bone-anchored prostheses.³ Radiotherapy in combination with surgery is the treatment generally used for malignant tumors in the craniofacial region. The osseointegration procedure is an alternative system to conventional reconstructive surgery, whereby craniofacial prostheses are anchored to the bone tissue by titanium implants. If the bone tissue is compromised in any way, early implant failures can occur.⁴

Clinically, the most common cause of compromised bone tissue in the craniofacial region is radiotherapy. It is well known that implants are lost more frequently in these patients.^{5,6} Some studies have pointed to the possibility that hyperbaric oxygen therapy (HBO) can be used to improve the bone quality and potential for osseointegration in these situations.^{4,7,8} Knowledge concerning peri-implant bone tissue reactions after radiotherapy is still insufficient. The present study was undertaken to histomorphometrically analyze early peri-implant bone tissue reactions that occur after

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radiotherapy and to determine whether HBO affects bone tissue at the microscopic level by altering bone morphology.

Materials and Methods

The study design was approved by the Göteborg University (Sweden) laboratory animal ethical committee. Twelve adult (over 9 months old) New Zealand white rabbits were used in the study. The rabbits were group housed, and all animals were females or neutered males.

Implants. A total of 48 screw-type endosseous implants was manually manufactured from commercially pure titanium by Meditech, Göteborg University, Sweden. The total length of the implants was 10 mm, with an outer diameter of 3.7 mm and a square head. After manual grading, the implants were cleaned in n-butylalcohol (Merck, Darmstadt, Germany) and absolute alcohol in ultrasonic baths. The cleaning procedure was completed by autoclaving.

Anesthetic Procedure. Intramuscular injections of fentanyl and fluanison (Hypnorm, Janssen-Cilag, Buckinghamshire, England) at a dose of 0.5 mL/kg body weight and intraperitoneal injections of diazepam (Stesolid Novum, A/S Dumex Denmark, Pharmacia-Upjohn, Stockholm, Sweden) at a dose of 1.5 mg/kg body weight were used for general anesthesia during irradiation and surgical procedures, but not during HBO treatment. Local anesthesia with 1.0 mL of 5% lidocaine (Xylocaine, Astra, Mölndal, Sweden) was administered to the tibiae and femora, where the implants were to be placed. The shaved skin of the rabbits was washed with a mixture of iodine and 70% ethanol prior to surgery. As an adjunct to surgery, the animals received an antibiotic, benzyl-penicillin (PenoVet, Boeringer Ingelheim Agrovvet, Hellerup, Denmark), at a dose of 20 mg/kg body weight. Postoperatively, intramuscular injections of buprenorfin (Temgesic, Reckitt & Colman, Hull, England) were given in doses of 0.1 mg/kg body weight. At the time of sacrifice, intravenous injections of a mixture of saline and barbiturates (Mebumal Vet, 60 mg/mL, Nord Vacc, Sweden) 1:4 were given.

Irradiation Procedure. Four to 6 hours prior to implant placement, the rabbits received irradiation to the proximal tibial metaphysis and the distal femoral metaphysis of the right hind leg; the left hind leg served as a control. During irradiation, the femur and tibia were placed on a 5-cm-thick polystyrene phantom. Gamma irradiation (cobalt⁶⁰) was used to minimize the difference in the absorbed dose in soft tissue and bone. Source-to-skin dis-

tance was 60 cm and field size was 7.5 × 7.5 cm. The dose rate was approximately 0.5 Gy per minute. A 5-mm bolus was applied to ensure full buildup, and 15 Gy were given as a single dose.

Surgical Procedure. After irradiation, the animals were divided into 2 groups (A and B), with 6 animals in each group. All animals had 4 implants placed 4 to 6 hours after irradiation, with 1 implant in each tibial and femoral metaphysis. The implants were placed under aseptic conditions using a very gentle surgical technique with sharp drills and low drill speeds. Ample cooling with saline solution was provided when preparing the implant sites, as well as when the implants were carefully screwed into the tibial and femoral cortical bone sites. The fascia and the skin were sutured in separate layers. The animals were allowed full weight-bearing after surgery. The implants were left in place for 8 weeks and then removed. Group B received HBO treatment during the first 4 postoperative weeks.

Hyperbaric Oxygen Treatment. On the third postoperative day, the animals in group B were placed in a 75 L pressure chamber (Göteborg Diving Technique, Göteborg, Sweden) and subjected to pure oxygen (280 kPa for 2 hours). During this period, the first 10 minutes were used for successive compression up to 280 kPa; the pressure was then kept constant for 90 minutes, and decompression lasted 20 minutes. The chamber temperature was kept at 23°C by a water cooling system. Produced carbon dioxide was eliminated by a constant flow of oxygen with a flow rate of 1.5 L/min. The HBO treatment was performed once daily Monday through Friday, and a total of 20 treatments was given.

Preparation of Specimens and Histomorphometric Measurements. On the day of sacrifice 8 weeks after implant placement, the animals were again anesthetized according to the method described above. The skin and the fascia were opened, the implants with surrounding bone tissue were removed en bloc, and the animals were then sacrificed. The specimens were fixed in 4% neutral buffered formaldehyde and further processed to be embedded in light curing resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Exakt sawing and grinding machines (Exakt Apparatebau, Nordstedt, Germany) were used to reach a final thickness of about 10 µm for the embedded specimens. The sections were stained with toluidine blue according to Donath,⁹ and light microscopic investigations, including histomorphometric calculations, were performed with Leitz Microvid equipment (Leitz, Wetzlar, Germany) connected to an IBM computer (Armonk, NY). Measurements

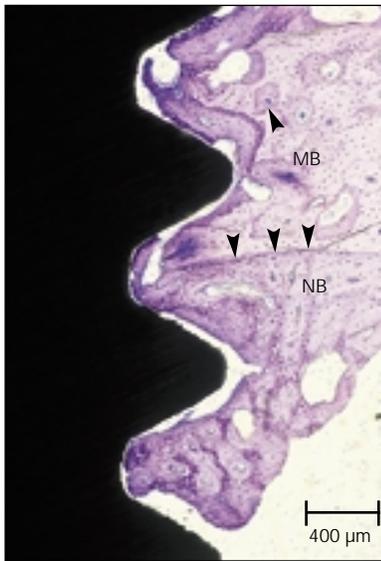


Fig 1 Specimen from a nonirradiated, non-HBO-treated implant. To get a better resolution of peri-implant bone formation, the bone was divided into 2 morphologically separable types. Mature bone (MB) was defined as the more compact and lightly stained bone. Newly formed bone (NB) was stained more darkly, more porous, and could be distinguished from the mature bone because of the broken lamellae (*arrowheads*) (toluidine blue stain, original magnification $\times 80$).

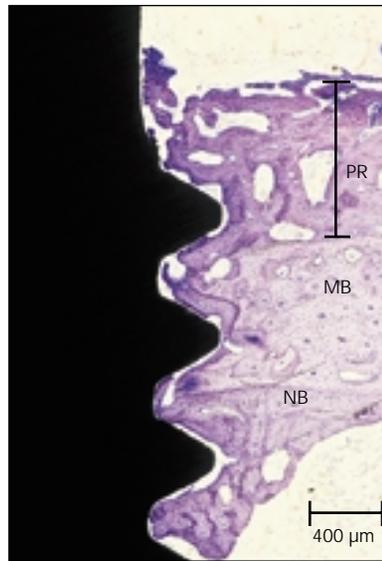


Fig 2 Specimen from a nonirradiated, non-HBO-treated implant (toluidine blue stain, original magnification $\times 63$). PR = periosteal bone; MB = mature bone; NB = newly formed bone.

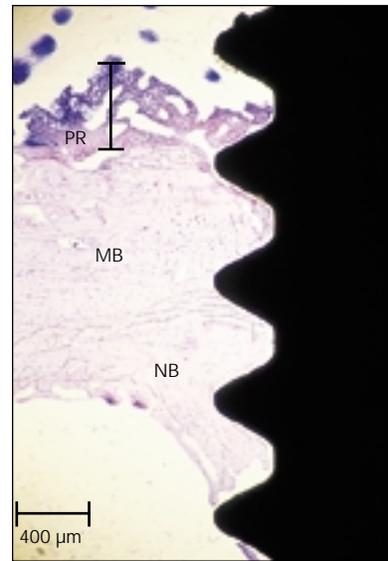


Fig 3 Specimen from an irradiated, non-HBO-treated implant (toluidine blue stain, original magnification $\times 63$). The most striking effect of radiotherapy on bone-forming capacity was reduced periosteal bone formation (PR) in the collar region of the implants. The irradiated implants also showed less newly formed bone (NB). MB = mature bone.

were performed in the eyepiece of the microscope using an objective of $10\times$ and a zoom of 2.5.

Histomorphometric measurements of bone-metal contact (BMC), the amount of bone in the thread area (TA), and the amount of bone in the area immediately “outside” the same thread—the out-folded “mirror image” (MA) area—were measured and calculated for the entire implant and for the 3 best consecutive threads. To get better resolution of peri-implant bone formation, the bone was divided into 2 morphologically separable types. Mature bone (MB) was defined as the more compact and lightly stained bone. Newly formed bone (NB) was stained more darkly, more porous, and could be distinguished from the mature bone because of the broken lamellae (Fig 1). The separation of bone tissue into mature and newly formed bone was based on findings by Sennerby et al.¹⁰

Mean percentages for the mentioned parameters were calculated for the implants on the irradiated side (tibia + femur) and compared to the mean per-

centages for the implants (tibia + femur) on the control side. Comparisons were also made between HBO-treated (group B) and non-HBO-treated animals (group A) for irradiated and nonirradiated bone tissue. The measurements were made in a blinded manner (the investigator did not know to which group the specimens belonged).

Statistical Methods. To assess the effect of irradiation, the Wilcoxon signed rank test was used in both groups. The effect of HBO treatment was evaluated by Fisher’s permutation test¹¹ for irradiated as well as nonirradiated bone tissue.

Results

The most striking effect of radiotherapy on bone-forming capacity was reduced periosteal bone formation in the collar region of the implants (Figs 2 and 3). Bone that had been cut during surgery often remained, without any signs of resorption or bone deposition around the implants that had been placed in irradiated bone tissue. Around several

Table 1 Detailed Histomorphometric Data

	Group A (no HBO, nonirradiated)	Group A (no HBO, irradiated)	Group B (HBO, nonirradiated)	Group B (HBO, irradiated)
BMC tot (%)	13 (9 to 20)	12 (5 to 23)	17 (14 to 20)	18 (11 to 36)
BMC MB (%)	0.15 (0 to 1)*†	1 (0 to 2)*	2 (0.5 to 3)†	2 (1 to 4)
BMC NB (%)	13 (8 to 20)	11 (5 to 23)	15 (11 to 20)	17 (7 to 35)
BMC tot 3b (%)	21 (13 to 34)	22 (10 to 43)	31 (27 to 36)†	30 (20 to 54)
BMC MB 3b (%)	0.3 (0 to 2)*†	3 (0 to 5)*	5 (1 to 9)†	5 (2 to 9)
BMC NB 3b (%)	21 (13 to 34)	21 (9 to 43)	27 (18 to 34)	27 (17 to 51)
TA tot (%)	52 (47 to 56)*	41 (32 to 56)*	53 (43 to 62)	49 (29 to 62)
TA MB (%)	4 (2 to 7)*	6 (4 to 9)*†	6 (3 to 8)	9 (7 to 12)†
TA NB (%)	48 (43 to 53)*	35 (26 to 50)*	47 (40 to 56)	40 (22 to 54)
TA tot 3b (%)	78 (69 to 86)	66 (53 to 78)	83 (76 to 88)*	75 (59 to 84)*
TA MB 3b (%)	9 (4 to 15)*†	17 (10 to 24)*	15 (10 to 23)†	21 (17 to 28)
TA NB 3b (%)	71 (64 to 81)*	54 (43 to 66)*	71 (61 to 75)*	60 (43 to 69)*
MA tot (%)	49 (40 to 60)	42 (33 to 56)	48 (38 to 58)	46 (25 to 56)
MA MB (%)	9 (4 to 21)	12 (7 to 15)	12 (8 to 16)*	15 (11 to 19)*
MA NB (%)	39 (33 to 52)	30 (21 to 42)	36 (28 to 44)	31 (13 to 40)
MA tot 3b (%)	83 (70 to 91)	72 (67 to 82)	82 (74 to 88)	77 (55 to 87)
MA MB 3b (%)	22 (10 to 37)	29 (17 to 37)	28 (20 to 35)*	33 (25 to 44)*
MA NB 3b (%)	64 (57 to 74)	51 (41 to 65)	58 (54 to 62)	53 (27 to 65)

* = significant difference in group; † = significant difference between groups.
 BMC = bone-to-metal contact; TA = bone within the thread area; MA = bone within the "mirror" area;
 tot = total amount of bone; MB = mature bone; NB = newly formed bone; 3b = 3 best threads.

implants in irradiated, non-HBO-treated bone (Irr-nHBO), persisting bone fragments were noticed; the woven bone was not replaced by lamellar bone, and the structure of the woven bone was sometimes very disorganized and immature compared to the specimens from the nonirradiated bone tissue (nIrr-nHBO). In one of the Irr-nHBO specimens, cartilage formation was seen.

The histomorphometric data are presented in detail in Table 1 and are graphically illustrated in Figs 4 and 5. The major portion of bone found in contact with the implants and within the thread areas was newly formed bone. There was less bone in the thread areas around the implants placed in irradiated bone tissue compared to the nonirradiated implants in both groups. The reduction was significant in the Irr-nHBO implants when calculating the entire implant ($P = .046$) and in the Irr-HBO implants when calculating the 3 best threads ($P = .046$). The implants placed in irradiated bone tissue showed less newly formed bone (NB) in the thread areas, compared to the nonirradiated implants in both groups. The reduction was significant in the Irr-nHBO implants, both when measuring the entire implant ($P = .046$) and the 3 best threads ($P = .028$) and in the Irr-HBO implants when calculating the 3 best threads ($P = .046$).

After HBO, significantly more bone was found in contact with the nIrr-HBO implants, compared to the nIrr-nHBO implants ($P = .032$), when calculating the 3 best threads. More mature bone was found in contact with the nIrr-HBO implants compared to the nIrr-nHBO implants, both when measuring the entire implant ($P = .043$) and the 3 best threads ($P = .0065$). There was a significant increase of mature bone in the thread area after HBO, both for the nIrr-HBO implants when calculating the 3 best threads ($P = .048$) and for the Irr-HBO implants when measuring the entire implant ($P = .035$) compared to the corresponding nHBO implants. When comparing nIrr-nHBO and Irr-nHBO implants, significantly more mature bone was seen in contact with the irradiated implants, both when measuring the entire implant ($P = .046$) and the 3 best threads ($P = .046$). There was also significantly more mature bone in the thread area in the Irr-nHBO implants, both when measuring the entire implant ($P = .046$) and the 3 best threads ($P = .028$). When comparing the amount of bone in the mirror areas, more mature bone was seen in the Irr-HBO compared to the nIrr-HBO implants, both when measuring the entire implant ($P = .028$) and the 3 best threads ($P = .046$).

Discussion

The method for histologic evaluation using light microscopy and computer analysis has been used in earlier studies.¹²⁻¹⁶ However, the follow-up time of 8 weeks in the present study and the calculation of mean percentages for the implants on the irradiated side (tibia + femur) compared to the mean

percentages for the implants on the control side differs from the mentioned studies. A follow-up time of 8 weeks was selected for calculating the early peri-implant bone reactions. The rabbits used in the present animal model have a bone turnover rate that is approximately 3 times faster than in humans, with integration of cortical implants occurring within 6 weeks.¹⁷ Both the follow-up

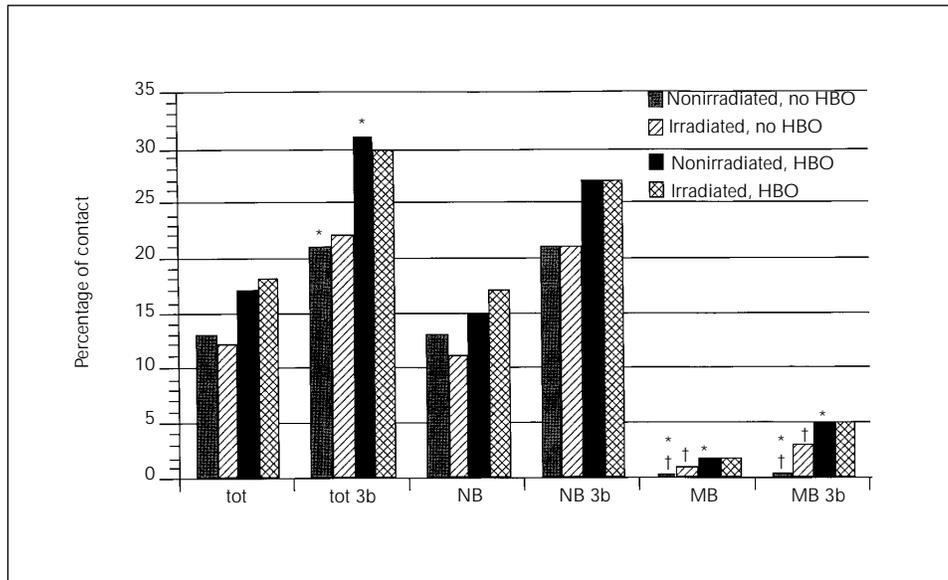


Fig 4 Graph depicting the bone-metal contact (BMC). Tot = total amount of bone; 3b = 3 best threads, NB = newly formed bone; MB = mature bone; * = significant difference between non-HBO and HBO group; † = significant difference between irradiated and nonirradiated implants.

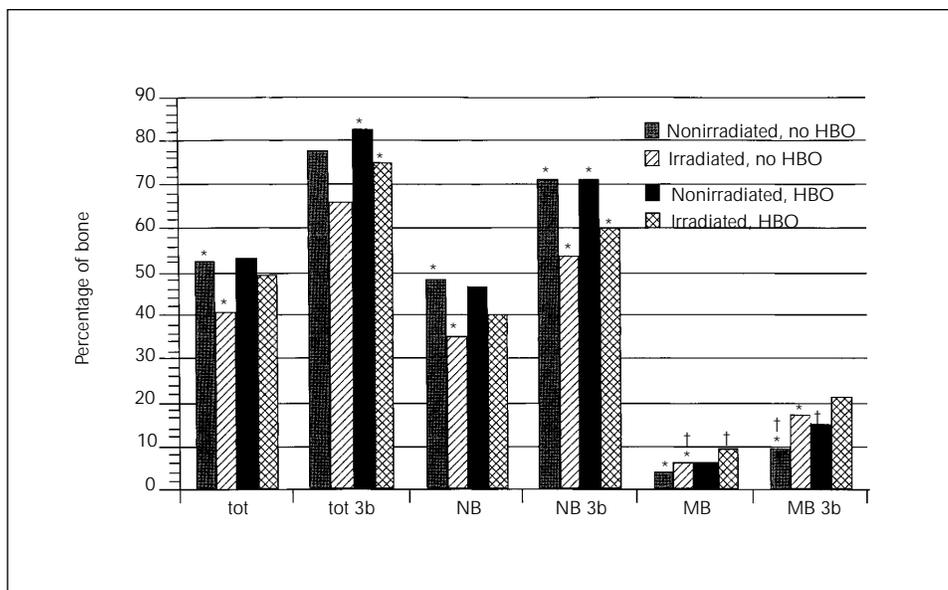


Fig 5 Diagram showing the amount of bone within the thread areas. Tot = total amount of bone; 3b = 3 best threads; NB = newly formed bone; MB = mature bone; * = significant difference between irradiated and nonirradiated implants; † = significant difference between non-HBO and HBO group.

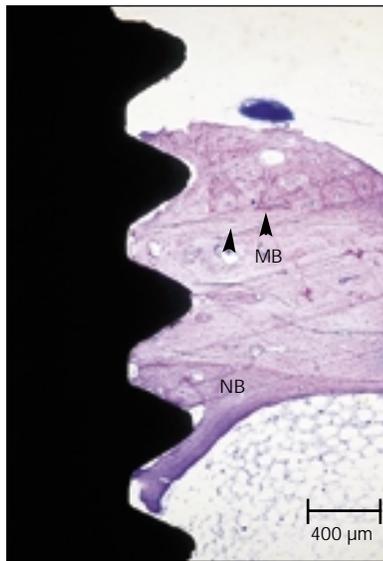


Fig 6 Specimen from a nonirradiated, HBO-treated implant. It was slightly more difficult to distinguish newly formed bone (NB) from mature bone (MB) in the HBO treated group especially in the periosteal region (*arrowheads*) (toluidine blue stain, original magnification $\times 63$).

time and calculation of the mean percentages for the tibial and femoral implants were chosen so that the results could be correlated with a previous study measuring removal torques.¹⁸

In the present study, the bone was divided into 2 morphologically separable types: mature bone and newly formed bone. This separation into different bone types was based on findings by Sennerby et al.¹⁰ In this study, Sennerby showed that bone formation around titanium implants can be seen 7 days after implant placement. Six weeks after implant placement, it was still possible to distinguish between the original bone and the newly formed bone, because of the interrupted lamellae. After 12 weeks, remodeling of the cortical bone was not apparent, and the bone close to the implant had the same degree of mineralization as the original bone. In the present study, mature bone could be distinguished from newly formed bone 8 weeks after implant placement. However, since the bone was not labeled, it was not possible to separate fully matured bone that was formed after implant placement from mature bone present at surgery.

Several studies have shown that bone healing in long bones of the rabbits is impaired after irradiation.^{6,19} A single dose of 15 Gy cobalt⁶⁰ was

used in this study, since severe inhibition of bone regeneration has been shown after this dose.¹⁹ The animal radiation tissue damage model used in the present study has been estimated to be equivalent to a clinically relevant dose in humans.¹⁹ However, the radiation dose and fractionation may not be sufficient to truly reflect the radiation pathology in humans, as the tissue response has been reported to be species-specific.²⁰ All implants in the present study were placed after irradiation. The clinical situation that corresponds to this experiment is that in which the patient is given preoperative radiotherapy followed by tumor removal. Tumor removal is undertaken approximately 4 to 6 weeks after completion of a radiotherapy course, ie, when the acute tissue reactions have declined. This is the ideal time to start the rehabilitation procedure, ie, to place the implants into the tumor cavity, since clinical data show higher implant losses in patients treated with radiotherapy long before surgery, and an increase in radiation tissue damage over time has been suggested.⁶

A significant reduction in the amount of bone within the thread areas around the implants placed in irradiated bone was seen compared to the nonirradiated implants in both groups (non-HBO and HBO). In both groups there was also significantly less newly formed bone in the thread areas around the implants placed in irradiated bone tissue. Author interpretation of these data is that the single dose of 15 Gy cobalt⁶⁰ reduced the bone-forming capacity, consistent with previous reports.^{19,21} There was significantly more bone-metal contact in the nonirradiated implants after HBO treatment. This result is consistent with other findings, where HBO treatment caused a significant increase in bone formation in nonirradiated bone tissue.²² There was significantly more mature bone, both in contact with and in the thread areas, around the nonirradiated implants after HBO. There was also more mature bone within the thread areas around the irradiated implants after HBO. It was slightly more difficult to distinguish newly formed bone from mature bone in the HBO-treated group (Fig 6). These findings suggest that bone remodeling occurred faster in the HBO-treated group.

Several studies have shown a positive correlation between bone-metal contact and removal torques.²³ In other studies, the most important factor determining removal torque has been stated to be the amount of bone around the implant at the cortical passage.¹³ In the present study, there was no significant difference (regarding total bone-

metal contact) when comparing irradiated and nonirradiated implants. The increase of bone-metal contact in the nonirradiated implants after HBO treatment supports the finding in a previous study,¹⁸ where use of HBO increased the biomechanical force necessary to unscrew titanium implants in nonirradiated bone.

For the Irr-nHBO implants, there was significantly more mature bone, with respect to both bone-metal contact and to bone within the thread areas, compared to the nIrr-nHBO implants. Since the bone was not labeled, it can only be speculated that the mature bone in the non-HBO group represents bone present at surgery and with an inferior quality and impaired remodeling capacity resulting from irradiation, and that the mature bone in the HBO group partially consists of new bone that has undergone continuous remodeling. Such quality parameters could have affected the removal torque measurements reported by Johnsson et al,¹⁸ where postirradiation use of HBO significantly increased the biomechanical force necessary to unscrew titanium implants in irradiated bone.

The damaging effect of radiotherapy on bone tissue is believed to be related to the effects on bone-forming cells, as well as on bone-resorbing cells, and on the periosteal and endosteal tissues.^{4,20,23} The osteoblasts and osteocytes are stationary cells, which after damage may die or stop producing bone matrix, whereas the osteoclasts are migratory cells that after radiotherapy is completed can recolonize the bone and continue the resorption. This might lead to bone imbalance between apposition and resorption. Whether such bone can accept and integrate endosseous implants, and the value of HBO in relation to osseointegration, is still under debate.^{24,25} The immediate effect of irradiation was used in the present study to simulate a clinical situation with reconstructive surgery in the early postirradiation interval. In earlier studies HBO has been shown to increase bone formation in bone harvest chambers,²² to increase the removal torque necessary to unscrew titanium implants after irradiation damage,¹⁸ and to improve histologic osseointegration.⁸ The present study supports the idea that HBO has an effect on bone metabolism and morphology that can be used to reduce some of the damaging effects from radiotherapy. The exact mechanism of this effect, however, remains to be elucidated. It could be an effect on the osteoprogenitor cells, causing an altered differentiation of osteoblasts, or it could be an effect on undifferentiated mesenchymal cells. Further studies are needed to resolve these questions.

Conclusions

The findings of this study compare favorably with other studies showing that irradiation reduces the capacity for osseointegration of titanium implants. Hyperbaric oxygen was shown, as in earlier findings, to improve bone formation in nonirradiated bone tissue. To some extent, HBO treatment also improved bone formation in irradiated bone tissue, and HBO could have positive effects on bone maturation after irradiation, as suggested in this investigation.

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