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Single-dose irradiation followed by implant insertion in rat bone. An investigative study to find a critical level for osseointegration

ABSTRACT

Aim No general consensus exists regarding the ideal time to insert implants in bone after irradiation or how the various irradiation doses influence implant success. This study aims at investigating integration of implants in pre-irradiated rat bone and find a critical level doses that cause disturbed osseointegration.

Materials and methods Single irradiation doses of 2, 5, 10, 20 and 30 Gy were given to one leg of adult rats 3 days prior to insertion of screw-shaped implants whereas the other leg served as a non-irradiated control. The follow up was 5 weeks. Bone implant contact (BIC) and bone area (BA) were measured on undecalcified cut and ground sections in the light microscope. The tissue quality was also examined in the light microscope.

Results Doses of 5 and 10 Gy resulted in 25% higher contact values for the irradiated samples compared to non-irradiated controls. The most impaired integration occurred when doses of 20 Gy were given, revealing a 50% difference between the irradiated (25%) and the non irradiated samples (50%). The bone area demonstrated no major quantitative differences albeit the qualitative observations differed substantially being most pronounced in the 20 and 30 Gy irradiated samples.

Conclusions The osseointegration was substantially impaired after radiation doses of 20 and 30 Gy. Quantitative data alone are insufficient to describe implant integration in situation like this. Qualitative observations are of utmost importance and require great attention. The importance of judging and describing various grades of tissue damage is complicated but necessary. Based on the results obtained in this study, full scale experiments are now ongoing.

KEYWORDS Augmentation procedure; Irradiation; Osseointegration; Quality; Rat-bone; Titanium implants.

INTRODUCTION

A patient with oral malignancy in the head- and neck region is usually treated with a combination of surgery and radiation therapy. Depending on tumor type and localization, the dose (0-70 Gy) and radiation field in the head and neck region may vary (0-70 Gy) both within and between the upper and lower jaw. Radiation has a significant effect/sequel on oral tissue (bone and mucosa) (1). An increased loss of implants in irradiated patients over time compared to non-irradiated patients has been reported and moreover, additional time after completed radiation therapy to implant surgery increases the risk for implant loss (2-4). From the patient's point of view that suffers from teeth loss in consequence to malignancy treatment, immediate rehabilitation is important. However, there is no general consensus on when the ideal time is to insert implants after irradiation or how various irradiation doses influences implant success. Several authors have reported on implant installation time varying between 6-18 months (3, 5-7). In general, animal studies related to "early" implant insertion in pre-irradiated rat bone tissue are not frequently found in the literature. One study reported on histomorphometrical data in a study design involving installation of implants one week after radiation of 15 Gy (8). The rats were followed for 1- to 12 weeks. These authors reported on approximately 50% greater bone to implant contact (BIC) values for non-irradiated controls compared to irradiated bone after 12 weeks of follow up. At 6 weeks the irradiated group had a BIC value of 20% and the control 31%. Corresponding percentages after 4 weeks were 14 and 27 respectively.

Late effects of single radiation doses between 10 to 35 Gy were studied in a rat model by Öhrnell et al. (9). Irradiation was carried out 8 weeks before implant

insertion. The follow up after irradiation was 12 weeks. However, their study did not report on interfacial percentages of bone area (BA) and BIC albeit estimations of bone thickness outside the implant threads were reported on. Some biomechanical tests revealed a significantly lower value for implants placed in irradiated bone compared to controls while shear stresses and shear moduli did not correlate to radiation doses.
Several studies have been performed on rabbit bone. Some have investigated late irradiation effects of implant integration both biomechanically and histomorphometrically. Irradiation with 15 Gy reduced both the removal torque and the bony contact compared to non-irradiated controls (10). Others have specifically focused on dose-dependent bone formation in harvested bone biopsies without implants (11). Impaired bone tissue formation and a dose-dependent deficit were observed in the irradiated biopsies compared to controls (11). Irrespective of study design, all studies required a network of various disciplines and were beyond routine work, and this might be the reason for the relative few studies conducted.
Since there is no general consensus existing on the ideal time to insert implants in pre-irradiated bone and what doses that are causing impaired osseointegration our aim was to perform an investigative study to find a critical level for osseointegration of implants placed in rat bone after single radiation doses of 2, 5, 10, 20 and 30 Gy. The other leg served as a non irradiated control. The follow time was 5 weeks.

MATERIALS AND METHODS

Animals
Nine male Sprague Dawley rats (mean age 14 weeks old, with a mean weight of 472 g upon arrival, Charles River Laboratories, Germany) were used in the study. The individual rat weight was recorded at arrival and once a week throughout the follow up time.
Three days after irradiation, two threaded implants (n=36) with an outer diameter of 2.2 mm and a total length of 3.2 mm were inserted in each tuberositas tibiae. The implant treatment followed the procedure for OsseoSpeed™ dental implants, i.e. TiO_2 blasting followed by treatment in a diluted hydrofluoric acid (AstraTech AB, Sweden). The follow up was 5 weeks. The study was approved by the animal ethics committee in Göteborg, Sweden.
Due to the low number of samples/irradiated group, no statistical tests have been performed.

Anaesthesia
A freshly prepared mixture of fentanyl and fluanizone + midazolam (2 parts sterile water, 1 part fentanyl/fluanizone, Hypnorm® Vet, Saunderton, England and 1 part midazolam, 5 mg/ml, Dormicum®, Roche, France) was used for intra-peritoneal injections to each rat initially with a dose of 2.7 ml/kg (12). Additional anesthesia was given when needed. Intra peritoneal injections of an overdose of Pentobarbitalium (Apoteksbolaget, Sweden) were used for sacrifice.

Irradiation
Before irradiation, each rat was anaesthetized as described above. External single doses of 2, 5, 10, 20 and 30 Gy were given using 4 MV photon irradiation from a linear accelerator (VARIAN Clinac 600 CD). For each dose level two rats were irradiated (except for the 2 Gy level with only one rat) with a dose rate of 3.4 Gy/min giving a total irradiation time between 0.6 min (2 Gy) and 8.8 min (30 Gy). Two rats were placed side by side in a supine position on the radiation table. A tissue equivalent bolus of 1.0 cm thickness was placed on one hind leg of each animal. The field size was 5 x 5 cm in an isocentric setup giving a source-bolus distance of 99 cm. The other leg was well outside the radiation field and served as a non-irradiated control leg. The distance between the field edge and the control leg was > 5 cm giving a dose to this leg of < 3% of the prescribed dose.

Implant installation
Three days post radiation the rats were anaesthetised. The hind-legs were shaved and washed with 70% ethanol-iodine solution. The skin and fascia layers were incised and closed separately. Periosteal layers were gently pulled away and not resutured. Each bone-site for implant insertion was opened with drills from small round drills to larger diameter drills during cooling with NaCl. One implant was inserted in each femur condyle region and 2 implants in each tibia (tuberositas tibiae region), about 0.5 cm below the tibia plateau, with a centre distance of approximately 5 mm apart. Low rotation drill speed was used and both legs were treated in the same surgical manner. The fascia was closed with 5-0 resorbable sutures (Vicryl, Ethicon, Johnson & Johnson, New Brunswick, NJ, USA) and the skin with 4-0 resorbable sutures (Monocryl, Ethicon, Johnson & Johnson, New Brunswick, NJ, USA).

Sample preparation
All animals were sacrificed after the 5-week uneventful healing period using an overdose of Pentobarbitalium (Apoteksbolaget, Uppsala, Sweden), whereafter soft tissues covering the implants were dissected and pined up on supporting cork and immersed in formalin fixative containing zinc (Histolab Products AB, Göteborg, Sweden). The legs were retrieved and immersed in fixative followed by

ordinary x-ray. Slices of bone without implants as well as individual retrieved implants with surrounding bone tissue were fixed for one week. The bone slices were decalcified in EDTA solution for about 3 weeks. The undecalcified samples and the soft tissues were processed to paraffin embedding, followed by cutting 5 µm thick sections. Haematoxyline-eosine (Htx-Eo) was used for routine staining. For initial trials of immunohistochemical methods, the endothelial cell marker Factor VIII (BioCare Medical, Concord, CA, USA) was used. The immunohistochemical protocol used followed the recommendations from Histolab Products, AB (Göteborg, Sweden) who was the supplier of the antibody. The extensive protocol for the immunohistochemistry will be reported elsewhere. The bone samples containing the implants were processed according to the so-called Donath / Exakt method (13). In brief the samples were dehydrated in ethanol (from 70% to absolute ethanol). Pre-infiltration in diluted resins followed by infiltration in pure resin (Technovit 7200, VLC, Kulzer, Germany). All steps were conducted using irrigation and vacuum. The polymerized samples were divided in the long axis of the implant. Undecalcified cut and ground sections of 10 µm thickness was prepared followed by histological staining with Toluidine-blue mixed in pyronin G (14).
Albeit the low numbers of animals used for each irradiation level the attempt was to treat the samples "routinely", i.e. conduct in-house histomorphometrical methods. The mean values presented should be regarded as "guide-lines" and not as "exact" values. All qualitative observations and quantitative measurements were performed "directly in the eye-piece" of a Leitz Metallux 3 light microscope using an objective of 16X. A computer based manual technique was used (15). The sections were inspected in a blinded manner and the investigators did not know the code. Histomorphometrical analyses involved BIC and BA measurements in all threads around the implant and in 3 consecutive upper threads placed in the original cortex. The same person performed all measurements.

RESULTS

Rat weight

The mean weight of the rats upon arrival was 472 g (range 431-560). At surgery, i.e. 3 days post radiation the mean weight was 482 g. All rats lost some weight after surgery and radiation. Except for a mean weight loss of 10 g between surgery and the first week post surgery, the mean weight-gain was approximately 20 g/week thereafter and the total mean weight was 547 g at termination of the study.

Observations – Ocular inspections

No rat needed to be re-sutured. Skin reactions consisted of hair-less areas only, and occurred one-week post radiation on the 20 and 30 Gy radiated rat legs only. These areas persisted throughout the follow up time.
The qualitative observations on the skin sections stained with Htx-Eo revealed an inflammatory reaction in almost all sections. Since these reactions may be due to the suturing material still in place (although resorbable sutures were used) no further attempts of describing skin reactions will be addressed in this material.
Preliminary immunohistochemical test with Factor VIII (von Willebrand factor) conducted on the paraffin sections from the skin demonstrated few endothelial cells (irrespective of test or control sections). However, other structures, such as glands and hair-follicles also stained positively and it was concluded that using this antibody alone would show unspecific staining.
The sections of the decalcified bone showed a larger amount of positive red stained megakaryocytes in the 20 Gy control group compared to the radiated test group (figures not shown). These findings were not observed in the 2 and 5 Gy groups. More extensive immunohistochemical tests are on-going and will be reported elsewhere.

Qualitative observations - Undecalcified cut and ground sections

In general, greater bone quality "disturbances" were found in the 20 and 30 Gy irradiated groups compared to the corresponding non-irradiated control bone and compared to the lower groups which received 2, 5 and 10 Gy.
In the following qualitative description, we have focused on various regions in the bone tissue surrounding the implants as well as the marrow cavity. In particular, the focus has been on:
1) the periosteal region, 2) the old cortical bone, 3) the endosteal region, 4) osteoid rims,
5) the marrow cavity and 6) the apical portion below the implant in the marrow cavity.
Table 1 summarizes qualitative observations on histologically stained undecalcified cut and ground sections. Figures 1 to 5 demonstrate some of the qualitative observations.

The 2 Gy

Irrespective of region, no major qualitative differences could be observed between irradiated test and non irradiated control samples. There was a periosteal bone up-growth on both test and control samples. The cortex revealed both woven and lamellar bone. Most of the cavities in this region revealed an ongoing remodelling. The endosteal bone tissue had formed around the entire implant. Irrespective of test or controls, the osteoid rims were thick and blue stained with rather round shaped dark

REGIONS	Test Radiated 2 Gy	Control 2 Gy	Test Radiated 5 Gy	Control 5 Gy	Test Radiated 10 Gy
Periosteal	Periosteal new bone tissue formation	More pronounced than test side	Extra cortical layer with great porosity in-between	Greater porosity and more remodelling cavities	Resorptive upper surface
Old cortex	Woven & lamellar bone. Interface new and old BIC. Clear cement lines Ongoing remodelling cavities	Similar to test side	Few remodelling cavities. Clear cement lines. Various sizes of osteocytes with- and without nuclei	More remodelling cavities. Similar cement lines and Osteocytes	Remodelling cavities but less than 5 Gy. Osteoblasts w. both empty- and filled lacunae
Endosteal	Endosteal new bone formation with trabeculae around the implant	Similar to test side	Similar with 2 Gy	Similar with 2 Gy Ctr	More endosteal trabeculaes
Osteoid rims	Thick, blue stained rim with round shaped and dark stained osteoblasts (clear nuclei)	Similar to test side	Fuzzy layered rim. Irregular shaped, light stained cells	Thick, blue stained rim. Osteoblasts light-stained	Thinner rim and fewer cells compared to 2- and 5 Gy tests. Elongated and both light and dark stained cells Similar to test side
Marrow cavity	Cell rich. Round fat cells, Several mast cells. Megacaryocytes observed	Similar to test side	Fat cells of various sizes. Few mast cells compared to control and lesser than 2Gy. Megac - difficult to detect	Homogenous fat cell sizes. Mast cells similar Megacaryocytes observed	Some mast cells observed. Fat cells various sizes. Positively stained megacaryocytes
Apical portion below implant	No fibrous tissue	No fibrous tissue	No fibrous tissue	No fibrous tissue	No fibrous tissue

stained osteoblasts observed. The marrow cavity revealed round fat cells, a great amount of mast cells as well as quite large round-shaped greyish cells with several nuclei, i.e. megakaryocytes, on both test and control samples.

The 5 Gy

Both test and controls revealed a great periosteal bone formation with an "extra cortical layer" formed on top of the old bone with a layer in-between, having great porosity. In this region, the control samples showed a greater porosity with more remodelling cavities compared to the test samples. The old cortical region seemed to have more remodelling cavities in the control samples compared to the test. Cement-lines were clearly observed in the cortical region as well as osteocytes with clear nuclei. Regarding the osteoid rim, the control sections from the 5 Gy sides were

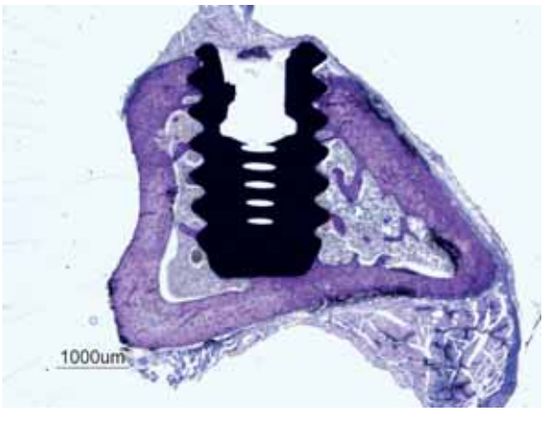

Fig. 1 *An undecalcified cut and ground section of a survey picture demonstrating an implant inserted in the proximal region of tuberositas tibia in rat bone (control non irradiate 10 Gy group). All sections were stained with Toluidine blue mixed in pyronin G. Bar = 1000 µm.*

REGIONS	Control 10 Gy	Test Radiated 20 Gy	Control 20 Gy	Test Radiated 30 Gy	Control 30 Gy
Periosteal	Greater periosteal new bone formation than test side	No/sparse amount periosteal new bone formation. Dystrophic calcification above periosteal surface	Some periosteal new bone formation	No periosteal reaction	Some periosteal new bone formation
Old cortex	Remodelling cavities	Few resorption cavities and no remodelling. No active bone formation sites. Fibrous tissue in the interface. Osteocytes w. empty lacunae. Old bone-flakes internalized in old cortex	Resorption-and remodelling cavities present. Osteocytes with-and without nuclei.	Thin cortical layer. No remod. cavities. Resorption cav. with micro-/monocytes. Fibrosis in the interface. More pronounced, "unsufficient mineralized bone" than control side. Blurry osteocytes with empty lacunae	Remodelling cavities observed. Cement lines clearly visible. Some "unsufficient mineralized bone". Osteocytes with and without nuclei
Endosteal	Similar reaction test & ctr	Thin rim-like coverage of implant	Thin rim-like coverage of implant. More trabeculae on ctr side	"Quiet" comp. to control side	New bone formation noted
Osteoid rims	More bone forming comp.to test side.	Difficult to detect "normal" rims. Thin bluish line with "smeared out" osteoblast like cells	Light stained rim and cells	Difficult to detect	Osteoblasts of various shapes and both light and dark stained
Marrow cavity	More mast cells compared to test side. Fat cells of same sizes. More pos. stained megacaryocytes than in test side?	Less fat cells than control sections. No pos. stained megacaryocytes	More fat cells comparedto radiated side. Pos. stained megacaryocytes	Difficult to detect megacaryocytes	Fat cells, mast cells & positively stained megacaryocytes observed.
Apical portion below implant	Similar as test side	Some fibrosis tissue formation	No fibrous tissue	Fibrosis on the radiated side only	No fibrous tissue, bone ar. apical porrt.

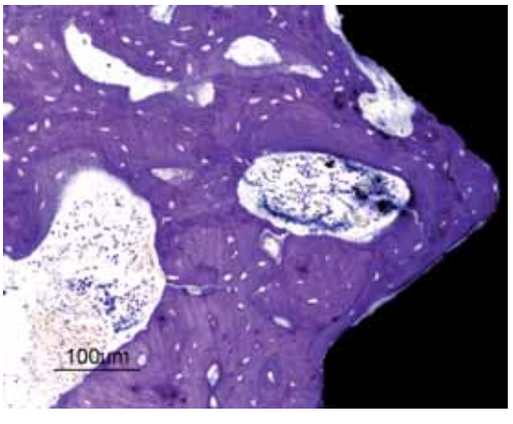

Fig. 2 *Higher magnification of bone tissue in a thread from a non-irradiated 20 Gy control group showing ongoing active bone remodelling cavities. Osteocytes, with and without nuclei, can be observed as well as cement lines demarcating old and new formed bone tissue. BIC and the bone area in the thread is greater compared to the test irradiated side shown in figure 3. Bar = 1000 µm.*

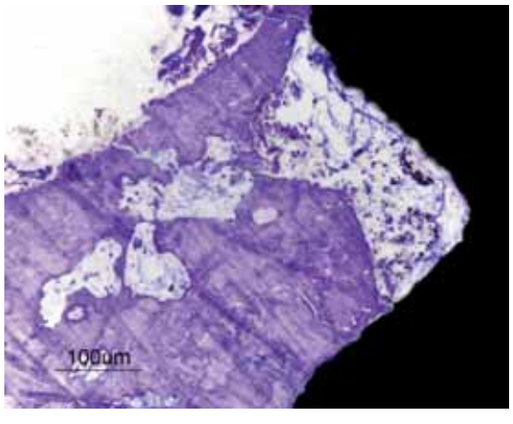

Fig. 3 *This figure illustrates a thread region sample from the irradiated 20 Gy group revealing less bony contact and bone area compared to the non irradiated control sample (Figure 2). No active bone remodelling cavities can be observed and no clear osteocytes. Cement-lines are difficult to detect. A great area of fibrous tissue can be seen in the thread region. Bar = 100 µm.*

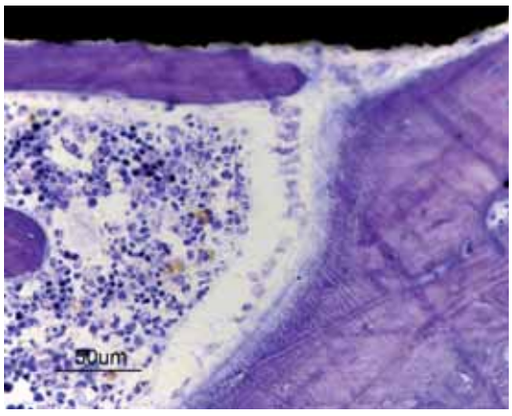

Fig. 4 *This figure illustrates the apical portion of an implant surrounded by new- formed bone facing the marrow cavity from the control non-irradiated 30 Gy group. Some bone to implant contact can be observed as well as bone forming regions. The ongoing bone formation is demonstrated by an osteoid seam covered with osteoblasts. Bar = 50 µm.*

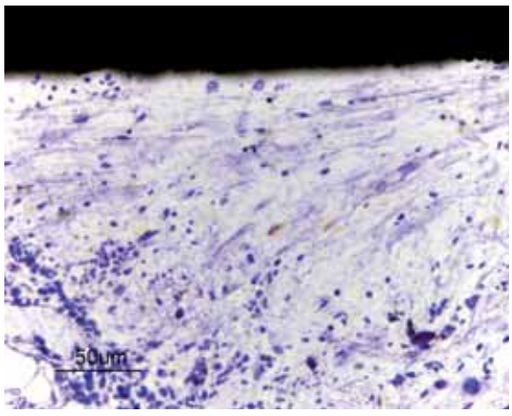

Fig. 5 *This figure illustrates the apical portion of an implant facing the marrow cavity from the irradiated 30Gy group. No bone tissue was formed around the implant in this area.*
Fibrous tissue was always observed in this region.
Bar = 50 µm.

rather similar to the control 2 Gy except that "all" osteoblasts on the former were lightly stained. In the test samples, the osteoid rims were more of a "fuzzy layer" (and not appearing like the 2 Gy test samples). The fat cells in the marrow had various sizes both in test and control samples. It seemed as the amount of mast cells were similar in on both sides whereas the megakaryocytes were difficult to detect on the test side. Some megakaryocytes could be observed on the sections from the control side.

The 10 Gy

The control samples revealed a greater periosteal new bone formation compared to the test samples, while the endosteal reaction seemed to be similar. The cortex contained several small remodelling cavities. The osteocytes in the cortex demonstrated both empty and filled lacunae irrespective of test or controls. Osteoid rims: the osteogenesis seemed to be more pronounced on the control compared to the test side. In the latter samples the rim was thinner compared to the 2 and 5 Gy irradiated samples and the cells were more elongated and darker stained. The fat cells in the marrow cavity seemed to be smaller in the control sections compared to various sizes of fat cells in the test group. The number of mast cells seemed to vary, being more frequently observed in the controls compared to the tests. This was also observed with the megakaryocytes – seemingly more in the control marrow cavity compared to the radiated samples.

The 20 Gy

While some periosteal new-formed bone could be observed on the control samples this was not the case for the irradiated ones. The latter samples had only sparse amount of periosteal bone tissue. Some dystrophic calcification regions were clearly observed above the cortex and at some distance away from the implant. The endosteal bone tissue formation close to the implant located in the marrow cavity revealed a thin rim-like coverage of the implants. There were also more trabeculae on the control side compared to the test. The old cortical region, on the control side, demonstrated both resorption and remodelling cavities, while the general finding on the test side was fewer cavities and only resorption cavities. Thus, no active bone formation sites could be observed on the irradiated samples. Fibrous tissue was generally observed in the interface region of the test samples. Osteocytes, both with and without nuclei, could be seen on the control sides. The test sides revealed more empty cells and a greater variation in cell size compared to the osteocytes on the control sides. The osteoid rims were difficult to detect on the test sides albeit sometimes a thin bluish line could be seen with "smeared out" osteoblast like cells. The control sides demonstrated lighter stained osteoid rims and osteoblasts compared to the 2, 5 and 10 Gy group. In the marrow cavity, the amount of fat cells seemed to be reduced on the test sides compared to the controls.

The 30 Gy

The non-irradiated control samples showed some periosteal new bone formation compared to test sides. The latter cortical layer was thin and some areas revealed resorbed bone. New bone formation in the endosteal part could be noted on the non-radiated sides and some in one of the test samples. The old cortical bone in the control samples showed remodelling cavities, which was not the case in the

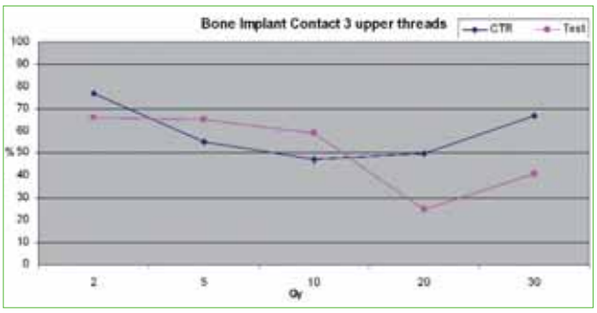

Fig. 6 *Mean values of BIC (Bone Implant Contact) in the 3 upper cortical threads. Blue = control non-irradiated samples and pink line = irradiated samples.*

test sides. The test sides revealed resorption cavities with micro-/monocytes. No giant cells were observed. The latter samples demonstrated fibrosis in the interface. Cement lines were clearly visible in the non-radiated old cortex however, no distinct cement lines were observed in the radiated bone. There were areas with "insufficient mineralized bone" visible in the control cortex albeit these regions were more pronounced in the radiated bone. While the osteocytes, with visible nuclei, were clearly observed on the control side this was not the case on the radiated side. In the latter side the osteocytes were more blurry and demonstrated empty lacunas. Osteoid rims with cells of various shapes, could be observed in the control samples. However, on the radiated test sides such rims were difficult to detect. The marrow cavity on the control side demonstrated fat cells, mast cells and megakaryocytes while on the test side these cells were not as easily detected and megakaryocytes could not be observed at all in the radiated samples. The apical region demonstrated fibrosis and reduced cell content on the test side but not on the control.

Quantitative data

In general the implants placed in the proximal region demonstrated greater BIC values compared to the distal sections. However, in the quantitative results mean values from proximal and distal samples are reported. Except for the 2 Gy rat with data from 2 implants in the irradiated leg and 2 in the control all other results were based on 4 control and 4 test samples/group.

BIC

In general, the non-irradiated implants showed greater BIC values compared to the irradiated samples and the lower the irradiation dosages were the higher BIC was found. In the 20 and 30 Gy groups the BIC was 20% higher for the non-irradiated samples compared to the irradiated ones. The mean values were 56% and 36% respectively. The other control samples (2, 5 and 10 Gy) revealed about 10% greater BIC compared to the test irradiated samples.

The mean values of BIC from proximal and distal implants in all threads were lower in all irradiated groups compared to the control sections.

The lowest BIC, when comparing all threads, was observed in the 20 Gy (mean 33%) rats followed by the 30 Gy group (mean 38%). The mean values in the corresponding control sections of 20 and 30 Gy groups were about 20% higher (i.e. mean 56%).

Comparisons of BIC in the 3 consecutive upper threads revealed a bit higher BIC in the 5 and 10 Gy irradiated samples (mean 62%) compared to their controls (mean 50%). The non-irradiated 2, 20 and 30 Gy demonstrated a greater BIC (mean 77%, 50% and 67%, respectively) than the corresponding irradiated sample (mean 66%, 25% and 41% respectively). A summary of the quantitative BIC data from the 3 upper threads is shown in figure 6.

BA

There were no major quantitative differences for BA in all threads when comparing the irradiated 2, 5 and 10 Gy samples, i.e. proximal and distal located implants revealed similar results within the test groups. The 20 and 30 Gy irradiated samples had greater BA in ≤ of the samples. This observation was reversed in the control group, where almost all samples located in the distal site had a greater BA compared to the proximal samples. The 3 consecutive upper threads revealed similar results as to BIC data, i.e. the 5 and 10 Gy irradiated groups showed a somewhat higher percentages (mean 49%) of BA compared to the corresponding non-irradiated controls (mean 44%). The 2 Gy control samples had greater BA than the irradiated ones (mean 73% and 69% respectively). The lowest BA was observed in the 20 Gy group. The corresponding mean in the control sections was 61%. The BA data from the 3 upper threads is shown in figure 7.

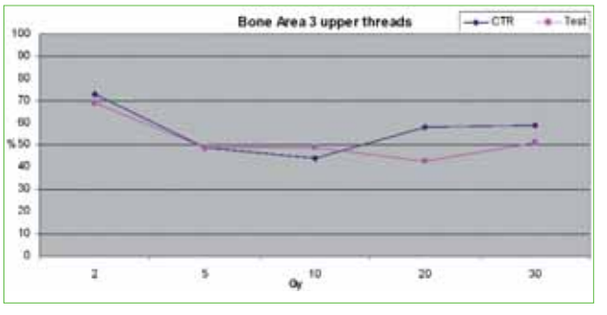

Fig. 7 *Mean values of BA (Bone Area) in the 3 upper cortical threads. Blue = control non-irradiated samples and pink line = irradiated samples.*

DISCUSSION

In this study single doses of radiation were used. The usage of single doses may be criticized but in general this method is in parallel with the clinical situation when applying brachy therapy, High Dose Rate (HDR). Moreover, single dosages are preferred to be used for animal ethical considerations.

Single doses were also applied in the rat study performed by Öhrnell et al. (9). However, that study focused on late effects of implant integration as measured by biomechanical tests compared to the present study focusing on quantitative and qualitative histomorphometrical analysis. Irrespective of study design, the common finding between that study and the present is the qualitative observation of impaired bone remodeling in irradiated bone compared to control non-irradiated bone.

Although a small number of animals were used in the present study, doses between 20-30 Gy reflected impaired integration of implants. Most samples in the 20 and 30 Gy radiated groups revealed disturbed and insufficient mineralization of the cortical bone. Some depressed bone formation could also be observed in the thread regions. The observation of changes in the osteoid and osteoblast rims with disturbed osteoblasts supports that conclusion. Resorption lacunas were observed in the upper cortical layer on the test sides. These lacunas contained a great amount of monocytes whereas the multinucleated cells seemed to be absent. Moreover, the 20 and 30 Gy irradiated samples revealed fibrosis in the apical part (i.e. in the marrow cavity) as well as reduced amount of bone marrow cells. Such findings could not be observed in the control samples.

The mean values of BIC and BA in the 3 upper threads were lower in all irradiated groups except for the 5 and 10 Gy in our study. Our interpretation of these observations is that these doses seemed to "stimulate" the integration and thus revealed some greater percentages. To the best of our knowledge these types of data have not been reported in other *in vivo* implant integration studies performed in rats. However, there are *in vitro* studies on osteoblast like cell lines being exposed to irradiation. Such studies report on elevated alkaline phosphatase (ALP) levels indicating an increase in osteoblast activity after dosages of 8 and 10 Gy (16, 17). Their observations are interesting, but one cannot compare *in vivo* with *in vitro* data. Further full scale *in vivo* studies are needed to explore the significance of our *in vivo* data.

The data from the present study is not in accordance with the study by Kwak et al., who reported on approximately 50% greater BIC values for non-irradiated controls compared to 15 Gy radiated, after 12 weeks of follow up. However, the follow up time in the present study was 5 weeks only compared to the 3, 4, 6, 8 and 12 weeks in the study performed by Kwak et al. and thus may not be fully comparable (8). The BIC data, when comparing all threads, were similar for the control and test in the 10 Gy group. The 20 and 30 Gy radiated bone demonstrated less BIC compared to the control non-irradiated side. The control had about 50% greater BIC compared to the test irradiated side that underwent 20 and 30 Gy irradiation.

The observation of greater BIC values around the implants placed in the proximal site is most likely due to the difference in anatomy, i.e. more spongeous type bone in the proximal region compared to the distal that consists of cortical bone. The non-irradiated bones demonstrated roughly a mean BIC value of 62%, which is in accordance with a previous study with similar implants performed in "healthy bone" (Johansson unpublished data).

While the inspection of the bone tissue quality seemed quite similar and thus did not differ much between control non-irradiated and irradiated bone in the 2 to 10 Gy groups, this was not the case in the 20 and 30 Gy groups. Instead, in the latter groups, the control non-irradiated samples differed "significantly" compared to the test ones. Therefore, the quantitative measurements must be accompanied with qualitative terms. It is difficult to judge and understand quantitative data alone in studies such as the present one and others dealing with irradiated bone tissue. The need to address additional qualitative and quantitative specific/specified tissue structures is of great importance. Also there is a need for more specific staining protocols. One example of this is the unsuspected finding when using the immunohistochemical staining with von Willebrand factor performed on decalcified bone, which revealed positive stained megakaryocytes on the control non-irradiated compared to no staining at all on the test 20 and 30 Gy sections. The megakaryocytes have a profound role for platelet formation; however, it was out of the scope in the present report to elaborate on these findings.

The importance of judging and describing various grades of tissue damage is complicated but necessary. Further studies related to the clinical problem associated with irradiation damage occurring around implants placed in bone shortly after irradiation is ongoing. These types of studies challenges at least five of the six important factors related to successful implant integration (18).

CONCLUSION

This paper aimed to find a critical level for osseointegration of implants in irradiated (2, 5, 10, 20

and 30 Gy) rat bone after five weeks of follow up. Impaired osseointegration was found both qualitatively and quantitatively in the 20 and 30 Gy groups. Further full-scale studies are needed in order to prove whether this is significant or not.

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