Effects of Local Irradiation on Osseointegration of Implants During Healing Stage in Rats

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(Directed by Prof. Moon Kyu Chung, D.D.S., M.S.D., Ph.D.)

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감사의 글

많은 분들의 격려와 응원 속에 작은 결실을 맺게 되었습니다. 먼저 본 논문이 완성되기까지 오랜 기간 격려해주시고 지도해주신 정문규 지도교수님께 진심으로 감사 드립니다. 또한 멀리서 또 가까이에서 세심한 지도와 가르침을 주신 금기창 교수님, 심준성 교수님, 정한성 교수님, 김성태 교수님께 감사 드립니다. 힘든 실험과정 동안 묵묵히 함께 해주고 시편제작과 계측에 큰 도움을 주신 박경미 연구원, 오성희 연구원, 주말을 할애하여 방사선 조사를 도와주신 방사선 종양학과 김주원 교수님, 김주호 기사님, 항상 가족같이 격려해주시고 물심양면 도와주신 통합진료과 교수님들께도 감사의 마음을 전합니다. 제가 치과의사로서의 역할을 잘 감당하고 지금까지 올 수 있게 가르쳐 주신 한동후 교수님, 이근우 교수님, 문홍석 교수님, 이재훈 교수님, 박용범 교수님, 김지환 교수님께도 감사의 말씀을 드립니다. 또 지면을 통해 일일이 언급하지는 못하지만 저에게 도움과 격려를 해주신 모든 분들께 다시 한번 진심으로 감사 드립니다.

저보다도 더 애써주시고 사랑으로 지켜봐 주셨던 아버지, 어머니, 시부모님, 이모할머니 감사합니다. 마지막으로 언제나 제가 의지할 수 있는 기둥이 되어주는 믿음직한 남편과, 바쁜 엄마에게 투정 한번 부리지 않고 잘 커준 첫째 아들 정우, 그리고 무탈하게 자라서 한 달 후면 만날 둘째 아들에게 사랑과 감사를 전하며 이 논문을 나누고자 합니다.

2012 년 12 월

도레미 드림

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Abstract

Effects of Local Irradiation on Osseointegration of Implants During Healing Stage in Rats

Re Mee Doh D.D.S., M.S.D.

(Directed by Prof. Moon Kyu Chung, D.D.S., M.S.D., Ph.D.)

Purpose: Reconstruction using oral implants has been a valuable treatment option for head and neck cancer patients after ablative surgery and additional radiotherapy. Complications of radiotherapy have been investigated for a long time, but there are few reports about the effect of post-implant radiotherapy on bone healing or osseointegration around the implant. The purpose of this study is to evaluate the effect of local irradiation on osseointegration of implants during the healing stage in the maxilla of rats.

Materials & Methods: Forty-eight Sprague-Dawley rats (body weight 130-140 g, 4 weeks old, male) were first divided into the radiation group and control group. The radiation group was again divided into 1-day and 4-week groups, which represents the interval between implant surgery and radiation. The maxillary first molars in each rat were extracted. At 4 weeks after extraction, custom-made implants were inserted bilaterally. Group 1, 2 and 3 received local radiation according to the experimental

schedule with a single dose of 15.0 Gy to the maxilla. The rats were allowed to survive for 2, 4, and 8 weeks after implant placement. To observe of bone formation pattern, fluorescence expression agents (Oxy TC, Calcein, Alizarin red) were injected intraperitoneally on the day of implantation, at 2 weeks later, and 1 day before sacrifice, respectively. The specimens were prepared for histological and micro-computed tomographic analyses.

Results: 1. Group 1 and 2, which were irradiated during the early healing stage, showed significantly lower bone mineral density (BMD) than non-irradiated groups. Group 3, which was irradiated during the late healing stage, showed similar BMD to the control group. 2. Irradiated groups showed a lower bone-to-implant contact (BIC) ratio but without significance. 3. Irradiated groups (group 2 and 3) showed significantly lower bone volume (BV/TV) and higher empty lacuna count (EL/BV) than control groups (group 5 and 6).

Conclusion: Within the limitations of this study, local irradiation resulted in retarded bone healing and reduced bone mineral density during the early healing stage. Local irradiation had relatively minor influence, but also caused retarded bone healing during the late healing stage. In summary, the timing of local irradiation has a critical influence on the bone healing mechanism, which is related to osseointegration around implants.

Key words: Postoperative radiotherapy, Head and neck cancer, Local irradiation, Implant, BMD, BIC, Empty lacuna, Bone healing, Bone volume

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I. Introduction

Patients suffering from advanced oral and maxillofacial tumors are usually treated by wide surgical resections, hard and soft tissue reconstruction and postoperative radiotherapy. Radiotherapy is largely used for treatment of head and neck cancer, as primary therapy, adjuvant to surgery, in conjunction with concurrent chemotherapy or as palliative treatment for late stage and unresectable head and neck malignancies[1]. After surgical treatment, oral functions such as mastication, swallowing, speech and oral comfort are often impaired[2]. Thereafter, the dental rehabilitation should be performed to

restore both the oral function and esthetics of the operated face[3], but this is often complicated by the patient's anatomical changes due to tumor resection and reconstruction. A decreased area of attached mucosa and limited movement of the tongue are the most frequently encountered problems[4]. Although radiotherapy can increase cure rates, postoperative radiotherapy causes reduction in salivary flow rates, resulting in xerostomia and rendering the oral mucosa more susceptible to injury[5]. In edentulous patients, trauma induced by prosthetic appliances is considered a predisposing factor of osteoradionecrosis[6]. Therefore, although radiation impairs the vitality of skeletal tissue, oral implants seems a valuable treatment option since these can minimize the trauma induced by prosthetic appliances.

The gross changes in the bone matrix after irradiation have been numerously reported, but it is still a matter of debate whether the altered bone remodeling activity is the result of direct irradiation injury to the cells of the remodeling system or the indirect result of irradiation-induced vascular injury, or a combination of both. Radiation injury to the fine vasculature of bone and its surrounding tissues first leads to hyperemia, followed by endarteritis, thrombosis, and a progressive occlusion and obliteration of small vessels[5]. Within bone, vascular injury results in a further reduction of the number of cells and progressive fibrosis. With time, the marrow exhibits marked acellularity and hypo- or avascularity, with significant fibrosis and fatty degeneration. The remodeling system of bone (osteocytes, osteoblasts, and osteoclasts) is also damaged. Some osteocytic lacunas become empty, devoid of osteocytes. The endosteum atrophies, with significant loss of active osteoblasts and osteoclasts. The periosteum demonstrates significant fibrosis, with a similar loss of remodeling elements[7,8]. Jacobsson et al.[9] have demonstrated that

irradiation has an acutely negative effect on bone regeneration. 70.9 % of regeneration activity was decreased within a 4-week period of irradiation in rabbits. In their study, there was a significant depression of osteogenesis around implants when inserted immediately after irradiation. Significantly reduced bone regenerative capability and reduced implant osseointegration in irradiated dog and human alveolar bone have also been reported in previous studies[10,11].

Dental implants can be inserted simultaneously with ablative surgery (primary) or after completion of tumor therapy including radiotherapy (secondary)[12]. The advantage of secondary placement is that the anatomical situation, residual function can be evaluated and prognosis of the tumor can be taken into account in the decision of whether to use implants. However, after radiotherapy, the vascularization and regenerative ability of the irradiated tissues can be decreased, and this may have a negative effect on osseointegration of the dental implants[13]. Surgical intervention in irradiated bone is also thought to increase the risk of osteoradionecrosis when a curative dose (>50 Gy) of radiotherapy is administered[14,15].

Therefore, aiming both to avoid bone radiation damage at the time of surgery and to minimize the delay between initial treatment and oral rehabilitation, some authors have advocated the opposite strategy of implant placement before radiotherapy (primary placement)[12,16]. Because of the complications of radiation mentioned above, it seems reasonable to insert implants prior to postoperative radiotherapy, preferably simultaneously with ablative surgery. Major advantages of implant placement during ablative surgery reported in the literature include[4,17,18], 1) A second surgical intervention can be avoided, which is a major advantage considering the psychological and

physiological exhaustion caused by tumor treatment, which can discourage patients from undergoing a second surgical intervention[2]. 2) Initial implant healing can be achieved before irradiation. 3) Implant-surgery in a due to radiotherapy compromised area is avoided thus reducing the risk of late complications or osteoradionecrosis may be reduced, which eliminates the additional therapy such as hyperbaric oxygen therapy. 4) Prosthetic reconstruction and rehabilitation can start early providing the patient with a more timely improvement in oral function. Among others this support is important for the rehabilitation of speech and swallowing.

It is currently unknown whether postoperative radiotherapy has a negative effect on the survival of primary placed implants located in the radiation field. There are relatively few studies addressing outcomes of irradiating already-placed implants. Some authors recommend to place dental implants before radiotherapy, if possible, during the ablative surgical session[12,18]. Schoen et al[19] reported a relatively higher success rate in the pre-implant radiotherapy group (90.5 %) compared with the post-implant radiotherapy group (83.4 % to 85.7 %), but still recommended inserting implants immediately after ablative surgery and prior to radiotherapy. This recommendation has been supported by Brogniez et al.[20] who demonstrated, in an experiment on dogs, that osseointegration is possible either before or after radiotherapy, but with higher bone-implant contact when the implants were placed before irradiation. There are a few animal studies about post-implant radiotherapy in maxillae or mandible[3,20-24], but only one study was focused on the effect of the early stage of healing procedure[23].

The purpose of study is to evaluate the biologic effects of localized irradiation on osseointegration of implants, which are administered during the early healing stage and the late healing stage in the maxilla of rats.

This study's hypotheses are as follows;

1) Irradiation has negative or minor effect on osseointegration during the early healing stage.
2) Irradiation has minor or no effect on osseointegration during the late healing stage.

II. Materials and Methods

2.1. Experimental animals

In this study, 48 Sprague-Dawley rats (body weight 130-140 g, 4 weeks old, male) were used. They were first divided into the radiation group and control group. The radiation group was again divided into 1-day and 4-week groups, which represents the interval of implant surgery and radiation (Table I).

All rats used the study were given free access to food pellets and tap water, housed and taken care of at the animal experimental laboratory of Yonsei University, College of Dentistry, Seoul, South Korea. All experimental procedures were performed in accordance with the guidelines for animal experiments of Yonsei University, College of Dentistry.

Table I. The experimental procedure between control and radiation groups.

Interval of procedure		Implant placement and radiation	Implant placement and sacrifice
Radiation groups	Group 1 (n:8)	1 day	2 weeks
	Group 2 (n:8)	1 day	4 weeks
	Group 3 (n:8)	4 weeks	8 weeks
Control groups	Group 4 (n:8)	-	2 weeks
	Group 5 (n:8)	-	4 weeks
	Group 6 (n:8)	-	8 weeks

2.2. Surgical procedures

The overall experimental protocol is presented in Fig. 1. All necessary surgical procedures were conducted under general anesthesia through intraperitoneal injection of an anesthetic cocktail composed of Rompun® (xylazine, 20 ㎎/㎖, 0.5 ㎖/㎏ body weight; Bayer, Leverkusen, Germany) and Zoletil (tiletamine and zolazepam, 100 ㎎/㎖, 0.5 ㎖/㎏ body mass; Virbac Lab. Carros, France)[25]. Then, both maxillary first molars were extracted. The extraction sockets were allowed to heal for 4 weeks before implant placement. At 4 weeks after tooth extraction, a small full thickness flap was elevated at each recipient site for dental implantation under general anesthesia. A 1.5 mm-deep implantation osteotomy was prepared with a low-speed Ø1.0 mm round bur and subsequently a Ø1.3 mm fissure bur and Ø1.45 mm twist drill. Customized sterile implants (Ø1.5 X 2.5 mm) made of grade IV titanium were inserted bilaterally into the drilled cavities by hand driver and tapped with a mallet so that their tops were situated just at the cortical bone surface or roughly 0.5 mm below the bone (Fig. 2 and Fig. 3). Then the flaps were repositioned carefully without suturing. After surgery, the rats were housed with free access to food pellets and tap water. The rats were allowed to survive for 2, 4, and 8 weeks, respectively, after implant placement. On the day of euthanization, the rats were perfused transcardially with 4 % paraformaldehyde under general anesthesia[26]. Then their maxillae, including implants, were removed *en bloc*, and immersed in the same fixative for an additional 24 hours.

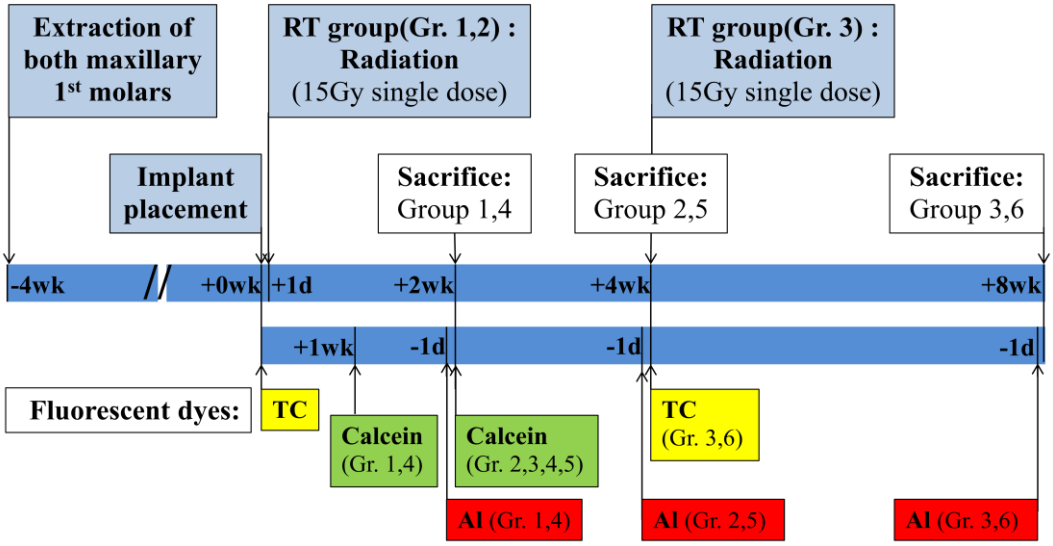


Fig. 1. Experimental design protocol.

TC : oxytetracycline HCl; yellow; Pfizer, Seoul, Korea.
Calcein : calcein green; Sigma, Tokyo, Japan.
Al : Alizarin red; Alizarin-3-mothylinimodiacetic acid; Sigma, Tokyo, Japan.

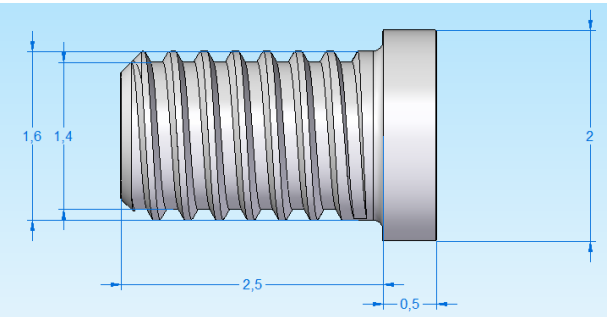


Fig. 2. Dimension of custom-made mini implant with smooth surface.

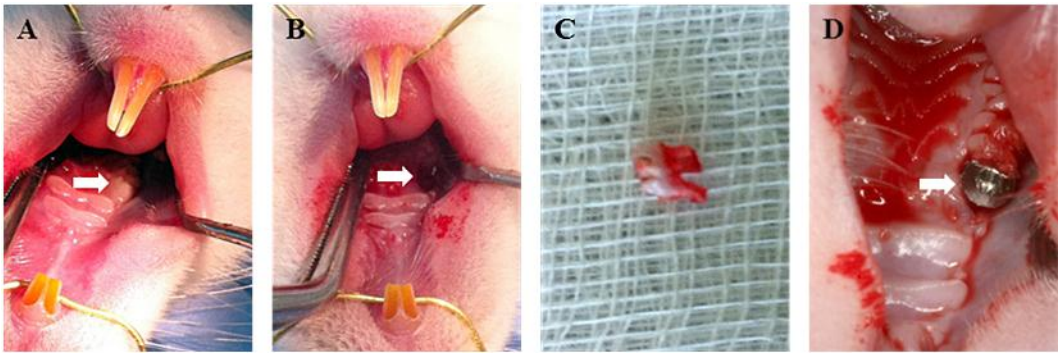


Fig. 3. Surgical procedures of extraction of maxillary first molars and implant placement.

A: Maxillary first molar of the rat (white arrow).
B: Extraction socket (white arrow).
C: Extracted maxillary first molar.
D: Implant was placed in the first molar space after 4-weeks later (white arrow).

2.3. Local irradiation

Group 1, 2, and 3 (radiation group) irradiated according to the experimental schedule under general anesthesia through intraperitoneal injection of an anesthetic cocktail composed of Rompun® and Zoletil. Rats were immobilized with a customized fixation device (Fig. 4.) and the radiation fields were verified using an external beam simulator (Nucletron, Veenendaal, the Netherlands). Rats received localized radiation with a single dose of 15.0 Gy to the maxilla using a 6.0 MV linear accelerator (Elekta, Stockholm, Sweden) commonly used for treatment in humans. This is biologically equivalent to 55 Gy delivered in 25 sessions of 1.8 Gy each administered 5 times per week within a 5-week period[27]. The treatment center was located at the midpoint between the first molars of the maxilla, and a field size of 2 x 2 cm was used to irradiate the implant site and

surrounding bone tissue (Fig. 5.). Radiation was delivered with parallel-opposed lateral fields at a dose rate of 4.19 Gy/min.

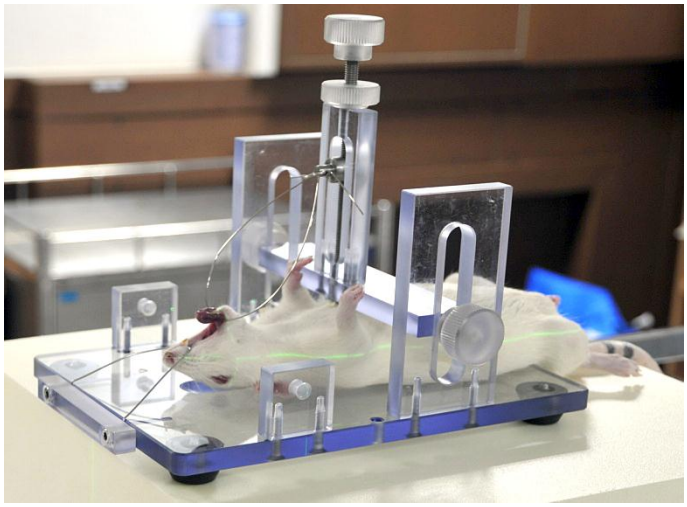


Fig. 4. Customized fixation device for local irradiation.

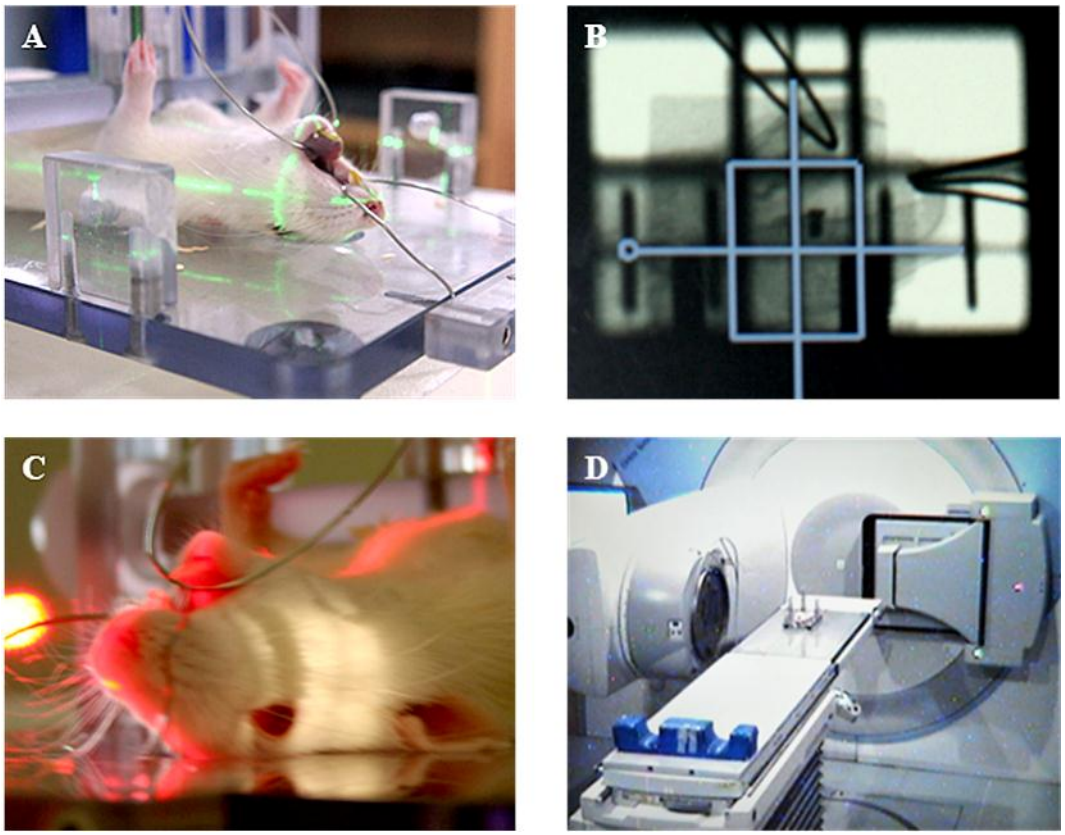


Fig. 5. Local irradiation procedures.

A & B: Verifying radiation fields using an external beam simulator (Nucletron, Veenendaal, the Netherlands).
C: Field size of 2 x 2 cm (see light window).
D: Radiation administration using a 6.0 MV linear accelerator (Elekta, Stockholm, Sweden).

2.4. Fluorescence analysis

Fluorescence expression agents were injected in a specimen for observation under a fluorescence microscope. Oxy TC (oxytetracycline HCl; yellow; Pfizer, Seoul, Korea; 20 mg/kg; intraperitoneal) was injected on the same day of implantation, Calcein green (calcein green; Sigma, Tokyo, Japan; 20 mg/kg; intraperitoneal) at 2 weeks later (1 week later of group 1, 4), oxy TC at 4 weeks later in group 3, 6, and Alizarin red S (Alizarin-3-mothylinimodiacetic acid; Sigma, Tokyo, Japan, 20 mg/kg; intraperitoneal) was injected 1 day before sacrifice. Fluorescence microscopic image (DM LB, Leica, Germany) were taken at a wave range of 543 nm ~ 617 nm (red filter) and 515 nm ~ 560 nm (green filter).

2.5. Histological analysis

After micro-CT taking, all specimens except 1 from each group were decalcified with 10 % EDTA at 4 ℃ for one month. The decalcified specimens were first embedded in paraffin wax using standard protocol, and then a series of 7 ㎛ thick sections were prepared. The specimens were stained with Hematoxylin and Eosin (H&E) stain and a Trichrome stain using Trichrome stain (Masson) kit (Signa, St. Louis. MO, USA). The stained specimens were observed with a light microscope (Leica DM 2500, Leica Microsystems, Germany).

The area of new bone formation was confirmed by Trichrome staining. Bone-to-implant contact (BIC) was measured at the distal surface of the implant, empty lacunae were counted in the ROI (region of interest) for the quantification of necrotic bone by using IMT i-solution lite ver. 8.1 program (IMT i-solution Inc., Vancouver, BC, Canada) (Fig. 6). Bone area was measured in the same ROI. According to Futami et al.[26], injured pre-existing bone during implantation is usually located within 100 ㎛ from the cavity surface. Kenzora et al.[28] suggested the possibility that the injured areas might be located 500 ㎛ beyond the bone cavity margin. In this study, the width of peri-implant ROI was set as 300 ㎛ from the surface of the implant used in Kim et al[29].

One rat from each group was fixed with 10 % neutral buffered formalin (pH 7.0) for 2 weeks. After that, it was dehydrated with ethanol and embedded in methylmethacrylate (Technovit 720VLC, HeraeusKulzer, Dormagen, Germany). The specimen was cut along the center axis of the implant by a cutting system (Exakt 300, Kulzer, Norderstedt, Germany). The central section of each specimen was cut 15 ㎛ in thickness by a microgrinding system (Exak, Apparatebau, Norderstedt, Germanay). The sectioned specimens were dyed with Hematoxylin and Eosin (H&E). Dyed specimens were observed under an optical microscope and fluorescence microscope (Leica DM 2500, Leica Microsystems, Germany).

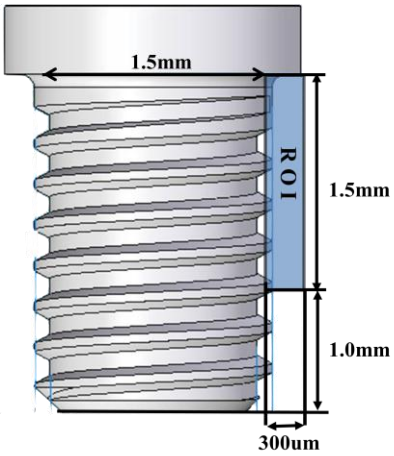


Fig. 6. The analyzed area in the proximity of the implant.

BIC (bone-to-implant contact) was measured at the distal side of the implant surface and the bone area was measured in the ROI (region of interest). The empty lacuna count was also measured in the same area as ROI.

2.6. Micro-computed tomographic analysis

Before decalcification of the specimen, a 3-dimensional micro-computed tomography (CT) image was taken for each rat using a micro-CT scanner (Polaris-G90, NanoFocus ray, Kwangju, Korea) at 50 ㎸ and 180 ㎂. This was reconstructed with OnDemend 3D (Cybermed Co., Seoul, Korea) software to obtain volumetric information and relative BMD (bone mineral density) in the proximity of implants. The density difference between bone and implant can allow separation of the bone compartment image from the image of an implant compartment. The threshold value for the separation was determined

by analyzing the gray-level distribution and picking up the intermediate gray-level value between the two peaks of the materials to be distinguished.[30]

2.7. Statistical analysis

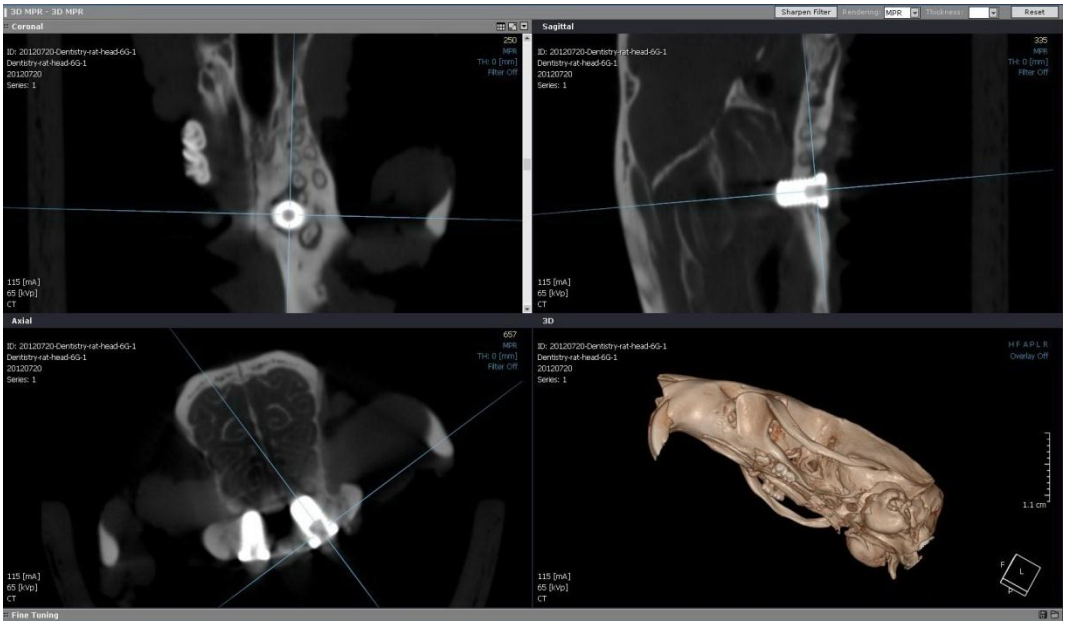
All data were expressed as mean ± standard deviation. According to test of normality, non-parametric analysis and parametric statistics analysis were implemented. If data followed normal distribution, mean values and standard deviations were calculated. The mean differences were verified with independent two-sample t-test and analysis of variance (one-way ANOVA) with a significance level of 5 % ($p<0.05$). If date did not follow normal distribution, median, maximal, minimal, 75 % percentile, and 25 % percentile values were determined for each group. Wilcoxon Rank-sum test and Kruskal Wallis test were used to evaluate the data. Bonferroni test and Dunn's test were used for post hoc multiple tests. All calculations were performed using a specific statistical program (SAS for Windows (version 9.2, Cary, NC, USA)).

III. Results

Among 48 rats, 4 rats died unintentionally during the study: one from group 1, 2, 3 and 6, respectively. A total of 18 implants were lost among the remaining 44 rats: 1 from group 1 and 4, 5 from group 2 and 3, 3 from group 5 and 6, respectively.

3.1. Micro-computed tomographic findings

3-dimensional micro-CT images were taken to obtain volumetric information and BMD (bone mineral density) data in the ROI (region of interest) depicted in Fig. 6. The implants were shown to be partially surrounded by trabecular bone. A representative image is shown in Fig. 7. There was a significant difference in BMD data between the control and experimental groups. When comparing the non-irradiated groups, BMD increased with the length of the healing period. Group 1 and 2, which were irradiated during the early healing stage, showed significantly lower BMD than the non-irradiated group (group 4 and 5). Group 3, which was irradiated during the late healing stage, showed similar BMD to the control group (group 6). The processed data are presented in Fig. 8.



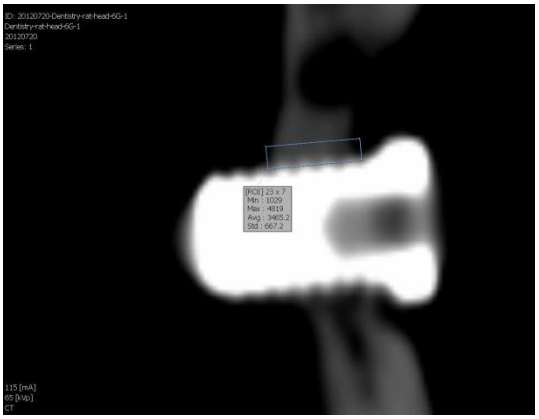


Fig. 7. Micro-CT images in implant sites.

Upper: 3D image reconstruction using Ondemend 3D (CyberMed Co., Seoul, Korea).
Lower: BMD (bone mineral density) measurement in the ROI (region of interest).

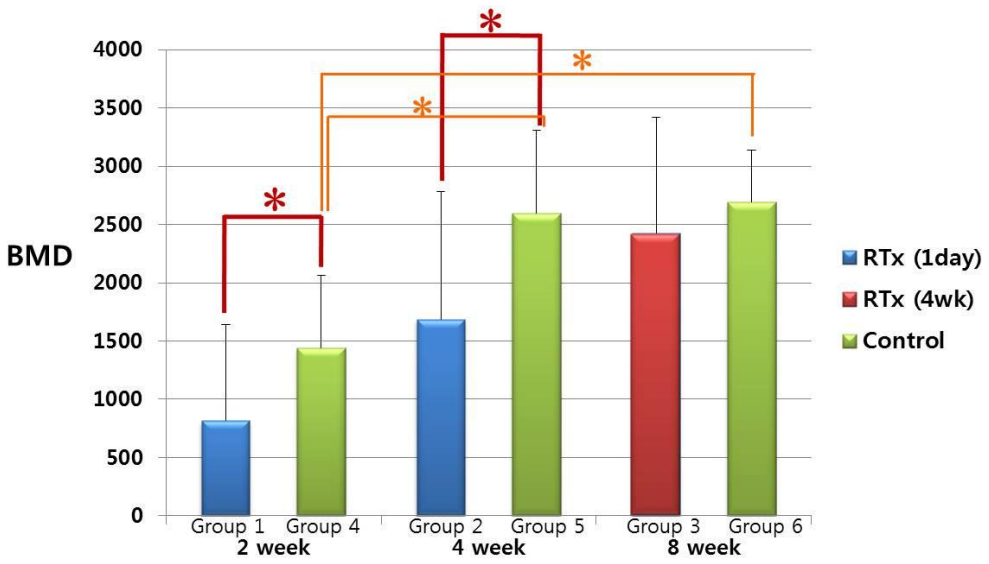


Fig. 8. Bone mineral density (BMD) in the ROI (region of interest).

* Red stars indicate that there was a significant difference between the radiation group and control group ($p<0.05$).
* Orange stars indicate that there was a significant difference within the control group ($p<0.05$).

3.2. Histological findings

To investigate the effects of radiation on peri-implant bone healing and remodeling at the histomorphometric level, H&E stained and Trichrome stained images containing the ROI (region of interest) at four magnification powers (12.5x, 50x, 100x and 200x), were obtained for each group (Fig. 11 - 14). First of all, the bone-to-implant contact (BIC) was measured over a 1.5 mm length of the distal surface of the implant. The total tissue volume, bone volume and empty lacuna count was measured within the ROI from the distal surface of the implant.

BIC gradually increased with the healing period, but without significance. Irradiated groups showed lower BIC compared to non-irradiated groups but there were no significant differences due to high data variation. Bone volume gradually increased with the healing period. When comparing the irradiated group and non-irradiated group, the irradiated groups (group 2 and 3) showed lower bone volume than non-irradiated groups (group 5 and 6).

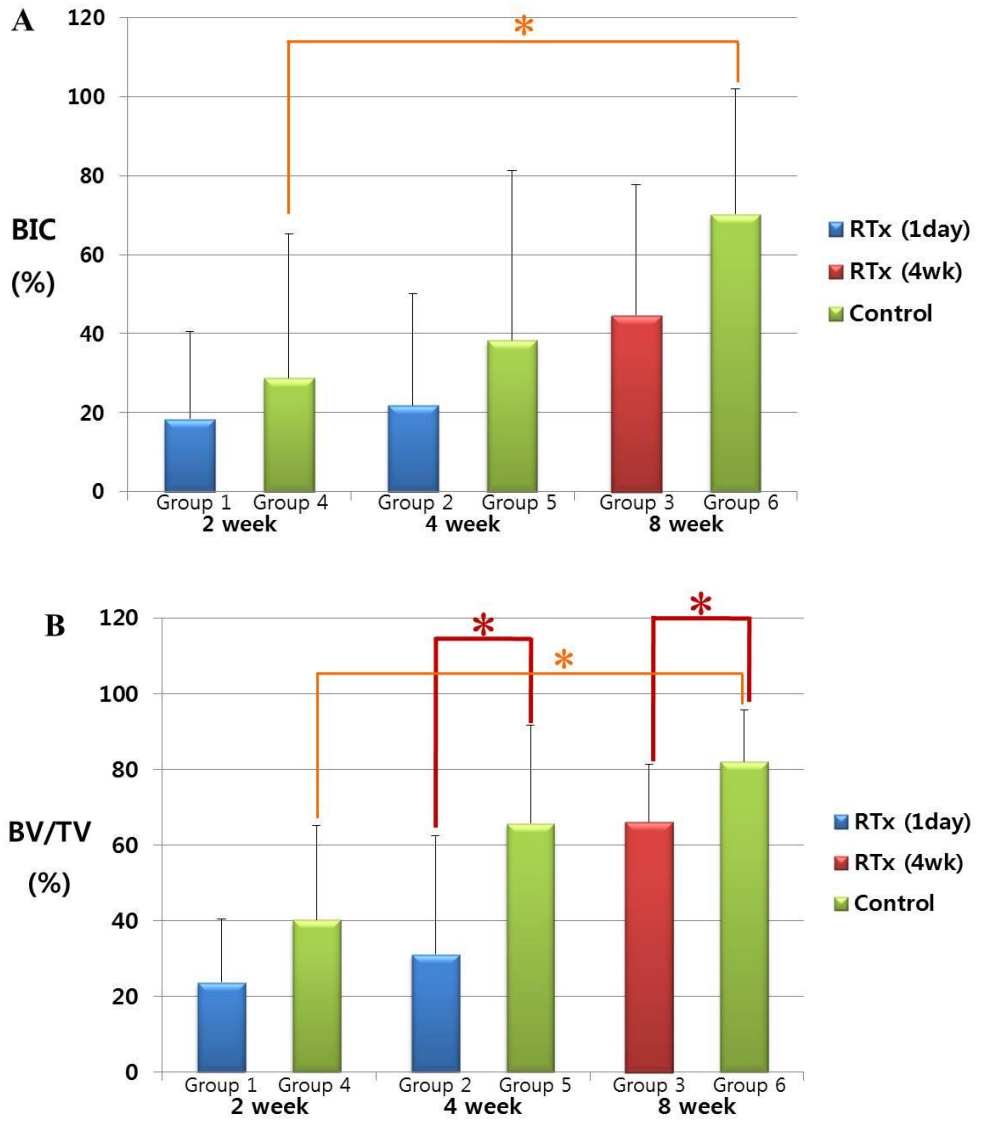


Fig. 9. Histomorphometric results in the ROI (region of interest).

A. Bone-to-implant contact (BIC) (%).
B. Bone volume/ Tissue volume (BV/TV) (%).

* Red stars indicate that there was a significant difference between the radiation group and control group ($p<0.05$).

* Orange stars indicate that there was a significant difference within the control group ($p<0.05$).

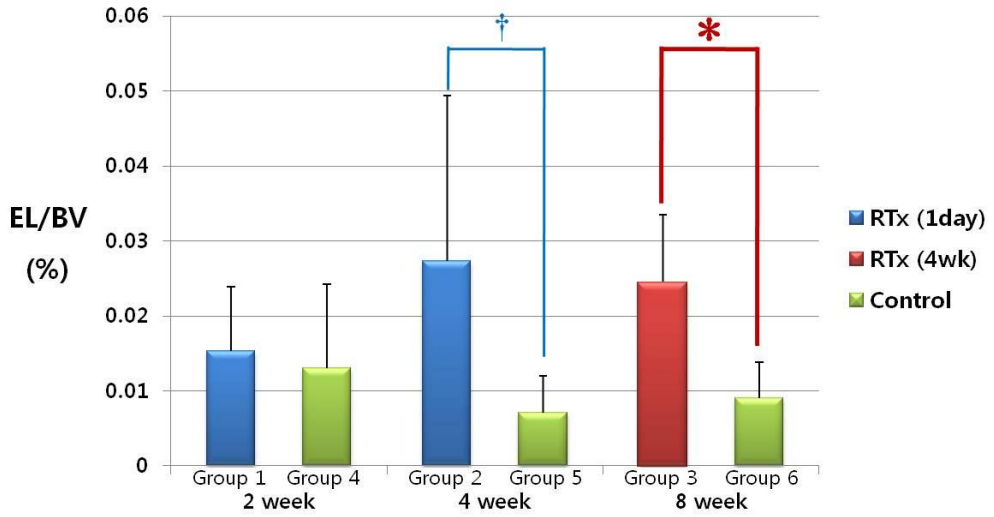


Fig. 10. The number of empty lacuna/ bone volume (%) in the ROI (region of Interest).

* Red stars indicate that there was a significant difference between the radiation group and control group ($p<0.05$).
† indicate that there was a significant difference between the radiation group and control group ($p<0.10$).

Regarding empty lacuna count, there were no difference within the control group that empty lacuna remained 8 week after implant placement. In the irradiated groups, empty lacuna count was higher in group 2 and 3 than group 1. When comparing the irradiation group and control group, there were significant differences between group 2 and 5 ($p<0.10$) and group 3 and 6 ($p<0.05$).

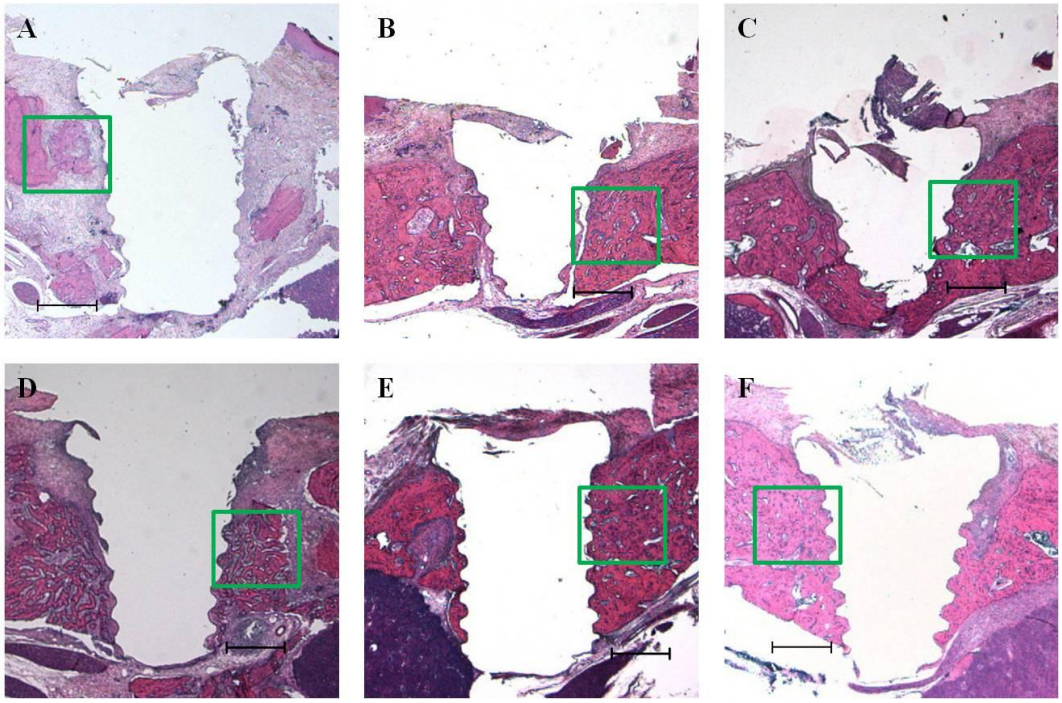


Fig. 11. H&E stained images of the implant sites at each group. Scale bar = 500 ㎛ (H&E stained image x12.5)

A: Group 1 B: Group 2 C: Group 3
D: Group 4 E: Group 5 F: Group 6

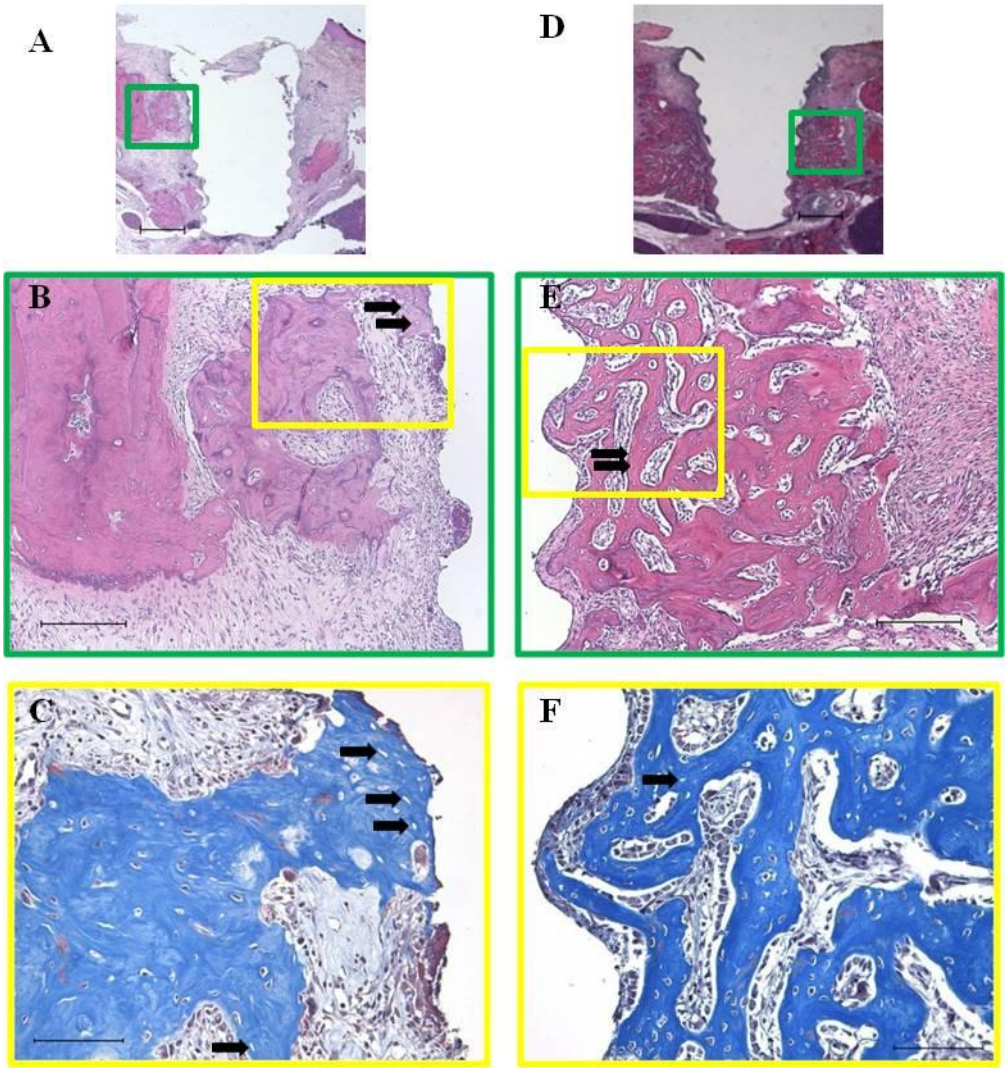


Fig. 12. Histologic images of implant sites of group 1 and 4.

A, B, C: Group 1. D, E, F: Group 4.
A & D: H&E stained images at lower magnification (X12.5). Scale bar = 500 ㎛.
B & E: H&E stained images of the green boxes in the A&D (X100). Scale bar = 200 ㎛.
C & F: Trichrome stained images of the yellow boxes in the B&E (X200). Scale bar = 250 ㎛.
* Black arrows: empty lacuna.

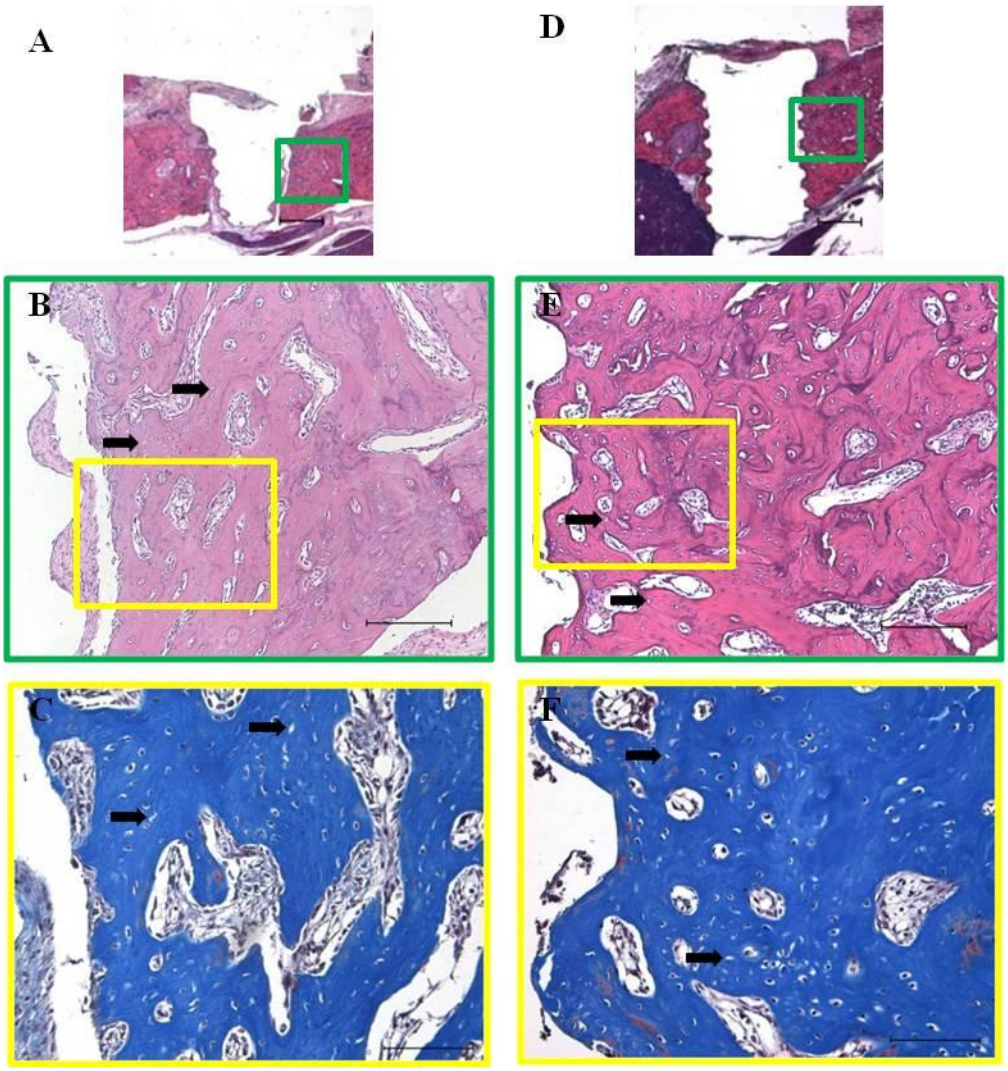


Fig. 13. Histologic images of implant sites of group 2 and 5.

A, B, C: Group 2. D, E, F: Group 5.

A & D: H&E stained images at lower magnification (X12.5). Scale bar = 500 ㎛.

B & E: H&E stained images of the green boxes in the A&D (X100). Scale bar = 200 ㎛.

C & F: Trichrome stained images of the yellow boxes in the B&E (X200). Scale bar = 250 ㎛.

* Black arrows: empty lacuna.

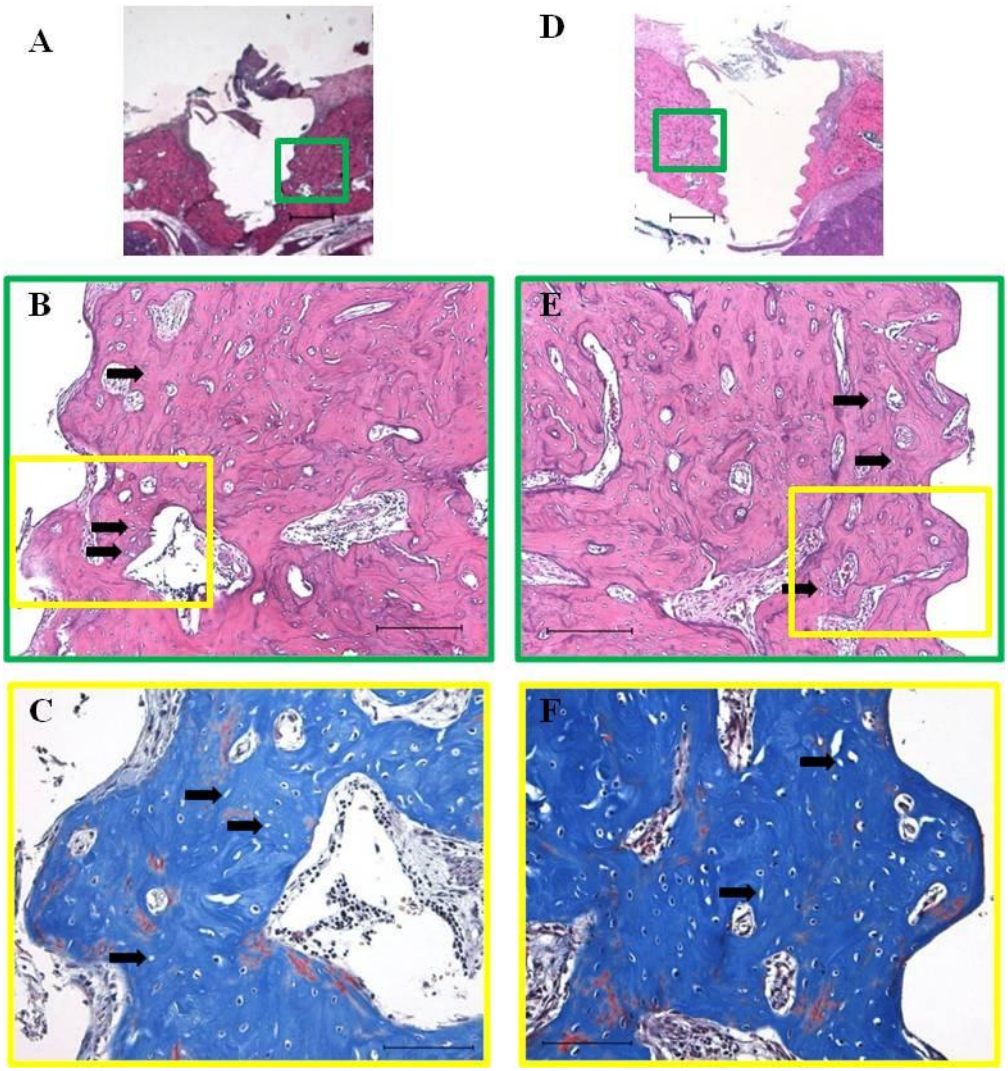


Fig. 14. Histologic images of implant sites of group 3 and 6.

A, B, C: Group 3. D, E, F: Group 6.

A & D: H&E stained images at lower magnification (X12.5). Scale bar = 500 ㎛.

B & E: H&E stained images of the green boxes in the A&D (X100). Scale bar = 200 ㎛.

C & F: Trichrome stained images of the yellow boxes in the B&E (X200). Scale bar = 250 ㎛.

* Black arrows: empty lacuna.

3.3. Fluorescence observation

Through fluorescence analysis, active bone forming area was observed. When seen through the green filter (515 nm ~ 560 nm wave length), tetracycline and calcein green emitted yellow and green, and under the red filter (450 nm ~ 490 nm wave length), alizarin red S emitted red.

Retarded new bone formation was noted in the irradiated groups compared to non-irradiated groups. In the group of 2 weeks after implant placement (group 1 and 4), there was more bright red fluorescent color than green fluorescent indication acceleration in new bone formation. In the group of 4 weeks after implant placement (group 2 and 5), red fluorescent color appeared at the interface of implant. In the group of 8 weeks after implant placement (group 3 and 6), green and red fluorescent color appeared at the border of the old bone.

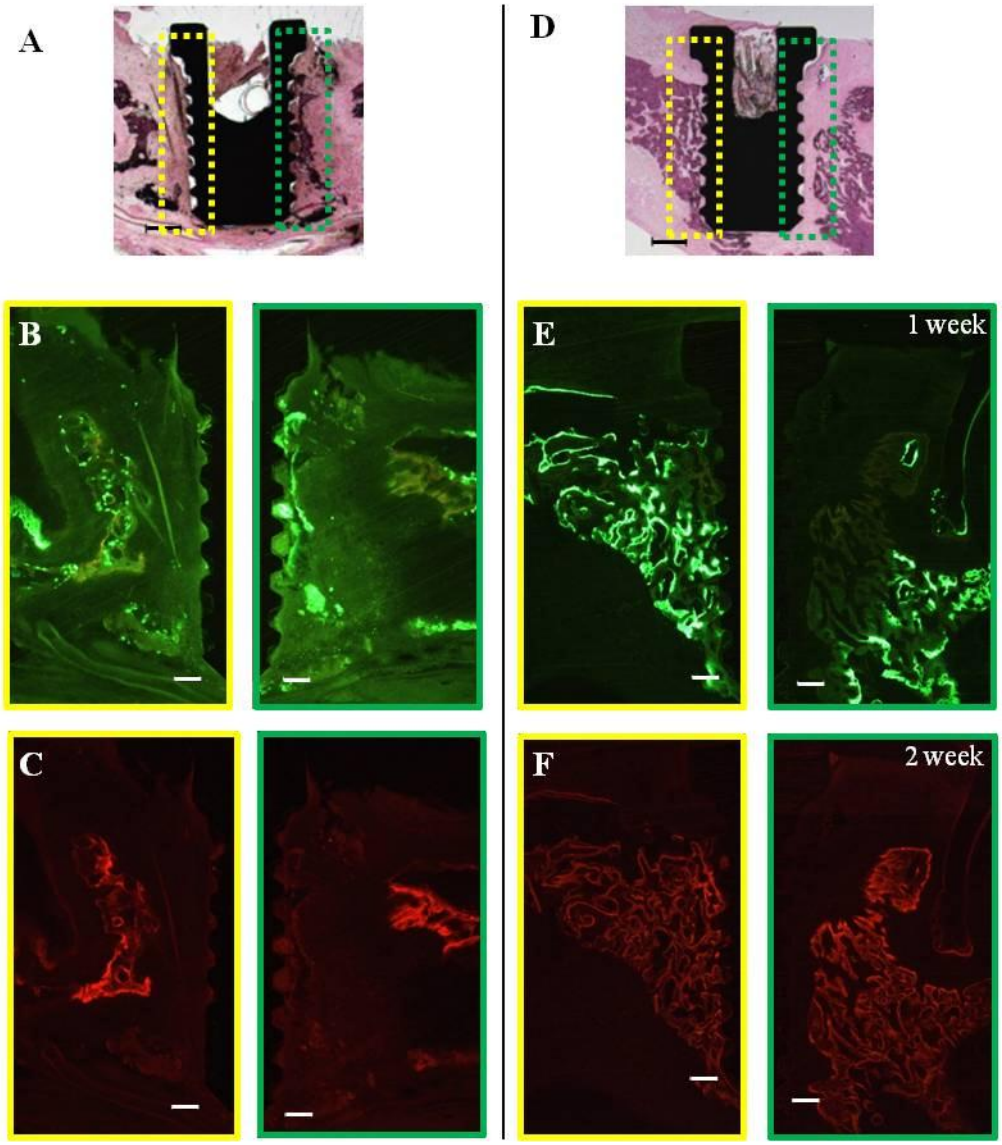


Fig. 15. Fluorescent microscopic images in the group 1 and 4.

A, B, C: Group 1. D, E, F: Group 4.
A & D: H&E stained images at lower magnification (12.5x). Scale bar = 500 ㎛.
B & E: Green emitting region (50x). Scale bar = 200 ㎛.
C & F: Red emitting region (50x). Scale bar = 200 ㎛.

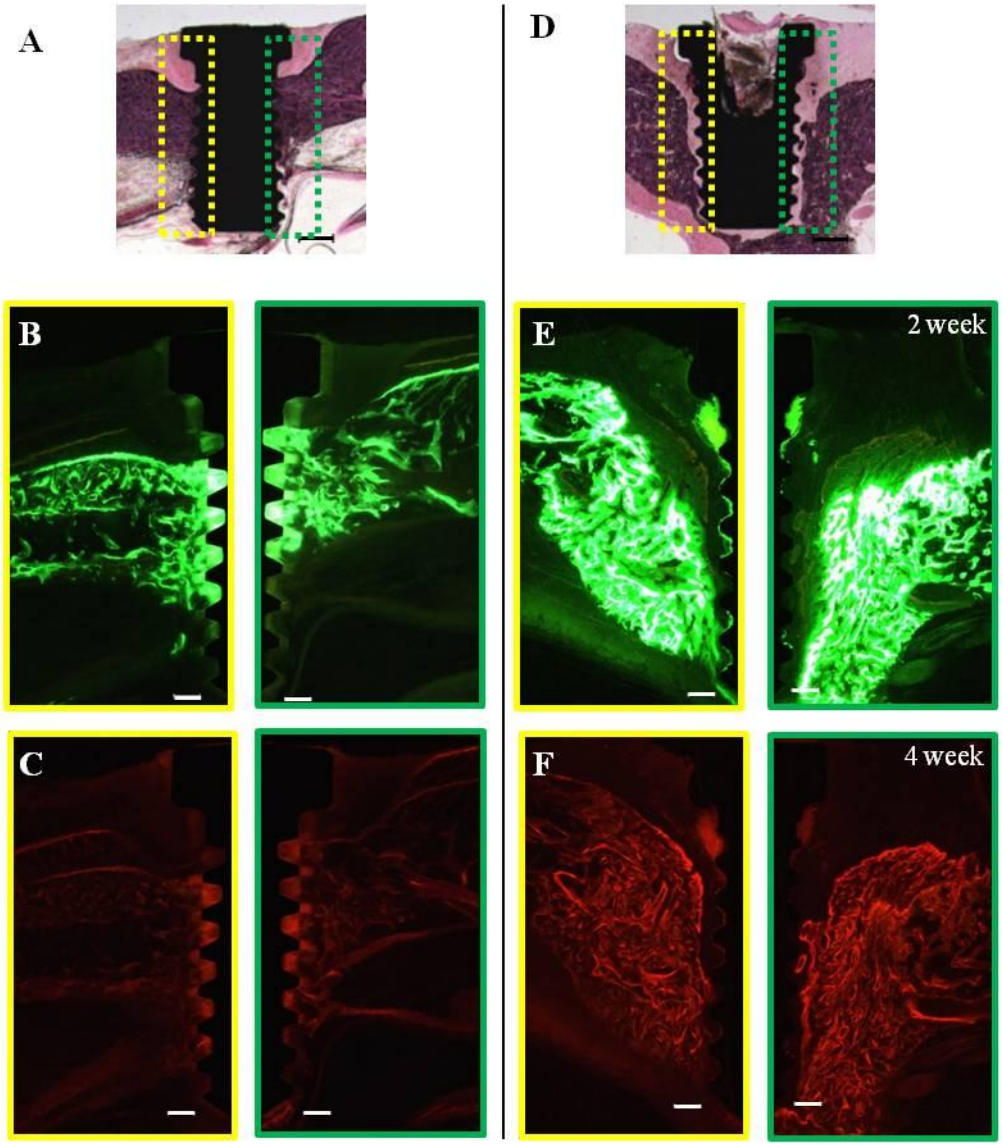


Fig. 16. Fluorescent microscopic images in the group 2 and 5.

A, B, C: Group 2. D, E, F: Group 5.
A & D: H&E stained images at lower magnification (12.5x). Scale bar = 500 ㎛.
B & E: Green emitting region (50x). Scale bar = 200 ㎛.
C & F: Red emitting region (50x). Scale bar = 200 ㎛.

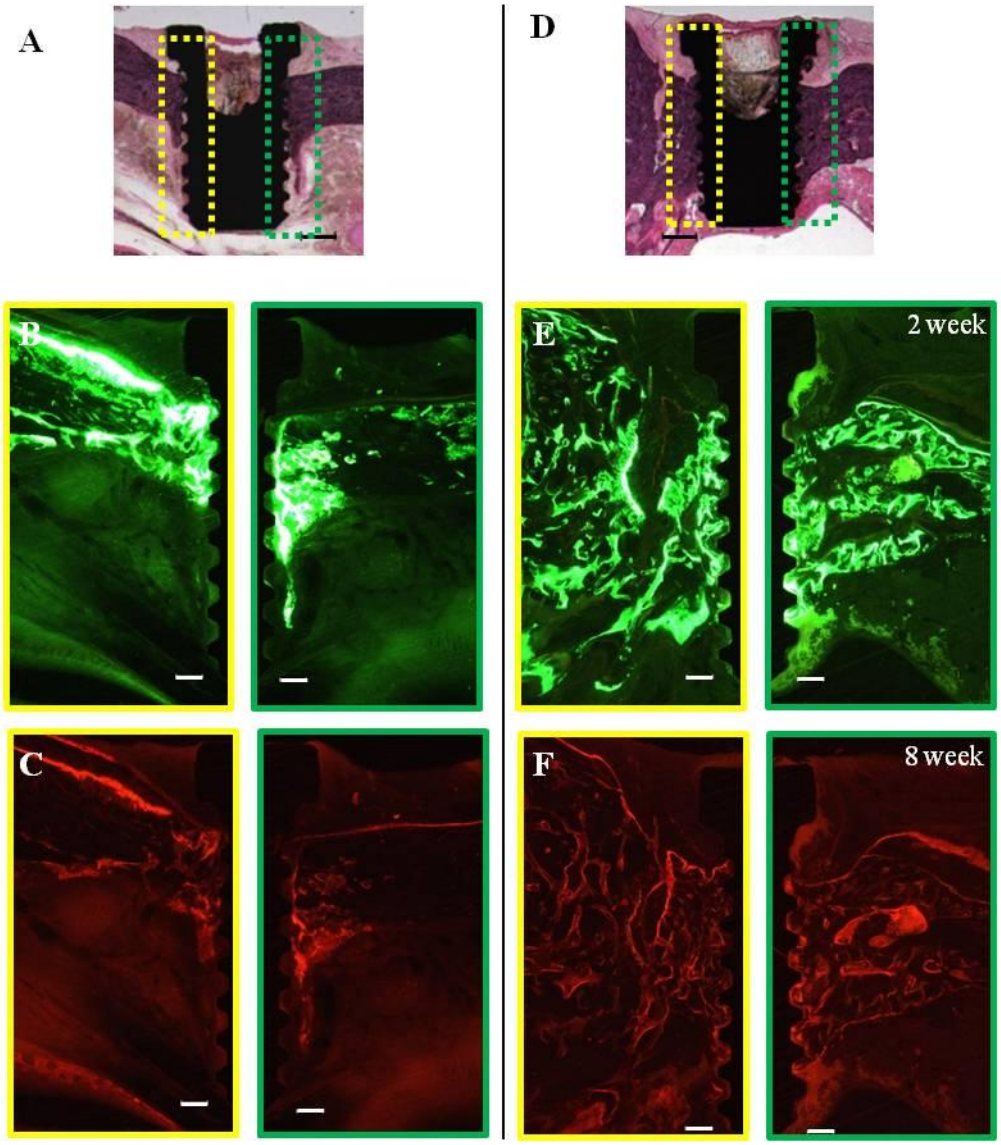


Fig. 17. Fluorescent microscopic images in the group 3 and 6.

A, B, C: Group 3. D, E, F: Group 6.
A & D: H&E stained images at lower magnification (12.5x). Scale bar = 500 ㎛.
B & E: Green emitting region (50x). Scale bar = 200 ㎛.
C & F: Red emitting region (50x). Scale bar = 200 ㎛.

IV. Discussion

Oral reconstruction in head and neck cancer patients using implants has mainly focused on implant placement after radiation therapy. This is because 1) implant placement during ablative surgery requires a thorough pre-surgical examination and multidisciplinary consultation for well-established treatment planning, 2) the excision border may enlarge during surgery, and 3) sometimes because second surgery for reconstruction is planned. However, late reconstruction worsens functional impairment and rehabilitation. Also the patient shows lack of self-esteem and loss of social ability. Implant placement prior to postoperative radiotherapy, preferably simultaneously with ablative surgery, not only obviates the need for additional surgical reconstructive surgery, but also advances prosthetic reconstruction. According to Schepers et al.[4], the interval between the end of tumor therapy and start of prosthetic rehabilitation was 4.8 months in their study. In studies with dental implant placement after tumor therapy the patients had to wait 17 - 44.5 months before the implants were placed and a delayed rehabilitation period could start[31-33]. Regarding survival rate, Schepers et al[4] reported a 97 % success rate of osseointegration in the post-implant irradiated group. In other clinical studies, although the sample size were not enough for statistical analysis, the reported success rate of implants before radiation was acceptable. The authors therefore recommended implant insertion during ablative surgery if postoperative radiotherapy is scheduled or possibly will be applied[13,18,19,34-36].

According to previous studies, extraction socket healing in rats takes 28 to 30 days[37]. There is new bone formation 5 days after implantation, and a thin layer of newly formed bone covered the surface of the implant, suggesting osseointegration at the light microscopic level after 28 to 30 days[26,37]. Compared to 3 to 4 months for osseointegration in humans, rats have a 3 to 4 times faster remodeling rate. Clinically, post-operative radiation therapy is commonly initiated 14-21 days after ablative surgery[23], which means that wound healing is not yet complete. In this study, several clinical situations were simulated according to the time of irradiation. Group 1 and 2 were irradiated 1 day after implant placement which represents the situation of irradiation during the early healing stage, group 3 was irradiated 4 weeks after implant placement which represents irradiation during the late healing stage.

Radiation could cause irreversible cellular and vascular damage resulting in hypoxia, hypocellular and hypovascular tissue[38]. Immature bone tend to be more sensitive to irradiation than mature bone[39]. Reduced regenerative capacity in bone after irradiation in rabbits has been reported, and the contacting bone may fail to lamellize within the normal time period and may have reduced adherence to the implant surface 4 weeks after irradiation with a single 15 Gy dose[23]. In this study, relative bone mineral density (BMD), bone-to-implant contact (BIC), relative bone volume and relative empty lacuna count was analyzed to evaluate the modeling and remodeling rate.

A recently developed technology allows evaluation of bone biopsies with 3-D micro-CT. The parameters computed by the micro-CT were bone volume, bone surface, trabecular thickness, trabecular separation, bone connectivity and bone-to-implant apposition. There are reports that showed data from micro-CT analysis is reliable, but the

titanium implant caused a blurred border of 45-60 μm along the implant surface[30,40]. It was affected by the difference in thickness between the titanium implants (2.0 mm and 3.5 mm). The thicker the titanium implant, the larger the influence on the quality of the micro-CT image. In this study, implants with a smaller diameter (1.5 mm) were used and BMD within 300 μm of ROI was measured. BMD is calculated by measuring Hounsfield units (HU) and relating those values to a calibration bone phantom with a pre-determined BMD [41]. However, absolute BMD values based on CT scans is not possible, unless calibration bone phantoms with a pre-determined BMD value are scanned[42]. Therefore, in this study, relative BMD, which is determined during CT scanning, was calculated.

Radiation damage has been considered to be the most important factor leading to decreased matrix formation and disturbance of bone mineralization[43]. In a previous study[42], an experiment using minipigs with the objective of comparison of bone mineral density in irradiation and non-irradiation groups was performed and the results showed that the BMD in the irradiated group was higher than that of the non-irradiated group 3 months after irradiation though the difference was not significant. The authors assumed that irradiation had a negative effect on bone vascularity and hence on bone sclerosis. In our study, groups irradiated during the early healing stage showed significantly lower BMD than the non-irradiated group. Group 3, which was irradiated during the late healing stage, showed similar BMD to the control group. When comparing the non-irradiated groups, BMD increased with the length of the healing period. This indicates that irradiation during the early healing stage significantly decreases bone remodeling but irradiation during the late healing stage showed no effect.

According to Haga and co-workers[44], at 1-month postimplantation in rat, part of newly formed woven bone contact particularly with of the implant surface and bone with empty oteocytic lacunae existed at the lateral wall of the cavity between the pre-existing and newly formed bone. After 1.5 to 2.5 months, almost all the implant surface was covered with the newly formed bone, even though the bone with empty osteocytic lacunae remained. Aitasalo et al[43] reported that a decrease in number of osteoblasts following irradiation decreases collagen production and increases the number of empty lacunae in the cortical bone. This increase was dose related since osteoblasts were destroyed after an irradiation dose of 10 Gy[23]. In animal studies, although statistically non-significant, lower BIC was reported in the post-implant irradiated group compared to the non-irradiated group, and subjective observation showed retarded bone formation and peri-implant resorption[23,24]. Brogniez and colleagues reported the effects of irradiation before implant placement and after implant placement in dog models[3,20,21]. In contrast to previous studies, bone healing capacity was relatively not affected by irradiation and BIC appeared to be better in the group of irradiation after implant placement than irradiation before implant placement. In our study, BIC and bone volume gradually increased with the healing period, indicating osseointegration takes 8 weeks or more, which coincides with previous study[44]. When comparing the irradiated group and non-irradiated group, the irradiated group showed lower bone volume whether irradiated 1 day or 4 weeks after implantation. It could reflect that irradiation affects bone healing both during the early stage or the late stage of the osseointegration process.

Regarding empty lacuna count, there was no statistical difference within the control group that empty lacuna still existed 8 week after implant placement. In the irradiated

groups, empty lacuna count was higher in group 2 and 3 than group 1, which is supported by previous study[43]. When comparing the irradiation group and control group, there is a significant difference between group 2 and 5 ($p<0.10$), group 3 and 6 ($p<0.05$). It indicated retarded bone healing after irradiation regardless of the stage of osseointegration, consistent with the bone volume analysis.

In the fluorescence analysis, the bone mineral apposition pattern was compared for various periods. The Haversian system diameter is the most optimal and diagnostic measurement to use[45], but due to poorly developed Haversian systems in rats and the small sample size, only subjective observation was done. In the group of 2 weeks after implant placement (group 1 and 4), there was more bright red fluorescent color than green fluorescent indicating acceleration in new bone formation. This is coincident with the result that new bone formation is accelerated 12-19 days after implantation[23]. In the group of 4 weeks after implant placement (group 2 and 5), red fluorescent color appeared at the interface of implant, which is also consistent with previous studies. In the group of 8 weeks after implant placement (group 3 and 6), green and red fluorescent color appeared at the border of the old bone indicating continuous bone remodeling. When the irradiated group and non-irradiated group were compared, retarded new bone formation was noted in the irradiated group.

Null hypotheses were partially accepted that not only irradiation resulted in retarded new bone formation around the implant during the early bone healing stage but also it partially affected bone healing during the late bone healing stage. This means that irradiation after implant placement may influence osseointegration of the implant though it does not increase implant failure. Some reports recommended implant submerging until

tumor therapy (including radiotherapy) is complete and a longer healing period than non-irradiation cases[12,35].

One thing that should be considered when choosing radiotherapy after implant placement is the backscattering effect of metal implants. There is no general agreement on explantation or preservation of metal dental implants in patients subjected to irradiation therapy[46]. Metal scattering could lead to three consequences: 1) a reduced dose of irradiation to the tumor if it is situated behind the implants, 2) a possible loss of osseointegration and implant failure because of a higher irradiation dose, and 3) an increased risk of osteoradionecrosis to develop in the bone adjacent to the implant[19]. In vitro studies of a monte carlo approach reported that directly in front of the implant the dose is 10 % higher and directly behind the implant, the dose is reduced by almost 16 % compared to the dose in the plain phantom due to differences in the density of the two material[46,47]. However, they compared plain water and titanium implant, not bone and titanium implant. Implant material or surface coating can also influence the scattering effect. Implants containing gold had dose enhancement on the bone-implant contact area compared to pure titanium or Ti-6Al-4V alloy[48]. One clinical study reported 3 cases of tumor recurrence that underwent simultaneous implantation with ablative surgery and mentioned that dose disturbance from radiation scatter could be one of the reasons[48]. On the other hand, Stoll et al[49] reported 12.5-16 % of dose increase at a distance of 0.45 mm from the metal specimen but there was no influence on the life of the implant, if there is sufficient thickness of soft tissue. Pekmezci et al[50] also reported that current radiation therapy regimens may be performed without additional harm using linear accelerators. In other words, in a real treatment plan, with several beams from different directions, the

overall effect will be spread over a larger volume and largely compensated for by the different beams. Until further data is available on the possible disadvantages of irradiation on titanium fixtures, avoiding abutment connection or removal of all prostheses or frameworks before radiotherapy is recommended[51].

Our study had several limitations. First, this study compared implantation before irradiation and non-irradiation. To evaluate the efficacy of implant placement before radiotherapy, a study that compares implantation before and after irradiation would be more relevant. Second, irradiation during the early healing stage groups (group 1, 2) and late healing stage group (group 3) had different sacrifice schedules which made it difficult to compare the histological or radiological analysis.

V. Conclusion

The purpose of this thesis was to evaluate the effects of local irradiation around implants during several healing stages. The following conclusions can be drawn:

1. Local irradiation resulted in retarded bone healing and reduced bone mineral density during the early healing stage.
2. Local irradiation had relatively minor influence, but also caused retarded bone healing during the late healing stage.
3. The timing of local irradiation has a critical influence on the bone healing mechanism, which is related to osseointegration around implants.

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국문요약

실험용 쥐 상악에서 방사선 조사가 치유단계의 임플란트 골유착에 미치는 영향

(지도: 정 문 규 교수)

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목적: 두경부 암의 수술적 제거 및 부가적 방사선 치료와 더불어 임플란트를 이용한 심미적 기능적 재건이 이루어지고 있다. 방사선 조사의 합병증에 관한 연구들은 오랫동안 진행되어 왔으나 재건을 위한 임플란트 식립이 선행된 후의 방사선 조사가 임플란트 주위 치유 및 골유착에 미치는 영향에 대해 체계적으로 진행된 연구는 많지 않다. 본 연구의 목적은 방사선 조사가 치유단계의 임플란트 골유착에 미치는 영향을 알아보는 것이다.

방법: 48 마리의 4 주령 실험용 쥐를 실험군 (방사선 조사)과 대조군으로 나누고 다시 임플란트 식립과 방사선 조사간 간격에 따라 1 일군, 4 주군으로 나누어 총 6 개 군으로 실험을 진행하였다. 모든 쥐에서 상악 양측 제 1 대구치를 발치한 후 4 주간의 치유기간을 가졌고 그 후 맞춤 제작된 미니 임플란트를 식립하였다. 군별로 식립 1 일, 4 주 후 15.0 Gy 단일선량 방사선

조사를 시행하였고, 골치유 및 골개조 양상을 관찰하기 위해 임플란트 식립 당일, 2 주 후, 희생 1 일 전 tetracycline, calcein, alizarin 을 희석, 복강투여 하였으며, 그 후 희생하여 방사선학적 분석과 조직학적 분석을 시행하였다.

결과: 1. 임플란트 식립 1 일 후 방사선 조사를 받은 1, 2 군에서 대조군과 비교해 통계학적으로 유의하게 낮은 골밀도 (BMD)를 보였고, 4 주 후 조사군인 3 군에서는 대조군과 유의한 차이를 보이지 않았다. 2. 방사선 조사를 받은 군에서 대조군에 비해 낮은 골-임플란트 접촉률 (BIC %)을 보였으나 통계학적 유의차는 없었다. 3. 방사선 조사를 받은 군 (2, 5 군)에서 대조군 (3, 6 군)과 비교해 유의차 있게 낮은 Bone volume (BV/TV)과 높은 empty lacuna count 를 보였다.

결론: 임플란트 식립 후 early healing stage 에서의 방사선 조사는 지연된 골치유, 골밀도의 저하를 일으켰다. 골유착이 진행된 late healing stage 에서의 방사선 조사는 초기 치유시의 조사보다 그 영향이 적었으나, 이 경우에도 방사선 조사를 받지 않은 군에 비해 저하된 골치유를 보였다. 본 연구의 한계 내에서 방사선 조사의 시기는 임플란트 골유착과 관련된 골치유에 중요한 영향을 주는 것으로 보여진다.

핵심되는 말: 술후 방사선 치료, 두경부 암, 국소 방사선, 임플란트, 골밀도, 골-임플란트 접촉, 상실된 골소강, 골치유, 골부피