

## **Effect of irradiation on autogenous bone transplantation in rat parietal bone**

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**Summary.** To determine the appropriate time for bone reconstruction after irradiation, the healing process after autogenous iliac bone transplantation in the irradiated parietal bone was examined by scanning electron microscopy and light microscopy. Bone transplantation was carried out at the second and the fourth weeks after Cobalt-sixty ( $^{60}\text{Co}$ ) irradiation with calculated dose and fractionation. Animals without irradiation were used as control.

The results show the appearance of mesenchymal cells and blood vessels around the transplantation to be extremely few one week after transplantation which was carried out at the second week after irradiation. These inhibitions were still seen two weeks after transplantation. Four weeks after transplantation, there were no differences in the bone formation among the experimental groups. Bone formation in the transplantation at the fourth week after irradiation was similar to that of the control group. Microvascularization in the transplantation at the second week after irradiation was inhibited one week after transplantation.

The delay in bone healing was responsible for the retardation of revascularization and caused micro-circulatory failures as well as the damage of osteogenic cells. It is quite clear that damaged cells and tissues recovered by the elapse of time under the irradiation procedure employed in this study and also that bone formation was carried out in the physiological process. We think that bone transplantation after irradiation should be done after recovery from the radiation damage to the periosteal cells and the blood vessels.

**Key words:** Radiation injury, Bone transplantation, Scanning electron microscopy, Corrosion cast, Rat

### **Introduction**

Radiotherapy for cancer is one of the most effective and efficient treatment methods and the retardation of

tissue healing after the irradiation is well known. This retardation of tissue regeneration is caused by direct damage to the cells and circular failure (Green et al., 1969; Jacobson et al., 1985). The protocols for combined therapy of irradiation and surgery are the most acceptable therapy for cancer in the oral and maxillo-facial region. Tumor resection including the jaw bone is performed after pre-operative irradiation and then the defects in the jaw bone and the soft tissue have to be reconstructed simultaneously. Bone grafts have been greatly employed for the reconstruction of the shape of the face and the oral functions. Timing for the bone graft after radiotherapy is of great importance for the clinicians.

There are many studies on bone healing after irradiation. The studies on bone reconstruction for irradiated bone are few. There are two contrary opinions on the appropriate time for bone reconstruction after irradiation. Jacobson et al. (1985) studied bone-regenerative activity after irradiation using a bone growth chamber which was implanted in the tibia of lap-eared rabbits. The inhibition of bone forming capacity one year after irradiation was less than that for immediately after irradiation. A longer period of time after the irradiation is needed for successful bone reconstruction. There are also some researchers who recommend bone reconstruction after a long period after irradiation due to cell injury to the connective tissue and radiation-induced circular failure which are severe soon after irradiation (Jacobson et al., 1985; Matsui et al., 1994). Lorente et al. (1992) suggested that surgery in the early period after irradiation produced better results in the study of bone graft for mandibular defects in Sprague-Dawley rats: these rats showed defective healing at 2 weeks after irradiation which was much better than that at 4 weeks after irradiation. The reason for the recommendation of early bone reconstruction was that endarteritis and fibrosis were caused by the late-soft tissue changes from the irradiation (Ostrup and Fredrickson, 1975; Altobelli et al., 1987). These controversies are responsible for the different doses and fractionations of the irradiation, a variety of bony-defects, graft site, various animals and experimental procedures.

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The goal of this study is to examine the appropriate time for bone reconstruction after preoperative irradiation. Prior to this study, we developed a study model using animals which postulated the protocol of combined irradiation-surgery and simultaneous reconstruction in the clinical stage. In our study model, the method of irradiation was characterized by employing Time Dose Fractionation (TDF) (Orton and Ellis, 1973) to calculate the dose and the fractionation with approximation of a human pre-operative dose. Bone transplantation was performed in the rat's calvaria after irradiation with the calculated dose and fractionation. To examine the difference of three-dimensional whole image of bone surface and microvasculature of transplantation area after the preoperative irradiation, we used a scanning electron microscopy and a conventional light microscope was also used for the observation of new bone formation, revascularization and cell-degeneration.

### **Materials and methods**

#### *Animals*

178 male Sprague-Dawley rats (aged 8 weeks, weighing about 320g) were used in this study. They were fed conventional commercial food pellets (CE-2, Clea Japan, Inc., Tokyo, Japan) and kept under optimum conditions (room temperature 22 °C; humidity 55%; lighting 300-500 lux; bad smell less than 20 ppm).

#### *Irradiation procedure*

The rats were anesthetized by intraperitoneal injection with sodium pentobarbital (40 mg/Kg). <sup>60</sup>Co source (Toshiba Co., Ltd. Tokyo, Japan) was used for irradiation (96.2 cGy/min) with a field size of 20x20 cm and source to surface distance of 80 cm. The experimental group received a total dose of 22.4 Gy (5.6 Gy x4 fraction/week) of <sup>60</sup>Co external beam irradiation to the head and neck areas. Total dose and the fractionation were calculated by TDF to approximate a human dose of 40 Gy in 2 Gy fractions given daily, five days per week for four weeks.

#### *Transplant procedure and tissue preparation*

To know the condition of the tissue after the irradiation, histological observation was carried out using a light microscopy. At the time points of 1-4 weeks after irradiation, five rats were used, respectively.

Experimental bone transplantation was performed at 2 and 4 weeks after irradiation. 32 rats were used for each group. The rats were anesthetized by intraperitoneal injection with sodium pentobarbital (40mg/Kg), and their parietal bones were exposed by ablation of the periosteum through a dermal incision. The parietal bone measured approximately 2x4 mm, and was resected by a dental bur for autogenous iliac bone transplantation.

The animals were divided into four groups of 8 rats (five rats for scanning electron microscopic observation and three for light microscopy), including two irradiated groups with each control group. At 1, 2, 4 and 6 weeks after transplantation, the animals of each group were anesthetized by intraperitoneal injection with sodium pentobarbital, and then sacrificed by transcardial perfusion with fixative containing 1.25% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/l phosphate buffer solution (PBS) (pH 7.4) for 20 minutes at room temperature. The transplanted area with peripheral host bone and soft tissue was removed.

For scanning electron microscopy, organic substances of the specimens were dissolved with 5% sodium hypochloride for 20 min at room temperature. They were rinsed in 0.1 mol/l PBS (pH 7.4) 3 times, and then they were postfixed in a 1% osmium tetroxide solution in 0.1 mol/l PBS (pH 7.4) for 90 minutes and dehydrated by a graded series of ethanol. After immersion in isoamyl acetate, specimens were critical-point dried with liquid carbon dioxide, mounted on stubs, coated by gold-platinum in a vacuum device and examined with a scanning electron microscope (S-4100, Hitachi Co. Ltd., Tokyo, Japan).

For light microscopy, the specimens were rinsed in 0.05 mol/l PBS (pH 7.4) 3 times after fixation. They were decalcified in 5% ethylenediamine tetraacetic acid at room temperature for 7 days and embedded in a water-soluble plastic media (JB-4, Polysciences Inc., Warrington, USA) and then 2 μm-thick serial sections were made. The other specimens were dehydrated with a series of graded ethanol solutions and embedded in Epon 812 resin and then 1 μm-thick serial sections were made. The sections were stained with hematoxylin and eosin or toluidine blue, and observed with a light microscope.

#### *The fine distribution of blood vessels*

For the resin replica of the blood vessels, the rats were anesthetized by an intraperitoneal injection with sodium pentobarbital (40mg/Kg) at 1, 2, 4 and 6 weeks after bone transplantation. A cannule was introduced into the aorta, and the vasculature was perfused immediately with 50 ml of physiological saline solution containing 0.1% heparin and subsequently 20 ml of Mercor resin (CL-2R-5, Dai-Nippon Ink Co. Ltd., Japan) was injected with manual pressure. The samples were kept for one hour after resin perfusion at room temperature for the polymerization and then they were immersed in a hot water bath (60 °C) to complete polymerization. The parietal bone was dissected as a sample. Organic substances of the specimens were dissolved by 10% sodium hypochloride for 2 days at room temperature. After dissolution of the tissue, the bone with the resin replica of the blood vessels was carefully rinsed in distilled water. They were postfixed in a 1% osmium tetroxide solution in 0.1 mol/l PBS (pH 7.4) for 60 minutes. The samples were dehydrated by a graded series of ethanol. After immersion in t-butyl

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alcohol, the samples were dried in the freeze-drier for 12 hours (ID-2, Eiko Engineering Co. Ltd., Mito, Japan). The specimens were mounted on stubs, coated by gold-platinum in a vacuum device, and observed with scanning electron microscopy (S-4100, Hitachi Co. Ltd. Tokyo, Japan).

### Results

#### General aspects

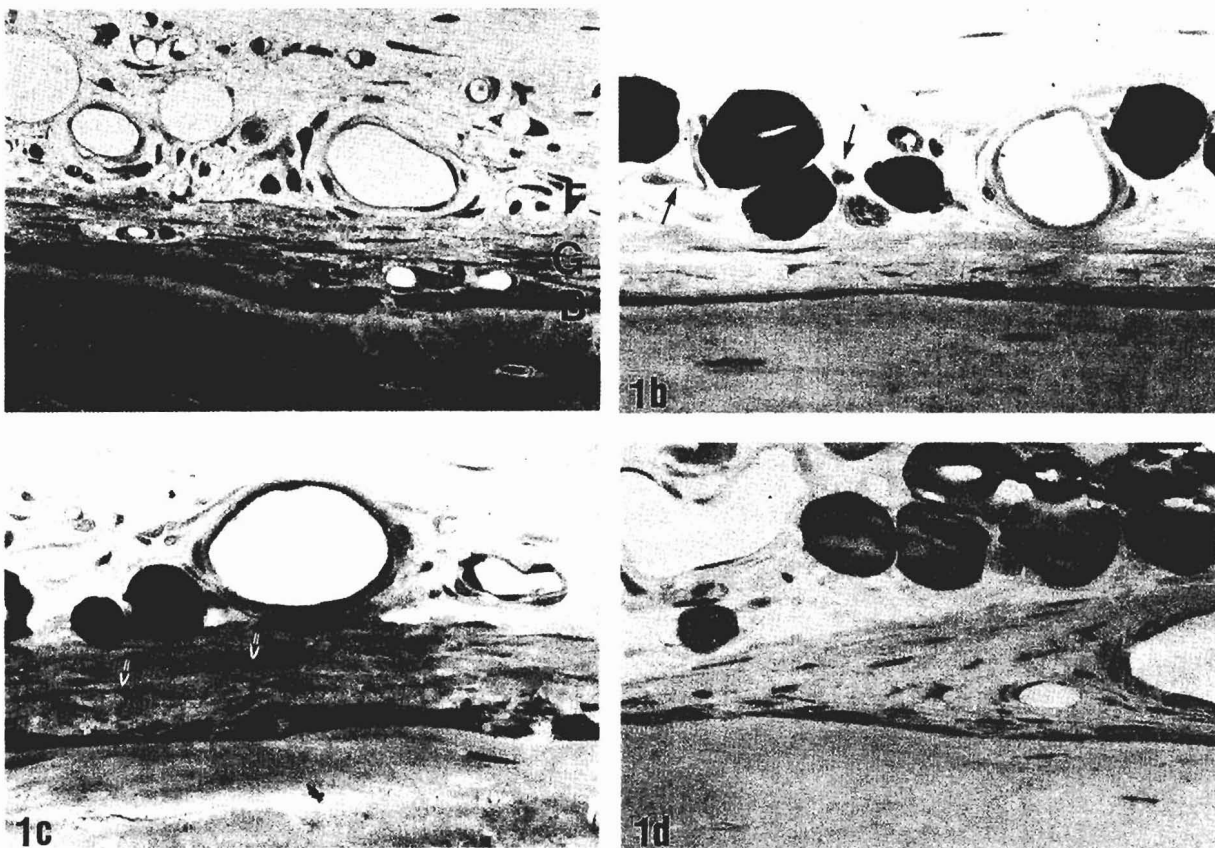
All animals remained healthy throughout the observation period. No wound infections were observed. In the irradiated areas, the redness of the skin and the mouth mucous membrane was seen at the maximum level in the 1st week after irradiation. Such damage disappeared after 2 weeks.

First we examined the physiological conditions of the periosteum in a normal adult rat's calvarium by conventional light microscopy. The periosteum of a normal rat consists of three layers, the first layer which is away from the bone surface is the layer rich in blood vessels but poor in cells, the second which exists in the central area of the layers is composed of slender-shaped

and spindle-shaped cells with a flat nucleus and the blood vessels are scattered among them. The third, which is attached to the bone surface, is composed of spindle-shaped cells with an elliptical-shaped or a round-shaped nucleus (Fig. 1a). In general, the periosteum in adult animals is divided into three layers, which are a fibrous layer, a cambium layer and an osteoblastic layer (Ham and Harris, 1971). Our observations were extremely similar to the histological finding which is generally observed in the periosteum of normal animals.

#### 1. Effects of irradiation on the periosteum

One week after irradiation, in the fibrous layer of the periosteum, the blood vessels became flattened and the number of the cells diminished (Fig. 1b). The shape of the blood vessels and the number of cells gradually recovered by four weeks after irradiation, but histological findings of the periosteum were still different from those of a normal periosteum (Fig. 1c,d). In the osteogenic layer of the periosteum, slender-shaped and/or spindle-shaped cells also showed low density one week after irradiation, and then the number of cells gradually increased (from 2 to 4 weeks after



**Fig. 1.** Light micrographs (LMs) of the periosteum after irradiation. **a.** Normal adult rat's calvarium. The periosteum is divided into fibrous layer (F), osteogenic layer (G), and osteoblasts (B). x 100. **b.** The blood vessels (arrows) are flattened at one week after irradiation. x 100. **c.** Slender-shaped cells (arrows) are in the osteogenic layer at 2 weeks after irradiation. x 100. **d.** The number of cells and blood vessels were increased in the osteogenic layer at 4 weeks after irradiation. x 100

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irradiation). Apparent transformation of the osteoblasts was not observed.

### Bone transplantation

#### Control

**1 week after transplantation.** Scanning electron microscopy showed that the transplanted bone was incorporated into the host bone by newly-formed bone which was produced on the host bone surface of the dura mater side (Fig. 2a). Under high magnification, new bone showed a spongy-like appearance with many vascular spaces. This was constructed by thin trabeculae, which twined around each other, with an aggregation of many small spherical mineral clusters (about  $1\ \mu\text{m}$  in diameter) (Fig. 3a). These findings were similar to those of the periosteal surface of primary bone as described by Boyde and Hobbell (1969). The bone resorption, which was characterized by depressed areas (Howship's lacunae) surrounded by sharp edges (Boyde, 1972), was observed on the skin side of the transplanted bone (Fig. 3b).

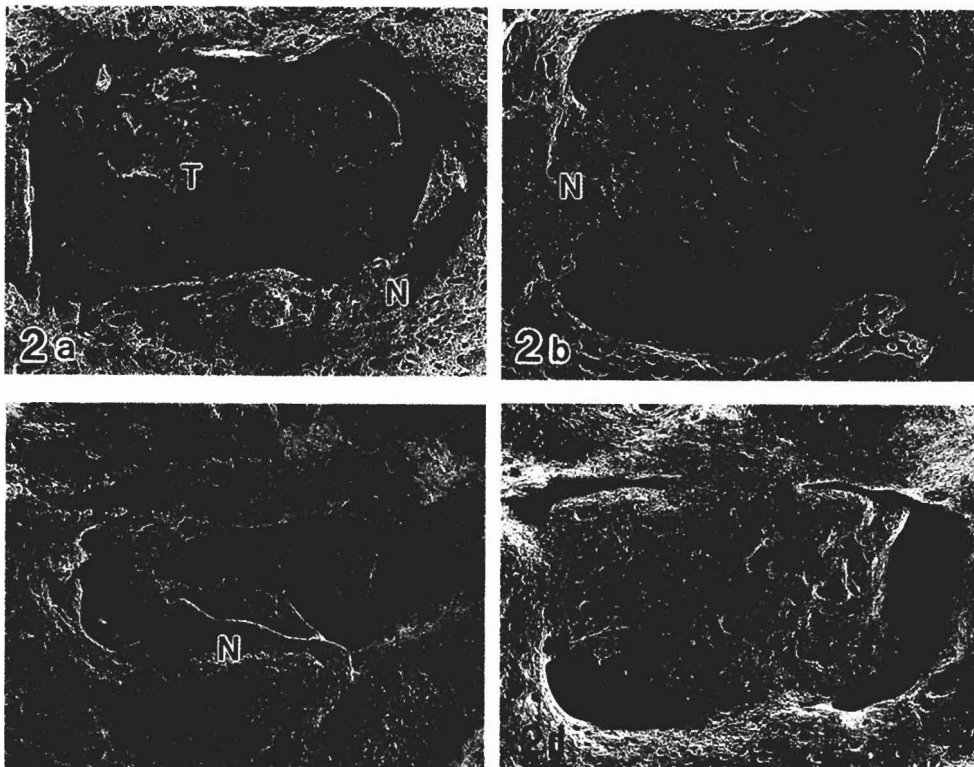
Under light microscopy, new bone formation was observed only on the dura mater side of the host bone (Fig. 4a). Thick trabecular-like new bone was enclosed by a layer of cuboidal-formed osteoblasts and many blood vessels. In the contact area between the host and the transplanted bone, many spindle- and round-shaped cells and blood vessels were observed (Fig. 4b). On the skin side, multi-nucleated large cells (osteoclast) were

observed in Howship's lacunae in both transplanted and host bone.

**2 weeks after transplantation.** Under scanning electron microscopy, spongy-like newly-formed bone sparsely covered the transplanted bone on the dura mater side, but it was not so extensive on the skin side. The incorporated area of the host and the transplanted bone spread (Fig. 2b). On higher magnification, in addition to the spherical mineral clusters, the regular arrangement of small nodules like rice-grains was observed on the trabeculae, and many lacunae, in which the osteoblast had existed (osteoblastic lacuna; Boyde, 1972), were observed on the spongy-like new bone surface. The lacuna was about  $15\ \mu\text{m}$  long and  $10\ \mu\text{m}$  wide, and the back wall of the lacuna was also constructed by small rice-grain-like nodules. Resorbing surfaces (Howship's lacunae) were observed on the host and the transplanted bone surface in the proximity of new bone (Fig. 3c).

Under light microscopic observation, the new bone on the dura mater side was thicker and denser than that at 1 week after transplantation. New bone with many blood vessels filled the space between the host and the transplanted bone. Many blood vessels were also observed around the new bone, but fewer mesenchymal cells were seen than that at 1 week after transplantation.

**4 weeks after transplantation.** Under scanning electron microscopy, bone unions were seen widely between the host and the transplanted bone (Fig. 2c). On both the skin and the dura mater side, the new bone matured and



**Fig. 2.** Scanning electron micrographs (SEMs) of autogenous bone transplantation. **a.** Transplanted bone (T) is united to the host bone by new bone (N) at one week after transplantation.  $\times 20$ . **b.** The spongy-like new bone (N) covers the transplanted bone and host bone at 2 weeks after transplantation.  $\times 20$ . **c.** Smooth surfaced new bone (N) covers the transplanted bone and host at 4 weeks after transplantation.  $\times 20$ . **d.** At 6 weeks after transplantation, areas without bone union still exist.  $\times 20$

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the bone surface showed a smooth surface. The smooth surface was characterized by an accumulation of small nodules like rice-grains with a few osteoblastic lacuna (Fig. 3d), and it was identical to the morphological findings of the forming surface of adult bone described by Boyde (1972). The vascular spaces on the bone surface came to have a uniform size and shape (about 50  $\mu\text{m}$  in diameter) and their number decreased as compared with 1 and 2 weeks after transplantation. Resorbed bone areas were observed widely on the matured new bone surface.

With light microscopy, dense and thick new bone surrounded the host and the transplanted bone, and relatively flat-shaped osteoblasts lined up on the matured new bone surface on the dura mater side. The new bone made bone unions between the host and the transplanted bone. Bone marrow cavities with many cells were observed in both the matured new bone and the transplanted bone.

*6 weeks after transplantation.* Scanning electron microscopy showed that the transplanted and the host bone were united by new bone, but the areas without bone union still existed (Fig. 2d). A mixture of "forming surface" and "resorbing surface" was seen on the matured new bone surface.

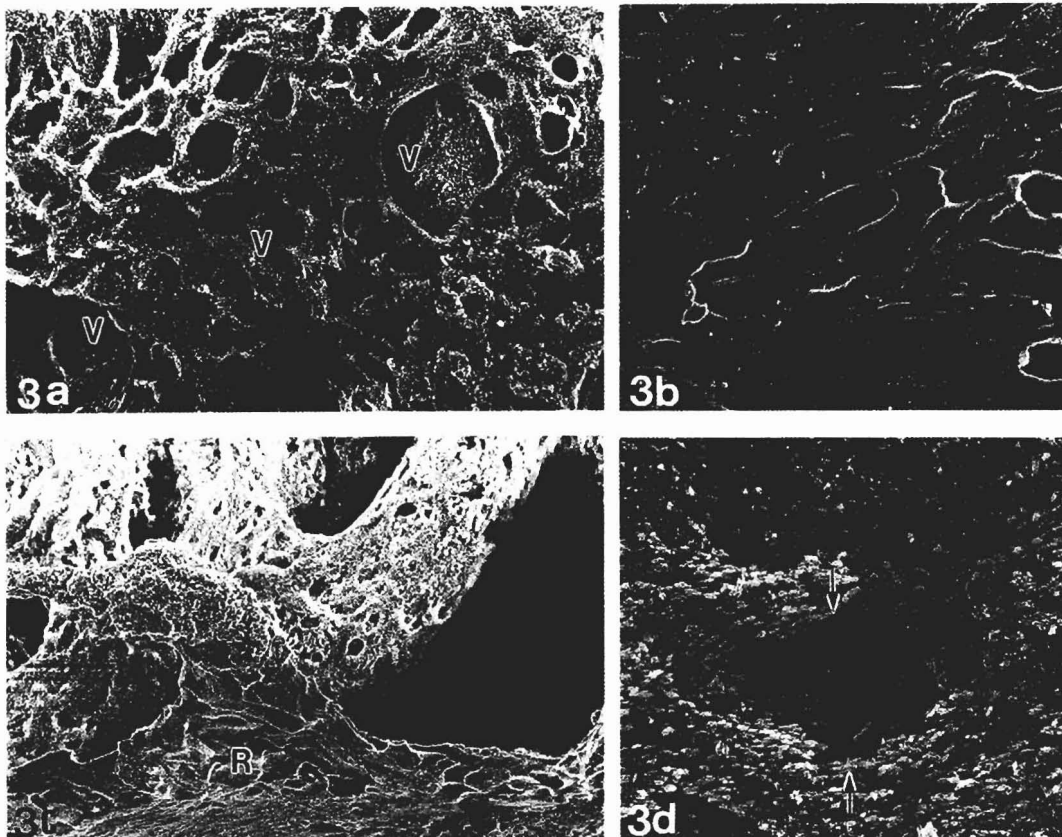
On light microscopic observation, the matured new bone filled the space between host and transplanted bone

(Fig. 4c). On the dura mater side, a layer of flat-shaped osteoblasts lined up on the matured new bone. The blood vessels around the new bone were few. The number of bone marrow cells in the marrow space of the transplanted bone increased.

#### Transplantation 2 weeks after irradiation

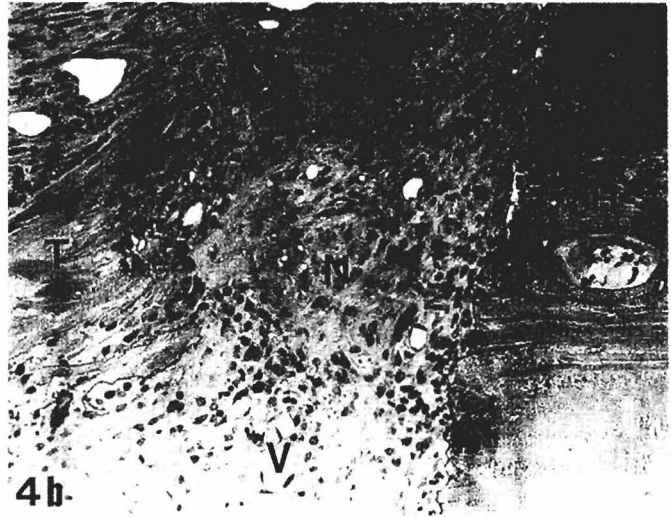
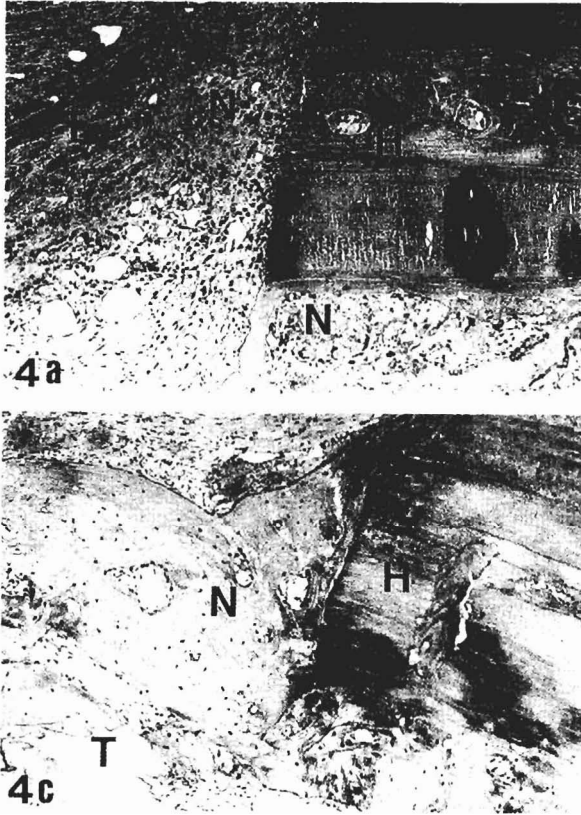
*1 week after transplantation.* There was no calcified union between the transplanted bone and the host bone; after anorganic treatment, the implanted bone detached from the transplant bed of the host bone (Fig. 5a). Under a scanning electron microscopy, band-shaped newly-formed bone, which looked like an embankment, was observed on the host bone surface far from the cut end of the transplant bed on the dura mater side. At higher magnification, small spherical mineral clusters arranged irregularly on the new bone were observed. Howship's lacunae were observed in the host bone surface adjoining the new bone. On the skin side, there was no new bone formation.

Light microscopically, thin trabeculae of the new bone were observed on the host bone surface on the dura mater side. The new bone formation was not in the circumference of the cut end of the transplanted bed (Fig. 6a). The range and the volume of new bone were less than that of the control. The periosteum of the dura mater side was composed of loose fibro-vascular tissue

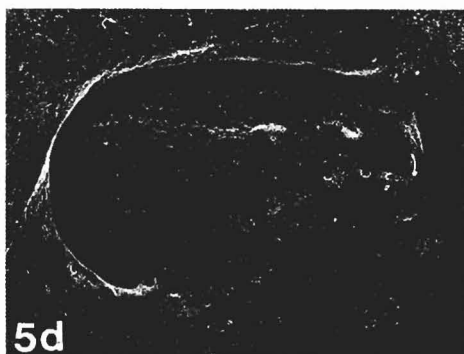
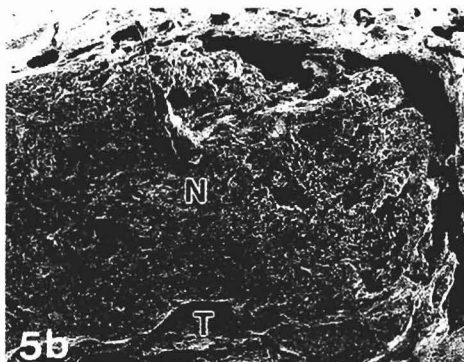
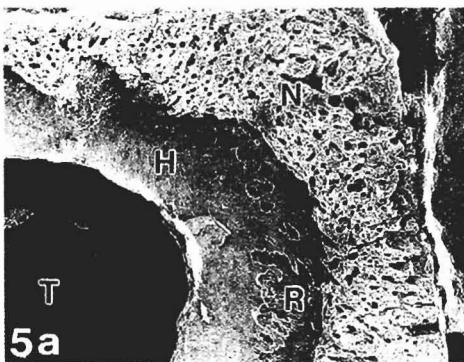


**Fig. 3.** SEMs of high magnification after auto-genous transplantation. **a.** New bone surface has a spongy-like appearance with vascular spaces (V) at 1 week after transplantation. x 600. **b.** Resorbed bone surface in the skin side of the transplanted bone at 1 week after transplantation. x 600. **c.** Resorbed bone surface (R) is in proximity to the newly-formed bone at 2 weeks after transplantation. x 300. **d.** Osteoblastic lacunae (arrows) of new bone 4 weeks after transplantation. x 2,000

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**Fig. 4.** LMs of autogenous bone transplantation. **a.** New bone (N) is on host bone surface in dura mater side and contact area between host (H) and transplanted bone (T) at 1 week after transplantation. x 30. **b.** High magnification of contact area in Fig. 4a. New bone (N) with many spindle- and round-shaped cells and blood vessels (V) united host (H) and transplanted bone (T). x 100. **c.** Bone union is seen between host (H) and transplanted bone (T). N: New bone. x 30



**Fig. 5.** SEMs of transplantation 2 weeks after irradiation. **a.** New bone (N) is far from the cut end of transplanted bed (T) at 1 week after transplantation. Resorbed surfaces (R) are seen in the host bone (H). x 20. **b.** New bone (N) widely covers host and transplanted bone (T) at 2 weeks after transplantation. x 20. **c.** Matured new bone (N) widely covers host and transplanted bone at 4 weeks after transplantation. x 20. **d.** 6 weeks after transplantation, new bone completely covers the host and transplanted bone. x 20

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(Fig. 6b). The number of osteogenic cells and blood vessels in the periosteum were less than that of the control, although blood vessels were seen around the new bone. The space between the host and the transplanted bone was only filled with fibrous tissue, and there was no new bone formation.

**2 weeks after transplantation.** Scanning electron microscopically, spongy-like new bone widely covered both the host and the transplanted bone on the dura mater side, but bone union was only seen in a part of the space between both bones (Fig. 5b). On the skin side, new bone covered the cut end of the host bone and a part of the transplanted bone. At higher magnification, spherical mineral clusters showing irregular arrangement and small nodules like rice-grains were observed in the new bone. The bone resorbed area existed widely on the cut surface of the host bone and the surface of the new bone.

Light microscopically, thick trabecular-like new bone that contained blood vessels appeared around the host and the transplanted bone on the dura mater side. Osteogenic cells around the new bone were seen in abundance as in the control. A part of the space between the host and the transplanted bone was incorporated by the new bone.

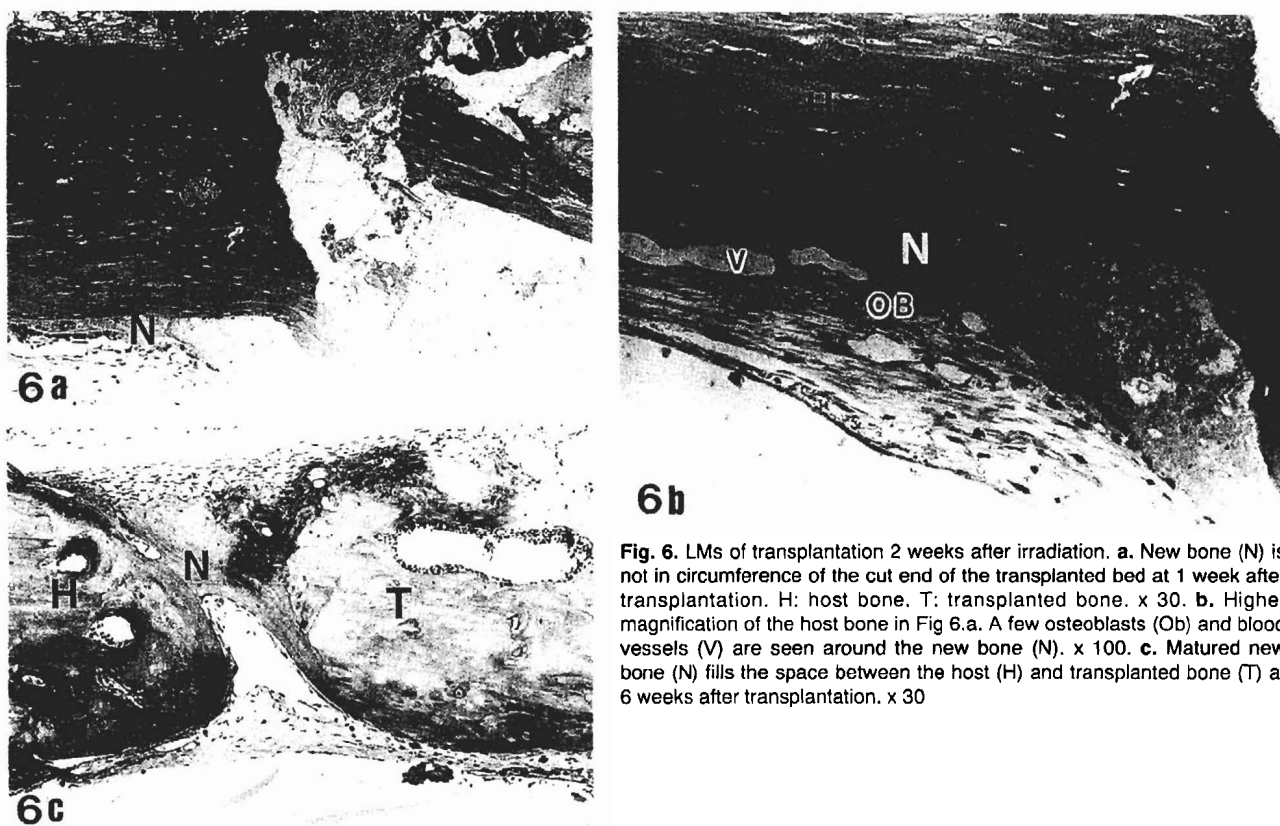
**4 weeks after transplantation.** Under scanning electron microscopy, the new bone on the dura mater side

covered the host and the transplanted bone, and they were united (Fig. 5c). On the skin side, new bone covered the cut end of the host bone and the surface of the transplanted bone. The new bone surface was similar to that of the control. The resorbed bone area was seen in a part of the matured new bone surface.

Under light microscopic observation, the transplanted bone was enclosed by new bone. Part of the space between the host and the transplanted bone was filled by the new bone, though there were spaces where fibrous tissue was still evident. Bone marrow space was observed in the center of the matured new bone.

**6 weeks after transplantation.** Scanning electron microscopically, the transplanted bone was completely covered by the new bone and it was impossible to distinguish the transplanted bone from the host bone on both the dura mater and the skin side (Fig. 5d). There were many samples in which the space was completely filled with the new bone; in a few samples, un-united areas in the space still existed. At higher magnification, most new bone surfaces showed the shape of a mature bone surface.

Light microscopic observations showed that the transplanted bone was surrounded by new bone that had a typical lamellar structure. The space between the host and the transplanted bone was completely filled with matured new bone (Fig. 6c).



**Fig. 6.** LMs of transplantation 2 weeks after irradiation. **a.** New bone (N) is not in circumference of the cut end of the transplanted bed at 1 week after transplantation. H: host bone. T: transplanted bone. x 30. **b.** Higher magnification of the host bone in Fig 6.a. A few osteoblasts (Ob) and blood vessels (V) are seen around the new bone (N). x 100. **c.** Matured new bone (N) fills the space between the host (H) and transplanted bone (T) at 6 weeks after transplantation. x 30

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### Transplantation 4 weeks after irradiation

*1 week after transplantation.* After anorganic treatment for scanning electron microscopy, the transplanted bone detached from the transplant bed because of no calcified union between the host and the transplanted bone. New bone formation was not observed on the skin side. On the dura mater side, spongy-like new bone formed on the surface of the host bone and protruded towards the transplant bed (Fig. 7a). Using higher magnification, newly-formed bone similar to that of the primary bone, as seen in the group of transplantation of 2 weeks after irradiation and the control, was observed.

Light microscopically, thick and trabecular-like new bone were observed in the dura mater side (Fig. 8a). Many blood vessels and mesenchymal cells assembled around the new bone and it closely resembled that of the control. In the space between the host and the transplanted bone, there were many blood vessels and osteoblasts, but there was no bone union.

*2 weeks after transplantation.* Scanning electron microscopically, spongy-like new bone widely covered both the skin and the dura mater side (Fig. 7b). The host and the transplanted bone were united by new bone on both sides. With higher magnification, the surface of the new bone consisted of spherical mineral clusters and small nodules like rice-grains which showed a regular arrangement, and additionally many osteoblastic lacunae were observed among the nodules. Resorbed bone areas were observed widely in both the host and the

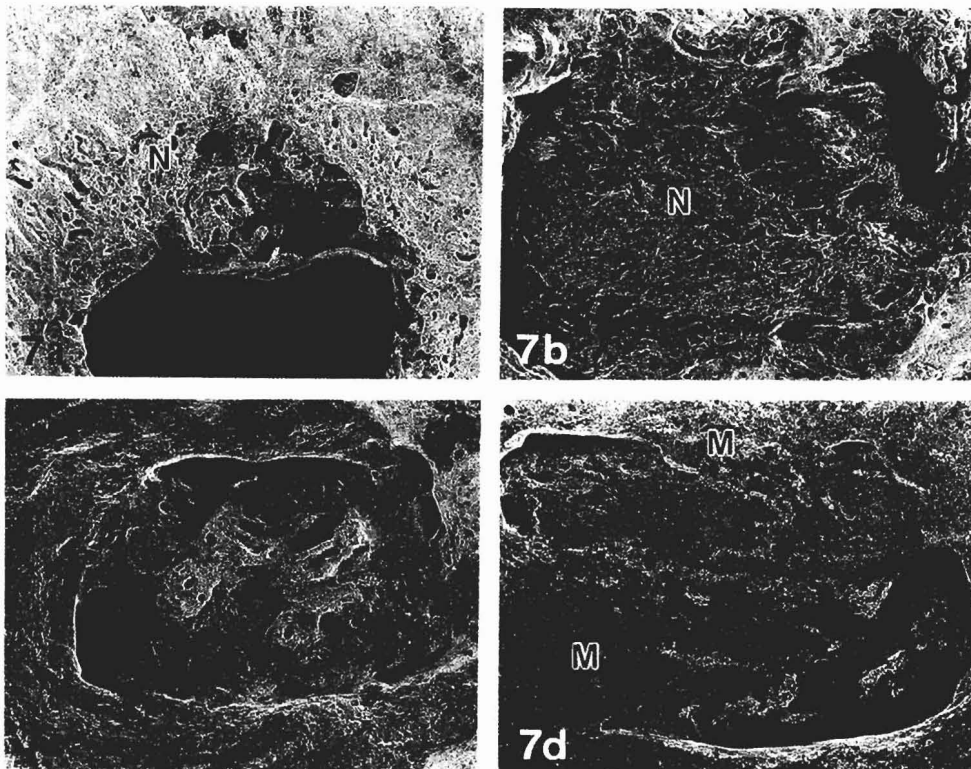
transplanted bone.

With microscopic observation, thick and trabecular-like new bone formed on the dura mater side of the host bone and both sides of the transplanted bone. Many blood vessels and mesenchymal cells were seen around the new bone. The space between the host and the transplanted bone was filled with new bone that contained blood vessels.

*4 weeks after transplantation.* Under scanning electron microscopy, the new bone incorporated with the host and the transplanted bone on both the skin and the dura mater sides, but non-united parts still existed (Fig. 7c). At higher magnification, a new bone surface close to that of mature bone was seen in the same week of the control. A resorbed bone area was more widely observed on the matured new bone surface when compared with that of 2 weeks after transplantation.

With microscopic observation, matured new bone covered the host and the transplanted bones, and both bones were united by them. Flat-shaped osteoblasts enclosed the new bone. Bone marrow cells filled in the marrow space of the matured new bone in the transplanted bed.

*6 weeks after transplantation.* In the scanning electron microscopic observations, the host and the transplanted bone surface were completely covered and united by new bone (Fig. 7d). The space between the host and the transplanted bone was filled with new bone, and some samples were united more extensively than that of the



**Fig. 7.** SEMs of transplantation 4 weeks after irradiation. **a.** Spongy-like new bone (N) covers the edge of host bone at 1 week after transplantation. Host bone surface is completely covered by the new bone. x 20. **b.** The host and transplanted bone are united by the new bone (N) at 2 weeks after transplantation. x 20. **c.** Mature-surfaced new bone is seen on host and transplanted bone surface at 4 weeks after transplantation. x 20. **d.** Matured new bone (M) is seen on the host and transplanted bone at 6 weeks after transplantation. x 20



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control. The surface of the new bone was similar to the matured bone surface; the bone resorption was more widely observed than that of 4 weeks after transplantation.

Light microscopically, the space between the host and the transplanted bone was filled with compacted bone (Fig. 8c). The bone marrow space with many cells was observed in the matured new bone.

### *The fine distribution of blood vessels by vascular corrosion casts*

#### Control

One week after transplantation, many capillaries and blood vessels (15-30  $\mu\text{m}$  in diameter) were observed in the new bone which was formed in the transplanted and the host bone (Fig. 9a). The blood vessels had various sizes and they were tortuous. On the transplanted bone after 2 weeks, the capillaries made many anastomoses and the thickness of the vessels became uniform, and the number of vessels was less than at 1 week after transplantation (Fig. 9b). Four weeks after transplantation, a thick vein about 100  $\mu\text{m}$  diameter) and a few meandering blood vessels intermingled on the surface of transplanted bone. On the host bone, the thickness of the blood vessels became uniform and the number of blood vessels was less than at 2 weeks after transplantation. 6

weeks after transplantation, a thick vein ( about 100  $\mu\text{m}$  diameter) was seen in the dura matter side of the bone, and the blood vessels in the transplanted bone showed less meandering and were uniform in thickness (Fig. 9c).

#### *Transplantation 2 weeks after irradiation*

One week after transplantation, there were few blood vessels in the host bone, and the area had no angiogenesis (Fig. 10a). Two weeks after transplantation, the number of blood vessels was lower than the control (Fig. 10b). In 4 and 6 weeks after the transplantation, the microvasculature was the same as the control (Fig. 10c).

#### *Transplantation 4 weeks after irradiation*

One week after transplantation, the transplanted bed was partly covered by blood vessels, the vascular anastomoses became denser and wider than the case of the transplantation of 2 weeks after irradiation (Fig. 11a). 2 weeks after transplantation, the blood vessels covered the transplanted bone (Fig. 11b). The number of blood vessels became lower than that of 1 week after transplantation, and the equalization of the thickness of the vessels was observed in the host bone. At 4 and 6 weeks after the transplantation, the microvasculature was very similar to the control (Fig. 11c).

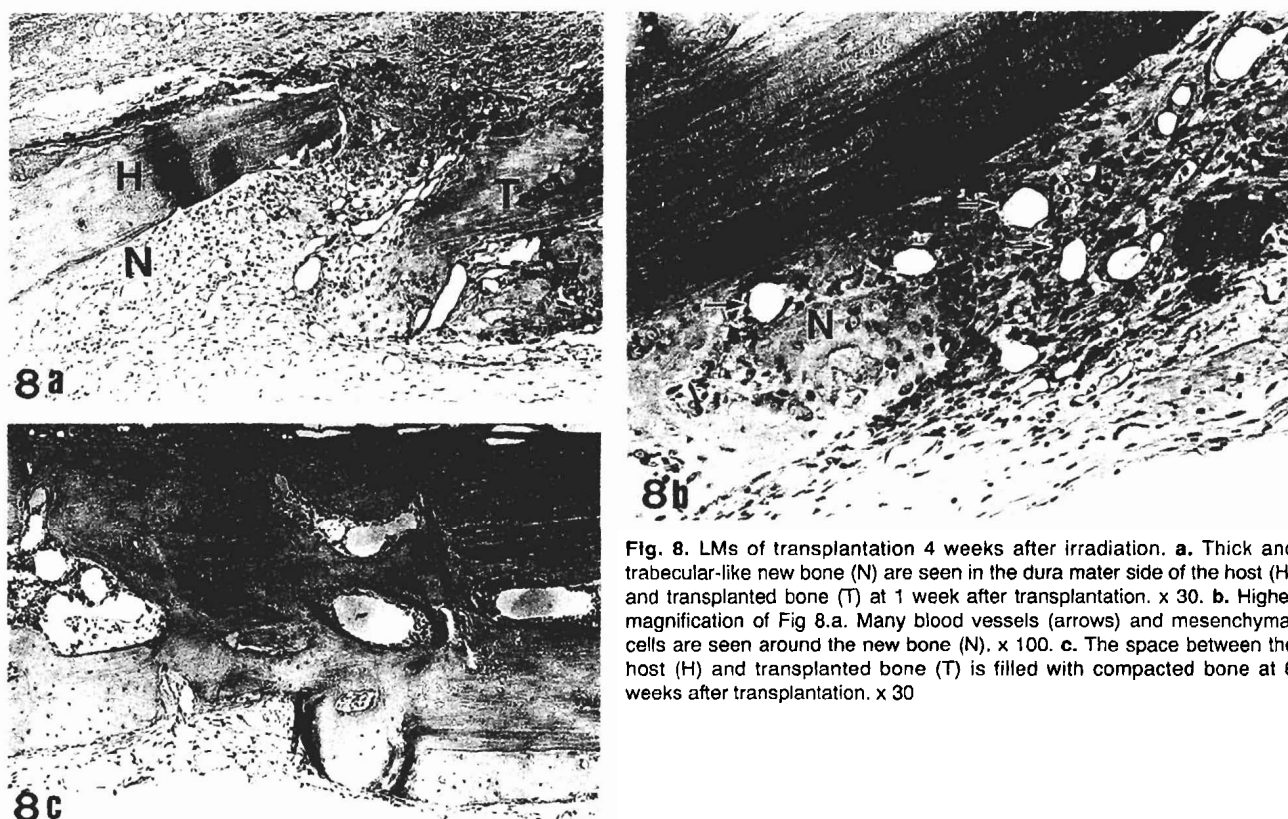


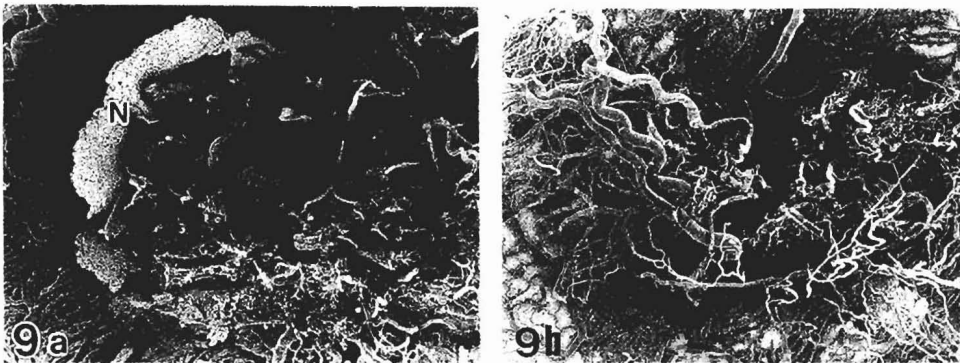
Fig. 8. LMs of transplantation 4 weeks after irradiation. a. Thick and trabecular-like new bone (N) are seen in the dura mater side of the host (H) and transplanted bone (T) at 1 week after transplantation. x 30. b. Higher magnification of Fig 8.a. Many blood vessels (arrows) and mesenchymal cells are seen around the new bone (N). x 100. c. The space between the host (H) and transplanted bone (T) is filled with compacted bone at 6 weeks after transplantation. x 30

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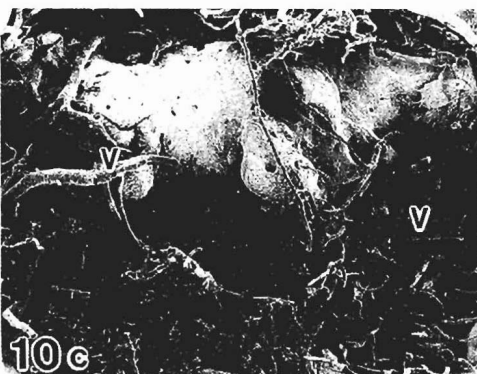
**Discussion**

Our results indicate that the healing process of bone transplantation in irradiated bone was retarded, as in

agreement with previous studies. The reduction of the new bone formation in the early period after transplantation was responsible for the retardation of bone incorporation with the host and the transplanted bone.



**Fig. 9.** Vascular corrosion casts of autogenous bone transplantation. **a.** Various-sized blood vessels are tortuous in the new bone (N) at 1 week after transplantation. x 30. **b.** The capillaries make many anastomoses and the thickness of the vessels becomes more uniform at 2 weeks after transplantation. x 30. **c.** At 6 weeks after transplantation, the thick veins (V) are seen on the new bone surface and blood vessels are uniform in thickness. x 30



**Fig. 10.** Vascular corrosion casts of transplantation 2 weeks after irradiation. **a.** At 1 week after transplantation, an area of no angiogenesis is seen in the host bone (H). x 30. **b.** The blood vessels around newly-formed bone (N) are fewer than the control at 2 weeks after transplantation. x 30. **c.** The thick veins (V) and uniformly thick blood vessels are seen at 6 weeks after transplantation. x 30

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With therapeutic doses of irradiation used in this study, the damaged cells and tissues recovered with time and bone regenerative processes became physiologically normal.

### Study model for the healing of irradiated bone

Until now, there have been many studies on the effect of irradiation on bone metabolism (Sela et al., 1982; Lorente et al., 1992). However, there is no universal agreement about the irradiation method for the experiment. Some researchers employed single large doses of the irradiation (Jacobson et al., 1985) and others used multiple divided doses without having constructed a dose-response curve (Hayashi and Suit, 1971). There is no study model that postulates the clinical protocols of combining the irradiation-surgery and simultaneous bone reconstruction. We had to determine the protocol of the irradiation method to reflect the pre-operative irradiation course in clinical treatment, and this was characterized by employing "Time, Dose and Fractionation (TDF)" (Orton and Ellis, 1973) to calculate the dose and the fractionation with approximation of a human pre-operative dose.

Under this protocol, the radiation damage, including the redness of the skin and the mucous membrane of the mouth, was seen at its maximum in the 1st week after irradiation. Such damage disappeared after 2 weeks. Histologically, the damage to the blood vessels and cells recovered from 2 to 4 weeks after irradiation. From these

results, we could determine the time point for bone transplantation of this study as 2 to 4 weeks after irradiation.

Our results were obtained from experiments using rats and it is thought to be possible to apply them to clinical treatment, because the protocol for the irradiation in this experiment was calculated using TDF from human pre-operative dose and fractionation.

### The effect of irradiation on transplanted bone healing

The reasons for the retardation of bone healing in irradiated bone have been thought to be as follows: (1) disruption of osteogenic cell differentiation; (2) impairment of revascularization; and (3) failure of micro-circulation in the irradiated area. Generally, a mature osteoblast is low in radio-sensitivity (Morales et al., 1987), but the undifferentiated cells like endosteal osteogenic cells with greater proliferative activity than that of old cells are more responsive to the irradiation at the dose levels (Tonna and Pavelec, 1970). In young rats, the activities of the proliferation of osteogenic cells in the periosteum are depressed by irradiation. This is caused by reproductive cell death, cessation of proliferation and differentiation of the cell which are caused by the damage to DNA and/or the intercellular organs resulting from the release of free radicals into the tissue and the cell yielded by irradiation (Withers and Peters, 1980).

In histological findings, the appearance of the

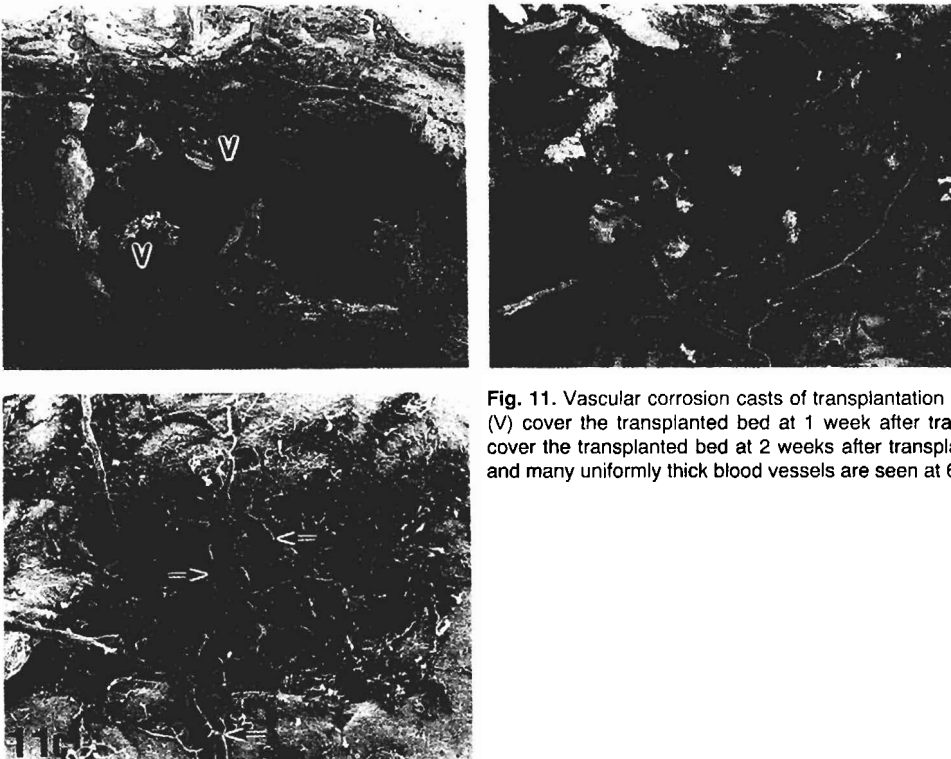


Fig. 11. Vascular corrosion casts of transplantation 4 weeks after irradiation. **a.** Blood vessels (V) cover the transplanted bed at 1 week after transplantation. x 30. **b.** The blood vessels cover the transplanted bed at 2 weeks after transplantation. x 30. **c.** The thick veins (arrows) and many uniformly thick blood vessels are seen at 6 weeks after transplantation. x 30

## *Effect of irradiation on bone transplantation*

mesenchymal cells around the transplant bed of the transplantation at 4 weeks after irradiation was equal to that of the control. In the transplantation 2 weeks after irradiation, the appearance of the cells was extremely low. The osteogenic cells in the periosteum with irradiation decreased one week after irradiation. These results suggest that retardation of bone healing after irradiation is responsible for the damage to the osteogenic cells and/or the mesenchymal cells in the periosteum rather than the effects of the osteoblasts.

Trueta (1963) showed that the blood supply played an important role in osteogenesis, and the vasculature and the osteogenic cells were closely related to each other for the bone formation (Caplan, 1989). Reinhold and Buisman (1973) reported that the radiation effect on the blood vessels reduced the proliferating capacity for capillary endothelium and that the revascularization was delayed. In this study, the scanning electron microscopy (SEM) corrosion casts revealed damage to the blood vessels which were avascularity and distortion of blood vessels in the fibrous layer of the periosteum at the first week after irradiation. These impairments continued for 2 weeks after irradiation. These results indicate that the blood supply in the periosteum and the revascularization of the transplanted area were injured by irradiation. The retardation of bone healing after transplantation in the irradiated areas was responsible for the irradiation effects on the damage to the revascularization and caused micro-circulatory failure as well as damage to the osteogenic cells.

### *The recovery from radiation injury and the appropriate time for bone reconstruction*

The damage to the osteogenic cells and the impairment for revascularization caused micro-circulatory failure after irradiation with a converted dose from the usual therapeutic doses that are recovered with time. The process of new bone formation after transplantation in the irradiated bone appeared to be normal. In the irradiated group without bone transplantation in this study, the damage to the mesenchymal cells and the blood vessels in the fibrous layer of periosteum, which were observed from the first week after the irradiation, started to recover the 2nd week after irradiation. In the 3rd and the 4th weeks after irradiation, the mesenchymal cells increased and the transformation of blood vessels returned back to normal. In the SEM of the bone transplanted group at the 4th week after the irradiation, revascularization in the early period after transplantation was similar to control, and it was superior to the bone transplanted group at the 2nd week after the irradiation. Finally, the damaged periosteal tissues of the bone transplanted group in the 2nd week after irradiation as well as the group in the 4th week after irradiation recovered from the radiation injuries. New bone formed and the transplanted bone incorporated with the host bone. As to the morphology of newly-formed bone, there were no differences

between the control group and the irradiated group except for the difference in the starting period for the new bone formation in the early stage of the transplantation. The SEM of the new bone was very similar to that of primary bone of the periosteal surface and matured bone surface, as described by Boyde (1972). There were no morphological changes in the new bone among the experimental groups at the 6th week after transplantation in this study, although there was a difference in the starting period for new bone formation. When bone formation was once started, even if it was retarded in the early stage of the transplantation of the irradiated groups, the new bone grew more rapidly than the bone growth in physiological condition. In fracture healing, the bone matrix proteins-messenger RNAs which are expressed in the bone formation phase under physiological condition and the bone morphogenetic protein-4 messenger RNA which is localized in callus-forming tissue before callus formation were transiently enhanced by the impact of fracture (Nakase et al., 1994). A similar phenomenon might occur on the healing process of bone transplantation.

We think the bone reconstruction after the therapeutic irradiation should be carried out with a considerable time elapse after the end of the irradiation therapy. Some obscure points in the mechanism of recovery from the radiation injury still remain, such as the restoration of the cell, the improvement of the circulation and the participation of the bone forming factor. These are the problems which should be solved immediately.

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