Reconstructive Ability of Prefabricated Vascularized Allo-bone Grafts Stimulated by Hydroxyapatite/collagen Composite Containing bFGF

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INTRODUCTION: In orthopedic surgery, vascularized or non-vascularized bone grafts are often selected for the treatment of massive bone defects. However, because the loss of normal bone tissue remains a major problem in the use of these methods, a new method to treat massive bone defects is needed.

To this end, we prefabricated vascularized allo-bone grafts in rat recipients and stimulated osteogenesis in these prefabricated bones. In this model, the saphenous vascular bundles of recipient rats are implanted into transplanted donor bones (1). Basic fibroblast growth factor (bFGF) is administered to stimulate bone formation in transplanted bones (2). Importantly, although bFGF stimulates bone formation and angiogenesis, it does not stimulate bone resorption. For bFGF to exert full biological activity, we used a hydroxyapatite/collagen (HAp/Col) composite as a carrier. In our previous study, we demonstrated the feasibility of using a bFGF-containing HAp/Col composite to enhance bFGF-induced osteogenic and angiogenic effects in prefabricated vascularized allobone grafts without enhancing bone resorption (2). However, whether these prefabricated allo-bone grafts have good bone union and reconstructive abilities for bone defects is yet to be examined.

The aim of the present study was to evaluate the bone union and reconstructive abilities of prefabricated vascularized allo-bone grafts implanted with the recipient's vascular bundle and a bFGF-containing HAp/Col composite.

METHODS: Donors consisted of 64 9-week-old female Sprague-Dawley rats, whereas the recipient group consisted of 64 9-week-old male Wistar rats. Graft bones were collected from the mid-shaft of the femurs of donor rats. Slits were made on the graft bones to implant the flow-through vascular bundles. Following heat sterilization, graft bones were preserved at -80 °C. Prior to the transplantation, 100 µg of bFGF was added to the HAp/Col composite. Bone grafts were then transplanted into the thigh region of the recipient rats, and the saphenous vascular bundles of the recipients were then passed through the medullary cavity of the bone grafts. The medullary cavity of the transplanted bone was filled with bFGF-containing HAp/Col.

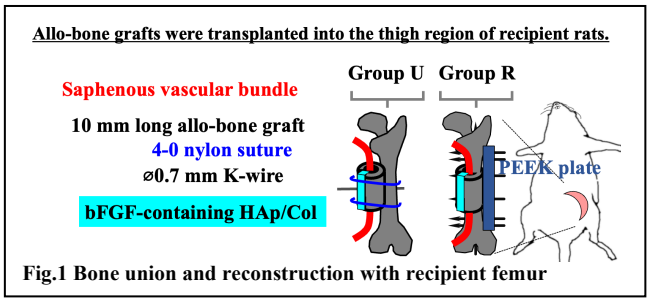

Fig.1 Bone union and reconstruction with recipient femur

For evaluating bone union ability group (Group U, n=32), prefabricated allo-bone was fixed to the femur of the recipient rat using ⌀0.7 mm K-wire pinning, followed by suturing using a 4-0 nylon suture (Fig. 1). The recipients were sacrificed 6 weeks after transplantation.

To evaluate reconstructive ability (Group R, n=32), after making 10 mm-long bone defects on the mid-shaft of the femora of recipient rats, allo-bone grafts were transplanted into the bone defects and fixed with a polyetheretherketone (PEEK) plate and K-wires (Fig. 1). The recipients were sacrificed 10 weeks after transplantation.

U-C	**R-C**	N-V allo-bone graft (Control)
U-V	**R-V**	V allo-bone graft
U-F	**R-F**	N-V allo-bone graft with bFGF-containing HAp/Col.
U-VF	**R-VF**	V allo-bone graft with bFGF-containing HAp/Col.

Table 1: Experimental group-subgroup. N-V: non-vascularized, V: vascularized

Table 1 presents the experimental groups and their subgroups. Fluorescence-labeled calcein was administered subcutaneously 2 days before animal sacrifice. After sacrifice, both the transplanted allo-bone grafts and femurs were collected from each recipient and soft X-ray images were acquired. After embedding half of the bone samples in methyl methacrylate, they were ground to a thickness of 100 µm. Subsequently, digital contact microradiographs (CMRs) were acquired and used to determine the percentage of new callus bone in each sample using the formula % CA = (total callus area/total bone area) × 100. This parameter was then used to observe callus formation. After initial evaluations, samples were further ground to 30 µm and used for microscopic evaluation of bone formation. The percentage of labeled bone surfaces [%LS = (length of labeled bone surface/total length of bone surface) × 100] were used to determine the presence of bone formation (non-decalcified specimens).

The other half of the samples were embedded in JB-4 and used for the histological evaluation of bone resorption (decalcified specimens). The percentage of osteoclast surface (%OcS: length of bone surface covered with osteoclasts/total length of bone surface ×100) and osteoclast number (N.Oc: total osteoclast number/total length of bone surface) were used to observe bone resorption (decalcified specimens: TRAP stain).

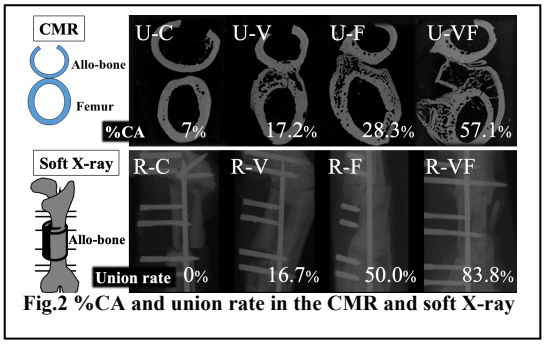

Fig.2 %CA and union rate in the CMR and soft X-ray

RESULTS:

Soft X-rays and CMR: We observed a slight increase in union rates and %CA values in subgroups V and F compared to that in subgroup C. In contrast, there was a significant increase in union rates and %CA values in the VF subgroup. (Fig. 2).

Histological Analysis:

***Calcified specimens*:** We observed a slight increase in the %LS values of subgroups V and F compared to that of subgroup C. In contrast, there was a significant increase in %LS values in the VF subgroup.

***Decalcified specimens*:** No significant difference in %OcS and N.Oc in any subgroup.

DISCUSSION: Several factors are necessary for bone regeneration, including an osteoconductive scaffold, osteogenic cells, growth factors, and mechanical environment, facets that form the "diamond concept of bone healing". (3). In the current study, allo-bone grafts were used as functional scaffolds, flow-through vascular bundles were implanted to supply osteogenic cells, and bFGF was used as a growth factor. Because our previous study showed that bone formation and angiogenesis are stimulated by bFGF without stimulating bone resorption (2), we used bFGF as a growth factor in combination with HAp/Col to prolong its effects. The mechanical environment was created via a rigid fixation using PEEK and a K-wire. The results of our radiological and histological analyses suggested significant stimulation of the bone-union ability of allo-bone grafts in which both a vascular bundle and bFGF-containing HAp/Col were added, compared to allo-bone grafts in which only a vascular bundle or bFGF-containing HAp/Col were added. Thus, this method is useful for reconstructing bone defects.

SIGNIFICANCE: This study demonstrated that both bone union and reconstructive abilities of allo-bone grafts in a rat recipient could be stimulated by adding a vascular bundle and bFGF-containing HAp/Col. Thus, the addition of a vascular bundle and bFGF-containing HAp/Col enables the prefabrication of an ideal vascularized allo-bone graft for the reconstruction of massive bone defects.

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