Prefabrication Of A Vascularized Allograft Bone In A Recipient Rat
- Combined Administration Of Basic Fibroblast Growth Factor And Bone Marrow-derived Mesenchymal Stromal Cells To Stimulate Bone Formation And Bony Union In The Transplanted Bone -

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Disclosures:
K. Yamaguchi: None. Y. Kaji: None. O. Nakamura: None. T. Yamamoto: None.

Introduction:
A previous study attempted to prefabricate a vascularized allograft bone in a recipient rat. In this model, the saphenous vascular bundles of recipient rats were implanted into transplanted donor bones. In addition, bone morphogenetic protein (BMP) was administered to stimulate bone formation in the transplanted bone. However, BMP also stimulated bone resorption in the transplanted bone. Therefore, anti-bone resorptive agents, such as bisphosphonates, were needed to prevent bone resorption caused by BMP. Unfortunately, bisphosphonates delayed bone union between the transplanted bone and recipient bone [1]. Basic fibroblast growth factor (bFGF) stimulates bone formation by promoting the differentiation of mesenchymal stromal cells (MSC) into osteoblasts. The current study evaluated whether the co-administration of bFGF and bone marrow-derived MSC (BM-MSC) stimulated bone formation and bony union in prefabricated vascularized allograft bone. In addition, we evaluated whether bFGF and BMP stimulates bone resorption.

Methods:
Twenty-four 7-week-old female Sprague-Dawley rats were used as donors, and 24 7-week-old male Wister rats were used as recipients. Seven-millimeter-long graft bones were collected from the mid-shaft of the femora of donor rats, and slits were made on the graft bones in order to implant the flow-through vascular bundle. Following heat sterilization, they were preserved at -80°C. BM-MSCs were isolated from the femora of recipient rats and cultured for 2 weeks. Two days before the bone transplantation, saline, 100 μg bFGF, $5 \times 10^4$ BM-MSCs, and a mixture of bFGF and BM-MSCs were added to the graft bone, (Table 1) and the bones were cultured for 2 days.
Next, the bone grafts were transplanted into the calf region of the recipient rats, and saphenous vascular bundles were passed through the medullary cavity of the bone grafts. At 4 weeks after transplantation, the prefabricated vascularized bone allografts were transferred onto the recipient femora, and fixation between these bones was performed using two nylon threads. Four weeks later, these animals were sacrificed. Fluorescent-labeled calcein was subcutaneously administered 2 days before sacrifice. After sacrifice, the transplanted bone allograft and associated femur were collected en bloc from each recipient.
After the bone collection, callus formation and the bony union between bone grafts and recipient bone were radiographically assessed. Both calcified and decalcified specimens were prepared for histological evaluation of callus formation and for assessment of bone formation and resorption parameters in the bone grafts. The percentage of labeled bone surface (%LS: length of labeled bone surface/total length of bone surface ×100) was used as a parameter of bone formation in the bone grafts (calcified specimens). The percentage of osteoclast surface (%OcS: length of bone surface covered with osteoclast /total length of bone surface×100) and osteoclast number (N.Oc: total osteoclast number /total length of bone surface) were used as parameters of bone resorption (decalcified specimens).

Results:
Revascularization of transplanted bone allografts
When the transplanted bone allograft and associated femurs were collected, bleeding was observed from all prefabricated vascularized bone allografts. In histological evaluations, blood flow from saphenous vascular bundles was maintained, and restructured small vessels were also observed in the bone marrow area.
Radiography
The bony union of bone grafts with saline, bFGF, and BM-MSC was insufficient, but the bony union of the bone grafts with bFGF and BM-MSC was effectively stimulated.
Histological Analysis
CMR: Significantly increased bone formation and bony union were observed in the bones in which both bFGF and BM-MSC were added (Figure 1).
Calcified specimens: %LS for the bFGF and BM-MSC groups were slightly increased when compared with the control group. On
the other hand, the %LS of the bone with the mixture of bFGF and BM-BMC was significantly increased.

**Decalcified specimens:** The %OcS of the bones of all groups did not increase. Similar observations were made regarding N.Oc.

**Discussion:**
These results suggest that the blood circulation to the transplanted bone was promoted by the implantation of the flow-through vascular bundle. Many reports suggest that many type of tissue were reproduced by bFGF, BM-MSC, or both in a variety of organizations, such as cartilage regeneration [2], but no study has utilized allograft bone in this context.

In this study, although bone formation in the transplanted bone was not stimulated sufficiently by either bFGF or BM-MSC alone, bone formation and bony union were significantly stimulated by the co-administration of bFGF and BM-MSC. This suggests that bFGF requires BM-MSC in order to effectively stimulate bone formation in the transplanted bone. Further, unlike BMP, bFGF did not stimulate bone resorption in the transplanted bone. This suggests that simultaneous administration of an anti-bone resorptive agent is not necessary when bFGF is used to stimulate bone formation.

**Significance:**
In conclusion, implantation of a flow-through vascular bundle with co-administration of bFGF and BM-MSC enables prefabrication of a better-vascularized allograft bone than that prefabricated with BMP.

**Acknowledgments:**

**References:**

| Table 1. Experimental groups (Each group : n=6) |
|-----------------|----------------|
| 1               | Saline         |
| 2               | bFGF           |
| 3               | BM-MSC         |
| 4               | bFGF+BM-MSC    |
Figure 1. Contact microradiogram shows that the increased callus formation and bony union were especially significant in the vascularized bones treated with FGF and BM-MSC.