Prefabrication of a vascularized allograft bone in a recipient rat — implantation of a flow-through vascular bundle —
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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 transected and implanted into the transplanted donor bones. Although blood circulation had been established in the transplanted vascularized bones and bleeding from the bones was observed at the time of bone collection, occasional occlusions of vascular bundles were observed in the deep area of transplanted bone. In the current study the vascular bundle was implanted in a flow-through manner in order to improve this problem. In addition, this study evaluated whether the combined treatment with bone morphogenetic protein and bisphosphonate is effective for the stimulation of bone formation and the inhibition of bone resorption in the transplanted bone.

Materials and Methods: Thirty 7-week-old female Sprague-Dawley rats were used as donors, while 30 7-week-old male Wister rats were used as recipients. Seven mm long graft bones were collected from the mid-shaft of the femora of the donor rats and slits were made on it in order to implant the flow-through vascular bundle. Following heat sterilization they were preserved at -80°C. Two hours before the bone transplantation, saline, 20 µg recombinant human bone morphogenetic protein-2 (rhBMP-2), 10^-4 M alendronate and a mixture of rhBMP-2 and alendronate were added to the graft bone. Next, the graft bones were transplanted into the calf region of the recipient rats and flow-through saphenous vascular bundles were implanted into the transplanted bones. Following bone labeling with calcein at 3 weeks after the surgery, the transplanted bones were collected from the recipients at 4 weeks after the surgery. After the bone collection, both undecalcified and decalcified specimens were prepared for histological evaluation. Undecalified specimens were stained with Villanueva bone stain and used for evaluation of bone formation using contact microradiography (CMR) and a histomorphometric analysis. The percent of labeled bone surface (%LS: length of labeled bone surface/total length of bone surface) was used as a bone formation parameter. The decalcified specimens were stained with tartrate resistant acid phosphatase (TRAP) stain and then they were used for evaluation of bone resorption using a histomorphometric analysis. The percent of osteoclast surface (%OcS: length of bone surface covered with osteoclast/total length of bone surface) and osteoclast number (N.Oc: total osteoclast number / total length of bone surface) were used as bone resorption parameters.

Results: Blood circulation had been established in the transplanted vascularized bones and bleeding from the bones was observed at the time of bone collection. CMR: Increased bone formation was observed in the vascularized bones. The increase was especially significant in the bones in which rhBMP-2 was added.

Histomorphometry: No vascular occlusion was observed in all transplanted vascularized bones. The %LS of vascularized bone with saline was increased in comparison to the non-vascularized bone. The %LS of the vascularized bone with rhBMP-2 as well as the mixture of rhBMP-2 and alendronate significantly increased. The %OcS of the vascularized bone with saline did not increase in comparison to the non-vascularized bone. However, the %OcS of the vascularized bone with rhBMP-2 was significantly increased. This increase was significantly suppressed in the bone with the mixture of rhBMP-2 and alendronate. N.Oc showed the same tendency as %OcS.

Discussion:
These results suggest that better circulation to the transplanted bone was obtained by the implantation of the flow-through vascular bundle. The bone formation in the transplanted bone also tends to be increased by this method in comparison to that by implantation of a transected vascular bundle. Although the bone formation was effectively stimulated by the addition of rhBMP-2, the bone resorption was also stimulated, simultaneously. Therefore, it may be necessary to use some anti-bone resorptive agents such as bisphosphonate to inhibit bone resorption caused by rhBMP-2.

In conclusion, using the implantation of flow-through vascular bundle, rhBMP-2, and alendronate it is possible to prefabricate a vascularized allograft bone with a rich blood supply and to thereby stimulate bone formation and inhibit bone resorption in the transplanted bone.