Abstract. Vascularized bone graft (VBG) is a form of vascularized bone marrow transplant in which the bone marrow is surgically grafted with its microenvironment intact. Due to the preservation of cellular viability, VBG have significant advantages over non-vascularized bone grafts. Free vascularized fibula grafts have superior material properties and tolerate infection. Bone healing can be accomplished in a shorter period, even in an irradiated bed. In addition to these properties, VBG has other biological advantages that are not always familiar to oncological surgeons. Hypertrophic change can be divided into reactive and adaptive hypertrophy. Early hypertrophy is associated with donor-derived cells, whereas later remodeling is associated with recipient-derived cells. VBG has significant advantages in enhancing neo-revascularization of necrotic bone. We reviewed VBG from a novel viewpoint that stems from our basic research.

Reconstruction of massive skeletal deficiencies following tumor resection remains a challenging problem for musculoskeletal oncologists. Recent advances in vascularized bone grafts (VBG) have dramatically expanded the possibilities for reconstruction of massive extremity bone defects. For long-bone defects, the most appropriate donor source of VBG is undoubtedly the fibula. In 1973, the first free vascularized fibula graft (FVFG) was carried out for a patient with cancer by Ueba and Fujikawa (1). These workers used FVFG to correct a deficiency comprising one-third of the ulna in an 11-year-old boy following resection of a neurofibroma. Subsequently in 1975, Taylor et al. reported the first two cases of reconstruction for trauma cases (2).

Several articles have reviewed the advantages of VBG for oncological reconstruction (3-5). A long-bone defect of between 6 to 10 cm is generally considered justification for a FVFG by surgeons (6). Due to preservation of cellular viability, VBGs have significant advantages over non-vascularized bone grafts. Moreover, FVFGs have superior material properties, including strength, toughness and elasticity that is two- to four-times greater than non-vascularized fibula grafts (7). Bone healing can be accomplished in a shorter period, even in a difficult environment such as a scarred or irradiated bed. FVFG can tolerate infection and also has the potential for longitudinal growth in children (3). In addition to these properties, VBG has other biological advantages that are not always familiar to oncological surgeons. In the present review, we consider VBG from a novel viewpoint that stems from our basic research.

Blood Supply of Vascularized Bone Graft Contributes to Superior Survival of Bone Cells

The major biological advantage of VBG is maintenance of a blood supply to the bone through Anastomosis of the vascular pedicle. The fibula receives two sources of blood via the periosteal and nutrient vessels (5, 8). The predominant blood supply is from a nutrient artery which enters the medullary canal from the posterior surface just proximal to the midshaft to anastomose with the endosteal artery. The endosteal vascular system supplies the vast majority of bone tissue. The periosteum receives its blood supply by way of adjacent muscle, although this source appears to provide only a minority of the total bone nutrition (9-11). However, using a canine vascularized rib graft model, Berggren et al. concluded that a bone transplant with re-vascularization of only the periosteal vessels could still supply enough vascularity to the entire bone (12, 13).
In the non-vascularized fibula graft, only the surface osteocytes may survive transfer. This has been estimated by some investigators to represent just 5-10% of the entire bone stock harvested (14). The remaining bone becomes necrotic within a week and diffuse marrow fibrosis can be anticipated (14). Although VBG is sometimes referred to as ‘living bone graft’, the osteocytes within the graft do not survive perfectly. Arata et al. investigated the biological behavior of free vascularized bone segment using a canine tibia model (15). A 5-cm segment of the proximal tibia was used for the VBG and was transferred to the groin together with nutrient vessel derived from the tibial artery, where pedicle anastomoses were performed. Interestingly, the percentage of viable osteocytes was 52.4% in the vascularized bone segment, 28.5% in the non-vascularized segment, and 66.8% in the normal control at six weeks after transplant. These differences were statistically significant. Similar to Arata et al.’s study, our experimental model using rat vascularized tibia transplant also demonstrated that a proportion of osteocytes in the cortex showed decreased staining or empty lacunae (16-18).

The process of conventional bone graft incorporation involves ‘creeping substitution’, whereby gradual vascular ingrowth, resorption and replacement of necrotic bone progressively occur (19). Excellent cell survival reduces the need for creeping substitution.

Hypertrophy Phenomenon of Vascularized Fibula Graft

One of the major advantages of using VBG is its ability for hypertrophy. This structural change increases the mechanical strength of the VBG, but is never observed in non-vascularized bone graft. Although many clinical studies have discussed hypertrophy (20), it is not clear how and why this change occurs only in VBG.

In our rat model of vascularized tibia graft, we found remarkable bone hypertrophy apparent in the radiographs after transplant (16-18) (Figure 1). Histologically, three patterns of bone hypertrophy were observed: i) cortical bone thickening due to sub-periosteal new bone formation, ii) formation of a neo-cortex separating the new bone marrow space from the original cortex, and iii) marked cancellous bone structure in the marrow space (Figure 2A-C). The specimens sometimes exhibited mixed histological patterns. Surgical intervention and the blood flow of bone grafts may have a major influence on periosteal bone hypertrophy. Goshima et al. demonstrated that temporal ischemia for 2 hours led to irreversible bone necrosis using a rat model (21). Siegert et al. demonstrated in a dog model that the blood flow in VBG was lower than that in undisturbed control bone at two days post-transplant, but increased significantly after one week (22, 23). Periosteal bone formation in the early phase after transplant may compensate for the bone loss experienced following re-perfusion.

Hypertrophy following FVFG appears to be more common in children and in the lower extremity for adults. De Boer and Wood reviewed 62 FVFGs, of which 38% showed graft hypertrophy in excess of 20% of the bone diameter (24). In cases with lower extremity reconstruction, 80% of patients showed graft hypertrophy. VBGs have not been demonstrated to overgrow the recipient bone. We reported the clinical outcomes for six FVFG cases involving the reconstruction of massive femur defects (>10 cm) subsequent to resection of skeletal tumor (25). Interestingly, the intercalary inlay graft in this series showed marked hypertrophy compared to the onlay fibula. (Figure 2A and B) In three cases with double or folded grafts, the mean De Boer index for the inlay FVFGs was 30% (range=15-54%) while for the onlay FVFGs it was 11% (range=0-20%). Mizumoto et al. demonstrated that mechanical stress accelerated new bone formation in rat vascularized fibula graft, whereas non-weight bearing bone grafts were gradually resorbed (26). These results indicate that hypertrophy of the fibula graft is associated with mechanical stimulation. The high incidence of graft hypertrophy observed several months after transplant is probably related to the mechanical stimulation provided by weight-bearing.

Wolff et al. described how bones could develop and re-align themselves to adopt the most appropriate shape with a minimum expenditure of materials (27). A change in load is likely to be a major factor that influences FVFG remodeling, with increased load associated with hypertrophy and decreased load with resorption (28).

Reactive, Adaptive Hypertrophy and Cell Repopulation in VBG

Hypertrophy of FVFG may be divided into two phases according to the time after transplant. Tamai et al. studied
Figure 2. Histologically, three patterns of bone hypertrophy were observed: A: Cortical bone thickening (upper half of the cortex) due to subperiosteal new bone formation; B: formation of a neo-cortex separating the new bone marrow space from the original cortex; and C: marked cancellous bone structure in the marrow x100.
hypertrophic changes in an experimental rat VBG model (29). They referred to active new bone formation that occurs soon after transplantation as ‘reactive hypertrophy’ caused by operative intervention. The subsequent remodeling process, called ‘adaptive hypertrophy’, occurs in response to the new dynamic environment. Our histological study revealed that initial bone hypertrophy observed one week post-transplant comprised of periosteal bone formation. Thereafter, the bone grafts exhibited various patterns of hypertrophy. Some changed their original structure of cortical bone into an architecture that resembled cancellous bone. Sempuku et al. transplanted rat vascularized tail bone into the diaphysis of femur (30). Similar to our findings, they demonstrated that the cancellous tail bone structure gradually changed to resemble that of the cortical bone of femur. This phenomenon suggests the behavior of VBG follows the principle of “when in Rome, do as the Romans do”.

The remodeling process of VBG is likely to be influenced by still unknown factors. Only a few studies have so far examined the cell replacement process in VBG at the molecular level. The lineage of living cells in VBG and the regulation of cell turnover remain unclear and require further study. Replacement by cells from the circulation or from adjoining normal tissue is possible. Undifferentiated multipotent cells may reach the bone graft by direct migration from adjacent bone marrow or through nutrient arteries. Cellular differentiation or proliferation from within VBG is also possible because the graft contains fresh bone marrow cells. Hence, the new bone cells could originate from within the graft itself or could migrate from outside.

We have established a vascularized tibia graft model in the rat using syngeneic sex-mismatched pairs (16-18, 31). The cell replacement process within the bone graft was investigated by semi-quantitative polymerase chain reaction.

Figure 3. A 23-year-old man presented with a malignant fibrous histiocytoma of the femur shaft. A 12-cm femur defect was reconstructed by 11+15 cm folded free vascularized fibula graft (A). The inlay fibula graft showed marked hypertrophy at 2-year follow-up (B).
inlay fashion (41). Primary bone union was achieved in all femur treated by irradiated bone autografts and FVFGs in all.

Krieg reviewed 13 cases with primary bone sarcoma of the also useful for the salvage of refractory cases. Krieg number of revision procedures that were required and was FVFGs in nine patients. This combination reduced the successful bony union without late fatigue fracture of the tumor resection in 14 patients (39). All but one case achieved to reconstruct massive diaphyseal bone defects following devitalized bone grafts with VBGs (33).

Several studies have been published on this subject since 2000 (33-36). The most commonly used devitalized material for oncological reconstruction is allogeneic bone graft. In some countries, devitalized autogenetic bone grafts are more commonly used because of the difficulties associated with the use of allografts (37, 38). To enhance the neo-vascularization of necrotic bone, including vascular bundle implantation. In a canine wrist model, we demonstrated a significant neo-vascularization effect of VBG towards the devitalized bone autograft (33). The proximal third of the radiocarpal bone was removed bilaterally and frozen in liquid nitrogen. Bone blood flow at the proximal pole was significantly higher on the side of the vascularized graft. Quantitative histomorphometry of the avascular proximal segment demonstrated significantly higher levels of fluorochrome-labelled osteoid- and osteoblast-covered trabecular surfaces on the vascularized side of the graft. The combination of FVFGs with massive devitalized autografts and allografts in a hybrid complex was shown to provide adequate bone stock, immediate stability and mechanical support for weight bearing, as well as enhanced neo-vascularization of the avascular graft (43). This was observed in both clinical and research settings.

Hybrid Combined Technique Using Allo- or Autograft with VBG

In addition to superior cell survival and mechanical properties, VBG has other significant advantages, including the re-vascularization of necrotic bone and improved local blood flow. Several studies have been published on this subject since 2000 (33-36). The most commonly used devitalized material for oncological reconstruction is allogeneic bone graft. In some countries, devitalized autogenetic bone grafts are more commonly used because of the difficulties associated with the use of allografts (37, 38). To enhance the neo-vascularization of non-viable allogeneic or autogeneic bone grafts, dead bone must be converted into living bone. The simplest and most practical method of achieving this aim is to combine devitalized bone grafts with VBGs (33).

Chang et al. used a combination of FVFGs and allografts to reconstruct massive diaphyseal bone defects following tumor resection in 14 patients (39). All but one case achieved successful bony union without late fatigue fracture of the fibula graft. Belt et al. used irradiated bone allografts and FVFGs in nine patients. This combination reduced the number of revision procedures that were required and was also useful for the salvage of refractory cases. Krieg et al. reviewed 13 cases with primary bone sarcoma of the femur treated by irradiated bone autografts and FVFGs in inlay fashion (41). Primary bone union was achieved in all cases after 9 months without any complication and with a successful average MTS score of 85% (42). These authors concluded that devitalized bone grafts with concomitant VBGs represent a promising biological alternative for intercalary reconstruction. We reviewed six patients from our institute who underwent curative bone tumor resection followed by FVFG in combination with irradiated autograft (34). Radiological and functional outcomes were excellent in all five patients who underwent tibial shaft reconstruction; in the patient with an ulnar shaft tumor, the distal third of ulna was resorbed due to the short length of grafted scapula.

Table 1. Two phases of hypertrophy of vascularized bone graft in rat tibial transplant model.

<table>
<thead>
<tr>
<th>Hypertrophy</th>
<th>Terms</th>
<th>Time after therapy</th>
<th>Stimulation factors</th>
<th>Cell lineage</th>
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<tbody>
<tr>
<td>Early phase</td>
<td>Reactive reriosteal</td>
<td>Within 6 weeks</td>
<td>Surgical intervention, less blood flow Weight-bearing (lower extremity), age, circumstance (cortical or cancellous bone)</td>
<td>Donor (local factor)</td>
</tr>
<tr>
<td>Later phase</td>
<td>Adaptive</td>
<td>After 6 weeks</td>
<td></td>
<td>Recipient (systemic factor)</td>
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There have so far been several experimental studies of neo-vascularization of necrotic bone, including vascular bundle implantation. In a canine wrist model, we demonstrated a significant neo-vascularization effect of VBG towards the devitalized bone autograft (33). The proximal third of the radiocarpal bone was removed bilaterally and frozen in liquid nitrogen. Bone blood flow at the proximal pole was significantly higher on the side of the vascularized graft. Quantitative histomorphometry of the avascular proximal segment demonstrated significantly higher levels of fluorochrome-labelled osteoid- and osteoblast-covered trabecular surfaces on the vascularized side of the graft.

The combination of FVFGs with massive devitalized autografts and allografts in a hybrid complex was shown to provide adequate bone stock, immediate stability and mechanical support for weight bearing, as well as enhanced neo-vascularization of the avascular graft. This was observed in both clinical and research settings.

The Future: VBG as a Source of Bone Marrow Transplant

VBG may in the future be used as a source of bone marrow cells for transplant (44-47). Bone marrow transplant is currently one of the standard treatments for leukemia. Recently, the vascularized bone/bone marrow transplant was recognized as being a better source for the reconstitution of hematopoietic cells in the myeloablated rat model than transplantation of bone marrow cells alone (48, 49). VBG represents a vascularized bone marrow transplant in which the bone marrow is surgically grafted with its microenvironment intact. The donor bone marrow cells and
stroma can, therefore, function immediately upon transfer (44-47). This transplant model is likely to be a better source for bone marrow reconstitution than the transplantation of cellular bone marrow cells alone.

We previously described the cell reconstitution process of lymphoid tissues in the recipient using a rat limb transplant model (50, 51). Firstly, some type of recipient pre-transplant conditioning is required in order to create a ‘clean space’ for the engraftment of donor hematopoietic cells into the recipient bone marrow (47, 48). This can involve total-body irradiation or cytoreductive chemotherapy. To stimulate cell movement from the VBG, we administered granulocyte colony-stimulating factor to the pretreated recipient immediately after transplant. One year after transplant, the recipient bone marrow was reconstituted by donor-derived cells. This phenomenon, referred to as macrochimerism, is important for the induction of immunotolerance.

References


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