Histological Findings in a Human Autogenous Pasteurized Bone Graft

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Abstract. Background: Autogenous bone graft after pasteurization is one of the most valuable procedures for reconstruction of large bone defects following excision of malignant musculoskeletal tumors. To date, there have been no documented histological reports on pasteurized bone grafts, apart from short-term histological results. Case Report: We treated a 27-year-old male with a periosteal chondrosarcoma of the tibia by wide excision and reimplantation of the large pasteurized bone. Biopsy specimens harvested from the pasteurized bone over 3 years after reimplantation were evaluated histologically. The graft cortices remained totally necrotic with empty osseous lacuna, whereas the architecture of the acellular cortical bones was still maintained without microfractures. Deposited seams of woven bone existed focally on the surface of the acellular trabeculae. Conclusion: Our medium-term histological outcome suggests the limitations of incorporating a pasteurized bone graft, but also advocates its role as a useful temporary material for the reconstruction of massive bone defects.

In limb-sparing surgery for malignant musculoskeletal tumors, bone grafts are generally used for the reconstruction of large amounts of segmental bone defects, including fresh (non-vascularized) or vascularized autogenous bone grafts, allogenic bone grafts and varied oncologically sterilized autografts (1-8). A fresh or vascularized autograft is the ideal bone graft to preserve biological and biomechanical properties. But the application of this procedure is restricted by the limited resources and the adaptability of the donor bone to the recipient site. An allograft may well be the most common biological material for the restoration of massive bone defects in Western countries. The supply of allografts, however, is limited in Asian countries, because the concept of bone donation is not widely accepted for cultural reasons. There are also concerns about immunological response and transmission of diseases (human immunodeficiency virus and hepatitis virus).

These disadvantages can be overcome by reimplantation of autogenous bone grafts after extracorporeal sterilization such as heating and irradiation to devitalize sarcoma cells. This method has the definite advantage of being anatomically adaptable to the host bone. Numerous studies have attempted to develop an adequate procedure to sterilize tumor-infiltrated bones without compromising the biomechanical properties and osteogenic potential. Among methods of thermal sterilization, pasteurization involving the heating of resected bone segments at a low temperature of 60°C for 30 minutes has been proved to preserve bone-inducing ability, whereas autoclaving using high temperatures impairs bone healing (9-12).

Recent studies on the clinical application of pasteurized bone grafts for the treatment of malignant solid tumors described encouraging oncological outcomes and suggested incorporation of the pasteurized bone graft, based upon radiological evaluation (1, 5, 8). However, little is known about the underlying histological incorporation process of a pasteurized bone graft. We here present the medium-term histological results of a human pasteurized bone graft used for the reconstruction of substantial bone loss in limb-sparing surgery.

Case Report

A 27-year-old male had a slowly growing small mass in the lateral aspect of the left lower leg. Five months later, he was referred to our department because open biopsy performed at a nearby hospital indicated a suspected extraskeletal
chondrosarcoma. Plain radiographs showed flocculent calcifications around the lateral surface of the tibia (Figure 1A). Magnetic resonance images showed a well-demarcated soft tissue mass arising from the lateral aspect of the tibia (Figure 1B). The tumor was excised with a wide margin, which included the 19.5-cm-long proximal half of the tibia excluding the proximal epiphysis. The extracted tibia was stripped of the tumor and surrounding soft tissues and heated in a sterile water bath filled with saline for 30 minutes at a low temperature of 60°C. It was subsequently reimplanted into the original anatomic site and secured with an external fixation device. A 22 cm vascularized fibula from the unaffected limb was transplanted laterally along the pasteurized bone (Figure 2A). The pathological diagnosis was a grade 2 periosteal chondrosarcoma. No further adjuvant therapy was applied.

Serial follow-up radiographs did not show bone union at the pasteurized graft-host junctions even five months after the implantation. In salvage surgery, the yellowish pasteurized bone without blood oozing indicated dead bone. Ample amounts of cancellous bone chips from the iliac crest were impacted into the gaps of both the graft-host junctions. In addition, an ipsilateral fibula holding blood distribution was translocated along the preexisting free vascularized fibula to reinforce junction stability. Dynamic compression plating and external fixation was used for rigid immobilization. Seven months postoperatively, solid bony unions at both the graft-host interfaces were achieved radiographically (Figure 2B).

Three years and four months after the primary surgery, a pin-hole-sized wound erupted at the center of the anterior lower leg. Examination of exudate cultures confirmed a Staphylococcus capitis bacterial infection. Radiographs
revealed a round progressive radiolucent lesion in the distal portion of the pasteurized bone, located right below the wound (Figure 2C). The infected graft was treated by removing sequestrated bones, by 3 weeks of continuous irrigation throughout the lesion and by administering systemic antibiotics. Microscopic investigation of the specimens indicated chronic osteomyelitis in the distal part of the pasteurized bone. At this time, with the patient's informed consent obtained prior to the procedure, several biopsy specimens were harvested from the proximal site of the pasteurized bone, where no finding of infection was verified histologically. The graft cortices remained totally necrotic with empty osseous lacuna. There was no evidence of revascularization and new bone formation, whereas the architecture of the acellular cortical bones was still maintained without microfractures (Figure 3A). Fibrovascular tissues occupied the medullary cavity and focally deposited seams of woven bone were observed on the surface of the acellular trabeculae (Figure 3B).

After this, the primary tumor was well controlled, but he later suffered from an astrocytoma in the brain, which consequently caused his death 8 years following the resection of the extraskeletal bone tumor.

Discussion

A number of experimental studies have been undertaken to histologically evaluate extracorporeally heat-treated bone grafts in animal models (9-12). Revascularization and new bone formation were observed in the grafted bones treated at a temperature < 70°C. Shimizu et al. (11) found newly formed bone even in the center area of the grafted bone. However, those animal studies used small bone fragments of less than 10 mm in length, which are expected to differ from clinically relevant massive pasteurized bones in the process of incorporation and remodeling. Just one study on the histological analysis of a retrieved human pasteurized bone has been documented in English (13). A whole knee joint was treated by extracorporeal pasteurization in a patient with an osteosarcoma of the proximal tibia. The patient developed an infection, resulting in thigh ablation 9 months after the implantation. Histological examination of the graft showed incorporation of the pasteurized bone with the host cortical bone through the continuous lamellar structure, and osteocytes and microvascular migration into the pasteurized cortical bone. Newly formed bone was obvious inside or adjacent to fibrous tissue in the medullary space, suggesting that the first step in bony formation in the pasteurized bone might be the migration of mesenchymal stem cells from the contiguous normal medullary cavity. Several authors reported histological results in human allografts that had been in situ for a long period (14-17), because block allografts are widely applied not only for skeletal reconstruction after malignant tumor resection but also for acetabular reconstruction during revision arthroplasty. Enneking et al. (14) detailed a retrospective study of a large series of massive human allografts, retrieved from 2 to 156 months after implantation, with radiographic and histological techniques. They stated that repair of a necrotic graft matrix occurred in two fashions;
surface (or external) repair and internal repair. External repair consisted of the apposition of a thin seam of host bone on the outer surface of the graft, coating about 80% at two years. Internal repair was confined to the ends and the periphery of the cortices and penetrated so slowly that only 15% to 20% of the graft was repaired by five years, after which deeper repair seldom occurred. Such histological findings are consistent with those reported by other authors. On the other hand, it should be noted that in the Enneking's study this general picture was common to all repair grafts for two to three years, while specimens retrieved after three years had a much wider variation in the extent of cortical repair. In a few specimens, progress was steady until virtually the entire graft had been repaired.

The current specimen demonstrated total necrosis in the cortices of the pasteurized bone graft without any signs of repair at all. In contrast, in the narrow space, deposited seams of reparative bone between the fibrous tissues and the acellular trabeculae still remained revealing the incorporation potential of the pasteurized bone graft, as noted in the report on a pasteurized whole knee joint graft (13). Interestingly, the structure of the acellular cortices was well preserved in the absence of the microfractures observed in allografts (15, 16). The mechanical strength of pasteurized bone compares favorably to that of allogenic bone, when considering the risk of fatigue fractures. Our samples, taken from a massive pasteurized bone graft over a 3-year period after the implantation, indicate the limitations of incorporating a pasteurized bone graft, but also advocate its role as a useful temporary material for the reconstruction of large bone defects.

The medium-term histological outcomes determined by our study have provided some insights into the mechanism of pasteurized bone graft incorporation, although long-term follow-up biopsies would be needed to assess the behavior of pasteurized bone grafts.

References


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