Reconstruction with Pasteurized Bone and Joint Following Musculoskeletal Tumor Resection

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Abstract: For skeletal reconstruction after resection of malignant bone tumors, hyperthermia-treated bone has been used with more favorable results by the method of pasteurization (60-65°C for 30 min) over autoclaving or boiling. Experimental studies demonstrated clearly that pasteurization destroys malignant cells while preserving the bone-inducing properties. Clinical investigations reveal few recurrences related to pasteurized tumor tissues. Complications include non-union, fracture, absorption, and infection. The operation protocol, while not ideal, is superior to that of other reconstruction procedures because many fundamental complications, such as the low long-term survival of massive prosthesis, the lethal risk of transfusions, such as HIV, and immunological responses of the allograft, can be avoided.

Key words: bone and soft tissue sarcoma, pasteurization, limb-salvage, reconstruction

Introduction

In the treatment of malignant musculoskeletal tumors, recent advances in diagnostic imaging1,2), neoadjuvant chemotherapy3), and operative techniques4,5) have encouraged orthopedic surgeons to shift the surgical techniques from amputations to limb-salvaging procedures. The survival rate of patients with primary malignant bone tumors has improved to approximately 70%, and modern protocols of multi-disciplinary treatment with limb-salvage procedures reveal similar oncological results to those of previous ablative surgery6). From an oncological point of view, therefore, limb-sparing operations are widely used for musculoskeletal neoplasms. Since most of the affected bone must usually be resected, a resultant large osseous defect is usually left. The limb-sparing surgery used so far for the reconstruction of the osteoarticular structure has included arthrodesis with bone graft, endoprosthesis implantation, reconstruction using allografts, and immediate reimplantation of resected bone with extracorporeal treatments, such as freezing, heating, or irradiation. However, no golden standard exists, and the indication is still controversial7).

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Widely used reconstruction procedures and their complications

**Arthrodesis with bone graft**

Local resection and arthrodesis for tumors was first described by Lexer in 1908. Subsequent reports have revealed frequent complications including infection, non-union, and late fatigue fracture. To overcome these problems, several techniques have been developed. Enneking and Shirley utilized a customized bent, a fluted rod, providing a stable extremity that permitted resumption of a vigorous life-style within approximately one year. Arthrodesis of the knee with a vascularized fibular rotatory graft has been reported by Rasmussen et al. These researchers suggested that, although the routine use of vascularized grafting as the initial procedure is not indicated, a vascularized fibula transfer should be considered when there is massive loss of bone structure after a non-united arthrodesis or a resection of a malignant bone tumor. Usui et al. described the results of limb-saving surgery using a vascularized fibular graft for osteosarcoma and suggested that this method should be considered for limb rescue in young patients with osteosarcoma because of the high durability of the graft.

**Endoprosthesis implantation**

Surgeons who treat primary bone neoplasms of the extremities are primarily concerned with the survival of their patients and the functionality of the remaining limbs. To preserve the joint function after resection of a massive osteoarticular lesion, endoprosthetic replacement has been performed for juxta-articular skeletal malignant lesions. Modular and extendible prostheses have been devised for growing children. Although an excellent functional outcome was observed for a few years, there is a high rate of complications. A long follow-up revealed that the most common reason for implant failure was aseptic loosening, which occurred at a relatively high rate of 27% or 9.9% in distal femoral replacements and only 2.3% in proximal femoral implants. On the other hand, infection occurs at high rates, especially for proximal tibia lesions. Another major complication is fracture of the bone or implants. As a result, survival rate of massive prosthesis is suggested to unexpectedly be low. A recent report described that the use of extendible prostheses of the proximal tibia remains an experimental procedure.

**Allografts**

In the 1960s, there were several reports of clinical series with the use of frozen massive osteoarticular allografts. Since then, osteoarticular allografts have been widely used for the reconstruction of malignant bone tumors. Allograft reconstruction has some apparent advantages over prostheses, including a physiological joint surface that promotes biological integration, reduction of additional resection for size adjustment, and preservation of the opposite side of the joint, which offers advantages, especially, to children with a growth plate. However, several serious complications occur with allograft transplantation. Fracture and non-union are closely related to immune responses to allografts. These are augmented by adjuvant chemotherapy. Infection is also frequently observed. Recently, Rodl et al. suggest that allograft application in the management of malignant bone tumors should, at best, be considered a temporary solution, since low-rate of long survival of the graft. Most seriously, the lethal risk of transfections, such as HIV, still exists with an allograft.
Autologous reimplantation

These disadvantages can be avoided by using autologous reconstruction and immediate reimplantation of resected bone following extracorporeal treatments, such as irradiation, or heating, for devitalization of the tumor-infiltrated segment. The functional results have not been satisfactory because of insufficient bone restructuring, although the lethal effect on the tumor cells was evident. A specific problem is that autoclave-treatment results in the degeneration and absorption of grafted bone. To preserve the bone-inducible properties, the heat is reduced to 65°C, and hyperthermia-treated affected bone, designated as pasteurized bone, is used for the reconstruction of a malignant bone tumor. Reconstruction with pasteurized bone has been developed and implemented mostly in Japan. In this article, theoretical and experimental evidence on the efficacy of pasteurization is provided, and the clinical results are reviewed.

Cytotoxic effects of pasteurization

The cytotoxic effect of pasteurization has not been extensively investigated. There are two good reports describing the cytotoxic effects of pasteurization for neoplasms. Inokuchi et al. heated VX2 carcinoma cells and demonstrated the lack of tumorigenesis of the cells after heating at 50°C for 15 minutes. In addition, no viable cells were observed with the trypan blue exclusion test after heat treatment at 60°C for 10 minutes in FM3A and Raji cells, and neither cell line revealed colony formation in soft agar after heating at 55°C for 10 minutes. Rong et al. reported a sophisticated investigation, in which a radioresistant (heat-resistant) chondrosarcoma block was used. The tumor blocks were heated to 40°C-80°C for 10, 20, or 30 minutes. No tumor growth was found in the group at 50°C for >20 minutes or at >60°C, and the tumors treated in the 50°C for 10 minutes began to grow at 3 weeks after transplantation.

Another critical factor influencing the heating effects is the heat-transfer property in bone tissue. Manabe examined the intraosseous temperature curves of an intact human tibia and suggested that a 30-minute water bath at 60°C provides safe pasteurization. Inokuchi et al. investigated the rising temperature curve of a porcine tarsalia and showed that the curve plateaued after approximately 10 minutes in a water bath. In summary, it appears that 60°C is necessary to achieve a cytocidal effect and that a 30-minute water bath is required for the heat to be transferred throughout the entire bone segment.

Preservation of bone-inducible capability after pasteurization

Bone induction is thought to be produced by the response of the mesenchymal cells in the recipient bed to the bone-inducible proteins such as bone morphogenetic protein (BMP) left in the bone implant. Bone induction is assumed to occur as a response of the mesenchymal cells in the recipient bed to bone-inducible proteins, such as bone morphogenetic protein (BMP) left in the bone implant. In thermal exposures of more than 70°C or irradiation sterilization, the biological activity of the BMP is destroyed. Based on these biological features of bone induction, Inokuchi examined and clearly showed histological osteoinductivity after 60°C heating, which was not found after boiling at 100°C. Histological findings of a bone induction process after pasteurization have been investigated in detail by
Ahmed et al. After pasteurization, marrow stroma, endosteval lining cells, and periosteal lining cells which are the main contributors to osteogenesis of the grafts, were symmetrically distributed at the 8th week. At the 16th week, a moderate increase in the diameter of the trabeculae and bone remodeling by osteoclasts and osteocytes were found. There was no significant difference between the groups that were pasteurized and those that were not given heat treatments in terms of the reorganization of the grafts. These histological findings were confirmed by radiological findings showing that the bone was gradually resorbed, causing temporary porosity, and that long-lasting remodeling followed. At 12 weeks, the callus formation was evident with partial cortication, and at 16 weeks, complete union was obtained. The histological and radiological processes were also observed in normal temperature-treated bone without significant difference as compared with pasteurization-treated bone. These remodeling processes were not found in boiled or autoclaved bone.

Manabe investigated the mechanical strength of pasteurized bone compared to autoclaved, boiled, and fresh bone and demonstrated that the weight curves of bending tests and the torque curves of torsion tests revealed that the curve patterns and mechanical strengths of pasteurized bone were not different from those of intact bones but are markedly different from those of boiled and autoclaved bones. Preservation of bone induction properties in pasteurized bone may be superior to that in boiled or autoclaved bone from biological and mechanical points of view.

The origin of the mesenchymal cells is still unknown. Histological investigations of pasteurized medullary bone obtained from human materials demonstrated that fibrous tissue, and not adipose tissue, migrates into the medullary space. New bone formation was obvious inside or adjacent to this fibrous tissue. Thus, the first step of the bone formation in the pasteurized bone might be a migration of mesenchymal stem cells existing in the contiguous normal medullary cavity followed by proliferation of the bone precursor cells. The differentiation into osteocytes may then induce cancerous bone and cortical bone formation from within.

Effect of pasteurization for non-osseous tissues

Most malignant bone tumors are located in the juxta-articular position. This could cause surgeons to use pasteurized bone in conjunction with resurfacing prosthesis, or to limit the use of pasteurized bone to the diaphyseal region. However, Hernden and Chase demonstrated considerable success with an autologous whole knee-joint graft in 1952. Thus, the effects of the extracorporeal pasteurization method on autologous whole knee-joint grafts were investigated to determine its validity for reconstruction following treatments for bone tumors in the juxta-articular region. One month after surgery, the meniscus showed hypo-cellularity in both pasteurized and non-heated groups without significance, indicating that reimplantation but not heat-treatment results in dead condition. Cellularity recovered significantly at 4 months in both groups. A significant recovery of cellularity was also observed in the ligaments in both groups. There was no significant difference between the groups regarding the structure and cellularity of the ligaments.

Hernden and Chase reported destructive changes similar to osteoarthritis in the replanted autologous knee joint, suggesting the lethal effect of reimplantation of a joint on the articular cartilage. This destructive process in the cartilaginous structure has not been observed one month after treatment; in the
later phase, however, the histological score decreased in the heat- and non-treated groups, suggesting that
degenerative changes may occur according to how long the vascular system is disturbed 35). On the other
hand, two months later numerous vascular spaces just underneath the calcified zone appeared in both
group commonly 35). These findings provide reason for surgeons to believe that a regeneration of the
cartilage by newly formed chondrocytes is derived from these vascular spaces, as suggested by Campbell
et al.38). Further examinations are required to elucidate whether the newly formed structure is renewed
cartilage with an original function.

Clinical application of pasteurized bone reconstruction

Several clinical articles describing the utility of pasteurization for the reconstruction of malignant
musculoskeletal tumors have recently been published. Bones pasteurized for reconstruction were
mandibular bones 31), costal bones 39), long extremity bones 40–44), and those invaded by adjacent soft
tissue sarcomas 45). Oncological results are fundamental and most important. No recurrence was
reported in the literature examined 31) 36) 39) 41) 44) 45). On the other hand, Manabe reported a recurrent
tumor mass after reconstruction with pasteurized bone. However, the recurrent lesions did not involve
the pasteurized area, and the recurrence was then thought to be due to residual subcutaneous tumors in
the case treated with intralesional excision 40) or skip metastasis 42). Lethal effects on tumor cells were
directly demonstrated by histological examination 36). The cytocidal effect of pasteurization (65°C for 30
min) has been established for malignant bone tumors.

The bone union rates ranged from 62.5 to 91.7% 40 42–44). Callus formation bridging the host and
graft bones was detected 3 months after surgery 36). The radiological bone union was visible 6 months
after surgery 40 42). Complete union was considered to have occurred at 3 to 7 months 44) or 6 to 10
months 45) later. Ehara et al. examined radiographic and scintigraphic features after a pasteurized
intercalary autogenous bone graft and demonstrated two types of healing. One occurred without an
evident periosteal callus, and the healing was complete in a relatively short time (7-18 months). The
other was characterized by periosteal callus formation in an early phase and a slow progression of the
callus formation into the opposed host and graft bones 43). The serial scintigraphical examinations
suggest that the uptake at the cortical rim, but not the host-graft junction, may enable a prognosis because
all cases showed callus formation 43). In comparisons with an autoclaved bone graft, revascularization
and incorporation of the graft were suggested to occur earlier in the pasteurized bone graft 43) 45).

Major complications, other than non-union, include fracture 40 42-44), absorption of the grafts 40 44-45),
and infection 36) 40 42). In two of the 12 patients (16.7%), fracture occurred because of an accidental
trauma within one year of the surgery 44). Manabe et al. reported that 25 percent of the patients
developed fractures 42). Most of these fractures were treated with conservative or internal fixations,
indicating that the complications were controllable 40). On the other hand, a fracture is one of the most
serious complications of a bone allograft and was thought to occur as a result of revascularization of the
allograft cortex 28). Chemotherapy augmented the rate of fracture at the junction of the allograft 29).
This type of fracture may be due to allogenic immunological responses 34). In contrast to an allograft,
an excellent bone union without any immunological reactions at the graft-host junction has been
demonstrated by histological evaluation of pasteurized bone graft 36). The risk of fracture or non-union
at the graft-host junction may be less significant in pasteurized bone than in an allograft. However, fracture is still a major complication in pasteurized bone. Thus, additional mechanical supports, such as a vascularized fibula (Fig. 1), should be devised in the future.

Manabe et al. found infection in 40% of the patients with pasteurization (42). In this report, one of two patients with tibia osteosarcoma developed infection resulting in a thigh amputation (36). Infection is the most important and devastating complication in the reconstruction of proximal tibia bone sarcomas and is not unique for pasteurization. Mnaymneh et al. investigated 70 patients treated with osteoarticular allografts and showed that all infections occurred in the proximal tibia (25). Grimer et al. also reported a high incidence of infection in the limb-salvage procedure for malignant tumors occurring at the proximal tibia (17). However, they reported dramatic reduction in the infection rate by applying a medial gastrocnemius pedicle flap. We avoided infection by using this technique (30). An active application of a musculo-cutaneous flap should be introduced for reconstruction using pasteurization as well (42).

Articular cartilage destruction was observed in patients with pasteurized bone and cartilage one year later (42). This was also suggested by the experimental study (35). Further investigation for reconstruction of the degraded articular cartilage will be necessary when the pasteurization is applied for bone neoplasms arising in the juxta-articular area.

Conclusion

Pasteurization is not ideal. There are many problems to be overcome, such as the long time required for rehabilitation before gait independence is achieved. Endoprosthesis replacement provides functional results earlier without gait support. Grimer et al. reported that patients who died from their disease within two years of the initial operation were mobile and walking throughout that time and that the

Fig. 1. A 12-year-old boy with an osteoblastic lesion in the distal metaphysis of the femur with an extraskeletal lesion (arrow) (A). After chemotherapy, an autologous pasteurized distal femur with a joint surface was used for reconstruction (B). An arrow indicates the junction between the host and graft bones. A vascularized fibula was fixed by two screws along the medial side of the femur. Note the vascularized fibula fragment was implanted inside of the pasteurized bone graft. On the scintigraphical examination taken 4 months later (C), a clear uptake is shown in the vascularized fibula, suggesting the supporting effect of the fibula. No uptake was seen in the pasteurized bone at that time.
procedure was estimated to be quite successful for the patients. In addition, joint cartilage degradation may be unavoidable in the future, as shown in animal experiments. Salvage operations, such as surface-type total knee arthroplasty or arthrodesis, could be considered if necessary. Alternatively, transplantation of differentiated chondrogenic cells would be applied into the heat-treated cartilage tissue. However, reconstruction with a patient’s own bone, cartilage, and ligamentous structure may make this operation superior to other reconstructive procedures since many fundamental complications, such as the low survival rate with a massive prosthesis, the lethal risk of transfections, such as HIV, the many kinds of immunological responses in the allografts, and the lack of availability of allografts, can be avoided.

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骨軟部腫瘍切除後の再建におけるパスツール法

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要旨：骨悪性腫瘍の切除後における骨格再建に、体外温熱処理骨が使われている。近年、煮沸骨やオートクレープ処理骨に比べて、パスツール処理（60-65度、30分）骨がより好ましい結果が得られることが報告された。実験結果から、パスツール処理は骨誘導能を維持させるながら、悪性腫瘍細胞を死滅させることが明らかとなった。臨床例の報告には、パスツール処理組織に関連する再発の報告は認めていないう合併症として、癒合不全、骨吸収や感染が見られた。パスツール法は、現在のところ理想的な手術法とは言えないが、他の再建術に見られる、本質的な合併症、例えば人工物の長期生存が低いこと、同種移植に報告されるHIVなどの致死的な感染の危険や免疫異物反応をさけることができることを踏まえると、これらの再建術に優る方法と考えられる。