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Review on Bone Tumour Sterilization Using Pasteurization for Autologous Bone Reconstruction

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Introduction

Malignancy of mesenchymal in origin is referred to as sarcoma, and the most common primary bone sarcoma is osteosarcoma. It is an osteoid-producing malignancy and usually high-grade tumour. Primary bone tumours account for 0.2% of all malignant type tumours and of all primary tumours, 15% are bone tumours[1,2].

According to World Health Organization (WHO) in 2002, the estimated annual incidence of osteosarcoma was approximately 4–5 per million, occurring most commonly in the second decade of life (11–20 years old) [3]. Stiller et al reported that in European population, of 5572 cases, osteosarcoma was the most frequent subgroup, accounting for 52%. American Cancer Society reported over 1000 cases diagnosed per year in United States. A total of 5016 patients with osteosarcoma had been recorded from the Surveillance, Epidemiology, and End Results (SEER) program from 1975 to 2017[4] and the frequency has been increasing 0.3% each year over the last decade[2]. In Malaysia, study at University Malaya Medical Centre from 1997 to 2011 reported 128 cases of osteosarcoma treated in 14 years [5], and Wahidah et al. reported 128 patients diagnosed with osteosarcoma over period from 1995 until 2006 in Hospital Universiti Sains Malaysia[3].

Multidisplinary modern protocol was introduced in the treatment of bone sarcoma and this had improved the survival rate markedly, from less than 20% to 80% [2,3,6]. These protocols involve better diagnostic imaging, chemotherapy (neoadjuvant and adjuvant) and better surgical technique in achieving wide margin resection; hence making the limb preservation surgery as an alternative to limb amputation as previously done [2,6,7].

In osteosarcoma, limb preservation surgery does not increase the rate of recurrence provided that adequate margin surrounding the tumour is been resected [2,7]. Osteosarcoma in particular usually involves metaphyseal area of the bone (Figure 1), hence during surgery**,** the resection of the bone will involve the metaphyseal together with the joint (osteoarticular) [1,2]

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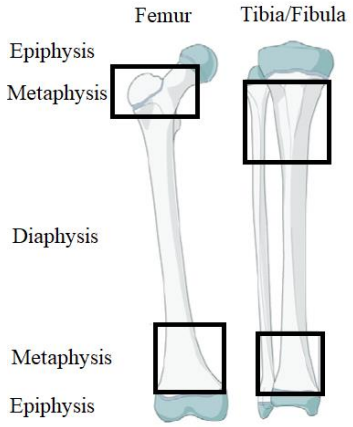

Figure 1: Diagram of femur and tibia bone showing epiphysis, metaphysis and diaphysis area of the bone.

After a bone tumour resection surgery, methods of bone defect reconstruction varies from endoprosthesis, bone allograft and bone autograft [8,9]. Each of the methods have their own advantages and disadvantages (Table 1). Loosening, breakage and wear are the problems faced by using prosthetic replacement especially in long-term[6]. Allografts bone reconstruction is another method frequently used after bone resection surgery. However, these allografts require a large scale bone bank system, risk of immunological response and transmission of diseases; and risk of graft fracture[10,11]. It takes a long time to incorporate due to denaturing of the osteoinductive proteins in the processing [10,11]. It is also a problem in countries where the deceased organ donor rate is low**,** especially in Asian countries[12].

Table 1: Summary of advantage and disadvantage of each bone tumour reconstruction methods

Methods of bone tumour reconstruction	**Advantage**	**Disadvantage**
Endoprosthesis	Widely available, modular, immediate stability	Implant failure due to loosening, breakage or wear, expensive
Bone autograft	Biologic reconstruction, readily available, cheap, no risk of disease transmission or immunologic response	Risk of infection, non union, graft fracture and resorption
Bone allograft	Biologic reconstruction	Bone bank facilities, risk of disease transmission and immunological response, risk of graft fracture, infection, non union and resorption

Bone autograft as reconstructive method

Bone autograft means using tumour bone that had been resected from the patient and subject the bone graft under certain method of sterilization to eradicate the tumour cells before reimplanting it back to reconstruct the bone defect. The process of eliminating the cancer cells is termed sterilization. Bone autograft can be used as intercalary reconstruction or intraarticular reconstruction; with other resurfacing method, primary arthrodesis or osteochondral especially in non weight bearing joints. They are usually reimplanted directly after sterilization process with suitable implant osteosynthesis [6].

Bone autograft using sterilized tumour bone has an obvious benefit in countries where organ donation is not widely accepted and practised especially in Asian countries. Furthermore, the dimensions of the

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autograft bone will precisely match the host bone [9]. It has no risk of transmission of diseases or issues with immunogenicity and graft rejection.

Bone autograft sterilization technique has been widely debated. The main concern of reusing a pathological bone in an oncology patient is the possible remnants of malignant cell in the resected bone. For autologous bone graft to work, total eradication of malignant cells in the graft is imperative. The choices of sterilization technique varied depending on the health care settings, availability of specialized equipment and level of operator specialty.

On the other hand, reimplanting sterilized tumour bone is not advisable in tumour bones which are structurally weak, osteolytic type of tumour and tumour bone with pathological fractures; and in these type of cases, other means of reconstruction were used such as endoprosthesis [13]. SY Lee et al in 2017 mentioned that they generally used autograft bone method in osteoblastic tumour, osteolytic tumour with less than one third cortical involvement and avoided when the tumour showed extensive osteolytic pattern [14].

Sterilization of the bone graft can be achieved by several techniques mainly irradiation, pasteurisation, cryotherapy with liquid nitrogen, boiling or autoclaving. Multiple studies had been conducted regarding the optimum sterilization technique in the oncologic orthopaedic setting. These studies demonstrated contradictory findings as comparisons of the methods used might not always be similar [15].

Among all sterilizing methods, pasteurization method is the simplest and least expensive. Furthermore, the inavailability of radiation services, equipment and room for cryotherapy and autoclave increases the significance of pasteurisation which can be carried out without the need for extensive infrastructure facilities[11,16–18].

The objective of this article is to gather data on sterilizing method of resected bone tumour autograft particularly on pasteurization as this method is belief to be able to solve many issue with regards to tumour reconstruction surgery. To date, there is no article review that is specific on pasteurisation technique only.

Pasteurization in medical field

History of heat therapy causing death of cancer cells has been long dated from 1928 where Friedgood HB experimented that heating at 44°C for duration of 30 minutes killed Walker rat sarcoma cells [11]. Subsequently, few literatures demonstrated that heat application (of nearly 50°C) can cause destruction of the tumour cells in animal model with minimal or no damage to surrounding normal tissues suggesting its potential use in tumour treatment [11,19].

Among the methods of bone sterilization, use of heat treatment is the simplest method to kill the tumour cells namely through boiled or autoclave but very high temperature may lead to poor bone remodelling due to loss of osteogenic potential[6,20]. The superiority of bone pasteurization, where the bone is treated with waterbath at 60°C to 65°C for 30-40 minutes has been proven by studies and applied clinically; in which it preserves the bone morphogenic properties to induce bone remodelling in comparison to autoclaving and boiling[6].

Urist and his colleagues in 1973 did an experiment using rat bone on how different environments affect bone morphogenic protein (BMP). He reported that bone which were exposed to temperature of 40°C and 50°C produced high bone yields. However, the yield reduced mildly after exposure to 60°C and dropped much further after being exposed to temperature of 70°C to 100°C as the bone morphogenic property of the matrix was lost[20].

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Multiple papers have described method of pasteurization, mostly using a waterbath (Figure 2) , in which they used physiologic saline warmed to 60°C to 70°C to submerge the resected bone tumour for a duration of 30 to 40 minutes [6,10,15,16].

Figure 2: Example of water bath method that can keep the temperature of the physiologic saline constant

Eradication of tumour cell with pasteurization

Jyoti Kode and teammates in 2014 did an in vitro study on efficacy of the pasteurization of bone tumour eradication prior to reimplantation using mice, showing that pasteurized tumour tissues did not grow in culture and did not exhibit clonogenicity. Cell viability, that was investigated using propidium iodide staining, showed positive dead cells significantly increased in pasteurized tumour bone. Development of tumour, proven by expression of antihuman nuclei and osteopontin by immunohistochemistry, was negative in pasteurized mice. They concluded that pasteurization was efficacious in ensuring tumour eradication from resected bone tumour specimens and can safely be used for bone reconstruction after the bone tumour has been resected[11].

Suwonda and team did an experiment on viability of tumour cells using osteosarcoma tissue and proved heating significantly increase in degenerated tumour cells and necrotic tumour cells using exposure of pasteurization for 40 minutes at 60^0C and 70^0C[21].

Histopathological study also showed 100% eradication of tumour cells in all samples of diseased bone treated with irradiation, boiling, pasteurisation and autoclave method[8]. Pasteurization can kill the cancer cells while preserving bone inducing property of the sterilized autograft[9].

Pasteurization effect on biomechanical properties

Bone collagen is related to the bone strength property and this property can be changed with heat[20,22]. The strength of the bone will not change if the temperature is below 60^0C[20,22].

S.Shin et al in 2004 did an experiment using rabbit bone to test the biomechanical properties of the bone treated with heat for 30 minutes in a 60^0C physiological solution or heated for five minutes in 100^0C physiological solution before retransplanted to the rabbit with external fixation. The heat-treated grafts were tested for compression and torsional strength before transplantation, at 18 weeks and at 48 weeks. In the compression test, the 60^0C heat-treated grafts showed a strength ratio of 97.3%, 63.5% and 94.5% respectively. On the other hand, the 100^0C heat-treated grafts showed significant lower strength ratio of 60.1% at 48 weeks[22]. The outcome was almost similar in torsional test[22]. The study supported earlier study done by Knaepler and his colleagues in 1991 that studied the compressive strength of pig cancellous bone treated with heat at 60^0C and 100^0C. The bone strength was not changed with 60^0C heat but reduced to 60% at 100^0C heat treatment[23].

Nor Faisal et al compared the radiological, histopathological and biomechanical differences in bones treated with irradiation, autoclaving and pasteurisation in rabbit. Pasteurisation shows superior radiographic and histopathological property compared to the other method [15].

A study by Singh VA and his team on the biomechanical and histopathological components of diseased bone which has undergone different types of sterilization including irradiation, boiling, pasteurisation and autoclave. They reported that boiling significantly reduced the mechanical strength of the bones, while autoclaving and pasteurisation reduced the strength of the bone to a lesser extent. On the other hand, irradiation does not significantly alter the mechanical property of the bone [8].

Clinical outcome of bone autograft with pasteurization method

Outcome of using pasteurized bone autograft has been reported in literatures. It has been showed to have positive outcome, but potential complications of pasteurized autograft such as infection, nonunion, fracture and bone resorption are also possible [9]. It is difficult to has a similar study as many co-founding factors may influence the outcome such as tumour type, tumour grade, location of the tumour, present of metastasis and different timing and experience of the surgeon in long term studies.

Although histological findings of human pasteurized bone graft showed graft cortices remained necrotic with empty osseous lacuna, the architecture of the cortical bone was maintained with woven bone deposited on the surface[24]. The result was in contracdiction with the study by Bahk WJ et al that mentioned there were areas of empty lacuna and lacunae contained nucleated osteocytes and some haversian system filled with fibrovascular tissue with lamellar bones[25].

Manabe and coworkers in 2004 reported primary bone union occurred in 20 out of 25 patients, where union started to occur at four months postoperatively with overall union rate was 77%. The mean Musculoskeletal Tumor Society (MSTS) functional rating was 86%. All patients had no local recurrence, 48% patients had no complications and achieved healing, graft fracture occur in 12%, infection in 20% and graft resorption in 8% of patients[6].

He also concluded that cancellous bone treated with pasteurization has an increased probability to develop resorption than cortical bone due to its structural bone property which is weaker than cortical, more porous; and easier neovascularization and vascular ingrowth. Rigid internal fixation, prolonged protected weightbearing and good vascular tissue coverage of the pasteurized bone graft with additional appropriate flap were very important for protection from infection, better bone incorporation and bone remodelling [6].

Liu T and teammates in 2012 reviewed patients with distal tibia bone tumour treated with limb salvage and used pasteurized bone graft to reconstruct the defect with mean follow up of 81 months. They had MSTS score of 74.3%. 100% achieved bone union at union time of 18.9 months (60% needed bone grafting at proximal site) and 10% had local recurrence [16].

In contrary, Jeon et al earlier reported worse outcome of nine patients, two developed deep infections, two fractures and two nonunions[18]. They also reported on intercalary pasteurized autograft for bone tumour reconstruction in lower extremity revealed overall 10 years survival rate of 74% with main reason for removal of the graft were fracture (4.7%) and infection (9.5%). They found out no clinical factors such as age, grade and length of the resection has correlation with graft survival. The mean union time was 15.5 months and mean MSTS score was 88%[18].

A study by Sugiura and friends in 2012 revealed outcome of pasteurized autograft alone or combined with vascularized fibula graft. They reported 93.5% ten-year survival rate with average bone union of 9.5 ± 4.3 months. Complications include infection (13% of cases), graft fracture (15%), non-union (17%) and bone

resorption (13%). The combination of vascularized fibula graft with pasteurized autograft significantly reduced non-union and resorption rate especially in intercalary and combination with composite graft cases [26].

Koyanangi and colleagues reported a long term result of pasteurized bone autograft over 165 months follow up. Five and ten-year survival rate of autograft bone was 78.6% and 47.6 %, respectively. There was significant difference between the size of grafts which survived in the long term and that of grafts which failed in the long term**,** with surviving pasteurized bone was in the range of 10-16cm while non surviving was 12.5-25 cm in length. Intercalary graft with wide bone junction surface and composite grafts with small size autograft has 100% survival rate. Hence, they suggest that smaller pasteurized bone and larger junction contact surface with the normal bone are advantageous to the survival the graft [10].

Another long term follow up of patients treated with pasteurized bone autograft with mean follow up of 113 months reported five, ten and twenty year survival of bone autografts were 73%, 59%, and 40% respectively. 38% were removed due to various complications; infection (13%), non-union (7%), graft fracture (6%), graft resorption (5%) and local recurrence (4%). They concluded that better outcome was in intercalary or distal long bone reconstruction and best outcome in long bone hemicortical resection. Higher complications associated with pasteurized graft-prosthesis composite, younger age (≤15years old) and pelvic location related to graft removal [14].

Conclusion

Pasteurization is a simple, convenient, and effective method of bone tumour sterilization. It has demonstrated satisfactory biomechanical and clinical outcomes in bone autograft for bone defect reconstruction after tumour resection. The advantages of pasteurization include being readily available, simple, less expensive, satisfactory bone remodelling, less risk of disease transmission such as HIV infection and viral hepatitis and no risk of allogenic immunogenicity. However, in few cases, it had showed deteriorating outcome during in long term follow up. Further studies that involved multicenter and larger scale of patients is needed to strengthen the benefits of this method and to suggest factors associated with good outcome.

References

1. Zhu W, Zhu L, Bao Y, Zhong X, Chen Y, Wu Q. Clinical evaluation of neoadjuvant chemotherapy for osteosarcoma. *J BUON*. 2019;24(3):1181-1185.
2. Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma Overview. *Rheumatol Ther*. 2017;4(1):25-43. doi:10.1007/s40744-016-0050-2
3. Wahidah T, Khattak MN, Wan-Arfah N, Naing NN. Five-year survival of osteosarcoma patients in hospital universiti sains malaysia (Husm): An eleven year review. *Res J Pharm Technol*. 2018;11(8):3534-3542. doi:10.5958/0974-360X.2018.00653.4
4. Cole S, Gianferante DM, Zhu B, Mirabello L. Osteosarcoma: A Surveillance, Epidemiology, and End Results program-based analysis from 1975 to 2017. *Cancer*. 2022;128(11):2107-2118. doi:10.1002/cncr.34163
5. Yasin NF, Abdul Rashid, Muhammad Lutfi, Ajit Singh V. Survival analysis of osteosarcoma patients: A 15-year experience. *J Orthop Surg*. 2020;28(1):2309499019896662. doi:10.1177/2309499019896662
6. Manabe J, Ahmed AR, Kawaguchi N, Matsumoto S, Kuroda H. Pasteurized Autologous Bone Graft in Surgery for Bone and Soft Tissue Sarcoma. *Clin Orthop Relat Res*. 2004;419(419):258-266. doi:10.1097/00003086-200402000-00042
7. Bacci G, Ferrari S, Lari S, et al. Osteosarcoma of the limb. Amputation or limb salvage in patients treated by neoadjuvant chemotherapy. *J Bone Joint Surg Br*. 2002;84(1):88-92.

http://www.ncbi.nlm.nih.gov/pubmed/11837839
8. Singh VA, Nagalingam J, Saad M, Pailoor J. Which is the best method of sterilization of tumour bone for reimplantation? a biomechanical and histopathological study. *Biomed Eng Online*. 2010;9:1-15. doi:10.1186/1475-925X-9-48
9. Zekry KM, Yamamoto N, Hayashi K, et al. Reconstruction of intercalary bone defect after resection of malignant bone tumor. *J Orthop Surg*. 2019;27(1):1-9. doi:10.1177/2309499019832970
10. Koyanagi H, Matsumoto S, Shimoji T, et al. Long-term results from use of pasteurized bone. *J Orthop Sci*. 2012;17(5):605-613. doi:10.1007/s00776-012-0258-0
11. Kode J, Taur P, Gulia A, Jambhekar N, Agarwal M, Puri A. Pasteurization of bone for tumour eradication prior to reimplantation - An in vitro & pre-clinical efficacy study. *Indian J Med Res*. 2014;139(APR):585-597.
12. Makmor T, Abdillah N, Raja Noriza RA, et al. Low organ donation rate in Malaysia: A survey. *J Heal Transl Med*. 2014;17(1):14-17. doi:10.22452/jummec.vol17no1.3
13. Nayak P, Gulia A, Puri A. Does Reconstruction with Reimplantation of Sterilized Tumor Bone Provide Survival Benefit in Diaphyseal Osteosarcoma? *Sarcoma*. 2020;2020(January 2006). doi:10.1155/2020/4092790
14. Lee SY, Jeon DG, Cho WH, Song WS, Kim BS. Are pasteurized autografts durable for reconstructions after bone tumor resections? *Clin Orthop Relat Res*. 2018;476(9):1728-1737. doi:10.1007/s11999.0000000000000100
15. Yasin NF, Singh VA, Saad M, Omar E. Which is the best method of sterilization for recycled bone autograft in limb salvage surgery: A radiological, biomechanical and histopathological study in rabbit. *BMC Cancer*. 2015;15(1):1-11. doi:10.1186/s12885-015-1234-9
16. Liu T, Guo X, Zhang X, Li Z, Zhang Q. Reconstruction with pasteurized autograft for primary malignant bone tumor of distal tibia. *Bull Cancer*. 2012;99(9):E87-E91. doi:10.1684/bdc.2012.1626
17. Sakayama, K., Kidani, T., Fujibuchi, T., Kamogawa, J., Yamamoto, H., & Shibata T. Reconstruction surgery for patients with musculoskeletal tumor, using a pasteurized autogenous bone graft. *Int J Clin Oncol*. 2004;9(3):167–173. doi:https://doi.org/10.1007/s10147-004-0391-7
18. Jeon DG, Kim MS, Cho WH, Song WS, Lee SY. Pasteurized autograft for intercalary reconstruction: An alternative to allograft. *Clin Orthop Relat Res*. 2007;456(456):203-210. doi:10.1097/BLO.0b013e31802e7ec8
19. Dickson JA. Destruction of solid tumors by heating with radio-frequency energy. *Radio Sci*. 1979;14(6):285-295. doi:https://doi.org/10.1029/RS014i06Sp00285
20. Urist MR, Iwata H. Preservation and biodegradation of the morphogenetic property of bone matrix. *J Theor Biol*. 1973;38(1):155-167. doi:10.1016/0022-5193(73)90231-2
21. Suwondo A, Mahyudin F, Edward M, Mustokoweni S. Effect of Temperature and Duration of Pasteurization Exposure on Tumor Viability in Osteosarcoma. *Int J Health Sci (Qassim)*. 2022;6(April):9042-9052. doi:10.53730/ijhs.v6ns4.10721
22. Shin S, Yano H, Fukunaga T, et al. Biomechanical properties of heat-treated bone grafts. *Arch Orthop Trauma Surg*. 2005;125(1):1-5. doi:10.1007/s00402-004-0746-6
23. Knaepler, H., Haas, H. & Püschel HU. Biomechanische Eigenschaften thermisch und radioaktiv behandelter Spongiosa. *Unfallchirurgie*. 1991;17:194-199. doi:10.1007/BF02588686
24. Kubo, T., Sugita, T., Shimose, S., Arihiro, K., Tanaka, H., Nobuto, H., Tanaka, K., & Ochi M. Histological findings in a human autogenous pasteurized bone graft. *Anticancer Res*. 2004;24(3b):1893-1896.
25. Bahk W, Kang H, Park S, Cho H, Cho Y SK. Histological findings of regeneration in retrieved pasteurized bone grafts. *J Orthop Sci*. 2019;24(4):737–741. doi:https://doi.org/10.1016/j.jos.2018.12.009
26. Sugiura H, Nishida Y, Nakashima H, Yamada Y, Tsukushi S, Yamada K. Evaluation of long-term outcomes of pasteurized autografts in limb salvage surgeries for bone and soft tissue sarcomas. *Arch Orthop Trauma Surg*. 2012;132(12):1685-1695. doi:10.1007/s00402-012-1606-4