

Editorial

Autoclaved bone graft usage revisited

Jeanne A. Pawitan^{1,2}

¹ Editor, *Medical Journal of Indonesia, Jakarta, Indonesia*

² Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

In this issue, Asmara, et al recommend avoiding the use of bone graft for limb salvage, as their study on rat showed that autoclaved bone graft was inferior compared to non-autoclaved bonegraft (control). Autoclaved bone autograft is initially thought to be an economical choice compared to prosthesis, may not cause disease transmission and rejection problems, and does not challenge socioreligional beliefs. The reason of the inferiority of autoclaved bone graft are due to some reasons, including denaturation of collagen into gelatin, mineral degeneration, inactivation of bone morphogenetic protein 2 (BMP-2), vascular ischemia, and damage of bone structure in autoclave temperature, which lead to bone resorption and minimal bone formation, and finally failure of bone healing. High temperature are intended to eradicate malignant cells in bone and soft tissue sarcoma, thus the autoclaved bone can be reimplanted for limb salvage.¹ High temperature also destruct the bone marrow, in addition to the various reasons already discussed in the study.

Bone marrow is rich in various kinds of stem cells, the majority are hematopoetic stem cells. In addition, bone marrow also contains mesenchymal stem cells that can differentiate into chondrogenic and osteogenic lineage, and other types of cells of mesenchymal origin,² which play a role in bone healing.

In bone repair, there is cartilage callus formation where chondrogenic cells play a crucial role, followed by the role of osteogenic cells that are involved in bone formation that will replace the cartilage callus.³ Therefore, destruction of bone marrow that contains mesenchymal stem cells will substantially impair bone formation and repair, which is crucial for limb salvage.

For the purpose of limb salvage in patients with a bone malignancy, eradication of malignant cells from autologous bone graft can be achieved by

various methods other than autoclaving, such as by irradiation, freezing, and pasteurizing. For the purpose of bone autograft, irradiation at 50 Gy showed that irradiated bone was biomechanically inferior compared to normal bone.⁴ Irradiation need a radiation facility, which is not always available in every hospital, and the need to transport to a remote facility causes an extension in sugery time. Moreover, irradiation at 50 Gy may not kill the malignant cells, as such a dose is also use to irradiate cells to be used as feeder layer in cell culture. A study used 6000 rad (60 Gy) irradiated human dermal fibroblasts as feeder layer for keratinocyte culture.⁵ A feeder layer is composed of living cells that loose their mitotic capacity. Therefore, bone irradiation for the purpose of bone autograft for limb salvage may contain living malignant cells, though devoid of proliferation potential.

Exposure to freezing temperature in liquid nitrogen may kill the malignant cells, as freezing causes the formation of ice crystals inside the cell, which kill the cell. Liquid nitrogen freezing is a simple procedure, do not require special equipment, economical, and was shown to eradicate osteosarcoma cells. Moreover, frozen bone showed adequate biomechanical properties, and maintenance of osteoinductive and osteoconductive properties. Therefore, liquid nitrogen freezing is suitable for bone autograft in limb salvage in bone malignancy cases.⁶

Pasteurizing uses heat to eradicate the malignant cells, but at much lower temperature compared to autoclaving. Adequate heat treatment may lead to tumor destruction. Several studies showed that heat treatment at 44°C for 30 minutes, or diathermy heating using a radiofrequency of 13.56 MHz caused tumor destruction, with minimal damage in surrounding normal tissues. Recently, pasteurization at 60-65°C for 40 minutes in a water bath eradicated malignant cells, which was shown by failure to grow in culture and lack of clonogenicity.⁴ Further,

bone structure is preserved after pasteurization, and use of pasteurized bone graft showed less negative effect on bone healing compared to autoclaved bone graft. This fact might be due other characteristics of pasteurized bone, such as sparing of osteoinductive growth factors in pasteurization compared to autoclaving.⁷

Therefore, pasteurization or liquid nitrogen freezing are superior compared to autoclaved bone autograft for limb salvage in bone malignancy.

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Correspondence author: Jeanne A. Pawitan, jeanneadiwip@gmail.com