

# The Effect of Gamma Irradiation on the Osteoinductivity of Demineralized Human Bone Allograft

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Received: 12 Jan. 2013; Accepted: 13 May 2013

**Abstract-** The gamma irradiation has been used for end sterilization of allograft bones and its effects with a 25 kGy dosage on the osteoinductive properties of demineralized bone allograft powder was studied. This work carried out using an experimental method in an animal model. In this study the demineralized bone allograft powder which had been sterilized and prepared with gamma irradiation in a 25 kGy dosage in 18 hours, was used as a study group and the demineralized bone allograft powder which had been prepared aseptically was used as the reference group. 30 mg of bone powder from each group were implanted into right and left paravertebral muscles of eighteen rats, separately. After four weeks, the implanted samples were harvested with a 0.5 cm border and then the osteoinductivity of implants in two groups were compared with histopathologic studies. In 94.4% of the reference samples a new bone formation was observed. In the study group, this difference was observed only in 27.7% of samples ( $P < 0.002$ ). It appears that using gamma irradiation may lead to a reduction in osteoinduction properties of demineralized bone allograft powder.

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*Acta Medica Iranica*, 2014;52(3):215-219.

**Keywords:** Gamma irradiation; Bone allograft; Osteoinduction; Osteoconduction; Sterilization

## Introduction

Nowadays, allograft bone transplantation has a wide utilization in different branches of medicine such as orthopedic surgery, neurosurgery, and maxillofacial surgery and even in dentistry. Allograft bone transplantation has a more than 100 years history. In the years between 1880-1980 the major problem was preparing the allograft bone and most transplantation was done using autograft method. In 1881 the first allograft bone transplantation was done by Mac Ewan. In this process a bone piece of a patient with osteomyelitis arm was replaced with allograft bone (1). As the utilization of allograft bone substitute are increasing rapidly, attention to the efficiency and safety is one of the critical issues in this field of medicine. The transmission possibility of some viral infections such as HIV1, 2, HTLV1, 2, Hepatitis B and C, CMV and prions

by using human tissues has been reported in recent studies. The transmission peril of such diseases in implanted tissues is closely related to preparing methods and utilized tissue's kind (2-4). These perils can be minimized with paying specific attention to analyze and corrective selection of donors, screening tests, and correct preparation and utilization of final sterilization methods (5). Different methods such as sterilization with ionizing radiations, high temperature sterilization or sterilization with chemical compounds have been reported for end sterilization of allograft bone (6-8). Many studies have been done regarding to hazardous affects of gamma irradiation on allograft bone in recent fifty years. However, it is important to know that the high dosage of gamma irradiation causes numerous physical and chemical changes in tissue which can lead to impairment of physical and biological properties of the bone. Despite, viruses have the most resistance

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against the irradiation. It has been proven that in cases which the dosage of gamma irradiation were high, HIV and HCV viruses existed in musculoskeletal tissues died (9). Hilmy and Lina studied the clinical efficiency of long bones which were irradiated with 25 kGy gamma radiation and according to their findings these bones were not different with aseptic bones (6). According to EATB and IAEA guidelines, the permissible dosage for gamma irradiation is 25 kGy and AATB suggests a dose of 15 kGy (8-10). By the way some scientists suggest a 35 kGy dosage (11). In the study conducted by Glowacki and Mulliken the affect of different preparation methods on implant osteoinductivity properties was examined (12). According to a study which was done by Urist and Hernandez an approximately 40 kGy of gamma radiation diminishes the osteoinductive properties of the demineralized bone allograft powder (13). In addition, it has been shown by Glowacki *et al.* that the result of a 20 kGy gamma irradiation was a 20 percentage decrease in osteoinduction with the control group (14). Moreover, Goclawska *et al.* reported that a 35-50 kGy gamma irradiation in the environment temperature could diminish the osteoinductive properties of the demineralized bone powder completely (14,15). Also biomechanical properties of the bone can be affected by gamma irradiation. Akkus and Rimnac demonstrated that the cortical bone pieces which were irradiated with a dose of 27.5 kGy were more vulnerable against the irradiated (16). Regarding to several opinions about gamma irradiation and biological properties of bone allograft, the objective of this study was the assessment of gamma irradiation effects on osteoinductive properties of bone allograft.

## Materials and Methods

This experimental study was done in Iranian tissue bank research center. Bone allografts were taken according to the standardized procedures of this tissue bank and after obtaining the informed consent from the next of kin. The samples were prepared from diaphysis of the femur bone and two horizontal sections from each sample were opted equally and were sent to preparation. Because of this study was fulfilled on human bone allografts and existed limitation in preparing these allografts for bone implant candidate and also existence a waiting list, only allografts which were serologically positive for Human T Lymphotropic Virus (HTLV) Antibody, were selected for this research. Accordingly, during the preparation procedure all safety principals

were considered and all instruments were sterilized by steam sterilization method. At first, the bone was cut in small rings using a bone saw (Stryker Company).

These rings (2–4 mm diameter) were cut into smaller pieces with less than 2 mm sizes obtained from them using a sternotom. Then, these bone pieces were converted to powder by bone miler instrument (IKA, Germany). In order to obtain particles with suitable size two kinds of stainless steel filter with 150 and 750  $\mu\text{m}$  pore size were used for separating particles in this range of size. In the demineralization step, in order to decrease the amount of  $\text{Ca}^{++}$  to less than 10%, the powder was immersed in 0.5 normality hydrochloric acid (HCl, Merck) for three hours. Then the residuals of HCl were eliminated using ultra pure water washing this step was repeated 6 times at least and in washing was stopped after reach to pH = 6-7.

Finally, tries buffer solution (ICN Company) was used for reach to this range of pH. For defatting process, a pure ethanol (Bidestan co. Iran) and chloroform (Merck) were prepared in equal volumes and the bone pieces were immersed in this solution in 4° C for twenty four hours. After this step, samples were washed 5 times with ultra pure water. Furthermore, no sterilization step was done for group a (reference group). In group B (study group), after a suitable packaging, all samples were sent to Atomic Energy Organization in dry ice. The sterilization process using Co60 source with a 25 kGy dosage of gamma radiation was performed for 18 hours. The prepared samples were preserved in a -80°c freezer (New Brunswick CO, USA). For samples implantation eighteen 6 weeks old female rats were used. All of these rats were hosted in cages with sufficient food and water, separately at the animal laboratory of Cancer Research Institute of Imam Khomeini Hospital. Before implantation, the rats were anesthetized for 35-40 minutes using diazepam and ketamine by intraperitoneal injection. Then 30 mg demineralized bone powder from each group was implanted in paravertebral muscle pouch in left and right side. In order to avoid from washing out samples, the muscular pouch was wiped up regularly and in the cases that sample was mixed with blood, replication procedure was performed again. After sample implantation process the muscles' fascia and skin was sutured by using 4.0 and 2.0 nylon sticks. At the all periods of the operation the rats were preserved on a suitable place with heater and daily preserving was performed for 4 weeks. After 4 weeks the rats were killed using CO<sub>2</sub> gas inhalation (1 atm) in 30 minutes.

After this step the sutured area was opened and the samples with a 0.5 cm borders were harvested

immediately. Each sample was replaced in formalin (10%) and then in order to performing a histopathologic study, these samples were sent to histopathology department of the Cancer Research Institute of Imam Khomeini Hospital. For further evaluation H&E staining method was used. All of the samples were evaluated in order to detect a new woven or lamellar bone formation, a new cartilaginous tissue, osteoblast cells or any bone marrow cellular element.

According to the obtained results, samples contain live bone tissue showed osteoinduction implant but samples contain demineralized bone powder not. The Fisher test (F-test) was used for further analyzing and comparison of the obtained results and *P*-values less than 0.05 were considered statistically meaningful.

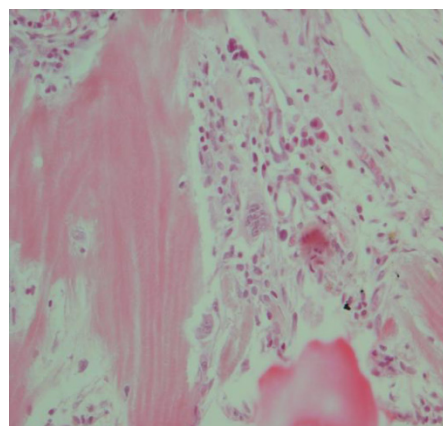
## Result

In histopathologic studies the presence of osteoblasts in tissue samples were considered as effective new bone formation. Also the existed layer precipitate in the death area of the replaced bone and layer bone formation with hyaline cartilage background compound or some bone marrow elements were considered as live bone tissue and this was a strong reason regarding to new bone tissue formation. After analyzing, new bone formation was observed in 17 (94.4%) reference samples (Figure 1).

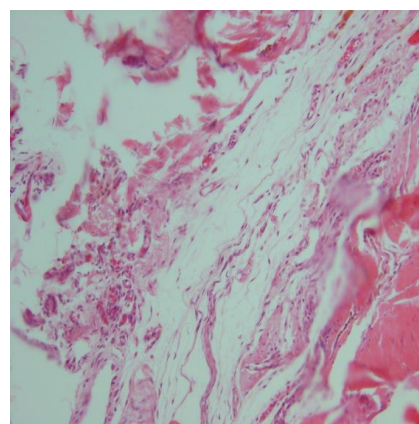
However, this bone tissue was observed only in 5 samples (27.7%) with gamma irradiation (Figure 2). This difference was statistically significant ( $P < 0.002$ ). Chondrocyte formation in the reference group was 5.5% and this was not observed in the study group. Moreover,

osteoblast formation in reference group was 88.8% and this amount in study group was only 50%. Also the difference between chondrocyte and osteoblast formation was meaningful too ( $P < 0.05$ ).

Bone formation, angiogenesis, chondrocyte and osteocyte formation in two groups (primary sterile and gamma irradiated) can be seen in table 1.



**Figure 1.** Bone formation in primary sterile demineralized bone allograft



**Figure 2.** Bone formation in gamma irradiated demineralized bone allograft

**Table 1. Comparison of bone formation, angiogenesis, chondrocyte and osteocyte formation in primary sterile & gamma irradiated demineralized bone allograft**

Histological results	Aseptically prepared powder	Gamma irradiated powder	<i>P</i> -value
New bone formation	17 (94.4%)	5 (27.7%)	<0.05
Chondrocytes	1 (5.5%)	0 (0%)	<0.05
Osteoblasts	14 (77.7%)	8 (44.4%)	<0.05
Angiogenesis	16 (88.8%)	13 (72.2%)	<0.05

## Discussion

Because of rapid increasing in utilization of allograft bone products in different surgical procedures,

sterilization methods of these products and obtained results was always a critical issue. Gamma irradiation is a simple, cost-effective and a very favorite technique. In contrary, it has some disadvantages. One of the disadvantages of the gamma irradiation sterilization is

its hazardous effects on physical and biological properties of tissues. For this reason, study of these adverse effects on osteoinduction implant properties was the major aim of this assay. On the other hand, based on some study results gamma radiation can reduce the shear, bending, and compression strength of cortical bone in comparison with ethylene oxide gas sterilization (17). Using ionizing radiation such as gamma irradiation with two direct and indirect effects on microorganisms. There are different options about using the ionizing radiations in allograft tissues sterilization. The source of such differences is usually nonsufficient information about effects of incorrect utilizations of such radiations. The irradiation effects on live tissues inside live bodies are completely different from these effects in laboratory environment. A gamma irradiation with a 3–5 kGy dosage has hazardous effects on live tissues inside the body. However, gamma irradiation with a 10 kGy dosage in laboratory environment does not have any unfavorable affect on the live tissue. Albeit viruses show the maximum resistance against irradiation, it has been shown in several studies that HCV and HIV viruses are completely vulnerable against using high dosages of radiation (4,5). Hilmy *et al.* showed that the clinical efficiency of long bones after irradiation process with a 25 kGy of gamma irradiation has no prominent difference with aseptic prepared bones (6). There are different views about favorable dosage of gamma irradiation sterilization. Some scientists in order to attain confidence safety suggest a 35 kGy dosage (11). Although using high dosages of radiation can after biological properties of tissue products in such cases hazardous effects of these methods is one of the most critical issues. Several methods have been reported for studying of osteoinduction implant properties in allograft bone derivative products. All of these reported methods performed using *in vitro* or *in vivo* methods. Numerous animal models have been used *in vivo* studies. In most cases rats have been used for these studies. There are several qualitative and quantitative methods for osteoinduction determination. Glowacki and Mulliken studied the effects of different preparation methods on the osteoinduction implant properties (12). They created a small subcutaneous pouch in 28 days old male rat and then implanted approximately 25 mg demineralized bone powder in the pouch. After passing 3 days they observed an increasing in connective tissue and then after 9 days observed a chondroblast cells cluster with background cartilage compounds. At last, after 14 days a calcified bone tissue with a row of osteoblasts penetrated into replaced bone powder (14).

Urist and Hernandez reported that a gamma irradiation with a 40 kGy dosage leads to 20% diminish in osteoinduction implant properties of demineralized bone powder (13). In addition, Glowacki *et al.* studied the osteoinduction implant properties after using gamma irradiation with a 20 kGy dosage in animal model (14). The major result of this study was a 20% decrease against the control group. Furthermore, it was found in this study that because of the gamma radiation eliminates most of existed antigens in the tissue the chance of foreign body reaction be minimized and therefore chance of a successful operation increases (12). It has been demonstrated by Dzeidzic-Goclawski *et al.* that a gamma irradiation with a 35–50 kGy dosage in the environment temperature can inhibit the osteoinduction properties of the rat demineralized bone powder completely (15). It has been found that biomechanical properties of bone can also change with gamma irradiation. This is important especially when a long bone is used in bearing position. Several studies have been done about effects of gamma radiation on increasing of long bone's crashing. Akkus and Rimnac showed that in comparison with the control group the cortical allograft bone withstand against pressing forces diminished after irradiation with 27.5 kGy dosage of gamma radiation (16). The results obtained in our study are same as some of previously attained results. In this work the decrease amount in osteoinduction implant properties is further than previous studies (> 60%). One of the probably major reasons of this difference can be longer irradiation time (approximately 18 hours). This long irradiation time with an increase in the temperature's can be hazardous for active osteoinductive proteins. However, using a new gamma source and lessen the irradiation time can decrease the osteoinductive property. Because of easy access and low costs of gamma radiation and its low affect on mechanical properties of long bones, in comparison to other means of sterilization, this method is more acceptable, especially among developing countries. Therefore, sterilization using such methods looks a logical means but referred to its adverse effects on osteoinduction implant properties of demineralized bone powder, further studies perform and protocols modify for utilization of this method seems an indispensable issue. In conclusion, despite the adverse effects of gamma irradiation on bone osteoinductivity it can be used as end sterilization method, especially for structural bone allograft we can use lower doses of gamma irradiation with implementation of aseptic tissue preparation and virus inactivation methods. We

recommend setting up another sterilization method when we need osteoinduction.

## Acknowledgement

Authors would like to acknowledge, Fereshteh Mohamadi-Jahani, Maryam Kavousi, and Niloufar Tirgar for their considerable assistance

## References

1. Tomford WW. Bone allograft: past, present and future. *Cell Tissue Bank* 2000;1(2):105-9.
2. Tomford WW. Transmission of disease through transplantation of musculoskeletal allografts. *J Bone Joint Surg Am* 1995;77(11):1742-54.
3. Sanzen L, Carlsson A. Transmission of human T cell lymphotropic virus type 1 by a deep-frozen bone allograft. *Acta Orthop Scand* 1997;68(1):72-4.
4. Cook Sd, Salkeld SL, Prewett AB. Simian immunodeficiency virus (human HIV-II) transmission in allograft bone procedures. *Spine* 1995;20(12):1338-42.
5. Conrad EU, Gretch DR, Obermeyer KR, et al. Transmission of the Hepatitis-C virus by tissue transplantation. *J Bone Joint Surg Am* 1995;77(2):214-24.
6. Hilmy N, Lina M. Effect of ionising radiation on viruses, proteins and prions. In: Nather A, editor. *Advances in Tissue Banking. The Scientific Basis of Tissue Transplantation*. 5th ed. Singapore: World Scientific Publication Co. Pte. Ltd.; 2001; p. 358-74.
7. Glowacki J. A review of osteoinductive testing methods and sterilization process for demineralized bone. *Cell Tissue Bank* 2005;6(1):3-12.
8. Radiation Sterilization of Tissue Allografts: Requirements for Validation and Routine Control, A Code of Practice, International Atomic Energy Agency. (Accessed in Jan 2014, 28, at <http://www.iaea.org>).
9. General standards for tissue banking. European Association of Tissue Banks (EATB). (Accessed in Jan 2014, 28, at <http://eatb.org>).
10. Standards for tissue banking. American Association of Tissue Banks (AATB). (Accessed in Jan 2014, 28, at <http://aatb.org>).
11. Dziejcz-Goclawska A, Kaminiski A, et al. Irradiation as a safety procedure in tissue banking. *Cell Tissue Bank* 2005;6(3):201-19.
12. Glowacki J, Mulliken JB. Demineralized bone implants. *Clin Plas Surg* 1985;12(2):233-241.
13. Urist MR, Hernandez A. Excitation transfer in bone. Deleterious effects of cobalt 60 radiation-sterilization of bank bone. *Arch Surg* 1974;109(4):586-93
14. Glowacki J, Kaban LB, Murray JE, et al. Application of the biological principle of induced osteogenesis for craniofacial defects. *Lancet* 1981;1(8227):959-62.
15. Dziejcz-Goclawski A, Ostrowski K, Stachowicz W, et al. Effect of radiation sterilization on the osteoinductive properties and the rate of remodeling of bone implants preserved by lyophilization and deep freezing. *Clin Orthop Relat Res* 1991;272(1):30-7.
16. Akkus O, Rinnac CM. Fracture resistance of gamma radiation sterilized cortical bone allografts. *J Orthop Res* 2001;19(5):927-934.
17. Zhou Z, Qin T, Yang J, et al. Mechanical strength of cortical allografts with gamma radiation versus ethylene oxide sterilization. *Acta Orthop Belg* 2011;77(5):670-5.