Validation of 11 kGy as a Radiation Sterilization Dose for Frozen Bone Allografts

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Abstract

A radiation sterilization dose (RSD) of 25 kGy is deleterious to bone allografts. This study aimed to establish a lower RSD for bone allografts using Method 1 of ISO 11137.2:2006. This provides a database to select a RSD corresponding to an allograft's bioburden, given that the bioburden's gamma resistance is equal to, or less than the standard. This can be verified by irradiating 100 allografts at a dose selected to provide a sterility assurance level (SAL) of 10^{-2}. The bioburden of our allografts was 0, which prescribed a verification dose of 1.3 kGy. After irradiating 100 allografts, sterility tests returned no positive cultures. We therefore validated a RSD of 11 kGy for allografts with that bioburden. According to the standard, this RSD provides a SAL level of 10^{-6} for bone allografts.

Key words:

Bone allograft, radiation sterilization dose, gamma irradiation, method 1 ISO 11137.2 – 2006, allograft bioburden

Running head:

Radiation sterilization of bone allograft at 11 kGy

Definitions

Bioburden: Population of viable microorganisms on or in allografts. In the case of tissue allografts, the bioburden is determined in the stage that allografts are already packed and ready for terminal radiation sterilization.

D_{10} value: dose required to achieve inactivation of 90 % of a population of the test microorganism under stated conditions

Sterility assurance level (SAL): probability of a single viable microorganism occurring on an item after sterilization

Verification dose: dose of radiation predicted to give a predetermined SAL $\geq 10^{-2}$ used in establishing the sterilization dose

Introduction

Due to its ability to penetrate through materials without opening the package, gamma irradiation is commonly used to terminally sterilise health care products encompassing medical devices, medicinal products (pharmaceuticals and biologics) and *in vitro* diagnostics. To establish a radiation sterilization dose (RSD) for their products, manufacturers have been guided by standards published by organisations such as International Standard Organisation (ISO), and the Association for the Advancement of Medical Instrumentation (AAMI). In these standards, developing a RSD is based on either the bioburden of the products (population of viable micro-organism on a product) or the gamma resistance of these organisms. To establish a radiation sterilization dose for health care products, International Standard Organisation (ISO) published a standard named "*ISO 11137.2-2006 – Sterilisation of Health Care Products– Radiation. Part 2: Establishing the sterilisation dose*" (referred to herein as "The Standard"). Accordingly,

establishment of a RSD is based on the bioburden of the product and the gamma resistance of the bioburden. The Standard (1) provides four main methods to establish a RSD for health care products: Substantiation of 25 kGy (VD_{max} 25) or 15 kGy (VD_{max} 15) for products with average bioburden of less than a thousand or 1.5, respectively; or selecting a particular RSD other than 15 and 25 kGy using methods 1 and 2 (being methods described in the Standard).

In tissue banking, the RSD of 25 kGy has been recommended to terminally sterilise tissue allografts. However, the average bioburden of allograft products varies from less than 0.1 (2) up to more than a thousand (3, 4). In addition, the species of contaminated organisms may also differ (5-10). In theory, the RSD may be varied from 25 kGy. Assuming that tissue allografts are a type of medical products, some authors have applied the standard to establish RSD (2, 11, 12). However, production volumes of tissue allografts are usually low compared to other medical products. Hence, Bone Banks have used VD_{max} 15 [1] or adjusted the sample size in Method 2B to make the Standard applicable for tissue banking (11, 12). Nonetheless, the Standard provides a method that is applicable to establish a relatively low RSD with a reasonable sample size. By applying this method, the current study aimed to establish a RSD lower than 15 kGy for frozen bone allografts.

Materials and methods

Bone materials

Bone samples for this study were provided by the Queensland Bone Bank under ethic approval from a local human ethics committee. These bones were transferred for research, with donor consent, as they were not suitable for transplantation.

Bone segments were processed at Queensland Bone Bank (Brisbane, Australia) using its standard operating procedures. Femoral heads were processed following procedures for single production batches, whereas cadaveric donated bones were processed under multiple production batch procedures. Bone allografts used in this study have different risk profiles for contamination because they were retrieved from either living donors during hip surgery (femoral heads) or from deceased donors (structural and morselized allografts). Therefore, they were allocated into two groups for dose validation. The number and type of bone allografts in each validation group are presented in Table 1.

Table 1: Types of bone allografts used in this study

Bone allograft type		Experimental stage	
		Bioburden determination	Verification dose
Femoral head (living bone allografts)		10	100
Cadaveric bone allografts	Morselized	10	20
	Structural	20	80
Total		40	200

Methods

Principally, the method is based on a probability model for the inactivation of microbial populations. The probability model, as applied to bioburden made up of a mixture of various microbial species, assumes that each such species has its own unique D_{10} value. The products were exposed to a series of radiation doses. The initial and survival number of organisms and D_{10} values of individual microorganisms were used to tabulate the standard distribution of resistance

of total microorganism population and then compute the individual doses required to achieve values of SAL of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} for certain levels of average bioburdens.

Practically, to establish a RSD one must first determine the average bioburden of the product and then select a verification dose from the database that gives an SAL of 10^{-2} for this average bioburden. This SAL has to be verified by irradiating 100 allografts at the verification dose, following which a test of sterility must return no more than two positives. If this condition is met, the allograft bioburden demonstrates a standard distribution of resistance. The database then can be accessed to select a sterilization dose that provides a SAL of 10^{-6} for the allografts having this defined bioburden. For example, the database indicates that a verification dose for allografts having an average bioburden of 1000 is 11 kGy (Table 5, page 14 of the Standard). If a sterility test of 100 allografts, irradiated at 11 kGy, returned 2 positives, it would imply that the gamma radiation response of the allograft bioburden was similar to those of the Standard. For this example, the database indicates 24.9 kGy as a necessary radiation sterilization dose to achieve a SAL 10^{-6} for allografts exhibiting a bioburden of 1000.

There are three steps to establish a RSD in this method as described below:

Bioburden estimation of processed-ready-for-irradiation allografts

Femoral heads (n =10) and structural/morselized bone allografts (n = 30) were selected to determine the bioburdens for living bone allografts and cadaveric bone allografts, respectively.

Details of the organism removal method were described and published previously by us (2). Briefly, thawed bone samples were rinsed with warm sterile saline and then the solution was removed and filtered through a filtering membrane unit (Millipore, USA). The membranes were placed on trypcase soy agar plates (Biomerieux, Australia). Incubation, enumeration and species

determination (if present) were carried out using standard microbiology protocols (Biotest – Australia).

The average bioburden of femoral heads was the average number of colony forming units (CFUs) in ten samples. The average bioburden for each of three batches of cadaveric bone samples (10 samples per batch) were separately calculated as above. The highest average bioburden among these three batches was used as the average bioburden for cadaveric allografts.

The bioburdens were normalised by recovery efficiency. The method to establish this efficiency was developed for each type of allograft as previously published (2). In the current study, the recovery efficiencies were 0.71, 0.51 and 0.74 for femoral heads, morselized bone and structural bone allografts, respectively.

Verification dose selection

Average bioburdens were used to determine verification doses at SAL of 10^{-2} using Table 6 of the Standard (13).

Verification dose experiment

One hundred bone segments from each allograft group were irradiated at the verification dose (Table 1). It is required that the maximum delivered dose must not exceed the verification dose by 10%, and the average delivered dose must not be less than 90 % of the verification dose.

Subsequent to irradiation, bone allografts were tested for sterility using filtration methods prescribed by the Therapeutic Goods Administration (TGA) (14). The results were recorded as sterile (negative) or non-sterile (positive).

If there were less than, or equal to, 2 positives out of 100 samples, then the gamma resistance of the bioburden was verified as a standard distribution. Consequently, a radiation sterilization dose

providing a SAL of 10^{-6} for bone allografts with the corresponding bioburden was determined from the Standard's database. If number of positives was more than 2, then the bioburden would have been more resistant than the standard, and the experiment would have failed.

Results

The results from Table 2 indicated that the bioburden of living and cadaveric bone allografts were 0. In Table 6 of the Standard, the closest bioburden to 0 is 0.1. At the SAL level of 10^{-2}, the verification dose for 0.1 bioburden is 1.3 kGy. Therefore, this dose was chosen as the verification dose for both types of allografts.

Table 2: Bioburden of frozen bone allografts (n=10)

Sample	Femoral head	Cadaveric donated bones		
		Batch 1	Batch 2	Batch 3
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
Average	0	0	0	0

Two hundred bone segments were irradiated at the Australian Nuclear Science and Technology Organisation (ANSTO, Luca Heights, Aus) with the required dose of 1.3 kGy. The delivered doses for each load are in Table 3.

Table 3: Delivered verification doses for each bone allograft type

Sample types		Delivered verification dose (kGy)		
		Minimum (≥ 0.94)	Maximum (≤ 1.43)	Average (≥ 1.17)
Femoral head	Run 1 (20 femoral heads)	1.07	1.41	1.24
	Run 2 (20 femoral heads)	1.06	1.39	1.23
	Run 3 (20 femoral heads)	1.05	1.39	1.22
	Run 4 (20 femoral heads)	1.06	1.39	1.23
	Run 5 (20 femoral heads)	1.07	1.40	1.24
Cadaveric bone	Run 1 (80 moserllised bones)	1.11	1.33	1.22
	Run 2 (20 structural bones)	1.17	1.38	1.28

According to ISO 11137-3 (15) , the maximum delivered dose did not exceed the required verification dose by more than 10 %, and the average doses delivered to all allograft items was higher than 90 % of 1.3 kGy. Therefore, delivered verification doses were acceptable.

Sterility tests of irradiated allograft samples returned no positives. Consequently, the verification dose of 1.3 kGy was accepted, or in other words the bioburden's gamma resistance was standard distribution. The radiation sterilization dose for both types of frozen bone allografts, at the required SAL of 10^{-6}, was 11 kGy (13).

Discussion

There is increasing demand in the orthopaedic community to deliver allografts in which the tissue quality has not been degraded by exposure to gamma radiation. The dilemma is to provide such allografts without jeopardising the sterility assurance level. The international Standards provide methods for validating radiation sterilization doses. These are mostly applicable to medical devices, but the Standard does provide methods that can be applied to tissue allografts. For products having very low average bioburden (e.g. less than 1.5), VD_{max} 15 is the first option for establishing a low sterilization dose. This method allows substantiation 15 kGy with minimum sample requirements of 20 (single production batch) or 40 (multiple production batch) (2, 16, 17). The drawback to this method is that regardless of the bioburden, e.g. even lower than 1.5, the preselected dose of 15 kGy cannot be changed.

In contrast to VD_{max} 15, Method 2 (18) is applicable for establishing a specific dose based on the gamma resistance of presenting micro-organisms. This method requires a huge number of more than 700 samples. Two authors have modified method 2B for use in tissue banking (11, 12). The modification resulted in a total of 400 samples being required for the validation. A RSD of 8.3 kGy (Moore (12) and 9.2 kGy (Baker (11) were developed. Even with this sample size modification, it is still beyond the capacity of many tissue banks. For example, the total annual production of all tissue banks in Australia is about 4000 (19). Obviously, the production available for research is considerably lower and so individual tissue banks in this country would not be able to apply this method.

Method 1 (13) in the Standard could be employed to offset the disadvantages of samples size and a dose lower than 15 kGy. Hilmy (20) suggested that to establish a radiation sterilization dose lower than 15 kGy for "*very clean product*", Method 1 can be used. In the case of products with

average bioburden less than 0.1, the verification dose of 0.1 bioburden could be used and the minimum radiation sterilization dose at SAL of 10^{-6} is 11 kGy. This approach adheres to the recommendation in the Standard that the next greater, given bioburden be used for selecting a verification dose if the determined bioburden is not listed in the database. This allows us to use Method 1 to set up a radiation sterilization dose for tissue allografts. The method requires 110 samples for single production batches or 130 for multiple production batches, much less bone specimens than that required for Method 2B.

In our validation of 15 kGy (2), frozen bone allografts were grouped into 3 product families: femoral head, morselized, and structural bone allografts. By adopting the definition of product family from other studies (11, 12, 20), morselized and structural bone allografts were grouped into one family as they are both from deceased donors, mainly retrieved in the morgue environment, and processed in the same process batch. We allocated living donated bones (femoral head) to a separate family group because the allograft comes from live donors, and is retrieved in the operating room during total hip replacement, and processed in individual process batches. Those two groups vary in their contamination rate and contaminated species (5-10). This product family classification reflects that a product family is ‘‘*a group of different products that can be given the same sterilisation dose*’’ and, in the manner of sterilization, is based on the number and types of organism present on or in the allografts, rather than graft’s density and packaging configuration (21). In addition, batch production of a tissue bank is much lower than a hundred. Therefore, to perform the verification dose experiment, instead of taking 100 samples from one process batch as required, we had to collect samples from more than one process batch as previously reported (12). With these minimal adaptations, this study has established a radiation sterilization dose of 11 kGy for frozen bone allografts manufactured at the Queensland Bone

Bank using a total of 240 allografts segments. This new processing procedure is an advance for tissue banks with low production volumes, and consistently low bioburden.

The radiation dose validated by this method is 10 to 13% higher than studies that used Method 2B of the Standard (11, 12). This difference is unlikely to affect the biological quality of bone allografts significantly because data from our mechanical study (22, 23) indicates that when gamma dose increased from 5 to 10 kGy, failure strain and modulus of toughness only decreased by 10 and 14 percent, compared with the control group, respectively.

Significant reductions in the radiation sterilization dose from 25 kGy to 15, and now 11 kGy, provides a clear benefit to the mechanical and biological performance of bone allografts, which is a principal concern of the orthopaedic surgeon. Data from our other studies [24, 25] indicated that the *in vitro* mechanical and biological properties of the bone allografts are significantly improved when the gamma dose is reduced from 25 kGy to 10 kGy. Compared to the fresh frozen allografts, bone allografts irradiated at 25 kGy lost about 26% of their ability to absorb the energy while allografts irradiated at 10 kGy lost only about 14 %. More significantly, osteoclast formation and fusion, *in vitro*, increased from 40% of the control group to almost 80% when the gamma dose decreased from 25 to 10 kGy. In addition, we found that the degradation of collagen molecules started to increase at 10 kGy, and became dramatically denatured at 25 kGy [25]. This alteration in collagen molecules may be a fundamental cause of mechanical and biological degradation.

The effect of gamma irradiation on bone morphogenetic proteins (BMPs) and growth factors is also controversial. Several authors report that gamma irradiation does not directly affect BMPs, but indirectly changes their performance by attacking their courier - the collagen molecules (24-26). A dose-dependent reduction in BMP-7 and CBFA1 expression in 3-week-postimplanted

allografts have also been observed using immunohistochemistry (27). In this case, the reduction was much more significant at 25 kGy than 15 and 0 kGy. Although more studies are required to investigate these issues of quality, a general conclusion can be drawn that the lower the gamma dose, the better the bone protein preservation. Clinically, establishing a lower dose that maintains the SAL results in better preservation of the collagen matrix, BMPs and growth factors leading to improved allograft-host bone incorporation.

Several studies have addressed the issue of gamma irradiation effects on viral contamination (28-30). They all agree that gamma doses of 40 – 50 kGy are needed to sterilize against viral infection. This high gamma dose essentially destroys the mechanical and biological viability of the bone allograft. Hence, it is not recommended for radiation sterilization of tissue allografts. To minimize the risk of viral infection, donor screening including social, medical and serology screening, in combination with processing techniques, are more important than radiation sterilization (31, 32). Currently, application of nucleic acid testing (NAT) in tissue banking is proving effective for early detection of viral infection (32-34). Therefore, gamma irradiation at low, or standard, doses is directed towards achieving a SAL of 10^{-6} with respect to the bacterial bioburden rather than the viral bioburden.

To the best of our knowledge, there is as yet no published standard to define the validation process for tissue allografts with extremely low, or even zero, bioburden. This will be particularly important for tissue banks whose allograft products are routinely irradiated at 25 kGy, but who wish to provide fresh-frozen bone allografts. The IAEA code of practice (35) adopts the Standard to establish radiation sterilization doses, but does not address validation of fresh-frozen products. Similarly, standards from tissue bank associations such as AATB, EATB, and APASTB mentions the use of fresh-frozen allografts, but do not indicate how their sterility can be

validated. The Australian Code of Good Manufacture Practice – Human Blood and Tissue does not rule out the necessity of terminal radiation sterilization if the product bioburden is zero. The issue will remain controversial until a standard validation for radiation sterilization dose for tissue allografts is established, and widely recognised among the tissue banking community.

Conclusion

The results from this study confirm that a radiation sterilization dose of 11 kGy is validated for the terminal sterilization of frozen bone allografts manufactured at the Queensland Bone Bank. The bone allografts sterilised at this dose will have a SAL of 10^{-6} as required in the ISO Standard.

Clinical relevance

A primary concern for orthopaedic surgeons using tissue allografts in joint surgery is the risk of allograft infection. The application of stringent donor screening, serology screening, aseptic techniques in retrieval, processing, packaging and handling of allografts, reduces the risk of viral contamination. In combination with terminal sterilization using a low radiation dose, this ensures provision of allografts with the required sterility assurance level of 10^{-6} that reduces the risk of infection.

Validation of a gamma dose lower than the current standard of 25 kGy also enables provision of allografts with greater bone strength and osteoconductive performance by preserving the integrity of collagen. Allografts sterilised at lower doses of radiation will have improved weight-bearing ability, greater potential for host cell attachment, activation and initiation of bone remodelling leading to incorporation. These characteristics will result in better clinical outcomes for joint surgeries using bone allografts e.g. less complication from allograft fracture, non-union, and prosthetic loosening or migration.

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