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Reducing the radiation sterilization dose improves mechanical and biological quality while retaining sterility assurance levels of bone allografts

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ABSTRACT

Background: Bone allografts carry a risk of infection, so terminal sterilization by gamma irradiation at 25 kGy is recommended; but is deleterious to bone quality. Contemporary bone banking significantly reduces initial allograft bioburden, questioning the need to sterilize at 25 kGy.
Methods: We inoculated allograft bone with *Staphylococcus epidermidis* and *Bacillus pumilus*, then exposed them to gamma irradiation at 0, 5, 10, 15, 20 and 25 kGy. Mechanical and biological properties of allografts were also assessed. Our aim was to determine an optimal dose that achieves sterility assurance while minimizing deleterious effects on allograft tissue.
Results: 20–25 kGy eliminated both organisms at concentrations from 10^1 to 10^3 CFU, while 10–15 kGy sterilized bone samples to a bioburden concentration of 10^2 CFU. Irradiation did not generate pro-inflammatory bone surfaces, as evidenced by macrophage activation, nor did it affect attachment or proliferation of osteoblasts. At doses $\geq$10 kGy, the toughness of cortical bone was reduced ($P < 0.05$), and attachment and fusion of osteoclasts onto irradiated bone declined at 20 and 25 kGy ($P < 0.05$). There was no change in collagen cross-links, but a significant dose-response increase in denatured collagen ($P < 0.05$).
Conclusions: Our mechanical and cell biological data converge on 15 kGy as a threshold for radiation sterilization of bone allografts. Between 5 and 15 kGy, bone banks can undertake validation that provides allografts with an acceptable sterility assurance level, improving their strength and biocompatibility significantly.
Clinical relevance: The application of radiation sterilization doses between 5 and 15 kGy will improve bone allograft mechanical performance and promote integration, while retaining sterility assurance levels. Improved quality of allograft bone will promote superior clinical outcomes.

Introduction

Allogeneic tissue transplantation has been used for over 50 years. Over the past two decades, around 10 million allografts were implanted worldwide [1]. While biomedical engineering searches for effective tissue replacements, allograft bone remains a necessary graft material in orthopedic surgery. The demand for allografts continues to increase. In the US, there were about 1.5 million allografts implanted in 2006, five times more than were implanted in 1996 [2]. The use of any allograft material carries with it the risk of transferring disease from the donor to the recipient. Among millions of tissue implantations, the American Association of Tissue Banks (AATB) reported an infection rate of 0.014% [1], some of which were fatal. Most of these infections occurred in allografts that were not subjected to terminal sterilization [3].

Allograft contamination is eliminated by donor screening, aseptic techniques during retrieval, processing and storage of the tissue [4]. Standards also ensure provision of allografts with a sterility assurance level (SAL) of 10^{-6} [5,6]. These standards require that donor screening includes socio-medical history and serology, and that aseptic techniques are used during procurement, processing and packaging. Above all, gamma irradiation with a minimum dose of 25 kGy, the "Standard Dose", was recommended in ISO standards for terminal sterilization of bone allografts to provide the prescribed SAL. That ensured that allografts (as medical products) are "sterile" with respect to bacterial infection, while donor screening is used to eliminate viral infection. At the Standard Dose, gamma irradiation not only sterilizes bone allografts

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but also weakens them mechanically and reduces their biological activity. For example, ultimate stress, strain, and toughness of bone allografts are significantly reduced after irradiation at 25 kGy or more [7–9]. Allograft irradiation at 25 kGy dramatically increases the rate of allograft fractures (39%) compared with non-irradiated allografts (18%) [10]. Irradiation at the Standard Dose also reduces the osteoinductivity [11] and osteoconductivity [12,13] of allografts, causing significant delays in host incorporation of the allograft [14].

During the past decade, a revolution in quality control in tissue banking has followed from the implementation of standards set by professional organizations such as AATB, International Atomic Energy Agency (IAEA), European Association of Tissue Banks (EATB), and government regulators such as the U.S. Food and Drug Administration (FDA) and Australian Therapeutic Goods Administration (TGA). As a result, the bioburden of allografts (being the number of microorganisms presenting on/in allografts prior to irradiation) following processing is extremely low, or even zero [15]. Processing refers to the preparation of allograft tissues after collection, and in most bone banks includes washing with sterilizing agents such as hydrogen peroxide and ethanol. Many such regulatory authorities also require that allografts undergo secondary sterilization. Recently, several tissue banks have successfully established radiation sterilization doses (RSD) for tissue allografts that are lower than the Standard Dose [15–17], with the lowest of these being 8.3 kGy [16]. The aim of lowering the RSD is to retain the prescribed SAL and to preserve the mechanical and biological properties of bone allografts, thereby leading to improved clinical outcomes for bone transplantation.

We hypothesize that tissue properties can be retained, with the required SAL of 10^{-6}, at a radiation sterilization dose between 10 and 15 kGy. Therefore, using a known bioburden, we sought to determine the threshold dose of radiation at which the SAL could be achieved, and its effect on the mechanical properties, biocompatibility and collagen biochemistry at different gamma doses. The broad objective of this work is to recommend a radiation sterilization dose providing the required SAL, while optimizing the biological and mechanical performance of the allograft.

Materials and methods

Bone sample preparation

With consent from the donor, or the donors' next of kin, 16 femora from 8 donors were provided by the Queensland Bone Bank (QBB). The Human Ethics Committee of the Queensland Health Forensic and Scientific Services approved the project.

Femora were processed according to QBB standard operating procedures in a clean processing room and stored at −75 °C. Each femur provided a femoral head, four cortical (diaphyseal) portions (l = 4 cm) and two cancellous blocks. It's important to note, here, that the improved procedures in tissue banking have led to significant reductions in the bioburden of processed bone allografts (i.e. prior to secondary sterilization). For example, a 12-month survey of QBB processing, demonstrated that bioburden of all types of bone allografts manufactured at QBB were zero [15]. Therefore, to establish valid recovery efficiencies and SAL, specimens were inoculated with bacterial species, consistent with ISO 11737-1, and described below.

Soft tissue and cartilage was removed from femoral heads, they were cut into small chips (0.3 g), washed with warm sterile saline, and soaked in 6% hydrogen peroxide and 70% ethanol for 10 min. One femoral head provided 36 bone chips for bacterial inoculation experiments. As detailed below, bone chips were inoculated with either *Staphylococcus epidermidis* or *Bacillus pumilus* at 10^1, 10^2 and 10^3 organisms/chip and allocated to 6 groups for irradiation (n = 16 per group). The bone chips reflect the volume of samples randomly taken from each pot of milled bone for micro culture at QBB, although the practical size of the milled bone may be smaller, depending on the required grade.

For 3-point bending tests, 3 cortical portions were sectioned into 6 beams (40 × 4 × 2 mm) per portion using a low speed saw (Leco VC50 diamond precision saw, USA). There was no significant difference in mechanical properties among cortical bone beams from the same portion (data not shown). Therefore, 6 cortical bone beams from the same portion were allocated into 6 irradiation groups. Eight donors provided 48 cortical portions, giving 288 cortical beams. The remaining cortical portion from each femur was sectioned longitudinally into cortical bone rods (40 × 5 × 5 mm). These were either sliced into 100-μm-thick sections for tissue culture or ground for biochemical analysis. Bone samples were wrapped in gauze moistened with saline, and placed in plastic containers before storing at −70 °C until testing.

Two cancellous bone blocks (60 × 20 × 20 mm) from femoral condyles were cut using the low speed saw to obtain 12 cancellous bone cubes (15 × 10 × 10 mm). Six specimens from each block were randomly allocated to 6 groups for compression testing.

Gamma irradiation (cobalt-60)

Specimens in gamma treatment groups were irradiated frozen at 5, 10, 15, 20, and 25 kGy at the Australian Nuclear Science and Technology Organisation, Lucas Heights, Australia. Control specimens (0 kGy) remained in a freezer. Dose-mapping verified that the delivered doses were 5.1 (4.7–5.5) kGy, 10.7 (9.9–11.5) kGy, 16.7 (15.5–17.9) kGy, 22.7 (21.1–24.3) kGy, and 26.6 (24.8–28.5) kGy.

Experimental methods

Sterility testing

The method was adopted from International Standard, ISO 11737-1.2006 [18], the Australian Therapeutic Goods Administration (TGA) guideline for sterility testing of medical products [19] and previous publications [20,21].

B. pumilus (ATCC 27142 — SGM biotech, USA) and *S. epidermidis* (ATCC 12228 — Australian Collection of Microorganisms — AMC) were supplied in stock suspensions. Working suspensions of 10^1, 10^2, and 10^3 organisms/0.1 mL were prepared according to the provider's instructions.

Bone chips were individually placed in 2 mL tubes according to organism type and population, and gamma dose; whereupon 0.1 mL of the bacterial suspension was added to the bone. Bone chips without bacterial inoculation, or irradiation, served as negative controls. The population in each working suspension was verified by direct agar plate inoculation (Table 1). Inoculated samples were returned to the freezer immediately after being inoculated and transported to the facility at the Australian Nuclear Science and Technology Organisation (ANSTO) under frozen conditions for irradiation.

After irradiation, sample tubes were filled with sterile saline, vortex mixed and the solution was filtered through Micropressure system membranes (Millipore, USA). The filter membranes were removed and placed onto either tryptic soy agar plates for *B. pumilus* or *Corynebacterium* agar plates for *S. epidermidis*. Plates were incubated at 30 °C (*B. pumilus*) or 37 °C (*S. epidermidis*) for 7 days. For the purpose of the sterility test, plates were observed and read as growth (non-sterile) or no-growth (sterile).

The TGA guideline for sterility testing [19] recommends that the efficiency of a removal method must be more than 50%. Efficiency of membrane filtration in our testing was 83% for *S. epidermidis* and 70% for *B. pumilus* (data not shown).

Table 1
The organism population (per 0.1 mL) of suspensions inoculated on to bone chips.

Species	10^3 suspension	10^2 suspension	10^1 suspension
S. epidermidis	2.0×10^3	3.7×10^2	4.7×10^1
B. pumilus	0.6×10^3	5.7×10^2	0.8×10^1