Microwave Sterilization of Femoral Head Allograft

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The potential shortage of allograft bone has led to the need to investigate other sources of bone for allografts. Some allograft bone donated from primary total hip arthroplasty recipients must be discarded or treated to become useable as a result of bacterial contamination. Femoral head allografts were contaminated with *Staphylococcus aureus* and *Bacillus subtilis*. A domestic microwave oven was used. The contaminated bone was exposed to microwave irradiation for different time periods. The samples were then cultured to attempt to grow the two bacterial species. The contaminated bone samples failed to grow any organisms after 2 min of exposure to microwave irradiation. This study shows that sterilization of femoral head allografts contaminated with *S. aureus* and *B. subtilis* can be achieved with microwave irradiation in a domestic microwave oven. This method of sterilization of bone allografts is cheap, easily used, and an effective way to process contaminated bone.

Femoral head allografts are collected from patients undergoing primary total hip arthroplasty. After the bone is frozen and confirmed to be free from bacteriological and viral infective agents, it can be released for use in operations such as revision hip surgery or spinal fusion. The increasing use of bone allografts, in particular, in association with impaction grafting for revision hip surgery, has increased the demand for bone allografts. This increased use of allograft bone has led Galea et al. (13) to suggest that the demand will soon outstrip the present supply from primary total hip replacements. Part of the shortfall occurs because femoral heads contaminated with bacteria are discarded. Bacterial contamination is found in between 10 and 15% of all available bone donations (13; R. Frame [Scottish National Blood Transfusion Service, Law Hospital, Lanarkshire, Scotland], personal communication, 1999). Before this bone can be used, it must be processed with gamma irradiation or ethylene dioxide. These expensive treatments are known to affect the quality of the bone (1, 4).

A simple, reliable, and cheap alternative for decontamination of donor bone would allow the use of previously contaminated bone and would help to address the shortage in allograft bone stocks. The project described here further examines the possible role of microwaves in decontamination of femoral head allograft bone.

MATERIALS AND METHODS

Femoral head allografts were obtained from the bone bank facility of the Blood Transfusion Service at Law Hospital, Carluke, Scotland. On harvesting of each of the femoral heads, bone chips were collected and swabs from the periosteal surface were taken and tested for contamination. All specimens were clear of bacterial contamination. The blood of all donors was tested and found to be negative for markers of infection with hepatitis B or C virus, human immunodeficiency virus type 1 or 2, or *Treponema pallidum*. To minimize the risk from Creutzfeldt-Jakob disease (CJD), none of the donors had a family history of CJD; had received growth hormone, human pituitary extracts, or gonadotrophic hormones; had undergone brain or spinal surgery to remove a tumor or cyst prior to 1993; or had any degenerative neurological condition. The bone was

frozen to −80°C. In all aspects the bone used was considered sterile by the Blood Transfusion Service at Law Hospital but was considered unfit for release because of a past history of neoplasia. Bones were stored before use at −30°C.

Each allograft specimen was thawed at 4°C in its sterile container prior to use. Under sterile conditions, remnants of the femoral neck were resected with a power saw to leave only the sphere of the femoral head. Four cores of bone were removed from the cut surface with a sterile trephine to give cores of bone approximately 2 cm long. The femoral heads were replaced in their sterile containers and stored at 4°C. Two cores of bone were then incubated in broth of *Staphylococcus aureus* and two cores of bone were incubated in broth with a *Bacillus* sp. for 24 h. After incubation one bone core from each of the incubation broths was replaced into the appropriate femoral head. The other core was immediately cultured. The femoral head was placed in a microwave oven (model M1713; Samsung) operating at 2,450 MHz. The specimens were irradiated for 0 (controls), 1, and 2 to 6 min at 800 W. The cores were then removed and cultured (see below). Three samples incubated with each species were irradiated for 1, 2, 3, and 4 min; and two samples incubated with each species were irradiated for 5 and 6 min (64 samples).

Microbiology. Two organisms were selected to test the effectiveness of microwave activity on sterilization of femoral bone cores. The organisms were *S. aureus* NCTC 6571 and *Bacillus subtilis* var. *globigii* (*B. subtilis*).

Overnight stock cultures of the two control organisms were prepared in 20 ml of Schaedler's broth. Optimum inoculation levels were established and consisted of 50 µl of stock culture for *B. subtilis* and 200 µl of stock culture for *S. aureus* NCTC 6571. Each of the organisms from the stock cultures was inoculated into 16 broth cultures (volume, 5 ml; two separate groups of broth cultures). All broth cultures were given a unique identifier to allow tracking of the individual femoral cores. Two femoral head cores were then placed in each broth culture and incubated aerobically at 37°C for 24 h to allow each core to become colonized with the appropriate organism. Two growth control broth cultures and a sterile control broth culture were also prepared and incubated as described above.

Following incubation, the femoral head cores were removed from the broth cultures. One core was immediately placed in 5 ml of sterile Schaedler's broth and the other was replaced in the original femoral head. Thus, two bone cores were inserted into each femoral head: one core inoculated with *S. aureus* and the other core inoculated with *B. subtilis*. At this stage Gram staining of each Schaedler's broth culture was performed to ensure the presence of spore-forming organisms and the presence of *S. aureus*. In addition, the *Bacillus* species chosen produced a distinctive red floccular appearance in the broth, which was evidence of growth of the organism in the broth.

The femoral head was then microwaved as described above. After irradiation both cores were removed from the femoral head and cultured separately in 5 ml of Schaedler's broth. The growth control and sterile control were subcultured onto Columbia blood agar (CBA) plates by using 300-µl sterile loops. All broth cultures containing the cores and the CBA plates were then incubated aerobically at 37°C for 24 h.

Following 24 h of incubation, all 64 broth cultures were subcultured onto CBA plates by using 300-µl sterile plastic loops to allow identification of growth. These plates were then incubated aerobically at 37°C for 24 h, and the broth cultures

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TABLE 1. Occurrence of growth in femoral head bone cores inoculated with bacteria and subjected to microwave irradiation[a]

Species and core no.	Growth after the following irradiation time (min)[b]:					
	1	2	3	4	5	6
S. aureus						
1	+	−	−	−	−	−
2	+	−	−	−	−	−
3	+	−	−	−	c	c
B. subtilis						
1	+	−	−	−	−	−
2	+	−	−	−	−	−
3	+	−	−	−	c	c

[a] All 32 control specimens were positive for growth.
[b] +, positive growth; −, negative growth.
[c] Insufficient bone samples were obtained to allow analysis of a third core at these times.

were reincubated. The broth cultures producing growth (identified by visible turbidity of the broth) were subcultured to confirm the presence of the relevant organism and then discarded. All other broth cultures were reincubated for up to 10 days, with daily inspection and subculturing of those cultures showing turbidity. The remainder of the broth cultures were terminally subcultured at 10 days. There was evidence of contamination in 1 of the 64 broth cultures.

RESULTS

Gram staining of the Schaedler's broth cultures after initial incubation but before irradiation identified *S. aureus* and a spore-forming *Bacillus* sp. in each of the cultures.

The results showed that no growth could be obtained in specimens subjected to microwave irradiation for 2 min or longer. No difference was noted for the two species of bacterium used as controls. All control (nonirradiated) specimens were positive for growth after 24 h, as determined by the method outlined above (Table 1). There was no growth in the sterile control. This suggests that no contaminants were present in the Schaedler's broth cultures before inoculation with the femoral head bone cores. The growth control for each species was positive. This suggests that the inocula from the stock specimens of the two species were sufficient to produce growth under the conditions used in this experiment.

DISCUSSION

The expected shortfall in allograft bone predicted by Galea et al. (13) presents a problem for all orthopedic surgeons, not just revision hip surgeons. Allograft bone is widely used. Therefore, every effort should be expended to maximize the retrieval of femoral heads from primary total hip replacements.

Even though femoral head allograft donations clear a rigorous screening program, up to 15% of donations are contaminated with bacteria (Frame, personal communication). This bone can still be processed for use. However, this processing is expensive, time-consuming, and not without potential risks to those working with and receiving the bone. In some centers the bone is discarded. A cheap, reliable, safe, easily used, and fast method of sterilizing allograft bone would be advantageous. Microwave irradiation could be such a technique. The effect of microwave irradiation has frequently been studied in the past.

However, only one report (20) to the European Federation of National Associations of Orthopaedics and Traumatology has suggested the use of microwaves to sterilize bone allografts. Microwave irradiation has been shown to be effective in the destruction of bacteria (2, 9, 11, 14, 15, 19–25), viruses (18, 21, 25), fungi (3, 8, 21), and parasites (6). To date most studies have examined the use of microwaves in the sterilization of laboratory equipment (2, 15, 19, 21–25) and surgical instruments (21, 22, 24). Douglas-Jones et al. (9) looked at the sterilizing effects of microwaves on *Mycobacterium tuberculosis* and their use for tissue fixation. All these studies showed that microwave irradiation sterilizes contaminated materials. The use of microwaves to sterilize bone allografts has not been reported in peer-reviewed journals.

Microwave irradiation has been shown to cause cell death in osteocytes, whose use in bone tumor surgery has been explored (10, 16). However, it appears that cell death occurs with exposures to microwave irradiation of durations shorter than those required to destroy bacteria.

This study has shown that femoral head allografts contaminated with bacteria can be rendered sterile by microwave irradiation.

The choice of *S. aureus* and *B. subtilis* was deliberate. *S. aureus* is a common pathogen and a common contaminant of femoral head allografts (5, 17). It is also relatively easy to grow from contaminated materials. *B. subtilis* is a spore-forming organism. It is commonly used in laboratories to determine the effectiveness of the sterilizing procedures used in hospitals and industry. Spores are more difficult to destroy than vegetative bacteria. Therefore, at the power settings used in this study, short exposures to microwaves were effective at destroying both forms of bacteria used in the experiment.

The method by which the microwaves destroy the organisms is not clear. The study clearly shows that short exposure to microwave irradiation achieves sterilization of experimentally contaminated femoral head allografts. Microwaves act on water, which absorbs microwaves. This presumably leads to heating of the water in the cytoplasm of the bacteria. This heating is presumed to kill the organism or render it incapable of cell division (7). Others suggest that microwaves have a direct lethal effect on the organisms (12). How the effect is produced is not clear, but it does appear to be real.

We wanted to perform the study with the same type of bone used for allografts. In our hospitals, this donor bone comes mainly from primary total hip replacement patients. We had considered the use of bone from hip fracture patients requiring hemiarthroplasty. However, we believed that the quality of the bone from these osteoporotic patients would be different and that the effects of microwaves on bone from these types of patients might not accurately represent the effects of microwaves on osteoarthritic bone. The bone samples used in the study were from our regional bone bank. They had been rejected because at the 6-month review the donor had indicated that he or she had a past history of one of the conditions highlighted in Materials and Methods that excluded the use of that particular bone specimen. In all other respects the bone samples used are exactly the same as those used for allograft bone. The selection process for bone donation is very strict and, therefore, this type of rejected bone becomes available very infrequently. We therefore had to wait a considerable

time to obtain the specimens that we used in this study. We accept that larger numbers of samples must be examined at each time interval to allow better investigation of the phenomenon that we have described in this report. However, the present study gives a clear indication that this intervention could prove to be useful for bone graft procurement in the future.

Further studies need to be performed with larger numbers of bone specimens to determine if the phenomenon that we observed is real. The biomechanical effects of microwave irradiation on bone were not addressed in this study. From the results of experiments with rat femurs, Liebergall et al. (16) have suggested that microwave irradiation of bones has minimal biomechanical effect. Whether this effect is consistent for larger human bones is not known but will be the subject of future experimentation.

The process of microwave sterilization of bone allografts is cheap and easy to use and appears to be effective for sterilization of contaminated femoral head allografts. If these preliminary data are confirmed on a larger scale, the use of microwave irradiation in this way could help to reduce some of the shortfalls in allograft stocks predicted by Galea et al. (13).

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