# High-pressure saline washing of allografts reduces bacterial contamination

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**ABSTRACT – 60 fresh-frozen bone allografts were contaminated on the operating room floor. No bacterial growth was detected in 5 of them after contamination. The remaining 55 grafts had positive bacterial cultures and were processed with three methods: soaking in saline, soaking in antibiotic solution or washing by high-pressure saline. After high-pressure lavage, the cultures were negative in three fourths of the contaminated allografts. The corresponding figures after soaking grafts in saline and antibiotic solution were one tenth and two tenths, respectively. High-pressure saline cleansing of allografts can be recommended because it improves safety by reducing the superficial bacterial bioburden.**

The use of allograft bone means not only the risk of viral transmission but also the much higher risk of bacterial infection (Chapman and Villar 1992, Deijkers et al. 1997, Aho et al. 1998, Aspenberg 1998, Bettin et al. 1998, Hirn and Krusius 1998, Journeaux et al. 1999). Allografts showing low virulence microorganisms in the samples taken at the time of donation can be sterilized by using chemicals such as ethylene oxide, heating or gamma irradiation (EATB and EAMST 1997). Each of these has some harmful effects on the structure or osteoinductive capacity of the bone (Ijiri et al. 1994, Fideler et al. 1995, Thoren and Aspenberg 1995, Currey et al. 1997, Aspenberg and Lindqvist 1998, Boyce et al. 1999). In addition, most of these methods are expensive and difficult to bring into clinical use, especially in smaller in-house bone banks. In the Tampere Bone Bank, we have used non-processed fresh frozen allografts. To obtain clean and safe material for clinical use, we have tried to find an inexpensive and easy method to reduce the bacterial contamination rate of allografts. The irrigation of contaminated wounds by high-pressure saline has proved useful in preventing infections (Rodeheaver et al. 1975, Stevenson et al. 1976, McDonald and Nichter 1994, Badia et al. 1996). We compared the cleansing effect of high-pressure saline on contaminated allografts by soaking allografts in saline and antibiotic solutions.

## Material and methods

15 clean fresh-frozen (–80 °C) femoral heads were selected from the hospital's own bone bank. They had been screened earlier by culturing small pieces of bone from the graft surface in 4 separate thioglycolate broth tubes and no bacterial growth had been detected. The femoral heads were split into 4 pieces to obtain 60 allograft pieces of bone. These 60 grafts had been contaminated on an operating room floor by rubbing one of their spongiosa surfaces against the floor and leaving them on the floor for 30 minutes. The operating room is normally used for arthroplasty surgery and is equipped with laminar flow. The floor had been cleaned 12 hours before for this study. Bacteria cultured from the floor were coagulase-negative staphylococci and Bacillus species. After contamination, swabs and small pieces of bone were taken from the most contaminated surface of the graft. The entire swabstick and the pieces of bone were cultured in separate thioglycolate enrichment broths for 14 days. A sample of the broth was stained with acridine orange. Positive tubes were cultured both aerobically and anaerobically.

Immediately after the specimens for bacterial

examinations had been taken, the grafts were cleansed using 3 methods. 55 grafts, which later proved to have positive bacterial cultures in at least one of the 2 primary bacterial cultures, were included in the final study. 18 of the contaminated grafts were soaked in physiologic saline, 19 in antibiotic solution (1.5-gram cefuroxim in 3 L of physiologic saline) each for 30 minutes and another 18 grafts were washed by high-pressure lavage, with approximately 0.9 L of physiologic saline per graft. Micro-Aire Pulse Lavage 4740 having an output pressure up to 7 bars (6 psi) was used. Then each of the 55 grafts was cultured separately as one piece in thioglycolate enrichment broth for 14 days.

## Results

Before cleansing, one or both bacterial cultures were positive in 55/60 of the contaminated grafts. The swab cultures were positive in 44 of the grafts and cultures of small pieces of bone were positive in 47. The bacteria were the same as those found on the floor before: coagulase-negative staphylococci and/or Bacillus species. In our preliminary studies, the amounts of bacteria from the floor and from the contaminated allografts were small. In the final study, an enrichment culture was used. The original amount of bacteria was not measured.

After cleansing, 2 of 18 grafts were found to be negative in bacterial culture after soaking them in saline for 30 minutes. 4 of 19 grafts were negative when an antibiotic was added to the saline. After high-pressure saline lavage, 14 of 18 grafts showed no bacterial growth. The difference between the high-pressure saline group and the other 2 groups was statistically significant, $p < 0.001$ ($\chi^2$-test).

## Discussion

Although the method of contamination in our study can be considered imprecise and the amount of contamination higher than in clinical work, the bacterial screening was unreliable. When only one bacteriological test was used, we found false-negative results in about 1/4 of the cases. Even with 2 tests, 5 of the grafts, which were obviously contaminated, were negative in cultures. If only grafts positive in culture are sterilized, there remains a risk of substantial contamination. Therefore, some cleansing of every graft seems necessary. In clinical work, contamination may derive from blood, air, or the patient's skin (Deijkers et al. 1997, Chowdhury and Pitt 1999). All donors are screened for any systemic hematogenous bacterial infection. The swabbing and culturing of small pieces of bone screen the graft superficially. However, fresh grafts can never be regarded as sterile and slight bacterial contamination may always exist. In some circumstances, it may reach a level causing clinical infection. According to our study, high-pressure saline lavage can reduce the superficial bacterial bioburden to a level which should improve safety. High-pressure saline was significantly more effective than soaking allografts in antibiotic solution, a method widely used (Deijkers et al. 1997).

High-pressure saline should be recommended for cleansing bone allografts, before packaging. To protect the environment from getting wet, the authors use a large sterile plastic bag in which the washing can be done. Bacteriological testing is performed after high-pressure lavage. The method presented is cheap and available in every hospital.

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