Why is the risk of disease transmission with bone allograft an important topic?

Approximately 1 million bone allografts are implanted every year in the United States. Although safety measures to prevent disease transmission have improved significantly over the past 2 decades, infectious complications remain a relevant concern. During informed consent, patients invariably take pause at the prospect of potentially contracting a serious and incurable condition from surgery. Although many are adequately reassured with the proverbial “1 in a million” estimated risk, astute clinicians should be prepared to provide the more incisive individuals a deeper understanding of the real and theoretical risks of allograft.

Are other products available for grafts?

In some situations, synthetic osteoconductive bone graft substitutes, such as calcium phosphate, can fill osseous voids and provide compressive support. Demineralized bone matrix consists of bone treated with hydrochloric acid, leaving collagen, noncollagenous proteins, and growth factors such as bone morphogenetic protein. Because at least 60% of the mineral content has been removed, the mechanical properties of demineralized bone matrix are poor, but with its osteoinductive properties, it is often combined with other allograft options, such as cancellous croutons. Demineralized bone matrix comes in different forms, including injectable paste, moldable putty, and flexible strips. Recombinant human bone morphogenetic protein products consist of an osteoinductive growth factor impregnated into an osteoconductive carrier, such as bovine collagen. Recombinant proteins are typically produced by genetically engineering a widely available and commonly used cell line, such as HEK293 (human embryonic kidney), and the risk of disease transmission from the biomanufactured protein itself is largely theoretical. Osteogenic allograft products are not available and can only occur if autogenous cells are used in conjunction.

How is allograft bone obtained and processed?

Allograft bone can be obtained from living donors (resected from the femoral head during total hip arthroplasty), multiple-organ donors (bone harvested in the operating room), and postmortem donors (bone harvested in the morgue). Two methods exist for allograft procurement: sterile surgical recovery (less than 12 hours after death) and clean, nonsterile recovery (less than 24 hours after death), in which the latter requires additional sterilization procedures. It should be noted that aseptic harvest alone does not render the tissue sterile, and simple antibiotic soaks do not eliminate clinically significant bacterial organisms.

The processing steps used by tissue banks vary, but typically involve a combination of mechanical lavage, biologic deter...
gents, antibiotic soak, and liquid sterilants. Alcohol and hydrogen peroxide have been shown to be virucidal and bactericidal, but a case report described hepatitis C transmission despite alcohol, antibiotic, and detergent soaks.\(^1,2\) One of the most important steps in reducing immunogenicity and disease transmission is fluid pressurization to maximally eliminate bone marrow and cellular debris. In massive osteochondral allografts, the epiphyseal cancellous bone and marrow are typically retained.

After packaging, terminal sterilization can be performed as a final decontamination step. Ethylene oxide has limited tissue penetration, is associated with adverse patient outcomes, and has been largely abandoned as a method of allograft sterilization. Gamma irradiation is often used in the range of 10 to 25 kGy (10 kGy≈1 Mrad). Although generally adequate for bacteria and spores, this level of irradiation is not necessarily virucidal for more resistant viruses. The reported range of effective irradiation is variable and dependent on the temperature during irradiation (colder temperatures require more irradiation), but a Gamma irradiation level as high as 34 to 50 kGy has been recommended for some types of the human immunodeficiency virus (HIV).\(^3\) Irradiation is not a substitute for careful donor screening and viral assays.

**How is allograft bone preserved?**

Fresh allograft has the highest risk of disease transmission, incorporates poorly due to immunogenicity, and is not used. Bone can be preserved 2 ways: freezing and lyophilization (freeze-drying). Frozen allografts are stored at a variety of temperatures ranging from 0°C in a freezer to −196°C in liquid nitrogen and typically have approximately 5 years of shelf life. Freezing the allograft does not significantly affect its mechanical strength or eliminate pathogens. Massive osteochondral allografts are typically deep frozen, and the articular cartilage is treated with dimethylsulfoxide or glycerol to maintain cellular viability.\(^4\) Lyophilized allografts are cooled to −70°C and then dried to less than 5% water content and stored at room temperature. The freeze-drying process reduces the bending and torsional strength of the graft, and lyophilized grafts must be rehydrated prior to use. Escalating doses of irradiation reduce the cyclic loading capacity of bone with a greater effect on freeze-dried bone at room temperature than deep-frozen bone.\(^1\) Lyophilization decreases the water content to levels that no longer support biological activity or chemical reactions, and it has been suggested that it decreases viral load to a subinfectious level, but an animal study dispelled this notion.\(^5,6\) Demineralized bone matrix is one example of lyophilized allograft.

**What additional safety measures prevent disease transmission?**

All musculoskeletal allograft donors undergo a detailed medical, social, sexual, and behavioral history to detect risk factors for diseases, including HIV, viral hepatitis, syphilis, prion disease, and bacterial sepsis. A physical examination is performed to detect evidence of transmissible diseases or suggestive findings of associated risky behavior, such as recent tattoos or other sexually transmitted diseases. Approximately 90% of donors are rejected based on exclusionary criteria for donor suitability.

Donor serological testing is performed to detect HIV, hepatitis B and C, syphilis, and human T-cell lymphotropic virus. However, because detectable levels of antibodies are not reached for 22 and 70 days after infection by HIV and hepatitis C, respectively, the risk still exists that the donor was infected just prior to death. With nucleic acid testing for the genetic material of the virus itself, the potential window is reduced to 7 days.

Aside from systemic donor disease, the other main source of infection is from postharvest contamination. Highly pathogenic bacterial organisms typically originate from the donor’s gastrointestinal or respiratory tract. Contamination can occur even in the surgical theater; swab cultures of sterile instrument packs are positive in 2.7% of tests. Swab and destructive cultures should be performed on the allograft tissue, including prior to antimicrobial suspension, to reduce false negatives. Because cultures are only 78% to 92% accurate, they should not be used as evidence of sterility, but only as a monitoring step for a previously validated sterilization process.\(^5\) Initial cultures are positive in up to 42% of cadaveric donor allograft compared with 1.2% to 22% of live donors. Reliance on terminal sterilization to fully decontaminate these specimens is questionable.

**Do regulatory bodies exist for bone allograft?**

The US Food and Drug Administration increased surveillance and auditing of allograft banks, and in 2007, new guidelines required nucleic acid testing for HIV and hepatitis C. In 2007, new FDA guidelines required nucleic acid testing for HIV and hepatitis C. Unfortunately, the FDA’s regulation of the tissue industry are largely unfunded mandates that limit its ability to enforce policy.\(^7\) The American Association of Tissue Banks is a voluntary accreditation organization that has its own set of tissue banking standards, but unlike the FDA, does not have disciplinary power. The Joint Commission on Accreditation of Healthcare Organizations regulates hospitals and established guidelines for working with allograft tissue. Because of the potential variability in processing methods and tissue sources, the American Academy of Orthopaedic Surgeons advises surgeons to know the tissue banker.

**What is the risk of disease transmission with allograft bone?**

For massive bone allograft, the risk of bacterial infection is approximately 12%,\(^8,9\) and the majority of the cases require resection of the graft or amputation to control the infection. For nonmassive allografts such as morselized bone, the risk is as high as 0.7%.\(^10,11\) Fortunately, not all contaminated grafts at time of surgery lead to clinically important infections. In comparison, the risk of transfusion-transmitted bacteremia is approximately 1 in 100,000 for platelets and 1 in 5 million for packed red blood cells.\(^12\) It should...
be noted that the Centers for Disease Control and Prevention reported 26 possible cases of bacterial musculoskeletal allograft infections, including 13 *Clostridium* spp and 11 gram-negative bacilli from 1996 to 2002. All cases could be traced to a single tissue bank processor, and only 3 grafts were gamma irradiated.\(^5\)

Because viral transmission is rare and allograft protocols have changed over time, the current risks are based on estimations rather than incidence statistics. The most recent reports were of HIV in 1985, hepatitis C in 2002, and human T-cell lymphotropic virus in 1991. The prevalence of hepatitis C in the general population is 1.8%, and 50% are unaware of their condition.\(^5\) The prevalence of HIV in the general population is less than 0.4%, and 20% are unaware of their condition.\(^4,15\) For tissue donors, the incidence rates are 0.03% for HIV and 0.012% for hepatitis C. The probability of false-negative serological testing (ie, in the window period) is approximately 18 in 1 million (1 in 55,000) for HIV and 24 in 1 million (1 in 42,000) for hepatitis C. With nucleic acid testing, the probability is reduced to 6 in 1 million (1 in 173,000) for HIV and 2 in 1 million (1 in 421,000) for hepatitis C.\(^16,17\) The risk is theoretically reduced further with virucidal tissue processing methods. Bone allografts containing marrow pose a relatively higher risk than cancellous chips that have been processed to remove the marrow. No reports have been published of disease transmission with demineralized bone matrix. In comparison, the estimated risk of HIV and hepatitis C transmission with nucleic acid testing for blood transfusion is 1 in 1.8 million and 1 in 1.6 million, respectively.\(^18\)

Iatrogenic transmission of prion disease has been reported with dural allograft, pericardial xenograft, corneal graft, and neurosurgical instruments and implants. Although no cases have been documented of prion disease from bone allograft to my knowledge, the concern is valid because the infectious particle has been isolated in blood products of affected individuals and since some graft material is bovine derived.\(^19,20\)

Additional factors should be taken into consideration regarding allograft safety. Estimated risks of disease transmission generally assume strict adherence to accepted protocols and do not take into account human error, immunovariant strains, and persistent antibody-negative carriers. Many cases of infection and disease transmission may go unreported.\(^21\) Rare infectious diseases, such as severe acute respiratory syndrome and monkeypox, are not screened for. The next new virus or unknown pathogen is always on the horizon. Less than 35 years ago, countless individuals were infected with HIV before anyone knew what it was or how it was transmitted.

**References**


