

## Current Concepts

# Overview of Safety Issues Concerning the Preparation and Processing of Soft-Tissue Allografts

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**Abstract:** With the increasing use of allograft tissue and the recent infections found in patients undergoing surgical procedures, the current practices that prepare grafts for implantation must be examined. Initially, most tissue banks harvest allografts aseptically. There are many steps in the different techniques of preparation and processing of allograft tissue. Before allograft tissue is ready for clinical use, it undergoes specific disinfection methods, according to the individual tissue bank's specific process. Tissue banks use in-process bactericidal and virucidal steps via physical cleaning, chemical treatments, or application of irradiation to the allografts (or some combination thereof). Gamma irradiation may also be used as a terminal processing method to reach an assurance of sterility after the allograft has been packaged. Because of the allograft toxicity potential, the use of ethylene oxide as a final tissue sterilization measure is really nonexistent. The role of the Food and Drug Administration and American Association of Tissue Banks in allograft tissue handling is presented, as well as the new rules that regulate tissue banks and affect their processing methods. **Key Words:** Allograft—Sterility—Tissue—Infection—Processing—Disinfection—Food and Drug Administration—American Association of Tissue Banks.

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In 2006 approximately 1.5 million bone and tissue allografts were implanted, most of which were bone-tendon-bone grafts.<sup>1</sup> Donors have increased from approximately 6,000 in 1994 to over 22,000 in 2005.<sup>2</sup> Currently, musculoskeletal allografts provide a solution to particular surgical procedures including revision surgeries where autograft is not available or as a supplement when an ample supply of autograft

tissue is not available.<sup>3-11</sup> The potential for viral or bacterial disease transmission is of great concern to clinicians and patients and is one of the limiting factors confounding the widespread adoption of allograft bone and soft tissue. As the use of allograft tissue increases, the safety of allografts will continue to be a critical issue and will hopefully be improved with advancing technology. This overview discusses the recently documented allograft infections and current regulatory changes for allograft tissue screening and processing.

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*0749-8063/06/2212-4439\$32.00/0*

*doi:10.1016/j.arthro.2006.10.009*

## CURRENT INFECTION ISSUES

### Viral Transmission From Musculoskeletal Allograft Tissue

Historically, musculoskeletal tissue transplantation has resulted in several reported cases of viral infection, specifically human immunodeficiency virus (HIV)<sup>12-14</sup>; however, this is misleading because 3 of these publi-

**TABLE 1.** History of Transmission of Viruses Through Allograft Transplantation

| Virus Transmitted                        | Year | Transmitting Allograft                             | Donor        | Reason for Viral Transmission   |
|--|------|--|--------------|---|
| HIV <sup>59</sup>                        | 1985 | 2 Femoral heads,<br>1 bone–patellar<br>tendon–bone | 22-year-old  | Within window period of testing positive for antibodies (anti–human T-lymphotropic virus III antibody, first-generation HIV antibody test).   |
| Hepatitis B virus <sup>16</sup>          | 1951 | Cancellous chips                                   | Living donor | Serologic testing to test for hepatitis did not exist (occurred outside of United States).  |
| HCV <sup>18</sup>                        | 1995 | Minimally processed,<br>cryopreserved              |              | Window period for HCV testing method used to screen donor. Organ/tissue donor was tested and found negative for HCV antibody. Organ recipient reported HCV infection 1 yr after transplant. Recipient of cryopreserved vein (tissue) also reported HCV infection postoperatively. Retest of donor sample via HCV NAT method revealed positive result. |
| Human T-lymphotropic virus <sup>17</sup> | 1991 | Femoral head                                       | Living donor | Living donor contracted human T-lymphotropic virus through blood transfusion during hip operation that resulted in donation of his femoral head.  |

cations refer to 1 tissue donor who transmitted HIV, viral hepatitis,<sup>15,16</sup> and human T-lymphotropic virus,<sup>17</sup> (Table 1). It should be noted that these transmissions occurred before the implementation of extensive donor screening for viruses and bacteria or the availability of validated serologic tests (or both). Some of these reported transmissions occurred outside of the United States.

In June 2002 the Centers for Disease Control and Prevention (CDC) reported a case of hepatitis C transmission from a patellar tendon allograft.<sup>18</sup> In the initial formal donor screening the serologic results indicated no detectable antibody to the hepatitis C virus (HCV), and no medical or behavioral risk factors were identified by history. During the ensuing investigation, a more rigorous HCV ribonucleic acid test was used on the donor's serum and confirmed that the donor was the probable source of HCV infection. Further investigation determined that the donor tissue was the source of disease transmission in at least 8 organ and tissue recipients from the 40 grafts that were transplanted.<sup>19</sup> It was noted that no cases of HCV transmission were reported in recipients of irradiated bone from the donor with the infection. The CDC stated that although HCV transmission is rare, assessing the frequency and risk of transmission is an important factor in determining whether additional preventative measures are warranted.<sup>18</sup>

### Bacterial Infection From Musculoskeletal Allograft Tissue

Historically, the transmission of bacterial infection from musculoskeletal tissue has been a rare event.<sup>20</sup> In March 2002 the CDC investigated 26 cases of possible allograft-associated bacterial infections that occurred over the preceding 5 years,<sup>20</sup> all of which could be traced to 1 tissue bank processor. The CDC investigations began after a 23-year-old man underwent reconstructive knee surgery in November 2001 in Minnesota and died 3 days after receiving a fresh femoral condyle bone-cartilage allograft. The blood cultures from this recipient were obtained before death and grew *Clostridium sordellii*.<sup>21</sup> Two other allografts from the same allograft donor, a fresh femoral condyle and frozen meniscus, were implanted into another patient also undergoing reconstructive knee surgery. Subsequently, septic arthritis developed. Eight additional tissues from the same donor were implanted, but no other infections were identified. This pattern of infection from certain allograft tissues but not others, originating from a single donor, suggested that improvements in tissue processing and in-process culturing methods may reduce the risk of disease transmission after transplantation.<sup>22</sup>

As shown in Table 2, of the 26 cases of bacterial infections reported by the CDC, 13 of the patients were infected with *Clostridium*. A single tissue pro-

**TABLE 2.** *Allograft-Associated Infections Investigated by CDC Transmitted by Musculoskeletal Grafts*<sup>20,24</sup>

|  | <i>Clostridium</i><br>spp | Other<br>Infections |
|--|---------------------------|---------------------|
| Tendons for anterior cruciate ligament | 8                         | 10                  |
| Femoral condyles                       | 2                         | 1                   |
| Bone                                   | 2                         | 1                   |
| Meniscus                               | 1                         | 1                   |
| Total                                  | 13                        | 13                  |

NOTE. Not all of these were probably associated or proven to be associated with the allograft; they were possibly associated with the allograft.

cessing facility processed 14 of 26 grafts. Whereas the *Morbidity and Mortality Weekly Report* referred to the tissue processor as Tissue Processor A,<sup>20</sup> the *New York Times* attributed those cases of bacterial infections to tissue processed by CryoLife (Kennesaw, GA).<sup>23,24</sup> Since the publication of the 2002 report, a few additional cases have been reported by the CDC.<sup>25</sup> Additional cases of allograft-associated infections were reported from 20 states involving 12 different tissue processors.<sup>25</sup>

A recent additional case of invasive bacterial disease involved a healthy 17-year-old male subject who became infected with *Streptococcus pyogenes* after reconstructive knee surgery.<sup>26</sup> This group A streptococcus infection was also found in 50 patients. This specific organism had not previously been reported with allograft-associated diseases. Although invasive group A streptococcus disease is most commonly associated with skin and soft-tissue infections, if allograft in-process culturing methods are not robust and able to detect contamination and processing methods are not validated to reduce or eliminate various levels of bioburden, this can result in contaminated allografts.<sup>26</sup> Allograft implants responsible for bacterial infections were all processed aseptically. Some were soaked in antimicrobial solutions, but no sterilization procedures, such as gamma irradiation, were used.<sup>20,26</sup> Although aseptic processing attempts to minimize contamination of donated tissue, it does not eliminate contamination originating from the donor.<sup>27,28</sup> If a tissue processor is accredited by the American Association of Tissue Banks (AATB), it is mandatory to adhere to a maximum allowable recovery time period for tissue recovery after asystole. A tissue processor should also assess the incoming bioburden of the recovered tissue from the donor so that the efficacy of its disinfection method or sterilization method (or both) can be evaluated. Failure to adhere to standard

tissue recovery times may result in contaminated donor tissues and increase the possibility of an infected processed allograft.<sup>28</sup>

## ALLOGRAFT TISSUE SCREENING AND PROCESSING FOR CLINICAL USE

### Aseptic Processing Techniques for Allograft Tissue

Aseptic processing refers to the harvesting of most allografts, beginning with the recovery of the tissue in an operative suite or morgue, followed by variable steps attempting to get the tissue ready for storage, before shipping and, eventually, implantation. This practice of soaking is the first step in allograft processing, whereby some tissue banks attempt to reduce the possible contamination to the allograft tissue that can come from the harvest environment, the working personnel, or the processing equipment. As stated in the CDC's *Morbidity and Mortality Weekly Report* from March 15, 2002, "aseptically processed tissue should not be considered sterile, and health-care providers should be informed of the possible risk for bacterial infection."<sup>20</sup> Organisms of high pathogenicity are generally endogenous, arising from the donor's gastrointestinal tract or respiratory tract.<sup>29</sup> Clinically significant organisms are not reduced by simple antibiotic soaks.<sup>30</sup> Therefore, the tissue industry relies on donor screening, which includes serologic testing for viruses, physical examination of the donor, and medical background review, as well as bacterial and fungal cultures, to accept or reject allograft tissue for transplantation.<sup>31</sup>

Culturing is performed on allograft tissue to detect bacteria and fungi after aseptic tissue processing. Studies have shown that cultures are only 78% to 92% accurate, and culturing is simply not an absolute method for ensuring allograft sterility.<sup>32</sup> In fact, the United States Pharmacopeial Convention, the standards-setting body for sterility testing and other quality-control procedures used in the medical industry, specifically states that cultures for sterility can only be used to monitor a previously validated sterilization process and should not be construed as definitive evidence of sterilization.<sup>33</sup>

Donor screening, although extremely effective in eliminating donors with active viral infections, has many limitations. With all viral infections, there may be a "window period" in which the donor does not have detectable antibodies to the virus.<sup>34</sup> The risk of implanting tissue from an HIV-infected donor via

current screening and testing protocols has been estimated to be as low as 1 in 1 million.<sup>22</sup> Recent work has estimated an overall risk of allograft-associated infections at about 4 in 1 million.<sup>35</sup> However, the risk of implanting tissue from donors with hepatitis B virus or HCV infection is significantly higher because of the greater prevalence of these viruses in the general population and the limitations of current testing methods.<sup>34</sup> The general population has a reported positive incidence of 1.8% for hepatitis C, and individuals may be hepatitis C carriers (50% with no history of hepatitis C) and not know it.<sup>35</sup> Because of the limitations of donor screening and microbial culturing, the current aseptic processing practices can reduce but not eliminate transplantation of infectious tissue.

Human errors in screening and processing have been shown to be a factor in the reliability of safe blood donation. The risk of bacterial infection from fresh platelet infusions is reported to be 1 in 2,172.<sup>36</sup> The incidence of postoperative infections currently estimated by the National Nosocomial Infection Surveillance System of the CDC is reported to range from 0.6% to 2%.<sup>37</sup> In light of these estimates, the risk of allograft infection to the average patient is much less.

### Allograft Tissue Disinfection

*Disinfection* is defined as the process of removing any possible contamination from the allograft tissue. *Sterilization* has been defined as the process or act of inactivating or killing all forms of life, especially microorganisms.<sup>38</sup> Studies published on swab cultures done on sterile instrument packs opened in an operating room setting showed an incidence of positive cultures averaging 2.7%.<sup>37</sup> Ideally, allograft tissue could be available sterile just like other medical devices, and disease transmission would be improbable. Unfortunately, human cadaveric tissue is not as easily sterilized as metals or plastics. For metal and plastic medical devices, the levels of bacteria and spores are measured for sterility. According to the Association for the Advancement of Medical Instrumentation, exposure to a validated sterilization process should achieve a sterility assurance level of  $10^{-6}$  microorganisms.<sup>39</sup> The Food and Drug Administration (FDA) considers a sterility assurance level of  $10^{-3}$  as adequate for implantable medical devices. However, for musculoskeletal allografts, the levels of bacteria, spores, and fungi must all be eliminated to ensure sterility. In addition, viruses offer unique challenges to sterilization

validation that can only be addressed by specific inactivation and testing protocols.<sup>22,40</sup> Blood is a significant pathogen reservoir and is reported to be the primary source for endogenous contamination of musculoskeletal tissue.<sup>22,40,41</sup> Consequently, tissue processing is a crucial component for ensuring allograft safety.<sup>22</sup> Given the complex physical surface structure of musculoskeletal tissue, this has proved to be a difficult task. A disinfection method, even if it can “sterilize” tissue, must not affect biomechanical properties, must completely penetrate the tissue, and must not affect the biocompatibility profile and graft incorporation characteristics.<sup>20</sup>

Currently, there is no one best way to clean, disinfect, or “sterilize” allograft tissue. There are several major processing companies with unique and specific methods of disinfection to “sterilize” allograft tissue. For example, Allowash formula (LifeNet, Virginia Beach, VA) uses irradiation (10 to 13 kilograys [kGy]), ultrasonics, centrifugation, and negative pressure in combination with reagents such as biologic detergents, antibiotics, alcohols, and hydrogen peroxide to increase the solubilization and removal of bone marrow, blood elements, and lipids. The BioCleanse tissue sterilization process (Regeneration Technologies, Alachua, FL) uses a low-temperature chemical sterilization process that perfuses the inner matrix of the tissue with liquid sterilants and then irradiates the tissue (25 kGy) (not bone-tendon-bone grafts). The Clearant Process (Clearant, Los Angeles, CA) treats tissue with 50 kGy of radiation, 2 to 4 times the dose recommended to avoid cell damage. This process claims to prevent radiation damage to cell proteins and devitalized tissue by freezing the sample, extracting the water, and adding dimethylsulfoxide as a pretreatment radioprotectant.<sup>42</sup> CryoLife uses a slow freezing rate via glycerol to remove water, a process known as *cryopreservation*. There is no subsequent disinfection method, such as gamma irradiation; rather, the cryopreserved tissue is incubated in a patented cocktail of bacterial antibiotics for 24 hours at 37°C before being frozen to  $-135^{\circ}\text{C}$ .<sup>43</sup>

There are other less commonly used methods of “sterility”/disinfection. Although all of these unique and different processes may be theoretically sound and some are even supported by scientific research, more studies are needed on the specific intricacies of each process and their effects on allograft tissue. Some banks offer freeze-dried (lyophilized [as discussed later]) tissue to surgeons.

**TABLE 3.** Comparison Between Aseptic Processing, Ethylene Oxide, Gamma Irradiation, and Chemical Soaking Methods and Their Sterilization Abilities and Properties

|  | Aseptic Processing <sup>63</sup> | Ethylene Oxide <sup>51,57,62</sup> | Gamma Irradiation <sup>28</sup> | Chemical Soaking <sup>5,70</sup> |
|--|----------------------------------|------------------------------------|---------------------------------|----------------------------------|
| Kills bacteria   | No                               | Yes                                | Yes                             | Yes                              |
| Kills fungi  | No                               | Yes                                | Yes                             | Yes                              |
| Kills spores   | No                               | Yes                                | Yes                             | No                               |
| Kills enveloped and non-enveloped viruses (e.g., HIV, hepatitis A) | No                               | Yes                                | Yes (dose-dependent)            | No                               |
| Removes blood and lipids   | Surface only                     | No                                 | No                              | Surface only                     |
| Preserves strength   | Yes                              | Yes                                | Decreases (dose-dependent)      | Yes                              |
| Preserves biocompatibility   | Yes                              | Yes                                | Yes                             | Yes                              |
| Penetrates into tissue   | Surface only                     | Thickness-dependent                | Full penetration                | Surface only                     |

### Storage and Packaging

Most of the major tissue banks follow their unique processing methods with the common storage guidelines of the AATB by deep freezing the tissue at  $-80^{\circ}\text{C}$ .<sup>28</sup> Tissue banks use a freeze-drying process (lyophilization) for bone tissue, which allows tissue storage at room temperature. CryoLife uses a unique cryopreservation technique for soft tissue.

The process of lyophilization freezes the tissue, and then the water content is reduced ( $<6\%$  of initial weight<sup>44</sup>), first by sublimation (referred to as the primary drying process) and then desorption (known as the secondary drying process) to values that will no longer support biologic activity or chemical reactions.<sup>43</sup> Two clinical reports have suggested that the freeze-drying process may decrease the viral load in infected musculoskeletal allograft tissues to a subinfectious level.<sup>45,46</sup> In these studies soft tissue from an infected HIV donor showed no detected antibodies to HIV in the serum of patients who received these freeze-dried allografts. A recent in vitro study has concluded that freeze drying did not inactivate the HIV retrovirus in an infected feline cortical bone and tendon.<sup>47</sup> Freeze-dried grafts are not commonly used nationally (roughly  $<5\%$ ), but they appear to be clinically safe when used in knee surgery.

Many tissue banks label their packaging and allograft contents as sterile. When the proposed good tissue practice (GTP) regulations are finalized (as discussed later), any label claims will be subject to FDA scrutiny and validated justification will be required for any claims made.<sup>48</sup>

### Terminal Processing Techniques

The terminal processing of allografts may be the last step in preparing the tissue for implantation, de-

pending on the culture swabs at the specific tissue bank. There are several terminal disinfection/“sterilization” techniques to inactivate bacteria and spores. Typically, tissue banks have used 1 of 2 methods to attempt disinfection—ethylene oxide gas<sup>28,49-58</sup> or gamma irradiation<sup>39,59-61</sup>—although various chemical antimicrobial solutions have been used as well. Some of these methods are more effective than others in eliminating microbiologic contamination and endogenous material such as blood and lipids while not adversely affecting the material properties of the allograft tissue (Table 3). Further study is required to evaluate these processing methods’ influence on sterility, as well as their effects on the biomechanical properties of orthopaedic soft tissue. In recent years ethylene oxide has fallen out of favor because of host tissue reactions found with ethylene oxide-treated grafts.<sup>51,57,62</sup> Most banks currently use radiation if terminal sterilization is attempted.

### RECENT REGULATION ISSUES AND CHANGES

With these current uncertainties, it is important that orthopaedic surgeons know the source of their allograft tissue. Allograft tissue processors are regulated and are required to follow mandatory federal and state regulations. The FDA and only 2 states, New York and Florida, currently require inspection and licensure. California currently requires licensing of tissue banks. Maryland and Georgia require registration of tissue banks.

Perhaps the most successful advances in allograft safety involve recent FDA rulings, 21 CFR parts 1270 and 1271, that address tissue bank registration, donor suitability guidelines, and proposed GTP.<sup>63-65</sup> This will improve past difficulties that the FDA has had

with tissue bank regulation.<sup>31,50</sup> GTP involves a similar approach to the current good manufacturing practices outlined for medical devices in 21 CFR part 820. The established GTP will increase the safety of manufactured human cells, tissues, and cellular and tissue-based products and help decrease any introduction, transmission, or spread of communicable disease through tissue banking. As of May 25, 2005, all manufactured or processed allograft tissues are subject to these new regulations. This final rule represents years of work by the FDA to ensure that there exists a comprehensive risk base system for regulating human cells, tissues, and cellular and tissue-based products. The FDA will now increase surveillance and auditing of tissue banks with strict enforcement through warning letters, recall of tissue, or possibly shutting down tissue banking at that establishment. These requirements aim to prevent the introduction, transmission, and spread of any communicable diseases, such as viruses, bacteria, fungi, parasites, and spongiform encephalopathy agents.

The FDA does not require that tissue undergo a specific informal sterilization process or technique. Any written representation that an establishment's processing methods reduce the risk of transmission of communicable diseases must be based on a fully verified and validated process. The FDA requirements apply only to tissues recovered on or after May 25, 2005. The FDA's GTP final rule comprises strong new changes for the tissue banking industry. The practicing orthopaedic surgeons who work with allografts must be aware of these new rules and regulations and be very familiar with their tissue processor and its compliance with these new rules.

The AATB is a voluntary professional accrediting organization for the tissue banking industry. It is a scientific nonprofit peer group organization that promotes high-quality transplantable human tissues. An AATB-accredited tissue bank undergoes onsite inspections every 3 years and, as a processor, must show that its procedures comply with AATB standards. As of March 9, 2005, the AATB also requires nucleic acid testing (NAT) screening for HIV and HCV.<sup>66</sup> NAT screening uses a highly sensitive polymerase chain reaction test to look for genetic material of viruses such as HIV and HCV. This screening increases the safety margin for allograft use by decreasing the "window period" not detectable by traditional antibiotic testing. This requirement does not apply to tissues prepared before March 9, 2005, and currently stored in inventory by AATB banks. It should be noted that the FDA's final rule does not

require tissue banks to use NAT screening. It is highly recommended that one obtain tissue only from banks that are accredited by the AATB.<sup>35</sup> The AATB, as a voluntary trade group, does not have disciplinary powers like the FDA.

The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) released new hospital standards and requirements when working with the tissue banking industry effective July 1, 2005.<sup>67</sup> These new standards are applicable to hospitals and apply to specimens that can be found in clinical laboratories, surgical centers, or outpatient centers. The JCAHO calls for responsibility by these institutions for overseeing their individual tissue program with regard to storage and tissue activity within the hospital setting. This includes validating tissue supplies and a better understanding of the tissue banks with which the hospitals work. There are guidelines for tissue ordering, transportation, and storage, including recording and documenting all of these activities within the institution. The tracking and identification of allograft tissue within the hospital will ensure staff and patient safety. The JCAHO also called for reporting of adverse events and prompt investigation of any adverse event.

## FINAL ALLOGRAFT CONSIDERATIONS

With the increase in allograft use in orthopaedic surgery, the recent FDA changes have produced a safer environment for our patients. Disease transmission is rare. Improved technology for tissue processing and preparation with stronger government tissue

**TABLE 4.** *American Academy of Orthopaedic Surgeons  
"Seven Simple Questions to Ask Your  
Tissue Processor"*

1. What are your standard procedures for evaluating potential contaminants on incoming tissue?
2. What procedures do you use to detect microorganisms?
3. What in-process steps do you use to reduce the biologic contamination of processed allografts?
4. Do medical professionals make the final determination on the release of allografts?
5. How do you address adverse event complaint reports from surgeons?
6. Are you in compliance with FDA regulations and accredited by the AATB?
7. Does your serologic evaluation of tissue include NAT for HIV and HCV?

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bank regulation and inspection provides better allografts. The American Academy of Orthopaedic Surgeons has recommended that surgeons should educate themselves if they choose to use allograft tissue and evaluate the specific processing methods of their chosen tissue bank.<sup>63,68</sup> It has outlined “Seven Simple Questions to Ask Your Tissue Processor” (Table 4) to help inform orthopaedic surgeons.<sup>69</sup> Improved education of patients and doctors will provide better soft-tissue allografts and better health care.

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