Current Safety Sterilization and Tissue Banking Issues for Soft Tissue Allografts

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KEYWORDS

• Allograft • Tissue bank • Processing • Graft • Safety

The number of recent grafts harvested and *implanted* in the United States has steadily increased. According to the American Association of Tissue Banks (AATB), the number of grafts distributed increased from 500,000 in 1998 to 1,300,000 in 2003.¹ This trend has been seen in other countries as well.²⁻⁴ Although they are used in other specialties, allografts are used predominantly in orthopedic sports medicine and reconstructive procedures. Orthopedic surgeons more often make daily decisions on the use and implantation of these tissue grafts. Many surgeons do not have a great understanding about allograft tissue and tissue banking. According to a recent survey by the American Orthopaedic Society for Sports Medicine (AOSSM), over 85% reported using allografts and over half of those surveyed did not know whether their grafts were sterilized or the specific sterilization process used.^{5,6}

The public is concerned regarding the safety of allografts and transmission of disease.⁷ As complications from allograft contamination have occurred, so has oversight from government agencies such as the Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC).

REGULATION ISSUES

Oversight of tissue processors is mandated by state and federal regulations and has greatly improved in the last decade. Human cells or tissues intended for another human recipient are classified as "human cell, tissue, and cellular and tissue-based products" (HCT/P). The federal code established in 2004 mandated the FDA Center for Biologics Evaluation and Research (CBER) to regulate HCT/P.⁸ These regulations required tissue banks to register and list their HCT/P with the FDA, to screen and test

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donors to reduce the transmission of communicable diseases, and to keep detailed records documenting the type of tissue processed, tests performed, results, and destination of the tissue.

Federal and state governments are not the only entities who have oversight of the tissue banks. The Joint Commission (JC), formerly known as the Joint Commission on Accreditation of Healthcare Organizations (JCAHO), is a regulatory entity independent of the FDA. In 2007, the JC released the latest version of Tissue Storage and Issuance Standards.⁹ These standards apply to implantable and transplantable products that are human or cellular based. The JC requires hospitals, critical access hospitals, ambulatory office-based surgery, and outpatient centers to develop procedures to address the critical areas of tissue recovery and storage, record-keeping and tracking, and adverse events/infection follow-up. These written procedures are required to describe protocols for tissue ordering, receipt, temperature-monitored storage, tissue handling, preparation for use, tracking the graft from receipt to implantation, and investigation/reporting of adverse events or possible infections. According to these guidelines, hospitals and surgery centers should be able to trace any tissue bidirectionally to report potential disease transmission to the recipient when notified by the tissue bank as well as report to the donor facility any adverse reactions. This improves record-keeping and adverse event monitoring. They have power of accreditation over hospitals and tissue banks, which, without these entities, would have difficulty billing state and private insurance companies, providing further new quality controls for current allograft tissue.

An organization critical to the regulation of tissue banks is the AATB. Founded in 1976, the AATB is a nonprofit organization to spread voluntary safety standards and ensure that human tissues intended for transplantation are safe and free of infectious disease, are of uniform high quality, and are available in quantities sufficient to meet national needs.

The AATB first published its Standard for Tissue Banking in 1984. Since then, it has been updated and revised, with the 12th edition released in 2008.¹⁰ Two years after the release of the Standard for Tissue Banking, the AATB began an accreditation program for institutions. This was followed 2 years later by a certification program for individuals. Accreditation is renewed every 3 years and is based on compliance with its standards. In addition, the AATB may perform surprise inspections to ensure compliance.¹¹

The AATB standards are stringent. Specifically, the AATB required nucleic acid testing (NAT) for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in March 2005. NAT reduces the window period, which is the time between infection and when the virus is detectable. The FDA later required this testing in August 2007.

The AATB is also strict regarding culture results. It requires that any processed allograft that tests positive for *Clostridium* or *Streptococcus pyogenes* be discarded.^{6,12} Furthermore, the AATB requires that a graft with positive final culture be discarded if there is no validated protocol to eliminate the identified organism.

Currently, the AATB has 106 accredited tissue banks, and it has been estimated that AATB-accredited tissue banks distribute 90% of musculoskeletal tissues in the United States.¹² Membership in the AATB is voluntary, and the AATB does not have any formal disciplinary powers outside of restriction or removal of AATB accreditation. The “committee on biological implants tissue work group” of the American Academy of Orthopaedic Surgeons have urged the orthopedic surgeons to work with AATB tissue banks and “know their tissue banker.”¹³ Other authors have stated that a tissue bank not accredited by the AATB should be “a red flag”¹⁴ with respect to quality.

INFECTION/DISEASE TRANSMISSION ISSUES

Over 10 million grafts have been implanted in the past 20 years, and relatively few incidents of disease transmission have been reported. Based on reports in the literature, the incidence of infections is estimated to be 0.02% from around 20,000 transplants a year and 0.0004% from around 900,000 allografts per year.¹⁵ A survey performed by the AATB reported the incidence of allograft-related infection for the years 2003-2004. There were 192 reports of suspected allograft infections overall in 1.35 million grafts for an incidence of 0.014%. Forty-two percent of these allografts were soft tissue grafts, and 59% of these grafts involved orthopedic/sports medicine procedures. During this period, there were only 2 probable/confirmed allograft-related infections for an incidence of 0.00015%.¹⁵ This is compared with a postoperative infection rate of 0.6% to 2%.¹⁶

PROCUREMENT AND PROCESSING

There are several critical steps taken during allograft procurement and processing to reduce the risk of transmission. These steps include donor screening, tissue procurement, tissue processing, and packaging/final sterilization. It is important to discuss the effects that these different tissue processing/sterilization techniques have on the graft properties.

Disease transmission with allograft transplants is possible from 2 main sources: an infected donor and contamination from tissue processing and packaging. To prevent transmission, Good Tissue Practices use several steps to reduce infections from allografts. The first is to prevent infected tissue from entering the donation pool. This is done by screening and donor selection. This process is further refined by cultures taken from the tissues themselves or their liquid environment. The next step is to prevent contamination. This is completed by performing the procurement in a timely fashion under aseptic conditions. The third step is to reduce or eliminate infecting agents. Newer processing techniques promise improved allograft safety without graft compromise. This is achieved during tissue processing as well as final sterilization.

One of the primary ways to prevent disease transmission by allograft transplantation is appropriate donor selection.^{10,17} The most important step is a careful history and identification of risk factors for infectious diseases. This should also include a careful review of the medical history from different sources, including surviving family members, hospitals, clinics, and private doctor's offices. Other records such as blood donation history or autopsy records can also be obtained. The next step in donor screening is testing for an active infection. There are limitations of tests that rely on the presence of antibodies or antigens, such that there is a window period where the donor is actively infected but exhibits no detectable immunologic response. NAT reduces the window period from 22 days (HIV) and 70 days (HCV) to 7 days.¹³ With current screening and testing protocols, the estimated risk of implanting tissue from an HIV-infected donor is 1 in 173,000 to 1 in 1 million. The estimated risk of implanting tissue from an HCV-infected donor is 1 in 421,000.¹⁸ The risk of contracting hepatitis is much higher than the risk of HIV. The estimated prevalence of 1.2 million with hepatitis B and 3.2 million with HCV¹⁹ is responsible for the increased risk. The incidence of HCV in the general population is 1.8%, and many of these patients do not know that they are carriers. Furthermore, 50% of carriers have no history of hepatitis or even know that they are seropositive.²⁰ The actual numbers of potential tissue donors that are infected with these viruses are unknown, and these numbers do not take into account the processing steps to reduce the risk of disease transmission.

Emerging pathogens have raised concern regarding the safety of allografts. There are very few data regarding the potential threat from the West Nile virus and the severe acute respiratory syndrome (SARS) coronavirus. Furthermore, there is no current screening test for prior diseases associated with spongiform encephalopathies. The risk of acquiring these diseases from a tissue allograft is unknown but is likely to be rare as is the overall incidence of these diseases in the general population.

Tissue recovery is the next step in minimizing the potential for disease transmission. According to the CDC, allograft tissue should not be considered sterile and the health care provider should be informed of the possible risk for bacterial infection.²¹ Recovery of tissue is performed under aseptic conditions, under standard sterile operating room techniques, yet contamination can be introduced by the human handling of the tissues. Time to harvest is also critical to reduce the risk of infection; according to AATB guidelines, tissue procurement must take place within 24 hours of asystole if the body is cooled or 15 hours if the body is not cooled.¹² Contamination has been documented from the breakdown of the donor's gastrointestinal or respiratory systems, and recovering tissue after this period has resulted in contamination and implanted infections.^{11,22}

As each tissue is procured, it is cultured, wrapped, labeled, and sealed in dedicated containers at wet ice temperatures. Surface swab cultures are performed to evaluate for the presence of bacteria and fungi; they are not used to establish sterility but to monitor previously validated sterilizing processes. Studies have shown that surface swab cultures are only 78% to 92% sensitive.²³

STERILIZATION ISSUES

After the tissues are procured, they must be processed and secondarily sterilized. Many tissue banks have their own proprietary sterilization technique. Ideally, a sterilization process should eliminate any microorganisms while maintaining the mechanical and biological characteristics of the allograft. Furthermore, any sterilization reagent used should permeate the tissue and be safely removed from the tissue without residue.

Sterilization is expressed as a mathematical probability of relative risk. The FDA requires a sterility assurance level (SAL) of 10^{-3} for implantation of a medical device. This means that there is a 1 in 1000 probability that a viable microbe exists in or on the implantable device. This is the acceptable level of SAL for HCT/Ps (human tissues). Many tissue banks, however, are applying AATB sterility standards required for medical devices at a level of SAL of 10^{-6} .

There are many different tissue processing methods used by tissue banks. Several tissue banks have a proprietary sterilizing method to process tissues, which must be validated and documented on site for any surprise inspection by the FDA regulators. **Table 1** outlines many of the processes used by major tissue banks.

Some tissue banks use gamma radiation to sterilize their products after packaging. This is referred to as terminal sterilization. Low-dose (<2 Mrad) gamma radiation is documented to be effective at sterilization, but studies have also shown that it generates free radicals that can adversely affect the structure of collagen and ultimately the mechanical properties of the graft. Higher radiation levels (>2 Mrad) have demonstrated biomechanical weakening of the collagen tissues.²⁴ Historically, ethylene oxide (ETO) gas was used for terminal sterilization, but its use was discontinued because of documented patient tissue reactions with ETO-treated grafts.²²

At this time, there is no one ideal sterilization technique for soft tissue allografts. The FDA does not specify which process or technique should be used. The FDA only

Table 1
Tissue banks and tissue-processing techniques

Tissue bank	Sterilization method
AlloSource	SterileR validated bioburden reduction cleansing system followed by low-dose terminal irradiation to provide SAL 10^{-6} . Package is labeled "sterile."
Bone Bank Allografts	GraftCleanse: proprietary blend of cleansing agents used to reduce bioburden and provide aesthetic white appearance. GraftCleanse: terminal low-dose gamma irradiation achieves package sterility.
Community Tissue Services (CTS)	Musculoskeletal grafts are soaked and rinsed in antibiotics, hydrogen peroxide, alcohol, sterile water, and AlloWash solutions. Low-dose terminal gamma irradiation is used to eliminate most bacteria.
LifeNet	AlloWash XG: rigorous cleansing removes blood elements followed by decontamination and a scrubbing regimen to eliminate bacteria and viruses. Tissue is terminally irradiated at a low dose to reach SAL 10^{-6} and is labeled "sterile."
Musculoskeletal Tissue Foundation (MTF)	MTF processes soft tissue allografts aseptically and treats the grafts with an antibiotic cocktail of gentamicin, amphotericin B, and imipenem and cilastatin sodium (Primaxin). Some incoming tissue is pretreated with low-dose gamma irradiation to reduce bioburden. No terminal irradiation used.
OsteoTech	OsteoTech processes allograft tissue using aseptic technique in class 100 clean rooms. Isolators are used to prevent cross-contamination.
RTI Biologics, Inc.	BioCleanse: an automated chemical sterilization process that is validated to remove blood, marrow, and lipids and eliminate bacteria, fungi, spores, and viruses while maintaining biomechanical integrity and biocompatibility. No preprocessing or terminal irradiation is used on sports medicine allografts. All tissues reach SAL 10^{-6} post-BioCleanse.
Tissue Banks International (TBI)	Clearant Process: pathogen inactivation process involving high-dose gamma irradiation at (5.0 Mrad) combined with radioprotectant that sterilizes tissue in the final packaging, significantly inactivates infectious agents, and maintains the function of the allograft. Process yields SAL 10^{-6} and package is labeled "sterile."

requires that claims of sterility be documented and validated. The 2002 guidelines state that these representations are subject to scrutiny by the FDA. Tissue studies are often performed on tissue immersed in the pathogen of interest and not from systemically infected donors. This is not the ideal infection model.

STORAGE OF ALLOGRAFT TISSUES

After procuring, processing, and packaging, allograft tissues are stored frozen. According to AATB guidelines, musculoskeletal tissues should be stored at -40°C or colder and can be held for up to 5 years.¹⁰ A temperature range of -20°C to -40°C is thought to be a more short-term storage condition and safe for up to 6 months. When shipped between supplier and end user, tissue should be kept on dry ice.¹²

DISCUSSION

Allograft implantation in orthopedic procedures has increased steadily over the last decade. Patient infections and widespread media coverage have raised concern

regarding the safety and efficacy of the allografts. Tissue banks, government agencies, and non-profit organizations have undergone multilevel changes to reduce the risk of disease transmission. Safely procuring and processing the grafts and thorough screening of donors have improved the quality of the allograft pool and decreased the risk of disease transmission. The regulatory agencies hope to ensure graft safety through improved regulation and standardized treatment methods. Finally, improved communication with a unique identification for each graft and donor leads to more efficient future monitoring and detection of infections and perhaps prevention of implantation of suspected tissues. All these steps make the clinical use of musculoskeletal allografts the safest it has ever been for the patient.

As newer methods of sterilization are developed, further biologic and biomechanical tests need to be performed. As tissue banks state claims of sterility and biomechanical properties, these statements should be independently evaluated. Oversight by governing bodies should provide monitoring and long-term follow-up of these new processes. As recommended by the American Academy of Orthopaedic Surgeons, surgeons need to be familiar with the tissue bank they work with and how their grafts are processed. Tissue banks and the processing of graft tissues still face challenges. Emerging diseases and pathogens reveal the need for more sensitive testing. Ultimately, the patients will continue to have improved tissue safety now and in the future.

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