Ensuring the Safety of Allograft Tissue

By Lloyd Wolfinbarger, Jr., PhD and Lila M. Eisenlohr, PhD, MBA

The risk of bacterial infection and disease transmission through tissue transplantation continues to cause significant concern among transplant recipients and implanting surgeons. Despite these concerns, musculoskeletal allograft usage has increased markedly in the past decade. The American Association of Tissue Banks (AATB) reports that in 2005 more than 1,300,000 musculoskeletal allografts were distributed in the United States. This is twice the number of allografts that were distributed in 1999. Overall, more than six million musculoskeletal allografts have been safely transplanted in the United States in the past decade. Preparation methods used by tissue processing facilities, which include screening for disease, microbiological testing, and aseptic processing, substantially reduce risk but do not completely eliminate the possibility of infections associated with allograft implantation. Sterilization has been adopted by several allograft processors as a method for eliminating microorganisms without adversely affecting the biomechanical and biochemical characteristics of allograft tissue. This article examines the current state of allograft safety and steps that are being taken by the tissue banking industry to minimize the risk of disease transmission through allograft tissue.

THE RISK OF DISEASE TRANSMISSION

Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human T-lymphotropic virus (HTLV) have all been reported to have been transmitted by tissue transplantation. According to a recent study that looked at data from the various review and testing procedures utilized by tissue banking organizations in the United States, the estimated incidence of viremia at the time of donation is as follows: 1 in 55,000 for HBV, 1 in 34,000 for HCV, 1 in 42,000 for HIV, and 1 in 128,000 for HTLV. The study concludes that the prevalence rates of HBV, HCV, HIV, and HTLV infections are lower among tissue donors than in the general population. The authors estimate that the prevalence ratios for tissue donors relative to those in the general population are 0.54 for HBV, 0.61 for HCV, and 0.46 for HIV. This lower finding is not surprising, as tissue donors are carefully selected based on medical history, physical examination, and interviews with next of kin.

Furthermore, blood samples from each tissue donor are tested for infectious diseases as required by the U.S. Food and Drug Administration (FDA) and American Association of Tissue Banks (AATB). Nonetheless, the concern of testing being performed during the so-called viremic window period, the period of time from infection until the virus can be detected by laboratory assays, is legitimate. Improved screening tests such as the recently introduced Nucleic Acid Testing (NAT) are implemented by many tissue banks as they become available and approved for use in donor tissue screening by the FDA. Table 1 provides an overview of the effect of NAT testing in the detection of HIV and HCV infection.

COMBATING LIMITATIONS IN TISSUE SAFETY

While the goal of allograft tissue processing is to provide the safest possible products to the surgical community while preserving the inherent tissue characteristics of the graft, even with adequate donor screening there remains a risk of allograft contamination. Oversight of tissuebanking practices has, however, become increasingly stringent to include monitoring by the FDA, the AATB, and individual state agencies. The FDA requires preparation, validation, and written procedures to reduce the probability of contamination during processing. The requirements under the Current Good Tissue Practices (CGTP) for human cells, tissues, and cellular and tissue-based products cover procedures, facilities, personnel, equipment, supplies, reagents, process and labeling controls, process changes and validation, storage, receipt and distribution, records, tracking, as well as handling of complaints. The AATB has established quality standards for procuring and processing tissue including the time limits for retrieval and for screening donors. The AATB also publishes recommendations for preservation, sterilization, preparation, evaluation, and labeling of tissues. Individual tissue banks can apply for voluntary accreditation by meeting AATB standards, which include use of aseptic techniques, microbiological testing (i.e., aerobic, anaerobic, and fungal pre- and post-processing cultures, as appropriate), and adverse outcomes reporting.

Despite these recognized guidelines, procedures for the preparation of allografts could further be enhanced for safety. Not all tissue banks, for instance, apply for AATB accreditation; in 2002, approximately 10% of musculoskeletal allografts were processed by non-accredited tissue banks. Kainer and colleagues demonstrated in a recent investigation that infections acquired through bacterial contamination of allografts have the potential to result in substantial complications or death. The study recommends that current regulations and standards for processing and testing allograft tissue need to be enhanced to prevent such life-threatening allograft-associated infections.

DEFINING STERILITY

Strictly speaking, a product should only be considered sterile when there is a complete absence of viable microorganisms; however, due to limitations in processing technology and environmental monitoring, no aseptic environment or aseptically produced product is provably sterile. A sterility assurance level (SAL) of 10 is comparable to the microbial survivor probability of aseptically produced products and is a level similar to the overall efficiency of an aseptic operation. A SAL of 10 is sometimes

**Table 1.** Window period and estimated risk of prevalence

<table>
<thead>
<tr>
<th>Virus</th>
<th>Window Period using FDA Licensed Tests</th>
<th>Blood Donor Estimated Risk (infect donor)</th>
<th>Tissue Donor Estimated Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV antibody – 22 days</td>
<td>with NAT* – 7 days</td>
<td>without NAT – 1,550,000</td>
</tr>
<tr>
<td></td>
<td>HCV antibody – 70 days</td>
<td>with NAT* – 1,2 million</td>
<td>with NAT* – 1,2 million</td>
</tr>
</tbody>
</table>

*Adapted from Jui et al. 2000*

- **Abbreviations**
  - AAMI: Association for the Advancement of Medical Instrumentation
  - AATB: American Association of Tissue Banks
  - ANSi: American National Standards Institute
  - CGTP: Current Good Tissue Practice
  - CGMP: Current Good Manufacturing Practice
  - FDA: Food and Drug Administration
  - HCV: hepatitis C virus
  - HIV: human immunodeficiency virus
  - ISO: International Organization for Standardization
  - HTLV: human T-lymphotropic virus
  - NAT: Nucleic Acid Testing
  - SAL: Sterility Assurance Level
  - USP: United States Pharmacopoeia

The United States Pharmacopoeia (USP) establishes in its standards that a sterility assurance level (SAL) of 10 is comparable to the microbial survivor probability of aseptically produced products and is a level similar to the overall efficiency of an aseptic operation. A SAL of 10 is sometimes
equated to culture negativity in microbiological testing. In contrast, physical sterilization technologies result in an SAL of $10^{-6}$, or lower, that is, whereas a $10^{-6}$ SAL provides a probability of one viable microorganism in a thousand units, products with a $10^{-6}$ SAL will have no more than a single viable particle in a million units. Consequently, the lower the SAL, the lower the chance of contamination by microorganisms and the greater the assurance of sterility.

In guidelines set forth by the Association for the Advancement of Medical Instrumentation (AAMI), the recommended SAL varies according to the intended use of the product. Sterilized medical devices that are not intended to be in contact with breached skin or compromised tissues are generally thought to be safe for use with an SAL of $10^{-6}$. Invasive and surgically implanted devices should have an SAL of at least $10^{-6}$.

Current regulations do not require tissue banks to eliminate bacteria present on tissues at the time of recovery or to use processing methods that guarantee tissue sterility. One way that tissue banks process allografts under aseptic conditions by treating the tissue with various chemical, mechanical, and detergent steps, using methods that prevent, restrict, or minimize the contamination with microorganisms from the environment, processing personnel, or equipment.

Asptic processing alone does not reduce the inherent microbial bioburden present in donor tissue but only minimizes the risk of additional contamination. Due to the limitations of processing technology and environmental monitoring, asptic processing does not eradicate microorganisms and spores, especially in tissue that is heavily contaminated at the time of recovery. Reduction of the microbial burden can only be accomplished through understanding of the bioburden of the processed product, asptic processing, use of a validated and disinfection process, a validated terminal sterilization process, and the correct interpretation of test results.

Tissue banks have developed methods for tissue sterilization with the goal of ensuring the maximum safety of allograft tissue. Sterilization of allograft tissue has associated challenges, however:

- Not all sterilants such as gases and liquids have adequate tissue penetration
- Musculoskeletal tissue may have a high incoming bioburden
- Tissue is an organic material that can serve to protect microorganisms, leading to a failure in the sterilization process
- The biomechanical and biochemical properties of tissue can be adversely affected

Numerous sterilants and sterilant combinations are used to eradicate microorganisms on allograft tissues. These include chemical sterilants, gas plasma, ethylene oxide (EO), gamma radiation, and e-beam radiation as well as sterilization systems such as those developed by several allograft tissue processors.

Ethylene oxide gas treatment and gamma irradiation are two sterilization methods that are typically employed by tissue banks and have known bacterial and viral effects. Even so, both methods have the potential to create technical problems with tissue. Ethylene oxide has a limited capacity to penetrate tissue and has been associated with adverse patient outcomes such as chronic synovitis. It therefore has been largely abandoned as a sterilizing agent for tissue. High doses of unprotected gamma radiation that are effective against viruses have been shown to adversely affect the biomechanical properties of allografts. Gamma irradiation is, nonetheless, the most popular option for the sterilization of allograft tissue.

To overcome the potential issues associated with gamma irradiation, several tissue banks have now developed controlled-dose, low-temperature sterilization approaches to eradicate vegetative microorganisms and spores while preserving biomechanical integrity and function of allograft tissue necessary for surgical applications. The Association for the Advancement of Medical Instrumentation (AAMI) has instituted standards and recommended practices for the radiation sterilization of health care products that have been adopted by the tissue banking industry. The sterilization of musculoskeletal grafts, both soft tissue and bone grafts, using gamma irradiation.

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Step Four–Rigorous Cleaning: Through treatment with hypotonic solutions and antimicrobial reagents and/or use of processes such as ultrasonication and centrifugation, blood elements as well as bone marrow and lipids are solubilized and removed from the tissue. Key solutions that contain a combination of detergents are forced into and through the bone matrix and then directed to waste, resulting in the lysis of cells and cleaning of the tissues.

Step Five–Disinfection and Rinsing: The tissue, freed from over 99% of marrow and lipids, is subjected to an intensive decontamination, disinfection, and cleaning regimen designed to remove and eliminate viruses, bacteria, and fungi. Tissue then undergoes water soak, medication treatment, followed by centrifugation and/or micro-absorption to remove excess water and processing residuals.

Step Six–Terminal Sterilization: The Allowash XG® process concludes with a validated controlled level dose of gamma irradiation administered at low temperatures after the tissue has been packaged. This final step results in tissue with a Sterilization Assurance Level (SAL) of 10^-6 without compromising the biomechanical or biochemical properties of the tissue needed for its intended surgical application.  

CONCLUSION

Using a validated methodology, controlled dose, low-temperature gamma irradiation can be used to obtain sterile allografts. Peer-reviewed literature, pre-clinical testing, and clinical outcome data all indicate that allografts processed using Allowash XG® exhibit no measurable detrimental effects in regard to the properties of the tissues needed for surgical applications. Whereas other tissue banks might claim sterility at a SAL of 10^-5, the Allowash XG® system delivers sterile allograft tissue to a SAL of 10^-6.

When making their choice among tissue suppliers, clinicians seek to find a balance between utmost tissue safety and greatest tissue efficacy in order to achieve the best patient outcome. With Allowash XG® technology, LifeNet Health is able to satisfy both needs. Today, it is more critical than ever that physicians and hospital administrators rely on sterile tissue provided by well-known, accredited tissue banks such as LifeNet Health. With Allowash XG®, LifeNet Health takes tissue safety to the next level. LifeNet Health’s Allowash XG® technology encompasses a comprehensive and validated process during which greater than 99% of bone marrow and blood elements are removed from the internal bone matrix. This step, along with a subsequent chemical sterilant treatment, has been shown to substantially reduce the bacterial and fungal bioburden and inactivate viruses. The Allowash XG® process concludes with a controlled terminal sterilization step that results in a SAL of 10^-6 without compromising the biomechanical or biochemical properties of the tissue as needed for its intended surgical application.

Since 1995, over 1.5 million allografts have been delivered to the medical industry and no incident of disease transmission has been directly linked to tissue screened and processed by LifeNet Health.

REFERENCES


16. The United States Pharmacopeia/The National Formulary. Sterilization Assurance Level (SAL) of 10^-6 is comparable to the microbial survivor probability of aseptically produced products and is a level similar to the overall efficiency of an aseptic operation. In: contras, physical sterilization technologies such as irradiation result in an SAL of 10^-12 or lower; whereas, a 10^-6 SAL provides a probability of one viable microorganism in an million units, products with an SAL of 10^-6 will have no more than one single viable particle in a million units. Consequently, the lower the SAL, the greater is the assurance of sterility.


