

Ensuring the Safety of Allograft Dermal Tissue

Mark Moore, PhD, Kristen Trost, MSS, Ralph Powers, DDS

BIO-IMPLANT BRIEF

Transplantation of human tissues carries an inherent risk of bacterial infection and disease transmission. Despite this risk, allograft usage has increased markedly in the past decade. The American Association of Tissue Banks (AATB) reports that in 2007 more than two million allografts were distributed in the United States, which is twice the number of allografts that were distributed in 2003.¹ Overall, more than ten million allografts have been safely transplanted in the United States in the past two decades.²

The risk of disease transmission is mitigated by standard practices in use by tissue processing facilities, which include screening for disease, microbiological testing, and aseptic processing. These methods substantially reduce, but do not completely eliminate, the possibility of infections associated with allograft implantation. As a further step, 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 dermal tissue safety and steps that are being taken by the tissue banking industry to minimize the risk of disease transmission.

The Risk of Disease Transmission

Virus, bacteria, and fungi have all been reported to have been transmitted by allograft tissue transplantation.³⁻⁶ According to a study that looked at data from the various review and testing procedures utilized by tissue banking organizations in the United States, with a focus on viruses, the current risk of viral transmission is thought to be exceedingly low.^{7,8} The study concludes that the prevalence rates of HBV, HCV, and HIV infections are lower among tissue donors than in the general population. This lower finding is not surprising, as tissue donors are carefully selected based on medical history, physical examination, and interviews with next of kin, with the intent of avoiding donation from those most likely to carry disease risk. As required by the U.S. Food and Drug Administration (FDA) and the AATB, blood samples from each tissue donor are tested for infectious diseases. However, there is still a legitimate concern of testing conducted during the so-called viremic window period, which is the time from infection until the virus can be detected by laboratory assays.

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 tissue-banking practices has, however, become increasingly stringent to include monitoring by the FDA, 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, donor eligibility determinations, screening and testing, process and labeling controls, process changes and validation, storage, receipt and distribution, records, tracking, as well as handling of complaints.⁹ 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.¹⁰ 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, and resultant inspections; for a current list of accredited banks, please go to the AATB website and search for accredited banks.¹¹

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.^{12, 13}

The United States Pharmacopeia (USP) establishes in their standards that a Sterility Assurance Level (SAL) of 10^{-3} is comparable to the microbial survivor probability of aseptically produced products and is a level similar to the overall efficiency of an aseptic operation. An SAL of 10^{-3} sometimes equates to culture negativity in microbiological testing. In contrast, physical sterilization technologies can result in an SAL of 10^{-6} or lower, that is, whereas an SAL of 10^{-3} provides a probability of one viable microorganism in a thousand units, products with an SAL of 10^{-6} will have no more than a single viable particle in a million units.^{12,13} Consequently, the lower the SAL, the lower the chance of contamination by micro-organisms and the greater the assurance of sterility.

In guidelines set forth by the Association for the Advancement of Medical Instrumentation (AAMI), the recommended acceptable SAL varies according to the intended use of the product.¹⁴ 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^{-3} ; 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.¹⁵ Most 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.⁹

Aseptic 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, aseptic processing does not eradicate microorganisms and spores, especially in tissue that is heavily contaminated at the time of recovery.^{13,16} Reduction of the microbial burden can only be accomplished through understanding of the bioburden of the pre-sterilized product, aseptic processing, use of a validated cleaning and disinfection process, a validated terminal sterilization process, and the correct interpretation of test results.^{12,17}

Tissue Sterilization Techniques

Several 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:¹⁸

- 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 heat, chemical sterilants, gas plasma, ethylene oxide (EO), gamma irradiation, supercritical CO₂, and e-beam radiation, and other sterilization systems developed by allograft tissue processors.

Ethylene oxide gas treatment and gamma irradiation are two sterilization methods that are typically employed by tissue banks and have known bactericidal and virucidal 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 synovitis¹⁹, therefore it has been largely abandoned as a sterilizing agent for tissue.

To overcome the adverse effects that high dose unprotected gamma radiation potentially has on the biomechanical properties of allografts, several tissue banks have now developed controlled-dose low-temperature sterilization processes.^{20,21} These approaches can eradicate vegetative microorganisms and spores while preserving biomechanical integrity and function of allograft tissue necessary for surgical applications.

Ensuring the Safety of Allograft Dermal Tissue

The AAMI has instituted standards and recommended practices for the radiation sterilization of health care products that have been adopted by the tissue banking industry.^{14,22} Based on the ANSI/AAMI/ISO 11137 Method 2B guidelines, Moore and colleagues at LifeNet Health® undertook a study to validate sterilization of allografts, both soft tissue and bone grafts, using gamma irradiation.²³ The sterilization method determines the minimum absorbed dose of radiation necessary to achieve an SAL of 10^{-6} for products with consistently low levels of microbial bioburden. Investigators demonstrated that Method 2B terminal sterilization validation can readily be transferred from the medical device industry to tissue banking by appropriately modifying the microbiological assessment methods to include testing for both aerobic and anaerobic microorganisms. Valid and reliable results are produced when appropriate considerations are taken into account.

It should be noted that the destruction of microbiological contaminants by physical or chemical agents follows an exponential law. The probability that a microorganism can survive is a function of a number of different factors; these include the number and types of contaminants on the product, the lethality of the sterilization method, and, under specific circumstances, the environment in which the organisms are situated during the sterilization process. Consequently, the sterility of a particular unit of product cannot be assured unconditionally.²⁰ Furthermore, the efficacy of the sterilization process cannot be verified by inspection or testing of the product itself. The sterilization process, even if it is validated and controlled, is not the only factor that assures that an allograft is sterile and suitable for implantation. The incoming bioburden of the donor tissue, a controlled environment in which the tissue is processed, packaged, and stored as well as the integrity and barrier properties of the packaging all contribute to the safety of the final product.²²

Preparing Dermal Tissue

LifeNet Health prepares its dermal tissue using a unique series of patented and proprietary technologies including decellularization with Matracell® and room temperature preservation with Preservon®, plus a final terminal sterilization step.

Decellularization. Matracell, a patented and proprietary decellularization process, was developed to reduce the impact processing reagents have on the biomechanical and biochemical properties of tissue, while still eliminating unnecessary cellular components.²⁴ Donor cells and DNA are removed from the allograft using a mild combination of an anionic, non-denaturing detergent, N-Lauroyl sarconsinate (NLS) in addition to Benzonase®, a recombinant endonuclease. This enzyme is used to efficiently degrade the DNA without introducing the disease risk, including prion transference, associated with other animal-extracted endonucleases. Subsequently, in a process utilizing USP grade normal saline, decellularization reagent residuals and donor cell remnants are removed from the allograft. The allograft dermal tissue is thus rendered acellular without compromising the biomechanical or desired biochemical properties for their intended surgical applications.

Preservation. Preservon, a patented and proprietary preservation technology, preserves the processed allograft in a solution comprised of USP Glycerol and USP Saline. This allows the decellularized dermis to be stored at room temperature while also avoiding freeze-drying processes that could cause tissue damage.

Sterilization. Removal of microorganisms through a validated, controlled low-dose, low-temperature, gamma irradiation step results in a sterile allograft dermal tissue with an SAL of 10^{-6} . Whereas other tissue banks might claim sterility at an SAL of 10^{-3} , LifeNet Health's terminal gamma irradiation step delivers sterile allograft dermal tissue at an SAL of 10^{-6} .

Conclusion

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 possible. With allograft dermal tissue processed using LifeNet Health's Matracell and Preservon technologies, LifeNet Health is able to satisfy both needs with the added safety provided by a terminal sterilization step. 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.

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