Managing biologics

Understanding tissue processing

Third in a series on managing bone allografts. In the October issue, articles included were Allografts: Overview of the process; and Donor screening: First step in safety. In the November issue, articles included Help in evaluating bone allografts; Bone allografts: Options for healing; and Making good choices of DBM products.

Tissue cleaning and sterilization provide an additional level of safety beyond donor screening and procurement for preventing the transmission of infection from allografts. Tissue processors need to strike a fine balance between eliminating microorganisms and preserving the integrity and quality of the graft. This is an overview of tissue cleaning/disinfection and sterilization methods with suggestions for evaluating processing claims.

Tissue processing is defined by the American Association of Tissue Banks (AATB) as “any activity performed on tissue, other than tissue recovery or collection, including preparation, preservation for storage, and/or removal from storage, to ensure the quality and/or sterility of human tissue. Processing includes steps to inactivate and or remove adventitious agents.” (“Adventitious” refers to agents that occur sporadically and are not inherent or innate.)

When sterilization is performed

Sterilization methods can often be completed during tissue processing with cleaning and disinfection steps or in the allograft’s final packaging with terminal sterilization. Sterilization is defined by the AATB as “a validated process used to render tissue free from all viable microorganisms, including spores.” If a sterilization step is employed by the tissue supplier, the process must be validated, a sterility assurance level must be set, and the process must reduce the allograft bioburden to the set level in its final packaged form.

Tissue banks use proprietary methods to process and sterilize tissue and must independently validate the processes employed. The US Food and Drug Administration (FDA) does not require tissue to undergo sterilization. The FDA and AATB do not recommend specific tissue processing or sterilization methods but state that all tissue processors must validate all tissue processing methods used.

Tissue processors must also ensure that cleaning/disinfection and sterilization methods maintain the tissue’s mechanical and biological integrity. An allograft’s strength is more important in some tissue forms than others. For example, soft tissue allografts must maintain strength to support the repair or replacement of the patient’s own tissue. Strength is also
critical when cortical bone is used in load-bearing applications, such as in spine surgery and large joint reconstruction.

**Tissue cleaning/ disinfecting methods**

Common chemical methods for cleaning/disinfecting allograft tissue use aqueous solutions of detergents or surfactants, hydrogen peroxide or other peroxides, organic solvents, acids, and alcohol. Chemical methods are often combined with mechanical methods. These processes must:

- completely penetrate the tissue
- remove endogenous materials such as blood, lipids, cells (for acellular tissue forms), and bone marrow
- reduce the level of microbiological and viral contamination.

Certain processing methods may have more deleterious effects on tissue properties than others. For example, extended exposure to hydrogen peroxide can significantly reduce the osteoinductivity of cortical bone; that is, the graft’s ability to induce the growth of new bone cells (DePaula et al, 2005).

**Sterilization of tissue**

Sterilization of allograft tissues is a controversial area. It is difficult to sterilize many allograft tissues because of their sensitive structure and components. It is also challenging to remove all of the potential organisms within tissue without disrupting the allograft's biological and structural integrity. The issue becomes even more challenging as technology advances, and tissue banks introduce more “fresh” or cellular tissue forms. Because cells cannot withstand sterilization, some tissue banks rely on donor screening and mild processing techniques to maintain safety.

**Evaluating cleaning/ disinfection and sterilization methods**

Asking questions of your tissue bank can help in understanding the complex relationships between tissue banks, tissue processors, and distributors. Hospitals should set criteria for evaluating tissue suppliers (sidebar, p 16).

More information on evaluating processing and sterility claims and on developing criteria for tissue suppliers are in *Hospital Tissue Management: The Practitioner’s Handbook* by the American Association of Blood Banks, AATB, and the Eye Bank Association of America.

The handbook advises that criteria include whether the tissue supplier’s facility and all related processors and intermediaries:

- are registered with the FDA
- hold state licenses (if applicable)
- are accredited.

Among other attributes the handbook suggests considering:

1. The transparency of the supplier’s organization and its willingness to provide information about its operations and tissues, including specific data on the efficacy of tissue processing or disinfecting methods.
2. The involvement and availability of the supplier’s medical director.
3. The supplier’s ability to provide the tissue needed and to meet any special requirements.

The handbook also recommends asking for a tissue processor’s inspection findings; FDA warning letters, if any; and any other related communi-
This information can be requested from the FDA directly. Warning letters are posted at www.fda.gov

Thomas E. Mroz, MD, a spine surgeon, and his colleagues in a 2008 article suggested questions to ask of tissue banks. Among these are: Is the graft at risk of being structurally or biologically compromised because of the type of sterilization? Does the bank validate its technique? Is there a sterility assurance level?

The safety and quality of the allograft are related not only to processing and sterilization but also to the initial donor screening and selection. The more stringent a tissue bank’s donor criteria, the gentler it can be on cleaning and disinfection, and the more biological properties the tissue maintains.

Ultimately, the techniques the tissue bank employs must yield safe, effective allografts that are processed and validated to maintain the tissue’s natural function to ensure the best possible outcome for recipients.

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References


Measuring tissue sterilization

Sterilization is measured by a sterility assurance level (SAL), which the Association for the Advancement of Medical Instrumentation (AAMI) defines as the probability of a single viable microorganism remaining on an item after sterilization. AAMI states that an SAL of \(10^{-6}\) is appropriate for medical devices, equating to a 1 in a million chance that a viable microbe remains on the device. AAMI also says that this level of sterility is appropriate if the medical device can withstand these sterilization methods without adversely affecting its essential safety and function.

The FDA considers an SAL of \(10^{-3}\) appropriate for biologic medical devices, which is an estimated 1 in 1,000 probability of the presence of a viable microbe (AAMI, 2007).

The FDA does not require a specific SAL level for tissue banks; however, both the FDA and the AATB state that if a sterilization process is used, and an SAL is claimed, it must be validated.

There are 2 methods to validate sterility of tissue:

**Sterilization for pharmaceutical-grade products**

The first method is per the US Pharmacopia (USP) guidance that governs sterilization for pharmaceutical-grade medical products that cannot undergo terminal sterilization (eg, injectable medications and sterile solutions). A tissue bank may choose to clean, disinfect, and aseptically process bone and soft tissue rather than use terminal sterilization because of the potential deleterious effect of terminal sterilization on tissue.

The tissue bank uses a validated cleaning and disinfection method with strict adherence to aseptic technique.

Sterility testing is then performed on the processed tissue in accord with the USP <71> Sterility Test. The test demonstrates that the tissue itself is not inhibitory to the growth of microbial contaminants. Tissue that is processed through aseptic means and passes all of the appropriate testing related to the USP guidance can be labeled as “sterile per USP.”

**Terminal sterilization**

In terminal sterilization, tissue is sterilized in its final package. Many tissue banks use a terminal sterilization step and claim to achieve a SAL of \(10^{-6}\). If this claim is validated, the FDA allows the bank to use the word “sterile” on its packaging.

Allografts may be terminally sterilized by several methods, including ethylene oxide, gamma radiation, electron (E)-beam radiation, and hydrogen peroxide plasma.

The most common sterilization technique used for tissue is gamma radiation. Many tissue banks use a low to moderate dose of gamma radiation to sterilize their tissues in their final packaging. High levels of gamma radiation have been shown to be detrimental to tissue integrity (Currey et al, 1997; Ijiri et al, 1994). Ethylene oxide has also been associated with negative effects on human tissue properties (Aspenberg et al, 1990).

The tissue bank should provide the hospital with detailed data on the effects of its sterilization method on tissue integrity.
Questions to ask about allograft processing

Some questions to ask tissue suppliers:

Registration and accreditation
• Is the supplier accredited by the American Association of Tissue Banks (AATB)?
• Is the tissue supplier registered with the Food and Drug Administration (FDA)? When was the last FDA inspection? What were the findings?
• Has the supplier received any warning letters from the FDA? If so, what action was taken in response?

Evaluation of incoming tissue
• What are the standard procedures for evaluating potential contaminants on incoming tissue?
• Does the serological evaluation of tissue include nucleic acid testing (NAT) for HIV and hepatitis C virus (HCV)? AATB requires NAT screening for HIV and HCV with FDA-licensed tests for human blood.

Tissue processing
• How is the tissue processed and/or sterilized? How are processing methods validated?
• If terminal sterilization is performed, what method is used and at what level?
• Does the tissue carry a label claim for sterility? If so, how is the claim validated?
• What are the effects of the processing methods on the tissue’s structural and biological integrity? How will this affect clinical outcomes?

Source: Musculoskeletal Transplant Foundation.