

# Allograft Safety: Efficacy of the Tutoplast® Process

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The need for hard- and soft-tissue grafts to treat the effects of disease and physical trauma has existed as long as human medicine. Historical reports describe facial reconstruction utilizing skin grafts as early as 600 BC<sup>1</sup>, and the first report of a successful bone graft occurred in 1682.<sup>2</sup> Today, allograft use for both dental and medical applications has rapidly expanded over the past decade. In the United States alone, nearly one million allografts are placed each year.<sup>3</sup>

**\_The early days of tissue banking** primarily focused on refining methods of tissue preservation. With improved methodologies, the focus has shifted to purification and sterilization technologies that help to ensure that an implant is safe from disease and pathogen transmission. While surgery-related risks are inherent with any invasive procedure, allograft processing methods have been very effective in minimizing the risks involved in utilizing biologic tissue for implantation. This article describes a proprietary tissue graft cleaning and preservation process using solvent dehydration (Tutoplast Process, Tutogen Medical, Neunkirchen a. Br., Germany), which virtually eliminates the possibility of disease transmission without compromising the biological or mechanical properties of the tissue.

## \_Background

The Tutoplast Process has been commercially available for over 30 years to process tissues utilized in all surgical disciplines, including dentistry, neurosurgery, orthopedics, ophthalmology, otolaryngology, gynecology, urology, and pediatric surgery. Over one million implants have been safely and effectively implanted without a single documented case of disease transmission. This is because the Tutoplast Process is the first comprehensive sterilization and preservation method that addresses all pertinent issues of tissue grafting and implantation. The process meets or exceeds all requirements set by the U.S. Food and Drug Administration (FDA) and American Association of Tissue Banks (AATB), and has been validated through numerous independent laboratory studies.



## The Tutoplast Process

### *Donor and tissue screening*

All prospective tissues are subjected to a comprehensive donor and tissue screening regimen to assure graft safety and eliminate the potential of using high-risk donors. The screening includes a medical/social history review, a detailed interview with next of kin, an extensive donor physical evaluation and comprehensive serological testing performed by third-party Clinical Laboratory Improvement Amendment (CLIA)-certified laboratories, using an FDA-approved test methodology. The substantive donor exclusionary criteria utilized in the pre-screening process surpass FDA regulations and AATB recommendations.

Following a thorough quality assurance data review and acceptance by a licensed physician, donor tissues are released for serological screening, which includes testing for the following transmissible diseases:

#### *Hepatitis*

- \_ Hepatitis-B surface antigen (HBsAg)
- \_ Hepatitis-B core antibody (HBcAb – IgG+IgM)
- \_ Antibodies to the hepatitis-C virus (HCV Ab)
- \_ Nucleic Acid Test (CV NAT)

#### *Human Immunodeficiency Virus (HIV)*

- \_ Antibodies to the HIV-1 & 2 (HIV 1 & 2 Ab)
- \_ HIV 1-p24 antigen (not required)
- \_ Nucleic Acid Test (HIV 1 NAT)

#### *Leukemia/Lymphoma*

- \_ Human T-Lymphotropic Virus 1 & 2 (HTLV-1 & 2 Antibodies)

#### *Syphilis*

- \_ Rapid Plasma Reagin (RPR/STS)

This blood sample screening is a significant step in reducing potential disease transmission by eliminating any donor that may have been involved in high-risk behavior. The manufacturer's medical director, a board-certified physician, oversees the implementation of the screening guidelines by tissue recovery agencies and releases the tissue for production. All documentation for released tissue is also reviewed by the FDA. Donor tissue is additionally tested for microbial growth prior to the commencement of preservation and sterilization procedures. This step determines the level of bacterial loading in the graft. Any tissue that exhibits unacceptably high levels of specific contaminants or highly pathogenic microbes is eliminated from processing. Tissues that pass this rigorous screening and testing process already present a very remote risk of disease transmission to the patient. This minute element is further reduced until it is virtually eliminated through additional processing that destroys, removes and/or inactivates any type of pathogen.

### *Tissue processing*

The Tutoplast Process is comprised of numerous steps, depending on tissue type. The following method is used for preparing tissues for use as bone grafts.

*Delipidization* Lipids are removed in an ultrasonic acetone bath. Removal of lipids is important, as they may interfere with the healing process, stimulate bacterial growth and, when irradiated, can become cytotoxic.<sup>4</sup> This step also inactivates enveloped viruses such as HIV and HCV, as well as reducing prion activity by two log.<sup>5</sup>

*Osmotic Treatment* Bacteria are destroyed utilizing a series of alternating hyperosmotic saline and distilled water baths. This process ruptures the cell membranes, killing bacteria, washes out cellular debris, removes antigens (usually found in the membranes) and exposes any intracellular viruses that may be present, which can then be addressed in the subsequent step.

*Oxidative Treatment* Soluble proteins are eliminated, and non-enveloped viruses and bacterial spores are destroyed using an oxidative treatment with hydrogen per-



oxide ( $H_2O_2$ ). This treatment has been confirmed to inactivate viruses, including enveloped and non-enveloped, DNA and RNA viruses.<sup>6</sup>

**Solvent Dehydration** A final acetone wash assures that any residual prions are removed and enveloped viruses are inactivated. The acetone wash, followed by vacuum extraction, dehydrates the tissue, allowing it to be stored at room temperature.

**Gamma Irradiation** After Tutoplast processing, tissue grafts are cut to shape and size and placed in double sterile packaging. They are then terminally sterilized using low dose gamma irradiation (17.8 kGy to 25 kGy). This step eliminates any microbial contamination that may result from post-Tutoplast process handling and packaging and yields a Sterility Assurance Level (SAL) of  $10^{-6}$ . This final step alone reduces the chance of viable microorganisms on Tutogen products to one in one million.<sup>7</sup>

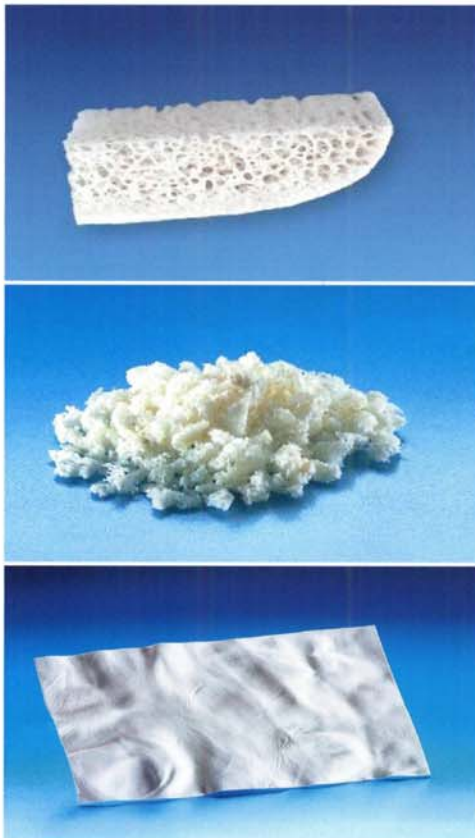
In addition to these processing steps for bone materials, soft tissue graft materials (e.g., fascia lata, pericardium, sclera, dermis) are further treated with 1N sodium hydroxide (NaOH) at room temperature for one hour. The current detection limit for prion infectivity is one log. This step in the Tutoplast Process is scientifically recognized as an acceptable and effective methodology for reducing prion infectivity by six log.<sup>8</sup> A validation study performed on the Tutoplast Process actually yielded a total prion reduction of eight log.<sup>9</sup> Prior to release, all processed tissue records are once again reviewed by Quality Assurance personnel, to ensure that all processing steps have been satisfactorily completed.

## Discussion

Over the years, numerous validation studies have been performed to support the effectiveness of the Tutoplast Process in eliminating pathogens and the potential for disease transmission. In one such study<sup>10</sup>, tissue was procured from patients

whose cause of death was AIDS/HIV and Hepatitis C. The tissue was subjected to the Polymerase Chain Reaction (PCR) test, one of the most sensitive available, to determine if the viruses were present. Those samples exhibiting the presence of viruses were then put through the many steps of the Tutoplast Process and retested for the presence of the viruses. All retested samples were found to be negative.<sup>8</sup> Another study, performed at the Institut Pasteur Texcell in Paris, France evaluated the Tutoplast inactivation capacity for all classes of viruses (enveloped, non-enveloped, DNA, and RNA).<sup>6</sup> Viruses were cultured to the highest possible concentration. Cancellous bone blocks were soaked in these virus suspensions before each step of the Tutoplast Process. After each step of the process was completed, the tissue was retested for viral concentration. With the exception of the osmotic treatment, which left a few viruses intact, all the other treatments were found to be effective in eradicating all viral content. The osmotic treatment aids the other steps by exposing and washing out most of the viral load, making the subsequent steps even more effective. This study confirms that the Tutoplast Process is effective against all types of viruses.<sup>6</sup>

An independent study reviewed several commercially available collagen grafts to determine if there was any intact genetic material contained in the graft after processing.<sup>10</sup> Ten samples from each of four major suppliers underwent a standard extraction technique to isolate genetic material. It was then subjected to the PCR test in order to determine if any DNA remained. It was determined that nine of ten Tutoplast Processed tissues exhibited DNA fragments up to 400 bp (base pairs), the remaining one had zero DNA fragments. This was 57% shorter than the next longest DNA fragment found in other allografts and much too short for replication, precluding viral disease transmission. The PCR test used in this study was not able to



measure DNA segments greater than 2,000 bp. Other non-Tutoplast processed samples contained DNA segments of 2,000 bp and possibly longer. The authors stated, "the presence of long intact DNA segments in the (other) products is a concern since the length of many DNA viral genomes (for example HPV and polyoma viruses) is 5,000 to 8,000 bp."<sup>10</sup>

In terms of prior infectivity, all Tutoplast processed grafts except sclera are classified by the World Health Organization (WHO) as Class IV, which means no detectable infectivity in the clinical state.<sup>11</sup> The current detection limit for prion infectivity is one log. Two steps of the Tutoplast Process have been shown to be effective in prion inactivation. Acetone can inactivate levels of over two log, while NaOH can inactivate levels of over 6 log.<sup>8,12</sup>

Prion inactivation capacity of the Tutoplast Process was tested on naturally contaminated dura mater, which is the tissue containing the highest level of infectivity after the brain (per WHO classification).<sup>11</sup> Infectivity reduction of 90–99% was found without the NaOH step of the process and no prion infectivity could be detected after the NaOH treatment.<sup>11</sup> An additional study also measured the efficacy of the different steps of the Tutoplast Process with regard to prion inactivation.<sup>9</sup> It further confirmed that one acetone treatment is effective in eliminating over two log of prion infectivity. The Tutoplast Process incorporates seven acetone treatments for each graft processed. The study reconfirmed previous work, including the efficacy of NaOH to inactivate over six log of prion infectivity.<sup>9</sup>

Bacteria and bacterial spores are eliminated by several steps in the Tutoplast Process. Cultures are taken from a reference sample, which has completed the process for each individual tissue lot, as a routine procedure. Any sample that exhibits the presence of a pathogen results in the rejection of the respective tissue.<sup>13</sup> Antigenicity is defined as the ability of a substance to produce specific antibodies, the result of which would lead to a potential graft rejection or rapid resorption without replacement. An animal study involving repeated implantation of Tutoplast Processed tissue was performed in Sprague-Dawley rats.<sup>14</sup> Histologic analysis showed

no signs of foreign body reactions even after four successive implantations, thus confirming that the ability of the process to remove antigenicity.<sup>14</sup>

Alternate tissue processing and preservation methods have been shown to deleteriously affect the biomechanical properties of grafts. For instance, lyophilization (freeze-drying) has been shown to disrupt the tissue fiber network, which detrimentally affects the strength and performance characteristics for the graft material.<sup>15</sup> This is also true for tissue products subjected to high-dose gamma irradiated. Both the gentle solvent dehydration process and the low-dose terminal gamma irradiation sterilization have been shown not to damage the inherent natural structure and biomechanical properties of bone and soft tissues.<sup>16,17</sup>

Since its commercial introduction more than 30 years ago, well over one million implantations of Tutoplast-processed tissues have been performed. In addition, the process has been evaluated and described in more than 450 clinical publications, involving more than 4,000 patients and long-term data spanning up to 15 years. The numerous validations performed by independent organizations confirm the efficacy and safety of the Tutoplast Process in inactivating prions, viruses and other agents responsible for transmittable diseases. Contaminant cells are ruptured and washed away during the process, exposing the RNA/DNA and enveloped and non-enveloped viruses. The Process also breaks down the RNA and DNA chains into fragments so short that they are not capable of replication and disease transmission, and of producing a tissue graft that has been proven to be effective in eradicating over 12 log of infectivity. Given that the highest level of contamination recorded in an end-stage AIDS patient was seven log, the Tutoplast Process provides a safety margin of five log or 100,000 times greater than necessary to eliminate this highest HIV viral load.

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