

The Tutoplast® Process: A Review of Efficacy

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Introduction

The use of allografts in recent years has increased dramatically. Use in the United States is now approaching one million grafts per year. Although historical reports describe facial reconstruction utilizing skin grafts as early as 600 B.C., the first report of a successful bone graft occurred in 1682. Earlier methods were primarily focused on the preservation of tissue. Newer methods, however, are more focused upon sterilization and purification, in order to provide a safe biocompatible implant. As with all surgical procedures, there is an inherent risk of complications in graft surgery. Tutogen Medical, Inc. (now RTI Biologics, Inc.) has concentrated on minimizing the risk involved in utilizing biologic tissue for implantation.

This paper describes the Tutoplast® Process, a proprietary tissue graft cleaning and preservation process using solvent dehydration that virtually eliminates the possibility of disease transmission, without compromising its biological or mechanical properties. It has been commercially available for more than 30 years and utilized in all surgical disciplines, including dentistry, gynecology, neurosurgery, ophthalmology, orthopedics, otolaryngology, pediatric and urology surgery. More than 1.5 million implants have been safely and effectively implanted without a single documented case of disease transmission. This is because the Tutoplast process addresses all pertinent issues of tissue grafting and implantation. In addition, RTI Biologics meets or exceeds all requirements set by the Food and Drug Administration (FDA) and American Association of Tissue Banks (AATB). The Tutoplast process has been validated through numerous independent laboratory studies.

The Tutoplast Process

Prior to beginning the Tutoplast process, all tissue goes through a comprehensive donor-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 CLIA (Clinical Laboratory Improvement Amendment) certified laboratories, using FDA approved test methodology. Following a thorough quality assurance data review and acceptance by a licensed physician, donor tissues are released for Tutoplast processing. The serological screening 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 (HBV NAT & HCV 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 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. RTI Biologics' medical director, a Board certified physician, oversees the implementation of our screening guidelines by tissue recovery agencies and releases the tissue for production.

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 to the patient. RTI Biologics, however, further reduces this risk until it is virtually eliminated by putting it through additional processing that destroys, removes or inactivates pathogens.

The Tutoplast process is comprised of numerous steps and is as follows:

1. Lipids are removed from all grafts 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.¹ This step also inactivates enveloped viruses such as HIV and HCV, as well as reducing prion activity by two log.²
2. Bacteria are destroyed in all grafts 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.

- Tutogen soft tissue products, such as Fascia Lata, Pericardium, Sclera and Dermis are also treated with 1N sodium hydroxide (NaOH), at room temperature, for one hour. This processing step is scientifically recognized as an acceptable and effective methodology for reducing prion infectivity by six log.⁵ A validation study performed on the Tutoplast process actually yielded a total prion reduction of eight log.⁶ This would be greater than that found in the brain of a Creutzfeldt-Jakob's Disease (CJD) patient in the final disease stage.
- Soluble proteins are eliminated from all grafts, and non-enveloped viruses and bacterial spores are destroyed using an oxidative treatment with hydrogen peroxide (H₂O₂). This treatment has been confirmed to inactivate viruses, including enveloped and non-enveloped, DNA and RNA viruses.³
- A final acetone wash for all grafts 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.
- After Tutoplast processing, all 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 20.1 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.⁴

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.

Literature Review

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, 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.⁷
- 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). 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.³

- An independent study reviewed several commercially available collagen grafts to determine if there was any intact genetic material contained in the graft after processing. 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 400bp (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 viable replication, precluding viral disease transmission. The PCR test used in this study was not able to measure DNA segments greater than 2000 bp. Other non-Tutoplast processed samples tested contained DNA segments of 2000 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."⁸

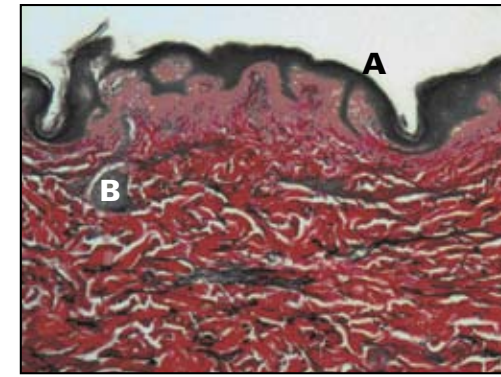
In terms of prior infectivity, all Tutogen processed grafts, except sclera are classified by the World Health Organization as Class IV (no detectable infectivity in the clinical state).⁹ The current detection limit for prion infectivity is one log.¹⁰ Two steps of the Tutoplast process have been shown to be effective in prion inactivation. Acetone can inactivate levels of over two log, while NaOH can inactivate levels of over six log.^{5, 10}

- 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). 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.¹⁰
- An additional study also measured the efficacy of the different steps of the Tutoplast process with regard to prion inactivation. 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.⁶

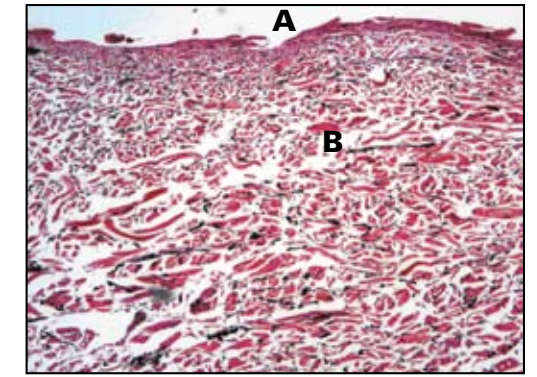
Bacteria and bacterial spores are eliminated by several steps in the Tutoplast process. Cultures are taken from a reference sample that has completed the process for each individual tissue lot, as a routine procedure. Any sample that exhibits the presence of a pathogen, results in the rejection of the respective tissue.¹¹

- 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. Histologic analysis showed no signs of foreign body reactions, even after four successive implantations, thus confirming that the Tutoplast process removes antigenicity.¹²

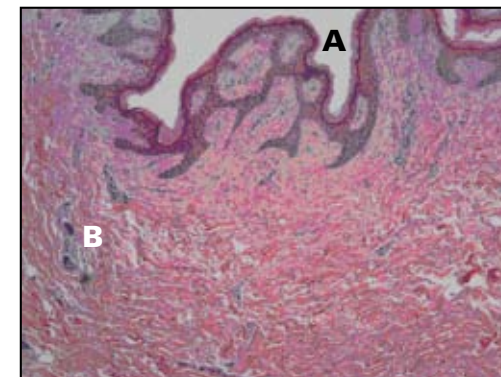
- Alternate tissue processing and preservation methods have been shown to deleteriously affect the biomechanical properties of grafts. For instance, lyophilization (freeze-drying) has been shown to disrupt the tissue fiber network, detrimentally affecting the strength and performance characteristics.¹³ This is also true for high-dose gamma irradiated products. 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.^{14, 15}



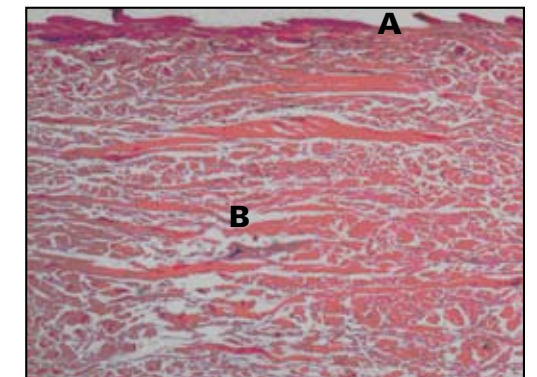
50X Verhoeff's Van Gieson stain (EVG)
Prior to Tutoplast® processing: Cells, elastin (dark brown/black), epidermis, and vessels are present.
A. Epidermis Present
B. Cells and Vessels Present



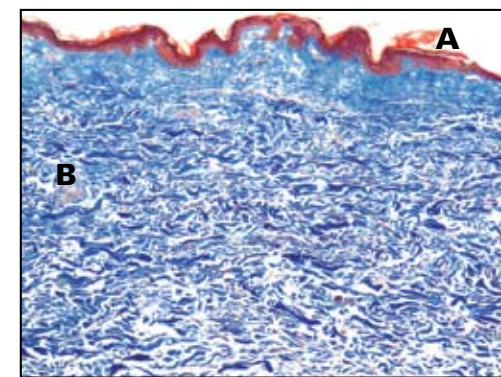
50X EVG Dermis post Tutoplast
After Tutoplast® processing: Cells, vessels removed and extracellular matrix (ECM), including elastin, remains.
A. Epidermis removed
B. Cells removed, Vessel channels remain



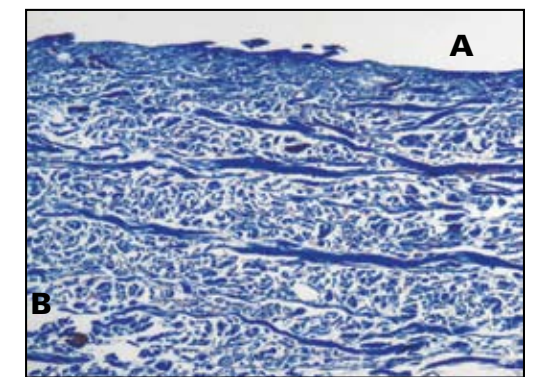
50X H&E
Prior to Tutoplast® processing: Cells, epidermis, and vessels are present.
A. Epidermis Present
B. Cells and Vessels Present



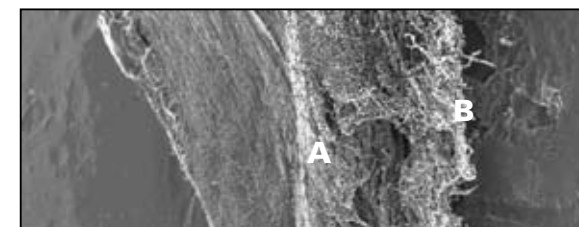
50X H&E
After Tutoplast® processing: Cells but ECM, including elastin, remains.
A. Epidermis removed
B. Cells removed, Vessel channels remain



50X Trichrome
Prior to Tutoplast® processing cells, epidermis, and vessels are present.
A. Epidermis Present
B. Cells and Vessels Present



50X Trichrome
After Tutoplast® processing: Cells, vessels removed and ECM remains.
A. Epidermis removed
B. Cells removed, Vessel channels remain



This SEM of a cross-section of Tutoplast Dermis demonstrates the denser superficial layer (A) on the left, progressing to the less dense reticular layer on the right (B).

Conclusion

Since the commercial introduction of the Tutoplast process, more than 30 years ago, more than one million RTI Biologics tissue implantations 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 quality and safety of the Tutoplast process and its efficacy 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. The process has been proven to be effective in eradicating more than 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.

References

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