TriCleanse™

Placental Extracellular Matrix Process

A proprietary processing method that balances thorough decellularization of tissue while preserving the native structure and functionality of the placental extracellular matrix (ECM)

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The Power of *Decellularization*

The proper decellularization of extracellular matrices (ECMs) is critical to the function of the final ECM product. The goal of decellularization is to remove the cells and eliminate residual genetic material, while preserving the ECM components and retaining the properties of the base tissue.[1] Incomplete decellularization has been shown to decrease the ratio of M2-activated macrophages to M1-activated macrophages when compared to more complete decellularization methods.[2] Further studies have demonstrated that higher ratios of M2-activated to M1-activated macrophages are more promotive of immunomodulation, constructive mechanisms, and remodeling activities.[3]

A Delicate *Balance*

The TriCleanse™ Process effectively removes cells and cellular debris while maintaining the structural proteins of the ECM.

Decellularization

ECM

Process Flow Chart

Material Source

Production begins with a carefully selected tissue that is sourced from a highly controlled, monitored, and exclusive facility. The site is certified to ISO 13485:2016 and ISO 9001:2015 standards and compliant with the FDA's Good Manufacturing Practices in 21 CFR 820. In addition, the raw placental tissues are compliant with ISO 22442-2 standards.

Physical Processing

The placental tissue undergoes thorough bulk washing, which removes surface contaminants, residual blood, and amniotic fluid.

TriCleanse™ Process

The placental tissue is subjected to a series of chemical baths and washes that disinfect and decellularize the tissue.

Preservation

The tissue is dried.

Final Processing

The dried tissue is processed to its final configuration.

Sterilization

The tissue is terminally irradiated to a SAL 10^{-6}.

Decellularization Efficiency
of the TriCleanse™ Process

Placental devices manufactured utilizing the TriCleanse™ Process were compared to raw untreated placentas to determine the decellularization efficiency of the process. Histologic evaluations were performed to assess the impact of processing on placental tissue.

Cell and Cellular Debris *Removal*

These images clearly show that prior to processing, the placenta is highly cellularized. However, following the TriCleanse™ Process, the placenta appears completely decellularized and void of any intact nuclei.

Figures on the left show untreated placentas, and figures on the right show TriCleanse™ Process treated placentas.

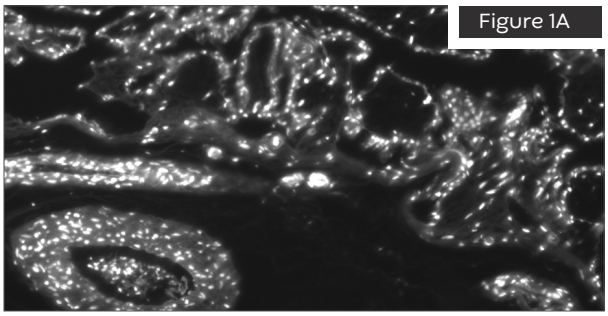


Figure 1A

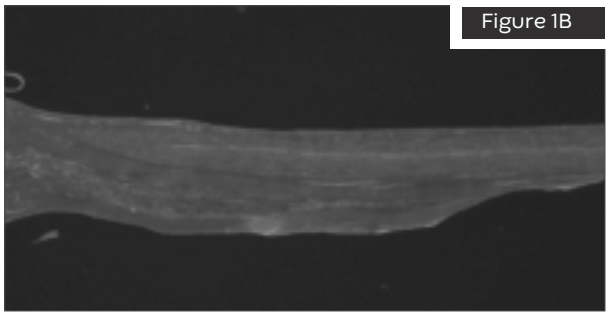


Figure 1B

DAPI (4′,6-diamidino-2-phenylindole) Staining (Figures 1A and 1B)

DAPI staining is a fluorescent DNA stain with high affinity for the AT region of double stranded DNA in either fixed or live cells.[4] DAPI has been demonstrated to efficiently label cell nuclei for easier quantification.[5]

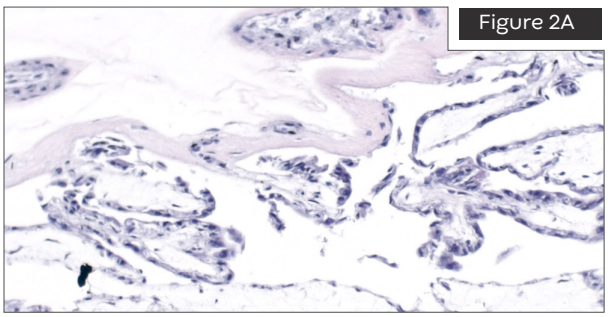


Figure 2A

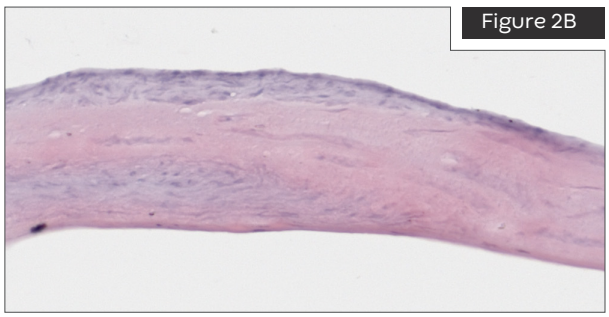


Figure 2B

Hematoxylin & Eosin Staining (Figures 2A and 2B)

Hematoxylin and eosin (H&E) staining is commonly used to stain tissue for histology evaluation.[6] H&E stains nuclei blue and extracellular matrix and cytoplasm pink and other tissues shades in between.

Because dehydration is part of the TriCleanse™ Process, images of processed tissue (Figures 1B and 2B) appear more compact and thinner.

These four stains confirm that the TriCleanse™ Process successfully removes the cellular components of the placenta while leaving the structural and modulating proteins intact in the final product.

This is the goal of efficient decellularization.

Preservation *of ECM*

These images demonstrate that Collagen I, Collagen III, and laminin are present in the placenta prior to processing and remain in the tissue after processing. This is an indication that the TriCleanse™ Process has preserved the native structural proteins of the ECM.

Figures on the left show untreated placentas, and figures on the right show TriCleanse™ Process treated placentas.

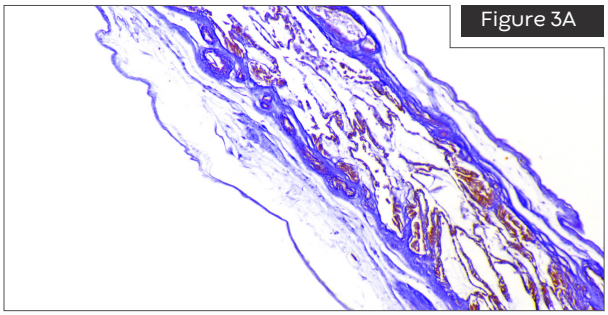


Figure 3A

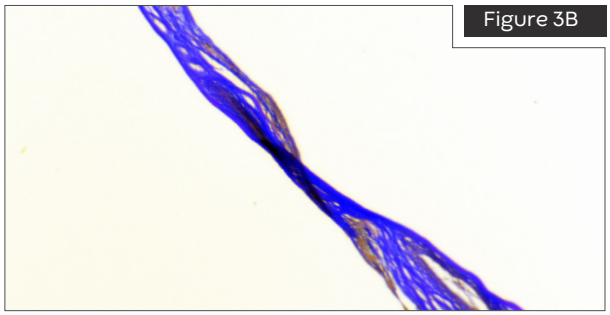


Figure 3B

Herovici Staining (Figures 3A and 3B)

Herovici staining is a technique that utilizes two separate preferential chromatic stains and image processing to calculate the ratio of Collagen Type III to Collagen Type I.

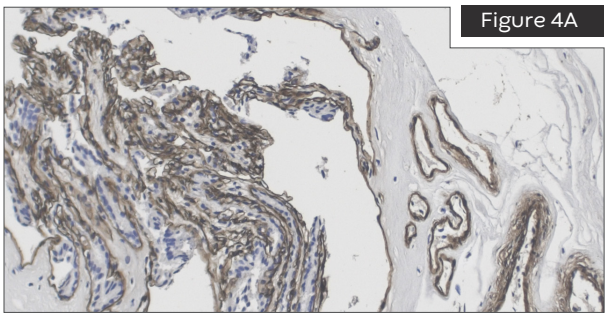


Figure 4A

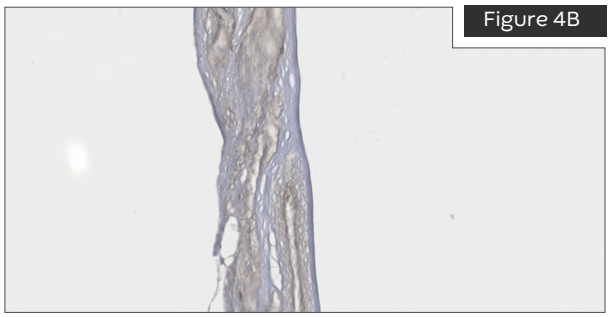


Figure 4B

Laminin Immunostaining (Figures 4A and 4B)

Immunostaining is a technique that allows for detection of specific proteins in tissue. The staining allows for determination of presence and location of the proteins. This particular stain was for laminin, a protein that is the major component of basement membranes.

Because dehydration is part of the TriCleanse™ Process, images of processed tissue (Figures 3B and 4B) appear more compact and thinner.

Convatec's production facility was specifically designed to meet the rigorous ISO 13485 standards, including ISO Class 7 clean rooms, and is staffed with highly trained technicians. This ensures each product not only meets regulatory requirements but also maintains Convatec's highest standards of safety and quality.

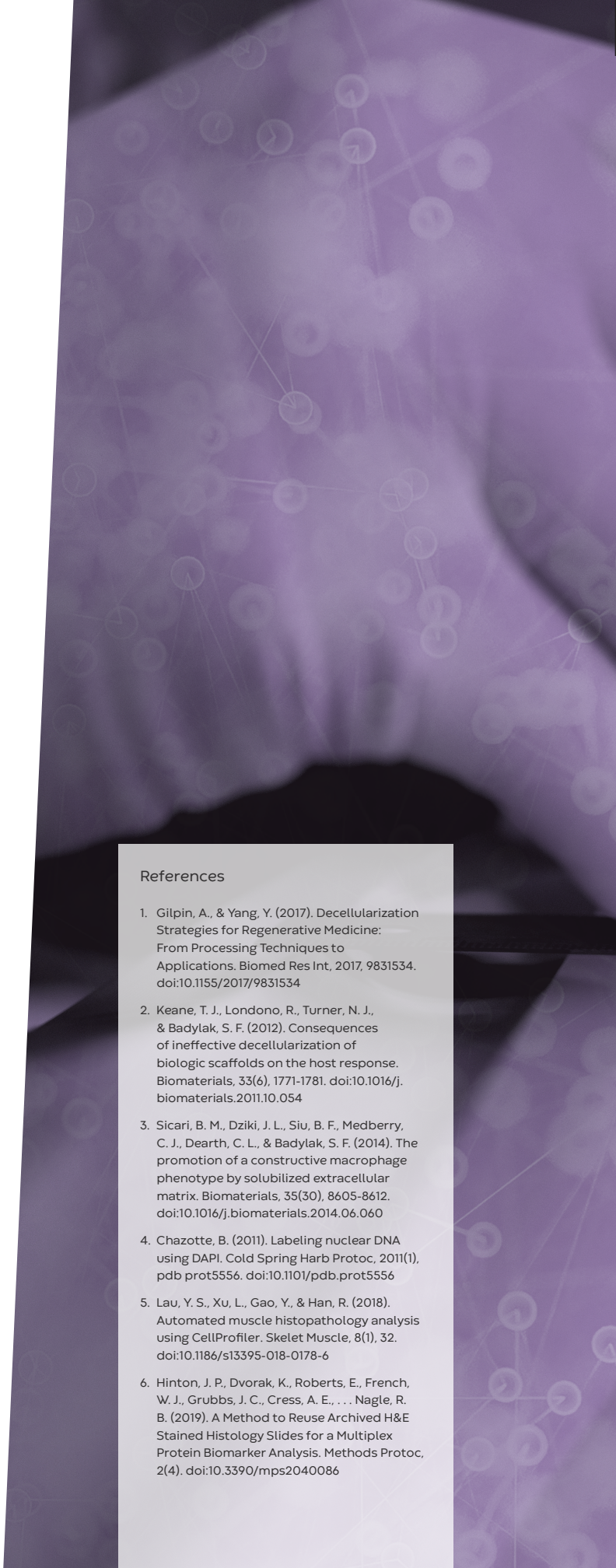
Processing *Matters*

Because tissue processing for ECM membranes has evolved over time and is performed by numerous processors, it is essential to understand that not all processes are similar.

Process variables can include:

- **Heated drying**
- **Antibiotics**
- **Fixation chemicals**
- **Oxidizing agents**

These variables may affect the processed tissue in multiple ways, including residuals from the processing chemicals, presence of antibiotics, and moisture content. Those variations can impact performance and safety of the graft.



References

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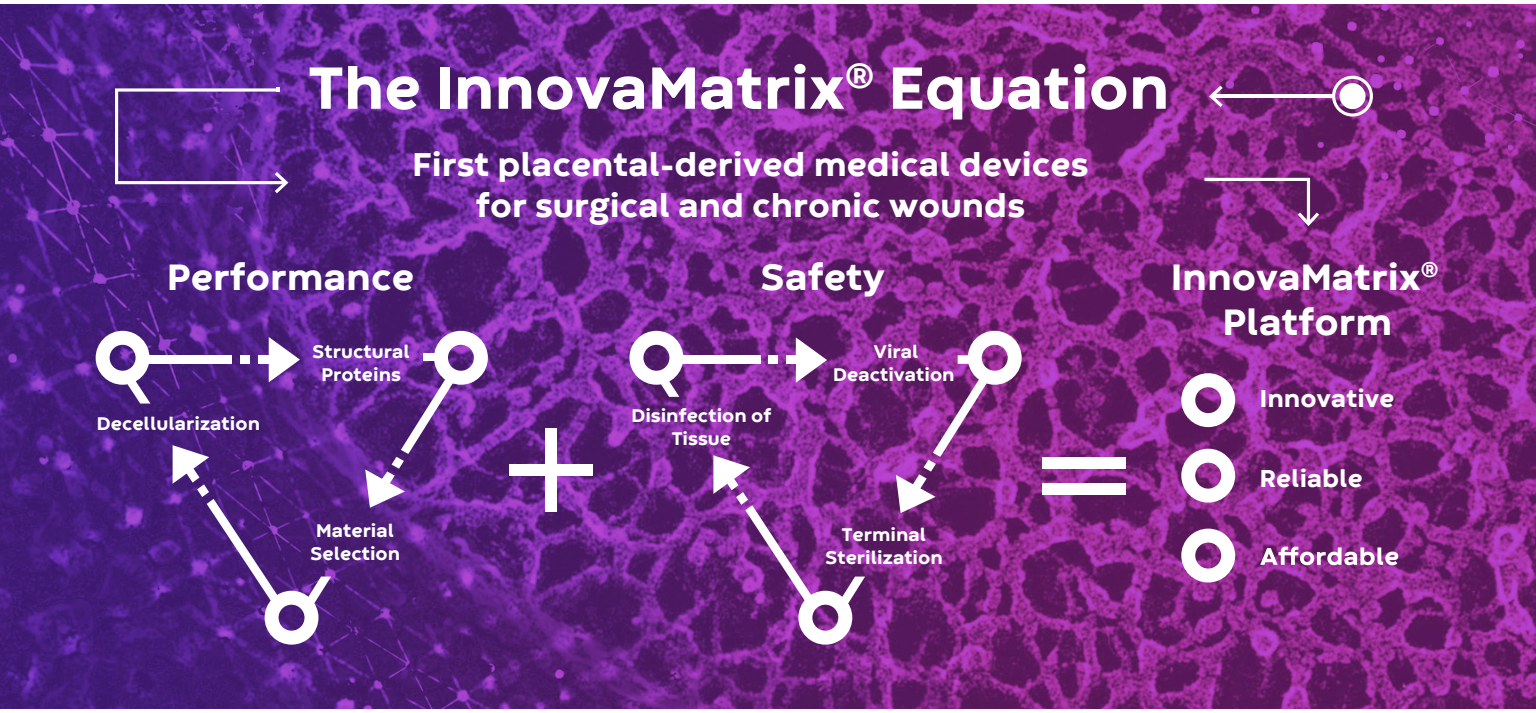

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