Retention Processing to Preserve Beneficial Components[3]

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Abstract

To date, processing has been primarily focused on the preservation of cells within a tissue or removal of all non-solid matrix components for the purpose of delivering stem cells and/or providing a substrate for regenerative growth. Since the development of these processes, scientific progress has been made, uncovering the value of factors within the placental tissue that are invaluable to wound healing. These factors do not rely on graft cellular content; hence preservation of cells is unnecessary. Additionally, removal of everything down to solid matrix essentially depletes the graft of these valuable factors. In order to provide safe, biocompatible, factor-rich grafts, we have developed a processing regime that cleans and prepares the tissues in a gentle and effective manner, while minimizing the risk involved in utilizing biologic tissue for implantation. Additionally, testing for retained factors is on terminally sterilized, final product grafts, mimicking the elution of those factors to the recipient tissue. These factors are reported in a standardized method: factor per cm^2 of product. [3,4]

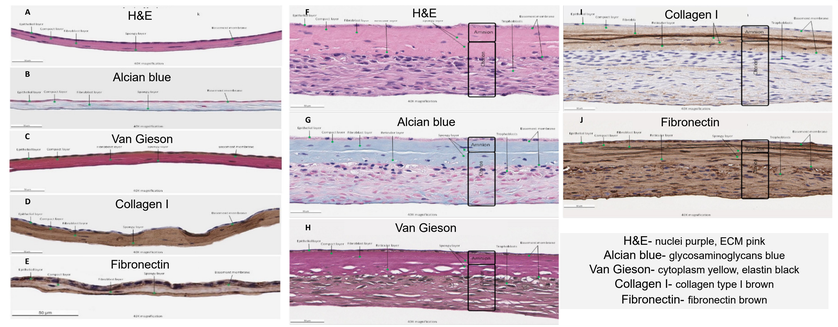
Introduction

The BioRetain® Process addresses the new data acquired in the regenerative field to maximize the advantages of placental tissue grafts. All tissue goes through a donor-screening process prior to beginning, cultures are collected for bioburden and comprehensive serological testing. Terminal sterilization of the grafts has been validated to eliminate the possibility of disease transmission. The BioRetain® Process utilizes hypothermic, isotonic, minimally disruptive procedures[5]. Conditions are maintained throughout the process to ensure that the membrane structure and inherent factors are protected to the greatest extent. The resulting dry membranes are examined histologically and biochemically to verify that structural components are retained, and inherent factors are present. We test for retained factors on terminally sterilized, final product grafts and utilize a testing methodology that mimics the elution of those factors to the recipient tissue. These factors are reported in a standardized method: available factor per cm^2 of product. [3,4]

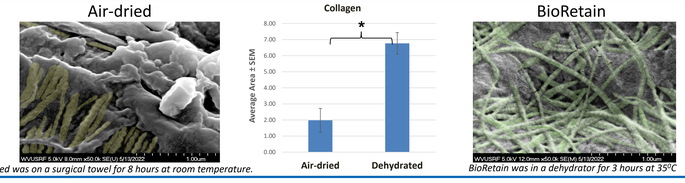
The BioRetain® Process

1. **Minimally damaging bactericidal, tuberculocidal, fungicidal and virucidal disinfection:**
   Placing the tissue in an effective and minimally damaging disinfectant for the required time gently kills any external contamination.
2. **Hand removal of blood/debris:**
   Blood and undesirable debris is removed from the membrane gently, ensuring the membrane integrity is not damaged or weakened thus providing retention of the favorable components within the tissue.
   - It is general practice to scrap and/or scrub all the material from the membrane, damaging the fibers and removing the favorable factors.
3. **Cold isotonic cleansing:**
   Low-temperature solutions with balanced pH limits the breakdown of tissue and growth factors during processing. No harsh chemicals are used.
   - It is general practice to use water and room temperature solutions.
     - Water is hypotonic to the membrane, causing cells to lyse, and valuable factors to move from the membrane to the water (osmotic equilibration), where it is discarded.
   - It is general practice to use NaOH, HCl and/or H_2O_2.
     - These have a negative effect on the tissue and factors due to high or low pH altering the structure of membrane proteins
     - Residual amounts of these can be left behind in the tissue.
4. **Gentle dehydration:**
   Slow drying of the membrane on specialized medical-grade platforms using physiological temperature preserves tissue structure and natural growth factors.
   - It is general practice to use freeze drying, air drying on towels or accelerated drying (heat) .
     - Freezing can tear up the crucial stromal support system within the membrane, higher temperatures may degrade factors and air drying usually requires placement on an absorbent material which tears the stroma when removed.
5. **Low-dose Electron beam sterilization:**
   The tissue is packaged and subjected to low-dose E-beam irradiation → terminally sterilized graft.
   - It is common practice to use gamma irradiation.
     - Gamma irradiation requires exposure of the tissue to extended periods of higher dose irradiation which cross-links and sometimes breaks down the factors in the tissue.

It Really Retains

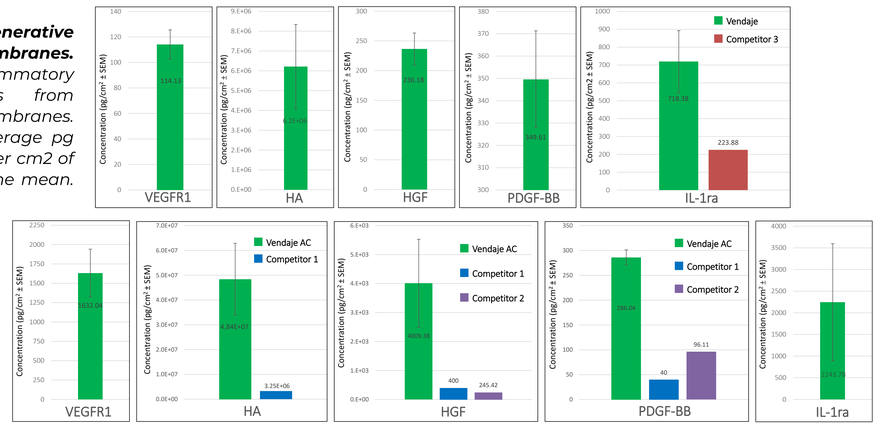


Histology of BioRetain-processed stroma. A-E *Amnion membrane,* ***F-J*** *Amnion/Chorion membrane* ***A,F****) Hematoxylin and eosin stain for structure.* ***B,G****) Alcian blue staining for glycosaminoglycans.* ***C,H****) Van Gieson staining for elastin and collagen.* ***D,I****) Staining for collagen I in brown.* ***E,J****) Staining for fibronectin in brown.* Performed by HistoWiz, Inc.



Collagen Structure of BioRetain-processed and air-dried stroma. *Each amnion/chorion was cut into 2 pieces. SEM Images from WVU Electron Microscopy Facilities, Marcela Redigolo, PhD. Collagen was colorized based in striation. Image J analysis quantified the collagen. N=3*

Air-dried was on a surgical towel for 8 hours at room temperature. *BioRetain was in a dehydrator for 3 hours at 35^{0}C*



Anti-inflammatory and regenerative factors delivered from membranes. *Elution of stromal, anti-inflammatory and regenerative factors from BioRetain-processed membranes. Results are presented as average pg eluted from the membrane per cm2 of product ± standard error of the mean. N=5.*

HA*=hyaluronic acid*
IL-1ra*= interleukin-1 receptor antagonist*
HGF*=hepatocyte growth factor*
PDGF-BB*=platelet-derived growth factor subunit B homodimer*
VEGFR1*=vascular endothelial growth factor receptor 1*

[3]Collection and Quantification of Elements per SQ CM of Tissue. Patent pending 63/391,031
[4]Sabol TJ, Tran GS, Matuszewski J, Weston WW. Standardized reporting of amnion and amnion/chorion allograft data for wound care. Health Sci Rep. 2022;5(5):e794. PMID: 36032519
[5]Placental Processing for Retention of Factors. Patent pending 63/391,032