Retention Processing Preserves Beneficial Stromal and Molecular Components[3]

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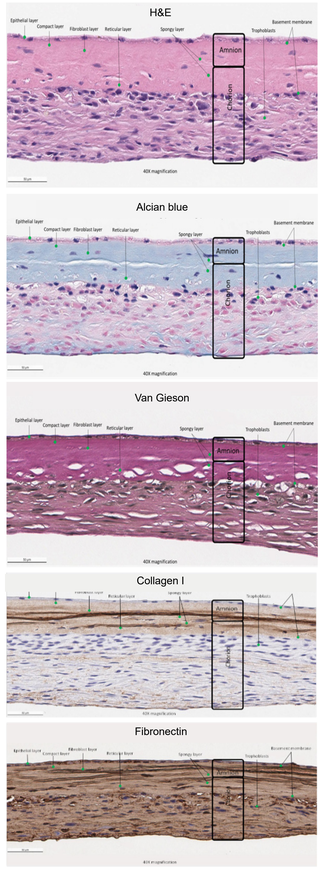
Abstract

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 to wound healing. These factors do not rely on cellular content, and removal of everything down to solid matrix essentially depletes the graft of these valuable factors. The clinical effect of specific endogenous factors within placental tissue grafts is well documented, hence retention of these factors in dehydrated, sterile grafts provides a safe and effective alternative to grafts that are merely structural or non-sterile. To provide safe, factor-rich grafts, we tested the stromal and molecular impact of a processing regime that prepares placental tissues in a gentle manner to minimize loss of beneficial components (BioRETain®). This process was hypothesized to demonstrate retention of structural and molecular components. Five separate lots of terminally sterilized, final product amnion/chorion grafts were tested for structural components and molecular factors by histology, scanning electron microscopy and cytokine analysis. Molecular analyses were reported as factor per cm^2 of product. This study revealed conserved structure, retention of GAGs, collagens, fibronectin, hyaluronic acid, IL-1ra, HGF, PDGF-BB, FGF2 and VEGFR1 in gently processed, dehydrated, terminally sterilized amnion/chorion placental tissue grafts. This study highlights the acceptance of the hypothesis that gentle (BioRETain®) processing demonstrates retention of structural and molecular components.

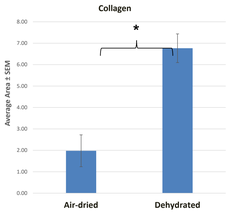
Methods

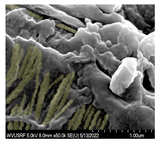
- Amnion/chorion (AC) was isolated from donated placentas and placed in minimally damaging bactericidal, tuberculocidal, fungicidal and virucidal disinfection.
- Blood and debris were gently removed ensuring the membrane integrity is not damaged or weakened.
- Membrane was placed in solutions for cleaning at low-temperature with balanced pH to limit the breakdown of tissue and growth factors during processing. No harsh chemicals were used.
- Cleaned membranes were gently dehydrated on specialized medical-grade platforms to preserve tissue structure and natural growth factors.
- Membranes were sterilized by low-dose Electron beam sterilization. Terminal sterilization of the grafts has been validated to eliminate the possibility of disease transmission.
- Histology: Final product membranes were sent to HistoWiz, Inc. for structural evaluation and stromal components.
- Scanning Electron Microscopy: Final product membranes were fixed in 4% paraformaldehyde for 24 hours, washed 3 times in DI water and sent to WVU Electron Microscopy Facilities, Marcela Redigolo, PhD..
- Multiplex cytokine assay: 10 mm punches were taken from final product membranes and placed in 500 ul DPBS$^{Ca\text{-}Mg\text{-}}$ at 37 °C for 72 hours. Supernatant was collected, clarified by centrifugation and used for ELISA's and BioPlex Pro cytokine assay on a BioPlex 200 multiplexer with high throughput fluidics.
- All testing was on terminally sterilized, final product grafts and utilized a testing methodology that mimics the elution of those factors to the recipient tissue.
- Reporting: Concentrations were multiplied by 0.5 ml (total volume of supernatant) and divided by the area of the 10 mm punch. This formula yielded pg/cm^2 of membrane.[4]

Retention of Stromal and Molecular Components



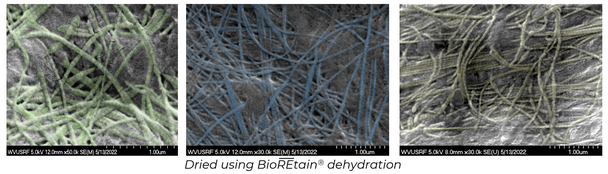
H&E- nuclei purple, ECM pink
Alcian blue- glycosaminoglycans blue
Van Gieson- cytoplasm yellow, elastin black
Collagen I- collagen type I brown
Fibronectin- fibronectin brown



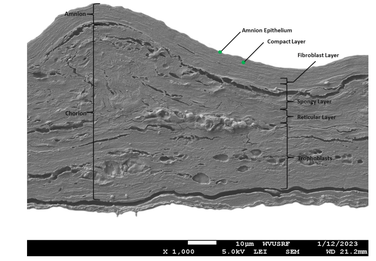


Air-dried on a surgical towel

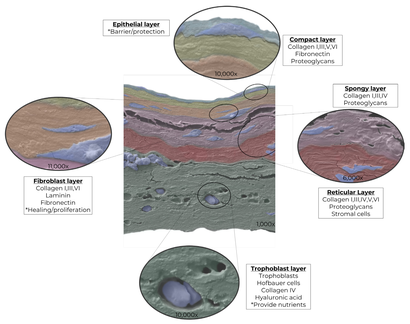
Collagen Structure of BioRETain®-processed and air-dried stroma.
SEM Images Collagen was colorized based in striation. Image J analysis quantified the collagen. N=3

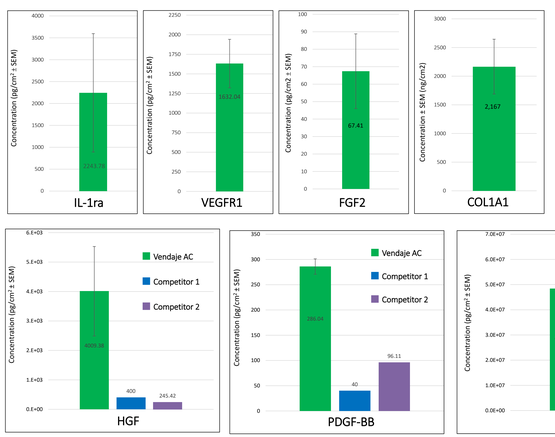


Dried using BioRETain® dehydration



West Virginia University, Electron Microscopy Services, Marcela Redigolo





Anti-inflammatory, stromal and regenerative factors delivered from membranes.
Elution of stromal, anti-inflammatory and regenerative factors from BioRETain®-processed membranes. 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*
***FGF2**= basic fibroblast growth factor*
***COL1A1**= Collagen, type I, alpha 1*

Conclusion

The increased utilization of birth tissues for wound healing has led to testing and better understanding of the valuable factors within placental tissue. To provide safe, factor-rich grafts, we developed a processing regime, the BioRETain® Process, that cleans, preserves, and sterilizes the tissues in a gentle and effective manner to maximize the retention of growth factors and stromal components. Terminal sterilization mediates the possibility of disease transmission. Our data demonstrates the retention of structure, GAGs, collagen I, collagen III, total collagen, fibronectin and HA, anti-inflammatory IL-1ra, regenerative HGF, PDGF-BB, and FGF2 and angiogenic VEGFR1 in dehydrated, terminally sterilized amnion/chorion placental tissue grafts when processed according to our BioRETain® method. Further studies are needed to compare this data to unprocessed tissue and grafts that are depletion-processed. Animal and clinical studies will ascertain the clinical efficacy of the factor-rich grafts.

[3] Placental Processing for Retention of Factors. Patent pending 63/391,032.

[4] Collection and Quantification of Elements per SQ CM of Tissue. Patent pending 63/391,031.