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RESTORIGIN™ Sx

A Natural Next-Generation Tissue Barrier

Preserving the Complex Natural Architecture of Amniotic Membrane

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Background

Regenerative healing of surgical wounds has been extensively studied since 1979 when Rowlatt first reported that fetal surgical wounds heal without scarring.[1] Compared to scar-mediated healing in an adult, regenerative healing in a fetus involves rapid re-epithelialization, the absence of an inflammatory response, preservation of tissue architecture, and the absence of scar tissue formation.[2,3]

Amniotic membrane (AM) is a thin, semi-transparent and resilient membrane that lines the inner cavity of the placenta. It has been used as a biomaterial for surgical reconstruction since 1910.[4] Studies on amniotic membrane transplantation report similar results as noted with regenerative healing in the fetus, namely re-epithelialization, reduced inflammation, and reduced scar formation.[5,6,7,8] With over 100 years of clinical history, the regenerative capability of amniotic membrane is well documented.

During gestation, AM provides both physical and systemic protection to the fetus. It is an immune-privileged protective barrier with inherent anti-microbial properties. The multi-layered physical architecture of AM includes an epithelial monolayer, resting on a basement membrane, which is in turn attached to a collagen-rich stromal layer. The stromal layer itself is made up of 3 additional layers – compact, fibroblast and spongy. The epithelial layer lies closest to the developing fetus, and the stromal layer is loosely attached to the chorionic membrane.[9] Figure 1 displays the natural structure of AM.

The AM contains multiple extracellular matrix molecules and growth factors. Table 1 displays a list of a few of the growth factors present in AM with their possible iterative functions. Although AM has inherent regenerative properties[9], these properties can be preserved, degraded or destroyed based on how the tissue is acquired and processed. Multiple commercial processing methods have been developed for AM, including cryopreservation and thermal drying. The novel process used for Restorigin Sx was specifically designed to ensure minimal disruption of the native tissue structure and properties. It has been demonstrated through physical and chemical verification testing that the processing methodology is able to preserve the AM's inherent structural properties.

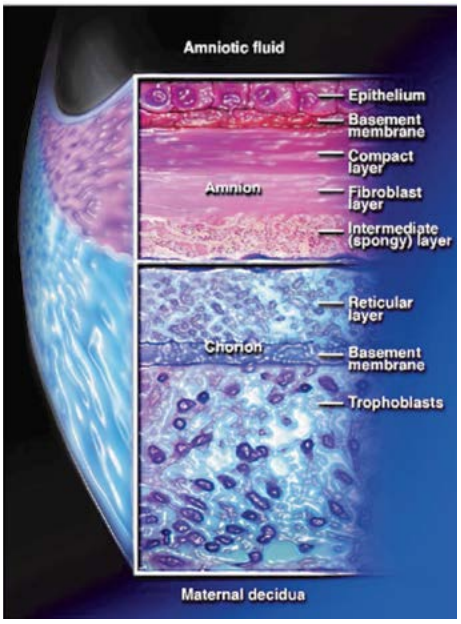


Figure 1 – Schematic Presentation of Amniotic Membrane Structure

Table 1 – Growth Factors present in AM and corresponding function

Growth Factor	Abbreviation	Growth Factor Function
Hepatocyte Growth Factor	HGF	Myogenesis, Wound Healing
Basic Fibroblast Growth Factor	bFGF	Angiogenesis
IL-1 Receptor Antagonist	IL1-RA	Anti-Inflammatory
Transforming Growth Factor–Beta 1	TGF-β1	Proliferation, Differentiation, Immune Modulation
Transforming Growth Factor–Beta 3	TGF-β3	Proliferation, Differentiation, Immune Modulation

Methods

Placental tissue was recovered surgically after Cesarean section birth. Once reviewed and released by a medical director in accordance with the Food and Drug Administration (FDA) and the American Association of Tissue Banks (AATB) regulations and standards, the placenta was manually separated into amnion and chorion. The amnion was then processed via gentle cleaning to remove blood components, and drying to allow for long-term room temperature storage. A total of 7 placentas were utilized for this study.

Tissue structure was evaluated histologically. Processed AM from 3 donors was embedded in paraffin, cut on a microtome into 5µm sections and air dried onto slides. The tissue was stained with hematoxylin and eosin in standard fashion and the structural integrity was analyzed by light microscopy (Nikon Eclipse 90i) at 20x and 40x magnification.

ELISA assays were used to analyze the content of key growth factors and cytokines released from the amniotic membrane. AM from 4 donors was placed in RPMI media and incubated for 24 hours at 37°C. The supernatant was evaluated to quantify the concentration of growth factors / cytokines, and the data was normalized with respect to the dried weight of the AM tissue. Testing was performed pre- and post-processing to ensure that select molecules were preserved after processing.

Results

Histology Results*

Representative histology of VIVEX processed AM is shown in Figure 2 demonstrating a continuous, intact single epithelial layer, underlying basement membrane and collagenous stroma similar to that observed in native AM tissue. These results indicate that the VIVEX processing methodology preserves the inherent structural integrity of AM. In contrast, two other methodologies described in published literature[10] (indicated as process A and process B in Figure 3) resulted in varying levels of disruption of the membrane structure. Process A resulted in nearly complete disruption of the epithelium monolayer and in Process B, the epithelium was partially disrupted.

*Results are prior to E-beam sterilization. All results have been verified post sterilization. See Restorigin Sx™ Growth Factor Data Post Sterilization.

Growth Factor Results

Figure 4 (next page) displays the average concentration of growth factors before and after processing. The five key growth factors and cytokines tested - HGF, FGF, IL-1RA, TGF-β1 and TGF-β3 - were detected in the AM post-processing. More importantly, statistical analysis showed that there was no significant difference in the concentration of these growth factors solubilized in 24 hours from the cleaned versus cleaned + dried AM. These results indicate that the inherent content of growth factors of the AM membrane are preserved during processing.

Figure 2 - Histology Analysis of VIVEX Processed Amniotic Membrane

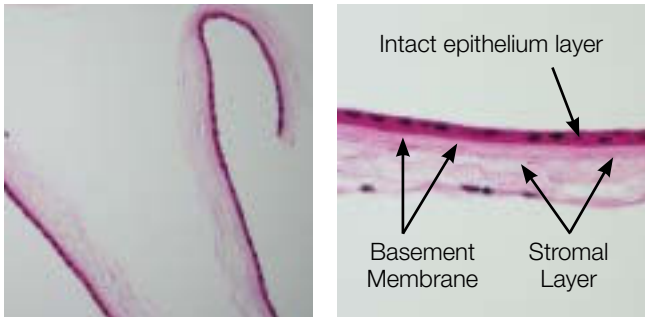
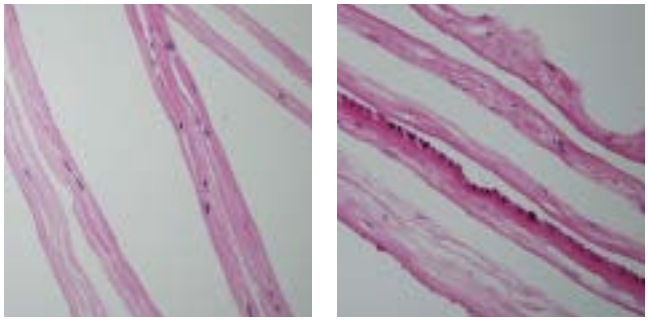


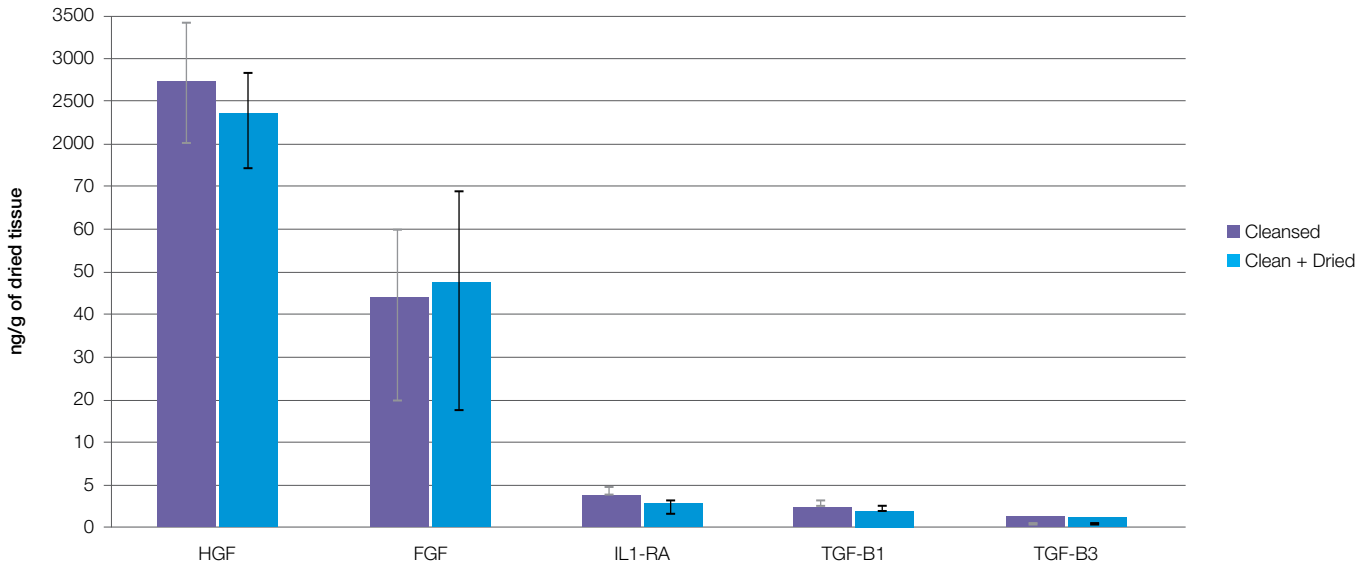
Figure 3 - Histology Analysis of Amniotic Membrane Processed via Published Methods



Process A
Very little remaining intact epithelium

Process B
Epithelium somewhat maintained

Figure 4



Conclusion

With over 100 years of clinical history, the regenerative capability of amniotic membrane is well documented. This resilient membrane has a multi-layered architecture, and a complex array of inherent growth factors and cytokines that provide both physical and systemic protection to a fetus during pregnancy. To ensure that these inherent properties are maintained during processing and preparation for clinical implantation, testing must be performed. The processing has been shown to successfully preserve both the complex natural architecture of the membrane, and the inherent levels of key cytokines and growth factors. AM has been successfully used clinically as a soft tissue adhesion barrier or surgical wound covering.

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