Multiscale Characterization of A Novel Dehydrated Full-thickness Amnion/Chorion Allograft

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ABSTRACT

Cellular, acellular, and matrix-like products (CAMPs) have gained recognition as adjunctive therapies for chronic wound management. Among these are processed, perinatal tissue allografts which act as protective barriers to wound sites. However, the methods used to process these allografts can significantly impact their structural and functional properties, underscoring the importance of comprehensively characterizing these allografts at a multiscale level.

To evaluate a novel, full-thickness amnion/chorion allograft—histological and biochemical analyses were conducted. Additionally, *in vitro* assays measuring human dermal fibroblast (HDF) proliferation, metabolic activity, and migration were employed to assess the bioactivity of graft-derived leachate.

Our results demonstrate that after processing, full-thickness amnion/chorion allografts maintain their microarchitecture and contain extracellular matrix (ECM) components and growth factors that may collectively influence HDF behavior.

INTRODUCTION

- Normal wound healing requires the coordination of several biological events, that when interrupted, can lead to the formation of chronic, nonhealing wounds[1]
- Advanced wound care products have emerged as effective, adjunctive options for the resolution of chronic wounds
    - These include perinatal tissue allografts, which serve as a covering and offer protection from the surrounding environment
- Perinatal tissues have been used for over a century as an allograft for their unique biological properties
    - Angiogenic, anti-inflammatory, anti-fibrotic, anti-microbial, and immune privileged[2,3]
- Human amnion/chorion-derived allografts are manufactured using a variety of processing techniques that can alter these biophysical characteristics

OBJECTIVE: To characterize a novel, dehydrated full-thickness amnion/chorion wound care allograft via histological, biochemical and *in vitro* cellular bioactivity evaluations.

METHODS

Dehydrated full-thickness amnion/chorion allografts were produced from placentas that were donated via informed consent following cesarean section deliveries. Tissue processing was performed in accordance with FDA's Good Tissue Practices and the American Association of Tissue Banks (AATB) guidelines prior to dehydration and terminal sterilization to a sterility assurance level of 10^{-6}.

1. **Histology:** Allografts underwent routine processing, staining and microscopic imaging.
    - Hematoxylin and Eosin (H&E) for general tissue architecture
    - Alcian blue for glycosaminoglycans (GAGs)
    - Masson's Trichrome for collagen
    - Verhoeff's van Gieson for elastin
2. **Biochemistry:** Dimethylmethylene blue (DMMB) assay was used to quantify sulfated GAGs, hydroxyproline assay was used to quantify collagen content, and growth factor content was quantified via a multiplex cytokine array (ELISA).
3. **Bioactivity:** Soluble full-thickness amnion/chorion leachate (1 mg/mL) was added to basal cell culture media and incubated with human dermal fibroblasts (HDFs) for 72 hours. HDF metabolic activity, proliferation and migration were evaluated.

*All analyses were performed by independent, third-party vendors.

HISTOLOGY RESULTS

- H&E staining (**Fig. 1A**) demonstrated a full-thickness amnion/chorion allograft containing all layers of the native perinatal membrane
- Alcian Blue staining (**Fig. 1B**) qualitatively demonstrated the presence of GAGs (blue) throughout the allograft
- Masson's Trichrome staining (**Fig. 1C**) demonstrated collagen (blue) throughout the allograft
- Verhoeff's van Gieson staining (**Fig. 1D**) showed positive (brown) staining for elastin in the chorionic mesoderm

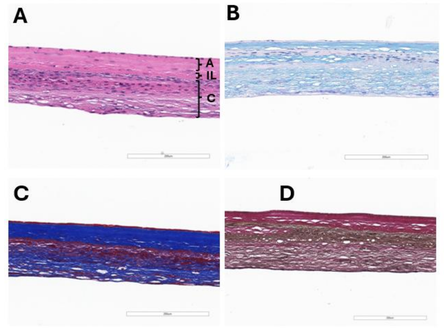

Figure 1. Qualitative evaluation of full-thickness amnion-chorion allografts for (A) H&E, (B) Alcian blue, (C) Masson's Trichrome, and (D) Verhoeff's van Gieson stains. A = amnion, IL = intermediate layer, C = chorion. Scale bars = 200 um.

BIOCHEMISTRY RESULTS

Table 1. Biochemical concentration of sGAG and collagen in full-thickness amnion/chorion allografts

Sulfated Glycosaminoglycan (sGAG) Content		
	Avg ug sGAG / mg dry weight	**St. dev.**
Full-thickness amnion/chorion	18.0	4.4
Fresh Amnion[4]	10.91	0.77
Fresh Chorion[5]	17.1	1.1
Collagen Content		
	Avg ug collagen / mg dry weight	**St. dev.**
Full-thickness amnion/chorion	179.7	46.6
Fresh Amnion[6]	313.5	27.9
Fresh Chorion[5]	74.8	18.5

Full-thickness amnion/chorion allografts retain native ECM biomolecules (Table 1):

- sGAG and collagen levels in dehydrated, full-thickness amnion/chorion allografts are similar to that of fresh, unprocessed chorion[5]

Select growth factors found in full-thickness amnion/chorion allografts include (Fig. 2):

- Angiogenic growth factors (ANG-1, Angiogenin, PDGF-BB)
- Inhibitors of matrix proteases (TIMP-1, TIMP-2)
- Matrix remodeling (ADAMTS9)
- Pro-regenerative growth factors (EGF-R, IGFBP-1)
- A total of 240 growth factors were detected

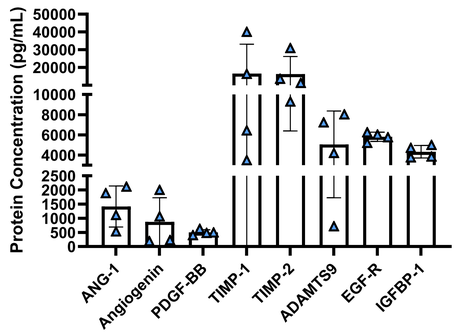

Figure 2. Select growth factors present in full-thickness amnion/chorion allografts. Results are presented as mean ± st.dev.

BIOACTIVITY RESULTS

Full-thickness amnion/chorion allograft leachate:

- Significantly increased HDF metabolic activity versus basal media at 48 and 72 hours (**Fig. 3A**)
- Led to similar responses in HDF proliferation (**Fig. 3B**)
- Increased HDF migration versus basal media at 72 hours (**Fig. 3C**)

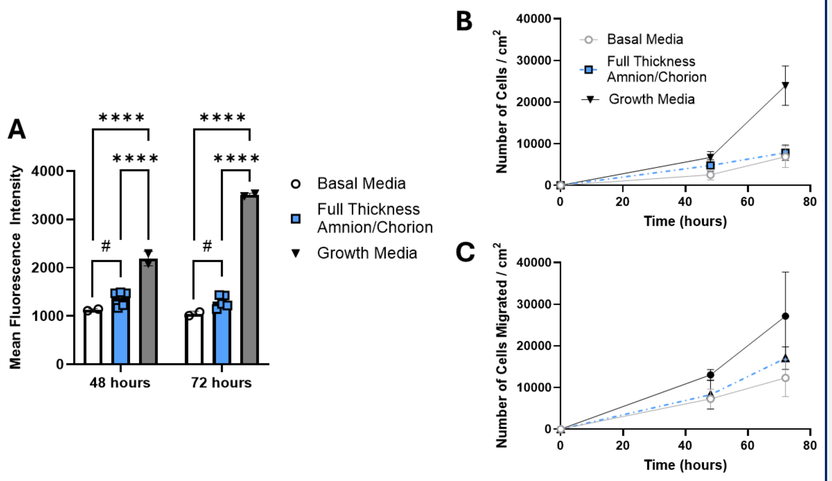

Figure 3. HDF (A) metabolic activity, (B) proliferation, and (C) migration when treated with 1 mg/mL leachate from full-thickness amnion/chorion allografts. Results are presented as mean ± st.dev. with * p≤0.05, ** p≤0.01, *** p≤0.001, and **** p≤0.0001 (One-way ANOVA) and with # p≤0.05 (t-test).

DISCUSSION

Our processing technique results in a perinatal tissue allograft containing:

1. An intact amnion/intermediate layer/chorion microarchitecture
2. ECM proteins, including GAGs and collagen
3. Growth factors and cytokines relevant to wound healing
4. Bioactive molecules that influence fibroblast metabolism and migration

CONCLUSION

Full-thickness amnion/chorion allografts are comprised of an intact perinatal membrane microarchitecture containing a variety of ECM components and growth factors that can influence HDF behavior.

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