Biological characterization of a novel air-dried human amniotic membrane (dHAM*) allograft for wound healing applications

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INTRODUCTION

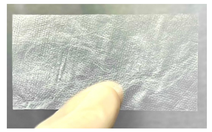
- Chronic wounds remain a significant clinical challenge, driven by persistent inflammation, impaired cellular proliferation, inadequate angiogenesis, and fibrotic tissue remodeling.
- Dehydrated human amniotic membrane (dHAM) is part of a growing class of Cellular, Acellular, and Matrix-like Products (CAMPs), designed to modulate the wound environment and support regenerative processes[1].
- Clinical studies have reported favorable healing outcomes associated with dHAM in chronic wound settings[2-3].
- Despite increasing clinical use, mechanistic understanding of dHAM's role in dermal repair remains limited.
- This study evaluates the structural and biological properties of air-dried dHAM*, including its extracellular matrix architecture, proteomic composition, and *in vitro* bioactivity to uncover its role in wound healing processes.

Product notation: *dHAM processed by FORMULA/5™ proprietary processing method (C5 Biomedical, FL)

METHODS

Processing

- Human amniotic membrane comprised of amnion, aseptically processed with proprietary methods (dHAM*).
- Air-dried with humidity and temperature control.
- Terminal sterilization by irradiation (SAL 10^{-6}).



dHAM Extracts

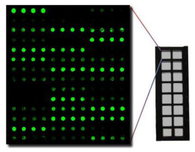
- dHAM (n = 3) was extracted in basal DMEM at 20 mg/mL for 24 h at 4°C
- Extracts were centrifuged at 13,000 rpm for 15 min, and the supernatant passed through a 0.22 μm PES sterile syringe filter.

Histology

- dHAM fixed, paraffin-embedded, sectioned (5 μm; 6 sections/lot), and stained with Hematoxylin & Eosin (H&E), Masson's Trichrome, Verhoeff's Elastin, and Alcian Blue.
- Immunohistochemistry (IHC) with antigen retrieval, blocking, primary antibodies (Collagen I/IV, Laminin, Fibronectin), HRP-conjugated detection, and hematoxylin counterstain. Imaged at 20× magnification.

QuantiBody Array

- dHAM extracts analyzed for regulatory proteins using QuantiBody® Q1000 and Human Angiogenesis Array GS3 (RayBiotech®); samples prepared at 20 mg/mL and run in quadruplicate.
- Fluorescent signals scanned (Cy3), with raw data quantified by RayBiotech and statistical analysis performed by C5.



Bioactivity Assays

1. **Proliferation: Human dermal fibroblasts** (HDFs) treated with serially diluted dHAM extracts. Intact cell DNA quantified after 5 d using CyQUANT® Proliferation Assay (Thermo Fisher®).
2. ***In vitro* Inflammation:** HDFs stimulated with 1 ng/mL IL-1β for 48 h, treated with serially diluted dHAM extracts for 48 h, assessed for inflammatory gene expression using qRT-PCR and protein expression via fluorescent ELISA.
3. ***In vitro* Fibrosis:** HDFs stimulated with 20 ng/mL TGF- β1 for 48 h, treated with serially diluted dHAM extracts for 48 h, assessed for fibrotic gene expression via qRT-PCR.

RESULTS

dHAM retains an intact extracellular matrix with scaffolding proteins

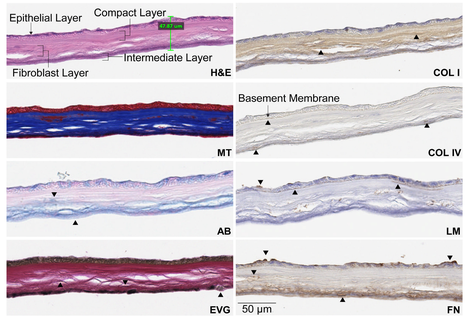
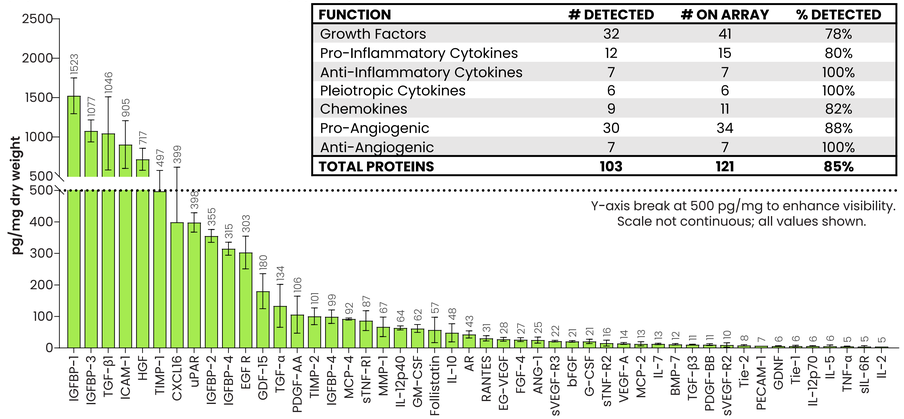


Figure 1. H&E reveals preservation of dHAM's anatomical layers. Collagen is densely distributed throughout all layers, shown by Masson's trichrome (MT) and type I collagen IHC. Non-sulfated GAGs (e.g., hyaluronic acid) detected with Alcian Blue (AB) is localized to the intermediate layer. Type IV collagen and laminin (LM) is stained in the basement membrane. Fibronectin (FN) is concentrated in the epithelial layer and intermediate layer.

dHAM preserves biological signaling proteins

(A)



(B)

FUNCTION	# DETECTED	# ON ARRAY	% DETECTED
Growth Factors	32	41	78%
Pro-Inflammatory Cytokines	12	15	80%
Anti-Inflammatory Cytokines	7	7	100%
Pleiotropic Cytokines	6	6	100%
Chemokines	9	11	82%
Pro-Angiogenic	30	34	88%
Anti-Angiogenic	7	7	100%
TOTAL PROTEINS	**103**	**121**	**85%**

Figure 2. (A) dHAM's proteomic signature reveals a matrix enriched in trophic growth factors and anti-inflammatory mediators that function to coordinate intersecting phases of wound repair. (B) Array detected 103 of 121 (85%) protein targets, categorized by function.

dHAM modulates inflammatory signaling *in vitro*

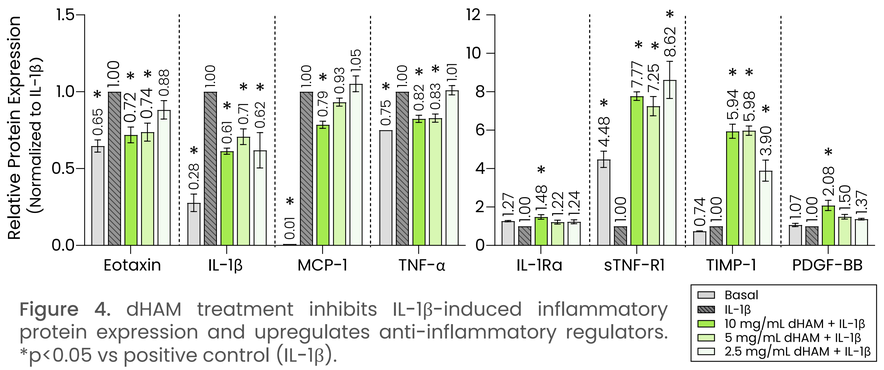


Figure 4. dHAM treatment inhibits IL-1β-induced inflammatory protein expression and upregulates anti-inflammatory regulators. *p<0.05 vs positive control (IL-1β).

RESULTS

dHAM treatment promotes *in vitro* HDF proliferation

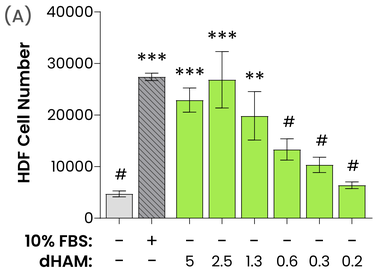
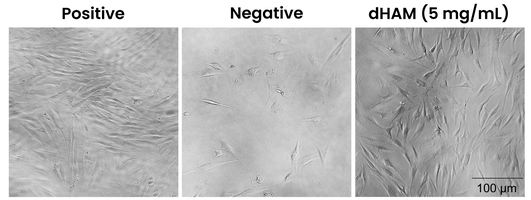


Figure 3. (A) dHAM treatment induced HDF proliferation after 5d. (B) HDF imaged 3d post-treatment. *p<0.05, **p<0.01, ***p<0.001 vs negative control (basal); #p<0.05 vs positive control (10% FBS).

(B)



dHAM downregulates fibrotic genes *in vitro*

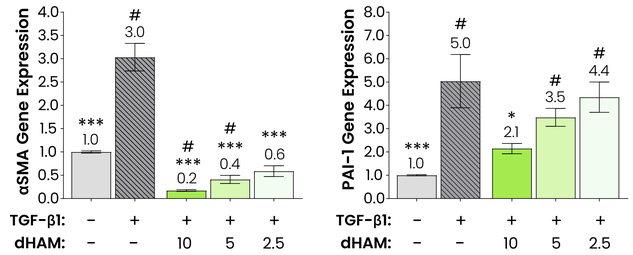


Figure 5. Following TGF-β1 stimulation and treatment with dHAM, dose-dependent reductions in αSMA and PAI-1 fibrotic gene targets was observed. *p<0.05, **p<0.01, and ***p<0.001 vs positive control (TGF-β1); #p<0.05 vs negative control (basal).

CONCLUSION

- This study demonstrates dHAM maintains a structurally intact and biologically active ECM.
- *In vitro*, dHAM promotes dermal fibroblast proliferation, modulates inflammatory signaling, and downregulates TGF-β1-induced fibrotic gene expression—highlighting its multifactorial regenerative potential.
- Future pre-clinical and clinical trials are essential to assess *in vitro* translation and real-world efficacy in chronic and acute wound care.

REFERENCES: 1. Wu S, et al. *Best practice for wound repair and regeneration use of CAMPs.* J Wound Care. 2023;32(Sup4b):S1-S31. **2.** Su YN, et al. *Human amniotic membrane allograft: A systematic review and meta-analysis of RCTs.* Int Wound J. 2020;17(3):753-764. **3.** Snyder RJ, et al. A prospective *RCT evaluation of dehydrated amniotic membrane vs SOC for the closure of chronic diabetic foot ulcers.* Wounds. 2016;28(3):70-7. **4.** Serena TE, et al. *Dual-layer amniotic membrane in the treatment of diabetic foot ulcers: an observational study.* J Wound Care. 2020;29(Sup9)S8-S12.

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Portions of this work currently under revision for publication.

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