Dehydrated Human Amnion Chorion Membranes Contain Key Extracellular Matrix Proteins, Resist Rapid In Vitro Degradation, and Function as a Scaffold

Katrina A. Harmon[1], Tayla A. Crear[2], Justin T. Avery[1], Kelly A. Kimmerling[1], and Katie C. Mowry[1]

[1]Organogenesis Discovery Center, Birmingham, AL; [2]University of Alabama at Birmingham, School of Engineering, Birmingham, AL

INTRODUCTION

Placental-based products have a long-documented use in various wound applications. They have been shown to contain growth factors and cytokines and an extracellular matrix (ECM) rich in collagens, fibronectin, and other ECM molecules. Here, a minimally manipulated dehydrated amnion/chorion membrane (dACMº) was evaluated for biophysical structure and composition as well as its ability to function as a scaffold using *in vitro* models.

ºNuShield®, Organogenesis, Canton, MA

METHODS

- Preservation of key regulatory proteins was assessed using a high-throughput multiplex array, while biophysical structure was assessed using immunohistochemistry (IHC).
- Quantification of key extracellular matrix proteins was evaluated using colorimetric assays.
- Reduction of proteolytic activity was evaluated using a fluorescent cleavage assay.
- Degradation characteristics, including mass loss and collagen content, were assessed using an *in vitro* simulated wound fluid (SWF) degradation model for up to 10 days.
- Scaffold functionality was evaluated using a fibroblast cell attachment and proliferation model on intact (non-degraded) and 3-day SWF-degraded dACM.

HIGH-THROUGHPUT PROTEOMIC EVALUATION

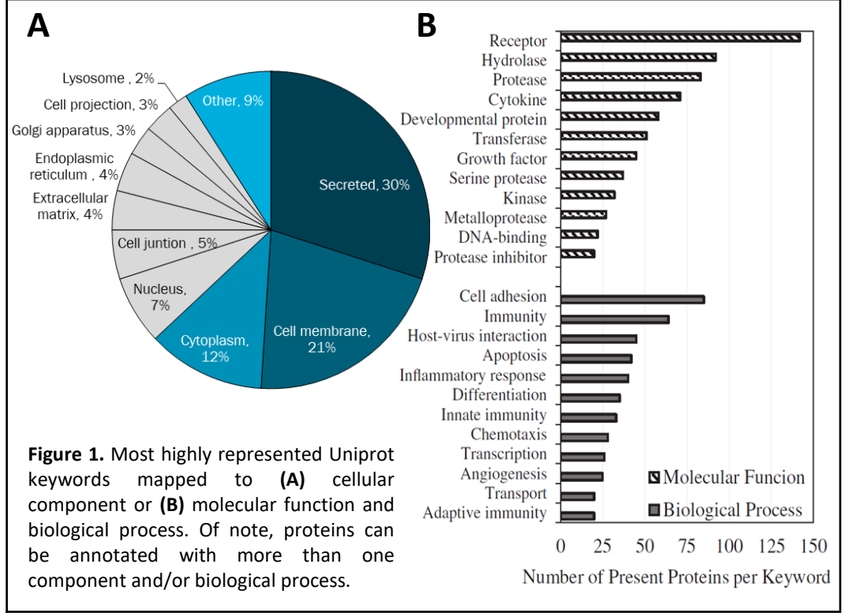

Figure 1. Most highly represented Uniprot keywords mapped to **(A)** cellular component or **(B)** molecular function and biological process. Of note, proteins can be annotated with more than one component and/or biological process.

BIOPHYSICAL STRUCTURE AND CHARACTERIZATION OF dACM

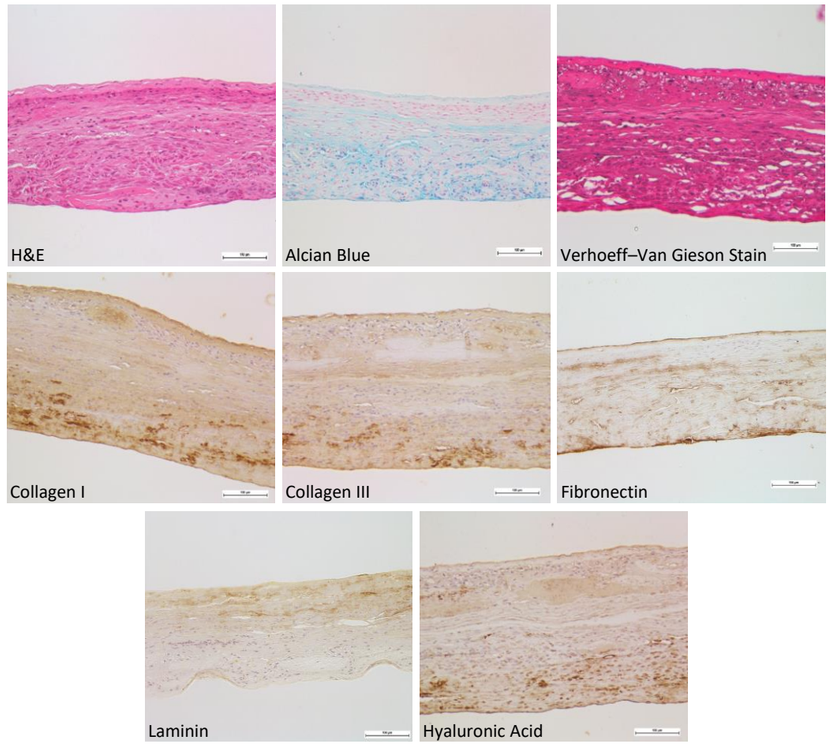

Figure 2. Biophysical structure of dACM. Histological evaluation of dACM including H&E, Alcian Blue, and Verhoeff-Van Gieson staining. Immunohistochemistry of key extracellular matrix proteins maintained in dACM. Collagens I and III and fibronectin were highly concentrated in the chorion layer (lower portion of grafts), while laminin was predominately found in the basement membrane of the amnion. Scale bars = 100 µm.

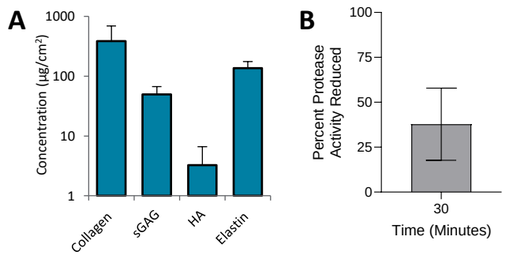

Figure 3. Characterization of dACM. **(A)** dACM maintains key extracellular matrix proteins, with collagens and elastin being present in the highest concentrations. **(B)** Reduction of protease activity with dACM resulted in ~40% reduction compared to uninhibited controls. Average ± stdev reported.

PHYSICAL SCAFFOLD PROPERTIES

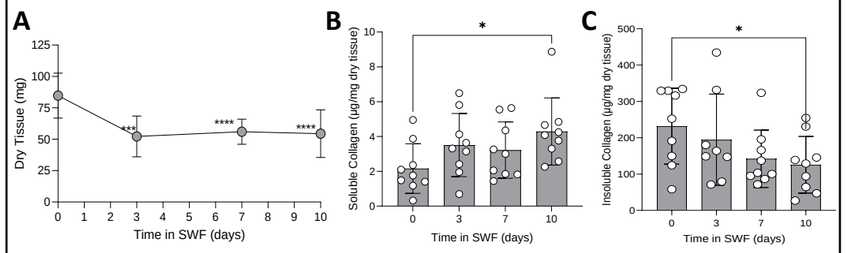

Figure 4. Physical scaffold properties after exposure to SWF in an *in vitro* model. **(A)** Dry tissue weight and **(B)** soluble and **(C)** insoluble collagen content were evaluated over 10 days. Average ± stdev reported. *P≤0.05, *** P≤0.001, ****P≤0.0001 compared to T0.

dACM SUPPORTS CELL ATTACHMENT FOLLOWING DEGRADATION

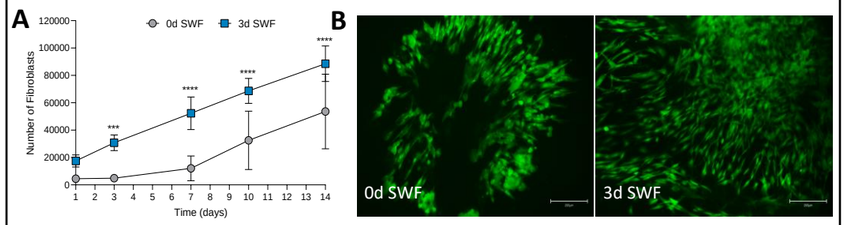

Figure 5. Cellular scaffold properties after exposure to SWF. **(A)** Fibroblast attachment and growth over 14 days on intact (0d) and 3-day (3d) SWF-degraded dACM and **(B)** representative Calcein AM-stained images of live fibroblasts after 3 days of attachment (20x; 200 µm scale bar). Average ± stdev reported. *** P≤0.001, ****P≤0.0001 compared to T0.

CONCLUSIONS

- Structurally, dACM maintained 640 regulatory proteins that were mapped to a variety of molecular functions and biological processes.
- The distribution and localization of key extracellular matrix proteins reveals that collagens I and III, in addition to elastin, fibronectin, laminin, hyaluronic acid, and glycosaminoglycans, are found throughout dACM.
- dACM reduced protease activity, with approximately 40% of activity diminished after 30 minutes.
- Exposure to SWF demonstrated that dACM is resistant to rapid degradation, as significant mass loss was only observed from T0 to day 3.
- dACM served as a scaffold for fibroblast attachment and proliferation, and these properties were maintained through degradation, with significantly greater attachment seen on partially degraded matrices compared to intact.
- These results demonstrate the complex structure of dACM, along with its ability to function as a scaffold for fibroblast attachment and growth.

ORGANOGENESIS