Hypothermically Stored Amniotic Membranes Retain Key Characteristics and Durability in an *In Vitro* Degradation Model

Katrina A. Harmon[1], Kelly A. Kimmerling[1], and Katie C. Mowry[1]

[1]Organogenesis Discovery Center, Birmingham, AL

INTRODUCTION

Placental allografts have a long-documented use in wound care. Maintenance of structure, retention of key components, and functionality of placental allografts may be significantly impaired due to harsh processing techniques. Here, hypothermically stored amniotic membrane (HSAM*), processed gently to maintain key characteristics, was compared to unprocessed amniotic membrane (uAM) and evaluated for retention of ECM components, degradation kinetics, and scaffold functionality.

*Affinity™, Organogenesis, Canton, MA

METHODS

- Qualitative structural and composition assessments were made using scanning electron microscopy (SEM) and histological staining.
- Durability was evaluated by subjecting membranes to simulated wound fluid (SWF) or SWF plus collagenases type I and II (SWF+).
- Degradation kinetics were assessed using histological assessment and changes in collagen content
- Functionality as a scaffold was assessed by the capacity of HSAM to support human dermal fibroblast attachment and proliferation *in vitro.*

HSAM MAINTAINS UNPROCESSED ARCHITECTURAL CHARACTERISTICS

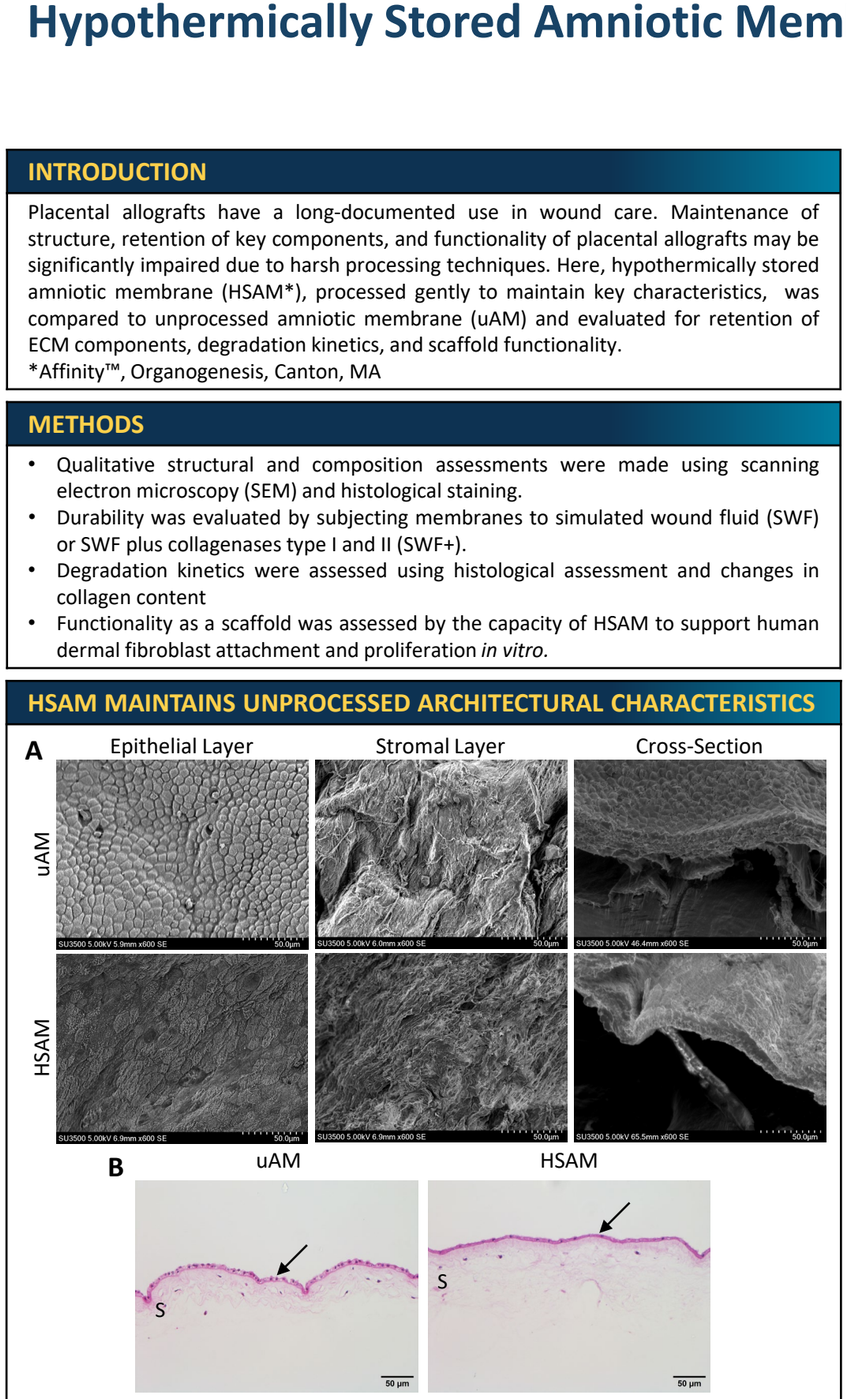

Figure 1: Structural assessment of uAM and HSAM using **(A)** scanning electron microscopy and **(B)** H&E staining. Imaging revealed maintenance of tissue architecture between uAM and HSAM. Scale bars indicate 50 µm; S=stromal.

HSAM MAINTAINS SCAFFOLD INTEGRITY FOLLOWING SWF EXPOSURE

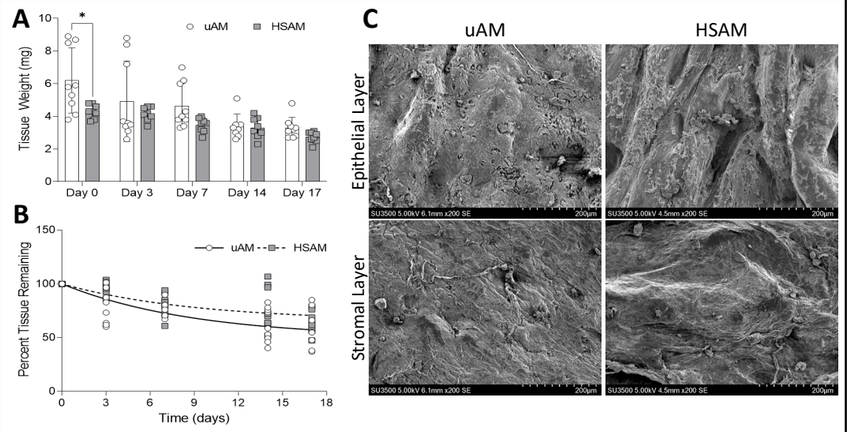

Figure 2: Scaffold integrity following exposure to SWF *in vitro*. **(A)** Dry weight and **(B)** rate of degradation were evaluated over 17 days. **(C)** Representative SEM images of epithelial and stromal layers after 17 days of SWF degradation. Average ± standard deviation reported, *p≤0.05; scale bars indicate 200 µm.

HSAM RESISTS RAPID ENZYMATIC DEGRADATION IN SWF+

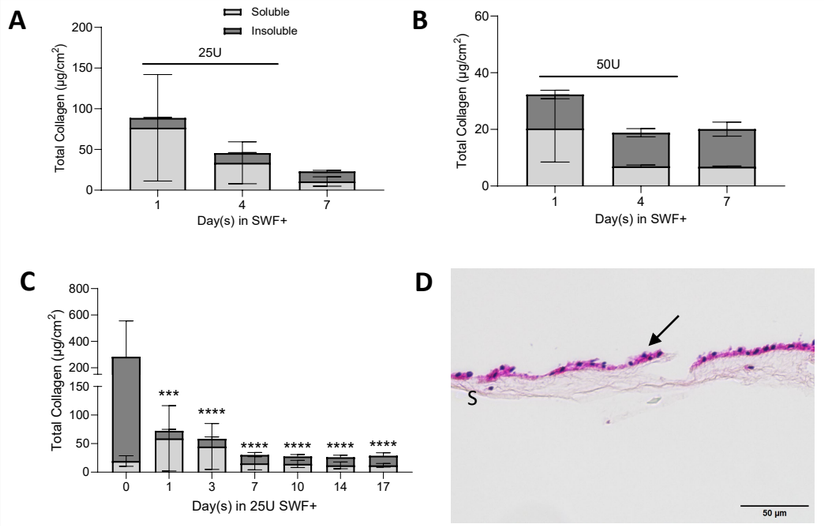

Figure 3: Scaffold integrity following exposure to SWF+ *in vitro*. Collagen content of HSAM following treatment with **(A)** 25U or **(B)** 50U of collagenases type I and II over 7 days. **(C)** Collagen content of HSAM following enzymatic degradation over 17 days. **(D)** Representative H&E images of epithelial (arrow) and stromal (S) layers after 3 days of degradation in 25U SWF+. Average ± standard deviation reported, ***p≤0.001, ****p≤0.0001; scale bar indicates 50 µm.

HSAM SUPPORTS FIBROBLAST ATTACHMENT & GROWTH

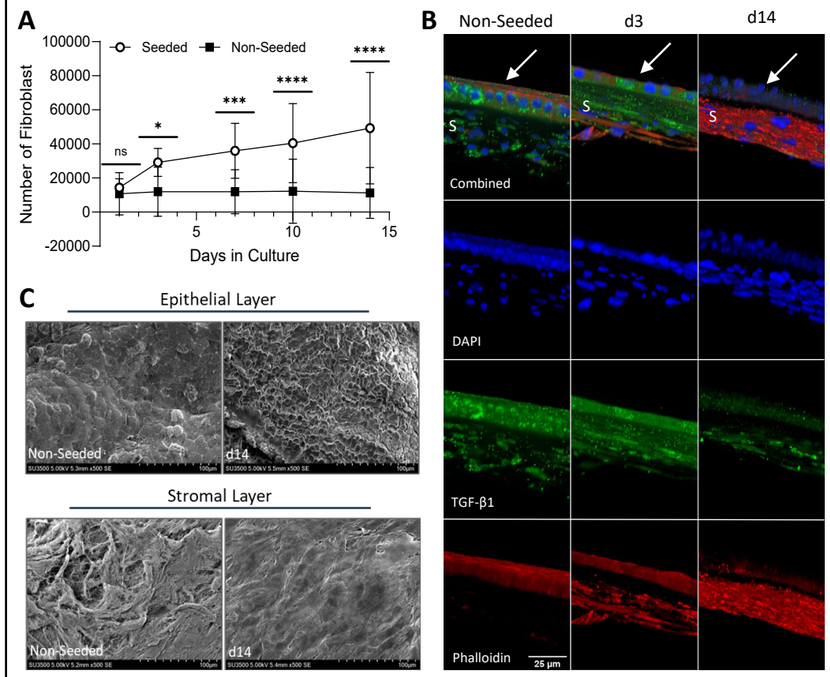

Figure 4: Scaffold functionality of HSAM was assessed via fibroblast growth. **(A)** Fibroblast number over 14 days was assessed by metabolic activity. **(B)** Representative immunofluorescence staining of non-seeded and day 3 and 14 seeded scaffolds. **(C)** Representative SEM images of non-seeded and seeded HSAM after 14 days of culture. Average ± standard deviation reported; *p≤ 0.05, ***p≤0.001, ****p≤0.0001. Blue: nuclei; green: TGF-β1; red: f-actin (phalloidin); arrow: epithelial layer; S: stromal layer; scale bar indicates (B) 25 µm and (C) 100 µm.

CONCLUSIONS

- Hypothermic processing and storage of amniotic tissue maintains native characteristics of unprocessed tissues
- HSAM had degradation rates that were comparable to unprocessed tissue and is resistant to rapid enzymatic degradation.
- HSAM supported fibroblast attachment and proliferation *in vitro* for 14 days.

Hypothermic storage of amniotic membranes maintains the native characteristics of unprocessed tissues, which preserves native ECM structure and scaffold functionality.

ACKNOWLEDGMENTS

The authors acknowledge the support of the University of Alabama at Birmingham's Pathology Core Research Laboratory, particularly Dr. Dezhi Wang (histology), the support of the University of Alabama's Optical Analysis Facility, particularly Dr. Kimberly Lackey (SEM), and the University of Alabama at Birmingham's High Resolution Imaging Facility, particularly Dr. Robert Grabski (fluorescent microscopy).

ORGANOGENESIS