Hypothermic Storage of Amniotic Membranes Conserves Structure, Composition, and Responses *In Vitro* through the Retention of Native Characteristics

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INTRODUCTION

Human placental tissues have an extensive clinical history, while preclinical studies have established that these tissues retain the extracellular matrix (ECM) and serve as protective barriers. A propriety hypothermic storage processing technique has been developed to preserve the native characteristics of amniotic membranes (HSAM*). In this study, it was hypothesized that the gentle HSAM process would result in the retention of characteristics found in native, unprocessed amniotic membranes (uAM).

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METHODS

- Donor-matched amniotic membranes were processed into either HSAM, which was stored at 1-10°C for up to 42 days before use, or uAM, which was used within 24 hours.
- Amniotic membranes were characterized using histology, scanning electron microscopy, and immunofluorescence.
- Durability was evaluated using an *in vitro* simulated wound fluid model.
- To evaluate retention of barrier properties, an *in vitro* human dermal fibroblast model was used to assess cell attachment via histology, immunofluorescence staining, and scanning electron microscopy.

HSAM MAINTAINS UNPROCESSED STRUCTURAL CHARACTERISTICS

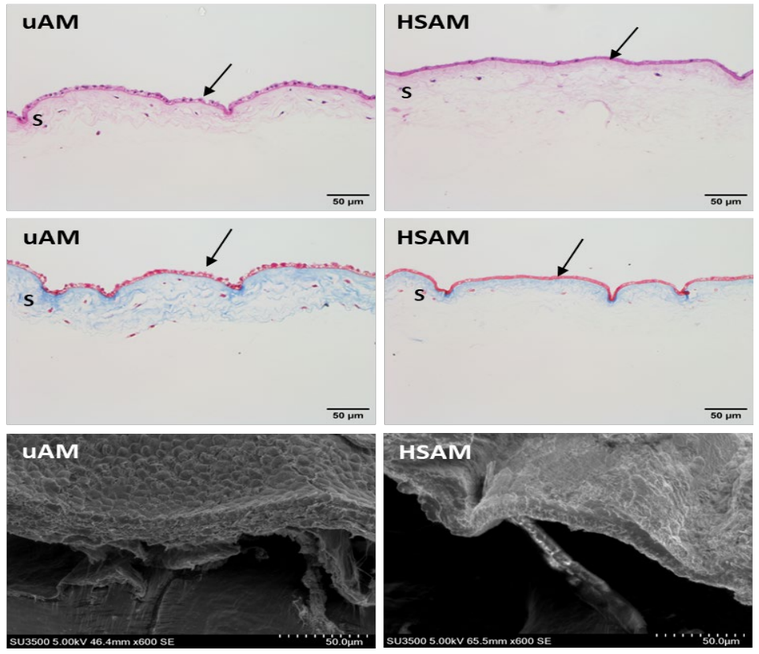

Figure 1. Structural assessment of uAM and HSAM. ECM structure was evaluated using H&E and Masson's Trichrome. Imaging revealed maintenance of tissue architecture between uAM and HSAM. Arrow: epithelial layer; S: stromal layer.

HSAM MAINTAINS UNPROCESSED STRUCTURAL CHARACTERISTICS

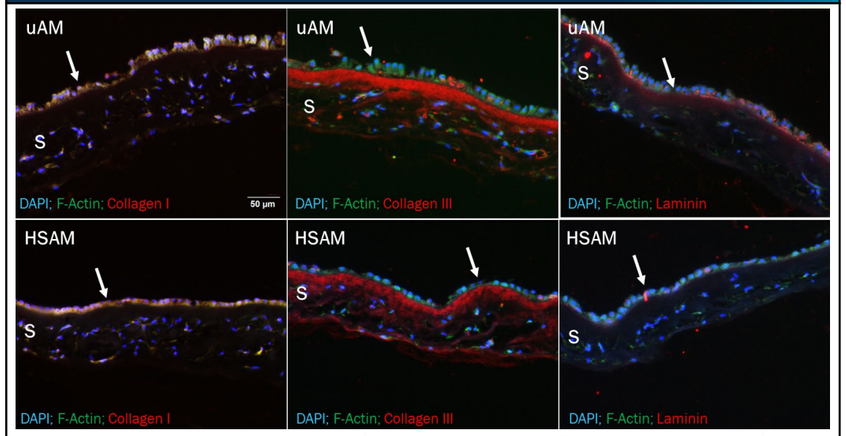

Figure 2. Structural assessment of uAM and HSAM. ECM structure was further assessed using immunofluorescence staining including collagen I, collagen III, and laminin. Arrow: epithelial layer; S: stromal layer.

HYPOTHERMIC STORAGE RETAINS UNPROCESSED TISSUE INTEGRITY

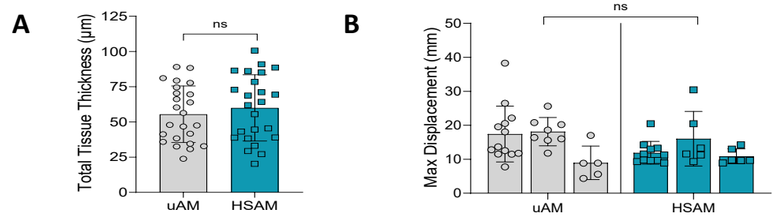

Figure 3. Characterization of unprocessed and hypothermically stored membranes. Retention of (A) tissue thickness and (B) tensile testing was evaluated in donor-matched uAM and HSAM. Average ± standard deviation reported; ns: not significant.

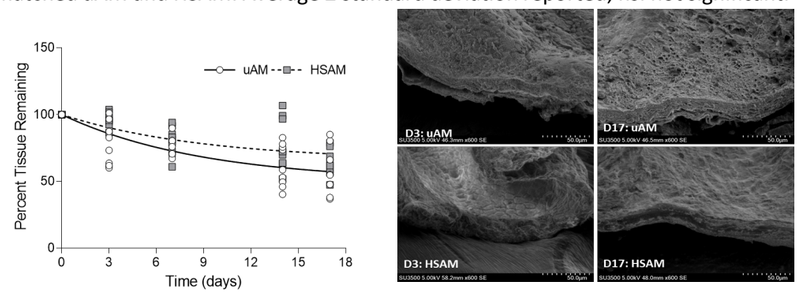

Figure 4. Durability of tissues following exposure to SWF *in vitro*. Rate of degradation and changes in ECM structure were evaluated over 17 days of *in vitro* degradation in SWF. Both uAM and HSAM retained over 50% of their initial tissue weight over the 17-day study, with no significant differences in the rate of degradation or tissue structure.

HSAM SUPPORTS BARRIER PROPERTIES

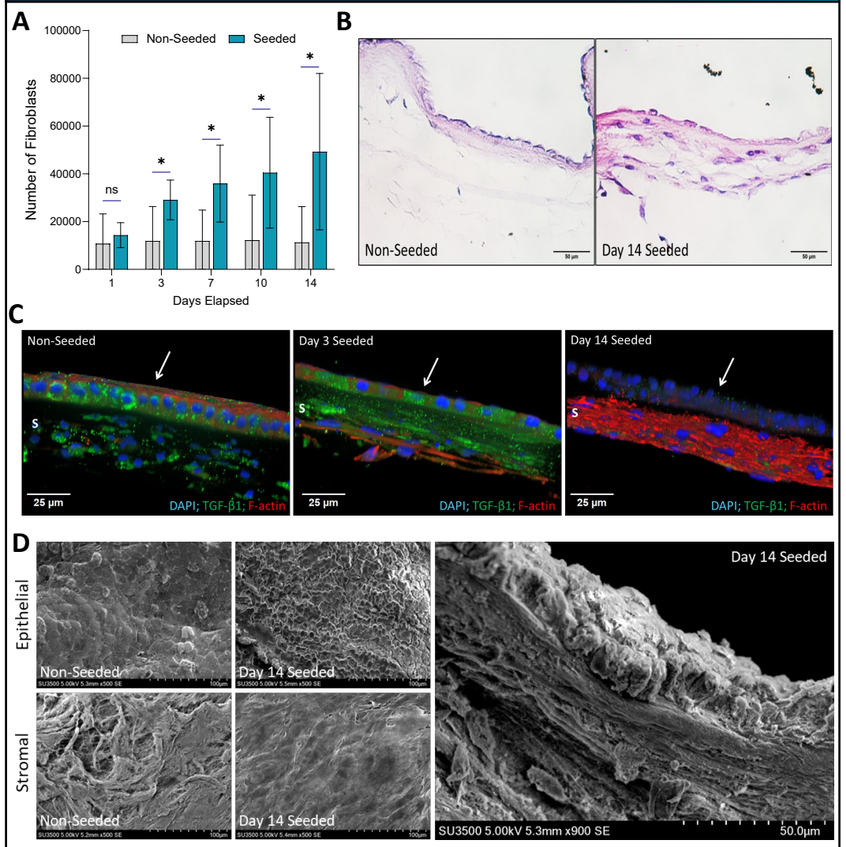

Figure 4. *In vitro* barrier properties. Fibroblasts were evaluated over 14 days as assessed by (A) metabolic activity, (B) H&E, (C) immunofluorescence staining, and (D) scanning electron microscopy. Average ± standard deviation reported; *p≤ 0.05. Blue: nuclei; green: TGF-β1; red: f-actin (phalloidin); arrow: epithelial layer; S: stromal layer, ns: not significant.

CONCLUSIONS

- HSAM is a minimally manipulated, protective barrier that retained the characteristics of native, unprocessed amniotic membranes.
- Tissue thickness, tensile strength, and *in vitro* degradation rates were comparable between unprocessed and hypothermically stored amniotic tissues.
- HSAM served as a protective barrier by supporting fibroblasts over 14 days of culture.
- Overall, all facets were conserved with HSAM, due to retaining the properties of uAM. These results highlight the hypothermic processing techniques as an effective method for processing and preserving amniotic membranes.