Hypothermic Storage Maintains Key Characteristics of Fresh Placental Membranes and Provides a Scaffold to Support Tissue Growth

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INTRODUCTION

Human placental tissues, consisting of amnion and chorion membranes, have been shown to support wound healing clinically. Pre-clinical studies have established placental membranes naturally consist of growth factors, cytokines, and extracellular matrix (ECM) proteins. A proprietary hypothermic storage processing technique has been developed to preserve and maintain the native characteristics of amnion (HSAM†) and chorion (HSCMº) membranes. In this study, we confirmed the minimal manipulation of the tissue by assessing how HSAM and HSCM retains key properties characteristic of fresh amnion and chorion membranes. To evaluate this, biophysical properties of fresh and hypothermically stored placental membranes were characterized, along with their response to degradation using a simulated wound fluid (SWF) model. Additionally, HSAM and HSCM were evaluated for their scaffold functionality in vitro.

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METHODS

- Structure and composition compared to fresh amnion and chorion membranes (fAM and fCM) were evaluated using scanning electron microscopy (SEM) and immunohistochemistry (IHC).
- Degradation was evaluated by mass retention in an *in vitro* SWF model.
- Functionality as a scaffold was assessed by the capacity for HSAM and HSCM to support human dermal fibroblast attachment and proliferation *in vitro.*

SEM ASSESSMENT SHOWED COMPARABILITY BETWEEN TISSUES

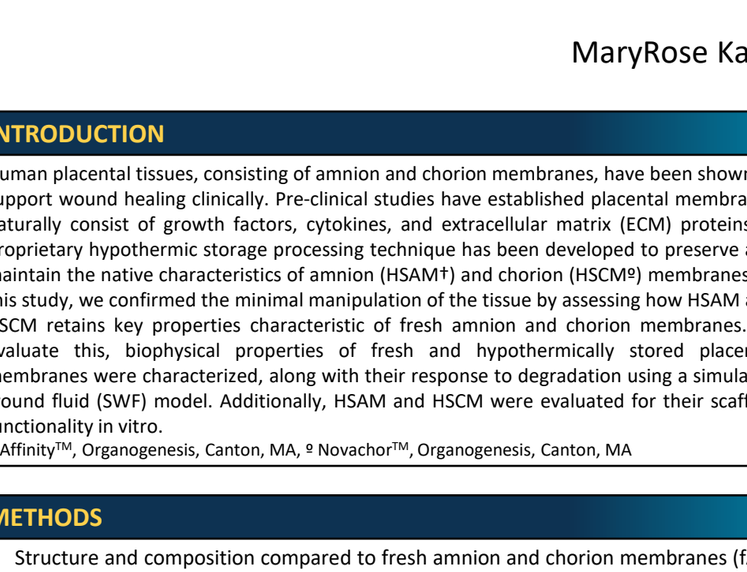

Figure 1: Cross-sectional assessment of fresh and hypothermically stored amnion and chorion membranes. Retention of all layers of each tissue and comparable thickness were observed for fAM and HSAM (compared to fresh); an expected decrease in thickness was observed in HSCM versus fCM due to debridement step during processing. Images captured at 600x.

EVALUATION VIA IHC SHOWED RETENTION OF KEY PROTEINS

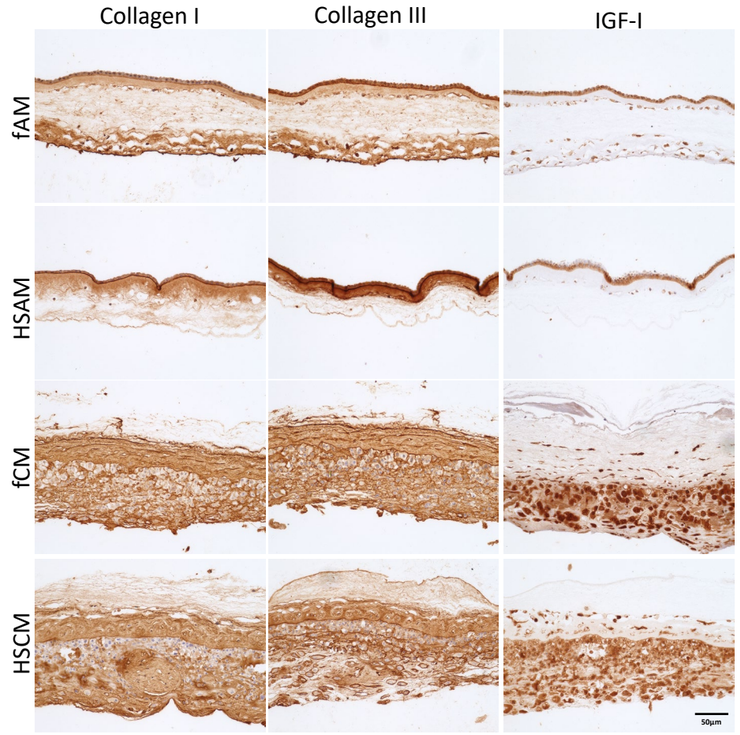

Figure 2: Biophysical composition of fresh and hypothermically stored amnion and chorion. IHC staining of key extracellular matrix proteins was maintained following hypothermic storage. Collagens I and III and IGF-I were distributed throughout HSCM and were highly concentrated in the trophoblast layer, while Collagen III and IGF-I were highly represented in the basement membrane of HSAM.

SCAFFOLD INTEGRITY FOLLOWING EXPOSURE TO SWF IS SIMILAR

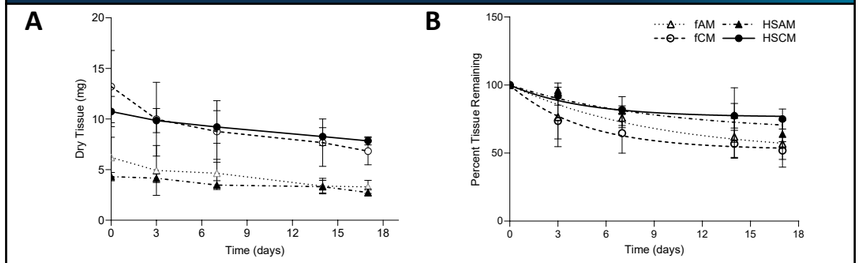

Figure 3: Fresh and hypothermically stored amnion and chorion integrity following exposure to SWF. Dry weight **(A)** and percent tissue remaining **(B)** were evaluated over 17 days. Average ± standard deviation. One-way phase decay fit for rate of degradation for each tissue is shown in **(B)**. Hypothermic tissues (HSAM and HSCM) resisted rapid degradation

HSAM AND HSCM SUPPORT FIBROBLAST ATTACHMENT & GROWTH

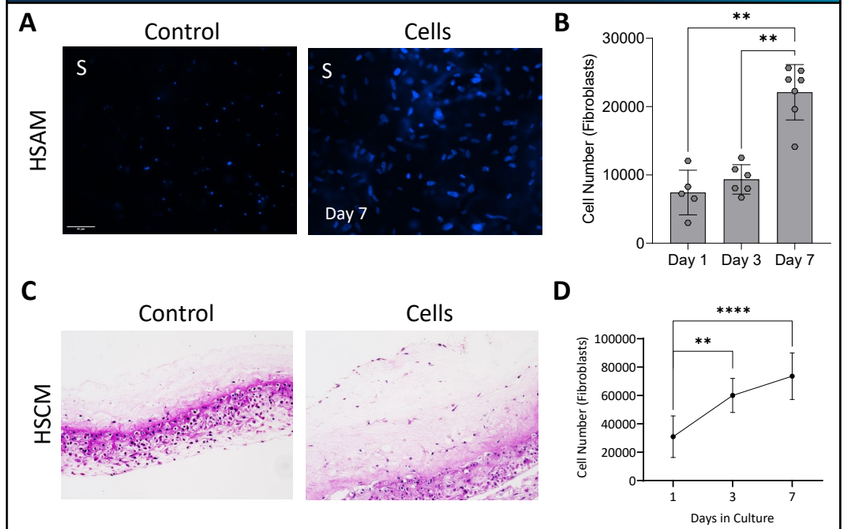

Figure 4: Scaffold functionality of HSAM **(A, B)** and HSCM **(C, D)** was evaluated via fibroblast attachment and growth. DAPI-stained nuclei on unseeded control and seeded HSAM **(A)** and H&E images of unseeded control and seeded HSCM **(C)** show cell attachment and infiltration into placental membranes. Cell number over 7 days demonstrate that HSAM **(B)** and HSCM **(D)** provide a scaffold that supports fibroblast cell proliferation. Average ± standard deviation reported. S=stromal side. **P≤0.01; ****P≤0.0001.

CONCLUSIONS

- Hypothermic processing and storage of amnion and chorion membranes maintain the structure of fresh tissue.
- Placental membranes retained native ECM proteins and key growth factors present in native tissue following hypothermic storage.
- Hypothermically stored placental membranes both withstood rapid degradation over time in a SWF model; the tissue remaining was comparable to fresh tissues.
- HSAM and HSCM supported the attachment and proliferation of fibroblasts *in vitro* for 7 days.
- Hypothermic storage of placental membranes maintains key characteristics and functionality of these membranes as both a protective barrier and a scaffold to support tissue growth.

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ORGANOGENESIS