MAINTENANCE OF TISSUE STRUCTURE AND INTEGRITY OF HYPOTHERMICALLY STORED AMNION AND CHORION MEMBRANES

Katrina A. Harmon, Ph.D.[1], Justin T. Avery, Ph.D.[1], Kelly A. Kimmerling, Ph.D.[1], and Katie C. Mowry, Ph.D.[1]

[1]Organogenesis Discovery Center, Birmingham, AL 35243

INTRODUCTION

Placental allografts have been widely utilized as wound covers in various applications, including diabetic ulcers, venous stasis ulcers, and traumatic wounds. Numerous processing techniques result in products with one or more layers, with differing impacts to final tissue structure and integrity within the product. In this study, hypothermic storage was utilized to maintain the native characteristics of human placental tissue, and samples of both amnion and chorion were evaluated for preservation of key components of tissue thickness and structure, along with mechanical properties.

METHODS

Placental tissue was processed and preserved using a fresh hypothermic storage method (AlloFresh™) into hypothermically stored amnion (HSAM†) and chorion (HSCMº). Structural assessments of HSAM and HSCM were made using hematoxylin and eosin (H&E) and Masson's trichrome staining, immunohistochemistry staining, and scanning electron microscopy (SEM). Tissue thickness was evaluated by sampling multiple areas from H&E slides, which were then quantified using ImageJ. Tensile testing using an Instron Model 3342 was completed to identify potential differences in tissue strength.

† Affinity®, Organogenesis, Canton, MA
º Novachor™, Organogenesis, Canton, MA

ARCHITECTURE OF HYPOTHERMICALLY STORED MEMBRANES

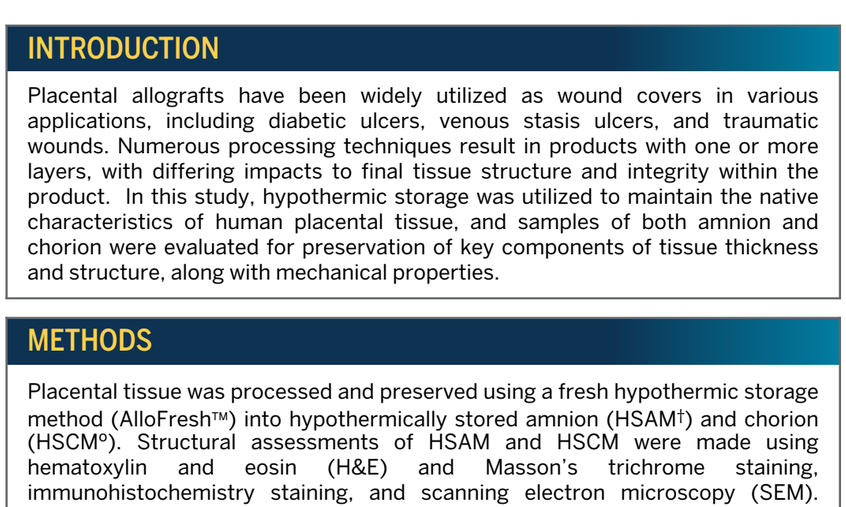

Figure 1: Representative SEM images of hypothermically stored placental membranes. **(A)** HSAM presents with an epithelial and stromal layer, consistent with native tissues. **(B)** HSCM does not present with an evident sidedness and contains all layers of native chorion.

HYPOTHERMIC STORAGE MAINTAINS NATIVE CHARACTERISTICS

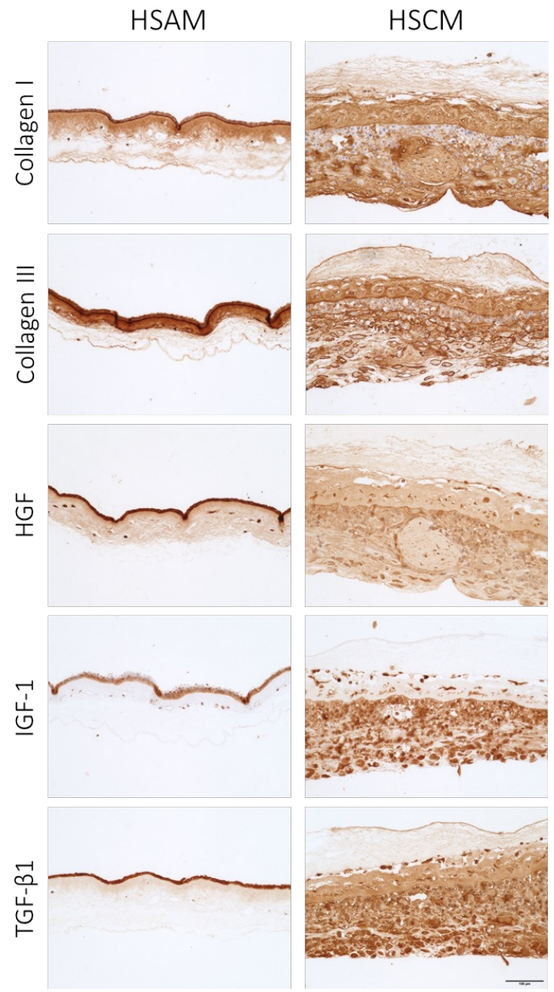

Figure 2: Hypothermically stored amnion and chorion membranes were evaluated for retention of natively expressed extracellular matrix proteins (Collagen I and III) and cytokines and growth factors (HGF, IGF, and TGF-β1).

As measured by IHC, the localization and relative staining intensity of targets on HSAM and HSCM are consistent with that of native amnion and chorion, respectively. This finding highlights how hypothermic processing and storage maintains native tissue architecture and layers, along with ECM, cytokines, and growth factors found in native fresh tissue.

HSCM IS THICKER THAN HSAM

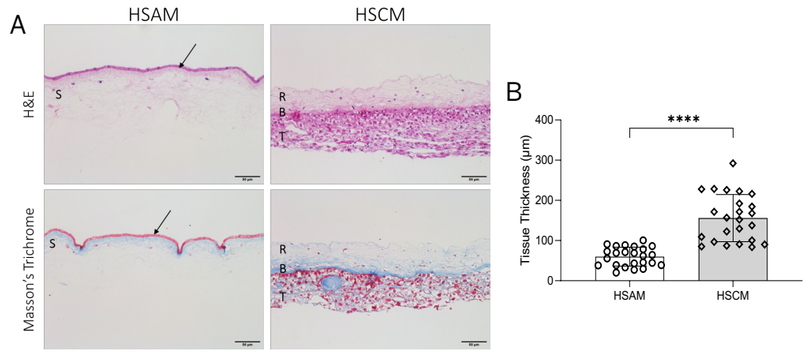

Figure 3: (A) HSAM and HSCM were assessed using H&E and Masson's Trichrome-stained cross sections of hypothermically stored tissues. Black arrows indicate epithelial layer. S=stromal layer, R=reticular layer, B=basement membrane, and T=trophoblast layer. **(B)** Tissue thickness was measured using Image J analysis of H&E slides. Average ± standard deviation reported.**** $P \leq 0.0001$.

MECHANICAL PROPERTIES OF HSAM AND HSCM

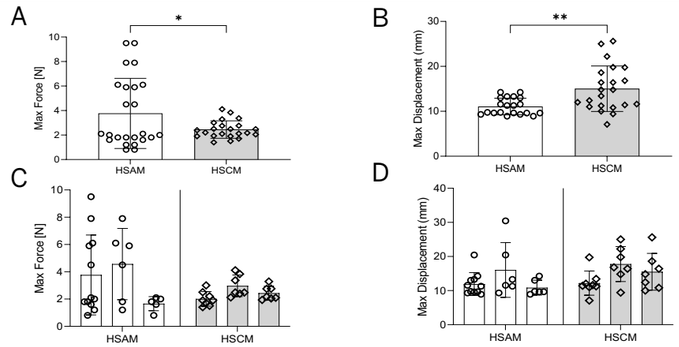

Figure 4: Differences in tissue integrity after hypothermic storage was evaluated using **(A)** max load and **(B)** max displacement. To evaluate intra-donor variability, nested **(C)** max load and **(D)** max displacement was also determined. Average ± standard deviation reported.* $P \leq 0.05$.** $P \leq 0.01$

CONCLUSIONS

Both HSAM and HSCM maintain the native tissue architecture and characteristics of amnion and chorion, respectively. While HSCM is thicker overall, mechanically, HSAM showed higher max load during tensile testing. These findings correlate with the physiological function of placental tissue in the role of maintaining the protective environment in utero and demonstrate the maintenance of key characteristics in both HSAM and HSCM.

ORGANOGENESIS