Title: AlloFresh Processing of Amnion and Chorion Tissues Maintains the Native Characteristics of Fresh Placental Membrane Tissues

Authors: Justin T. Avery, Katrina A. Harmon, Kelly A. Kimmerling, Katie C. Mowry

Placental tissues have an extensive history as wound coverings dating back to the early 1900s. They contain extracellular matrix (ECM) components, growth factors and cytokines, and act as a protective barrier to support healing. To preserve these components, several processing techniques have been utilized such as dehydration, lyophilization, cryopreservation, and hypothermic storage. The AlloFresh process, a hypothermic storage method, was designed to retain native characteristics of fresh placental membranes and is assessed herein.

All collection and processing were in accordance with the FDA's Good Tissue Practices and AATB standards. Briefly, amnion and chorion tissues were separated and stored as either unprocessed amniotic membrane or chorion membrane (uAM or uCM) or processed as hypothermically stored amnion or chorion membrane (HSAM or HSCM). Comparability assessments for retention of native characteristics included tissue thickness, hematoxylin and eosin (H&E) staining, mechanical testing, scanning electron microscopy (SEM) imaging, ECM composition through immunohistochemistry (IHC), and degradation in a simulated wound fluid (SWF) model *in vitro* for up to 17 days.

Hypothermic processing maintained the structural characteristics for both HSAM and HSCM compared to uAM and uCM, respectively, with similar cellularity and fibrous density. While structurally similar by H&E, HSCM was thinner than the uCM due largely to the mechanical debridement step required during processing. No statistical differences in maximum force or displacement were observed during mechanical testing when comparing HSAM and HSCM to their unprocessed counterparts, highlighting the retention of ECM.

Similar levels and localization of collagen I, collagen III, hepatocyte growth factor, insulin-like growth factor 1, and transforming growth factor beta 1 were observed between uAM/HSAM and uCM/HSCM. While statistical differences in starting weights were observed between membranes initially in degradation studies, these differences were lost over time. Assessment of degraded amnion membranes by SEM showed a similar loss of cellular continuity on the epithelial side, while the ECM fibers were slowly lost as the spongy layer degraded. Degradation of both uCM and HSCM was visible by SEM imaging, with deep pockets developing in the macrostructure, a loss of individual fiber clarity, and a loss of discernable definition across the surface of the membranes.

This study shows that hypothermic processing and storage of both amnion and chorion membranes maintains the key native characteristics and function of unprocessed placental membranes.

AlloFresh Processing of Amnion and Chorion Tissues Maintains the Native Characteristics of Fresh Placental Membrane Tissues

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ABSTRACT

Placental tissues have an extensive history as wound coverings dating back to the early 1900s. They contain extracellular matrix (ECM) components, growth factors and cytokines, and act as a protective barrier to support healing. To preserve these components, several processing techniques have been utilized such as dehydration, lyophilization, cryopreservation, and hypothermic storage. The AlloFresh process, a hypothermic storage method, was designed to retain native characteristics of fresh placental membranes and is assessed herein.

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ALLOFRESH PRESERVATION PROCESS

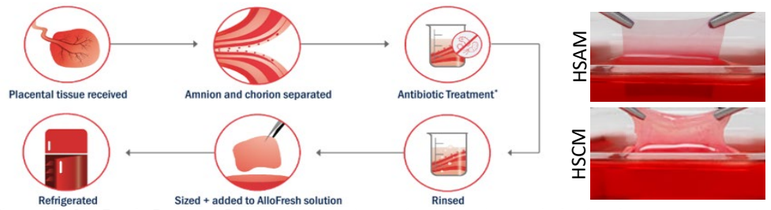

Figure 1. AlloFresh Process. Placental tissue is received, amnion and chorion layers are separated, samples undergo various processing steps (cleaning, antibiotic treatment, and rinses), are cut to size, and stored in hypothermic storage solution.

AMNION HISTOLOGICAL CHARACTERIZATION

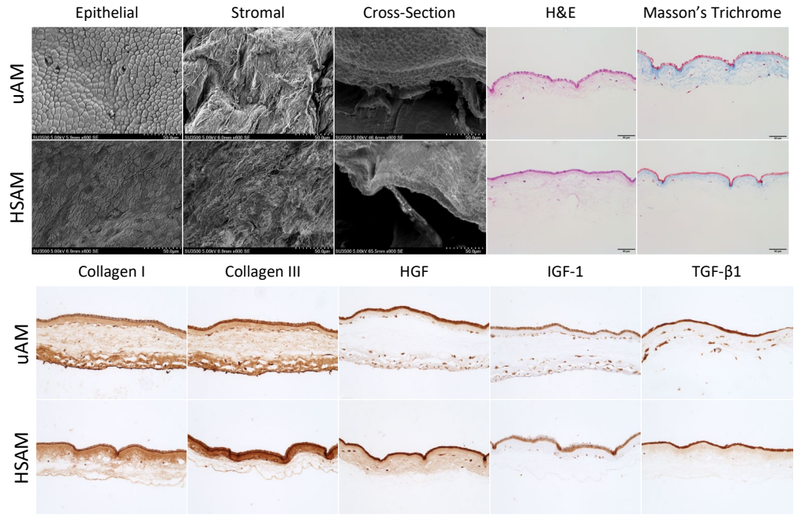

Figure 2. Histological characterization of unprocessed and hypothermically stored amnion membranes. SEM, H&E, Masson's Trichrome, and various IHC markers were all assessed. AlloFresh processing does not impact the retention of assessed ECM or growth factor retention. Scale bars=50µm.

CHORION HISTOLOGICAL CHARACTERIZATION

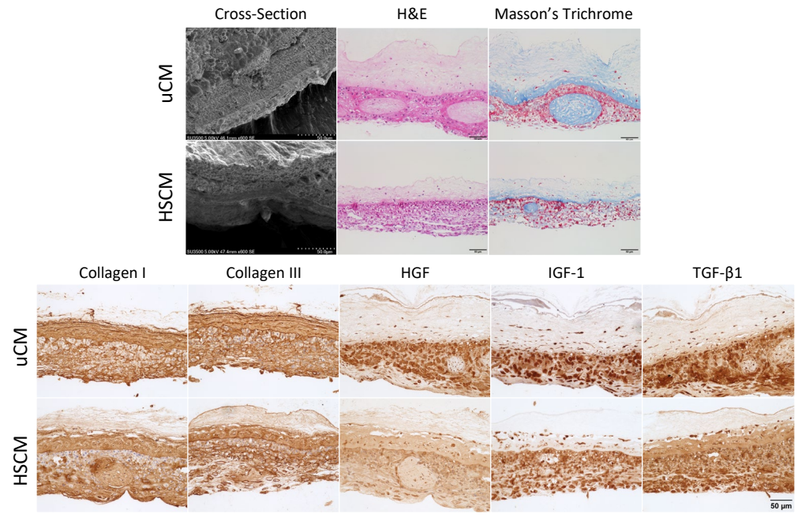

Figure 3. Histological characterization of unprocessed and hypothermically stored chorion membranes. SEM, H&E, Masson's Trichrome, and various IHC markers were all assessed. AlloFresh processing does not impact the retention of assessed ECM or growth factor retention. Scale bars=50µm.

AMNION STRUCTURAL AND DEGRADATION ASSESSMENTS

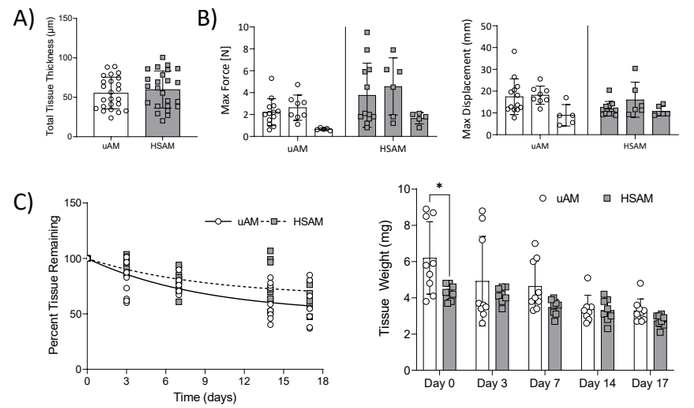

Figure 4. AlloFresh processing retains native tissue structure, integrity, and durability of amnion membranes. A) Tissue thickness. **B)** Mechanical testing for max force and max displacement; no statistical differences were observed. **C)** Percent tissue weight remaining and rate of degradation in simulated wound fluid after 17 days. * P ≤ 0.05.

CHORION STRUCTURAL AND DEGRADATION ASSESSMENTS

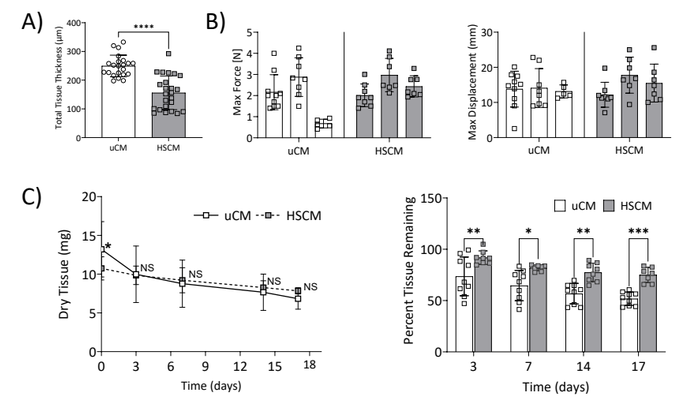

Figure 5. AlloFresh processing retains native tissue structure, integrity, and durability of chorion membranes. A) Tissue thickness; differences attributed to debridement during processing. **B)** Mechanical testing for max force and max displacement; no statistical differences observed. **C)** Percent tissue weight remaining and rate of degradation in simulated wound fluid after 17 days. Initial differences in mass at Day 0 drove percent differences. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001.

CONCLUSIONS

Hypothermic processing and storage of amnion and chorion membranes maintains the key native characteristics of unprocessed placental membranes.

ORGANOGENESIS