Hypothermic Storage of Amnion and Chorion Membranes Retain Fresh Placental Tissue Characteristics Compared to Dehydrated Tissues

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Introduction

Placental tissues have been utilized to treat a variety of wounds. Various processing methods have been used, including cryopreservation, dehydration, lyophilization, and hypothermic storage. Processing methods have been shown to impact tissue characteristics, including extracellular matrix (ECM) structure, graft thickness, and degradation characteristics. In this study, we evaluated the impact of a fresh hypothermic storage process (AlloFresh™) on both an amnion product (HSAMº) and a chorion product (HSCM†) and compared them to unprocessed and dehydrated placental tissues. We evaluated qualitative structural differences, quantified tissue thickness, and measured degradation characteristics using an *in vitro* wound model utilizing simulated wound fluid (SWF).

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

Methods

Amniotic tissue from at least two donors was prepared as 1) unprocessed placental membranes, 2) processed into HSAM or HSCM, or 3) processed into dehydrated membranes. Of note, HSCM processing includes a debridement step, which removes a portion of the trophoblast membrane. Qualitative structural assessments for HSAM, HSCM, unprocessed, and dehydrated placental membranes were made using hematoxylin and eosin and Masson's trichrome staining. To quantify differences in graft thickness, multiple areas from each slide were sampled and quantified using ImageJ. To evaluate degradation characteristics *in vitro*, membranes were exposed to SWF for up to 17 days at 37°C, and ECM degradation was evaluated. Degradation was evaluated by measuring collagen released into the degradation solution and taking dry weights of samples.

Assessment of Graft Thickness after Hypothermic Storage

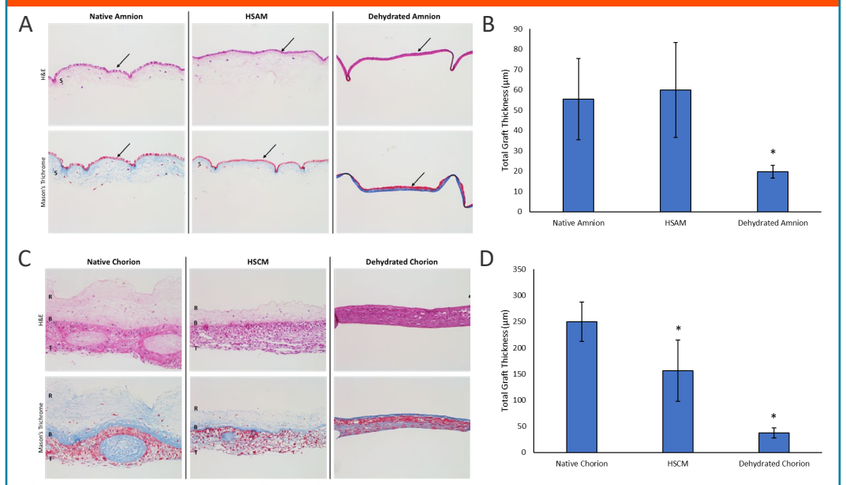

Figure 1: (A) Representative H&E- and Masson's Trichrome-stained cross-sections of fresh amnion and HSAM. Black arrows indicate epithelial layer and S=stromal layer, **(B)** Effect of processing on amniotic membranes on total graft thickness, **(C)** Representative H&E- and Masson's Trichrome-stained cross-sections of fresh chorion and HSCM. R=reticular layer, B=basement membrane, and T=trophoblast layer, **(D)** Effect of processing on chorion membranes on total graft thickness. * denotes $p<0.05$ compared to native tissue

IHC of Native and Hypothermically Stored Amnion & Chorion

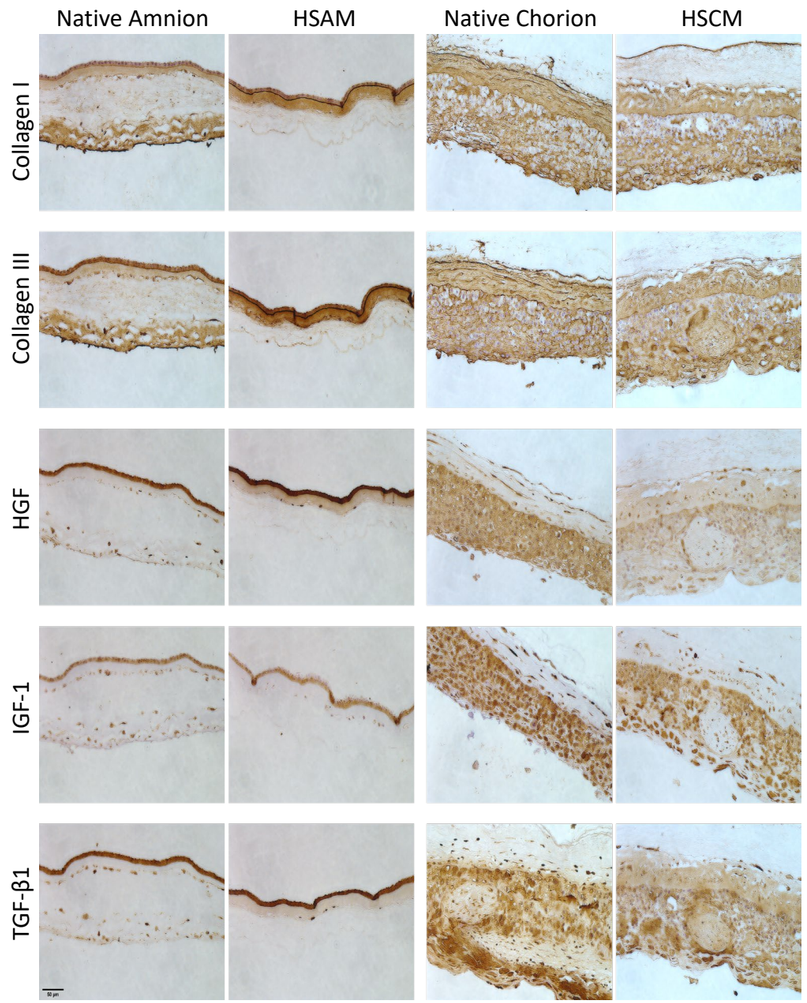

Figure 2: Immunohistochemical (IHC) assessment of native amnion and chorion compared to HSAM and HSCM, respectively. Donor-matched membranes were evaluated for retention of natively expressed ECM proteins (Collagen I and III) and cytokines and growth factors (HGF, IGF, and TGF-β1). Of note, the fresh amnion group appeared to have a processing/staining artifact where tissue was split.

As measured by IHC, the localization and relative staining intensity of HSAM and HSCM were characteristic of native amnion and chorion tissue, respectively. This finding highlights how fresh hypothermic processing and storage maintains the native tissue architecture, ECM, cytokines, and GF found in native human tissue.

In vitro Degradation Characteristics of Placental Membranes

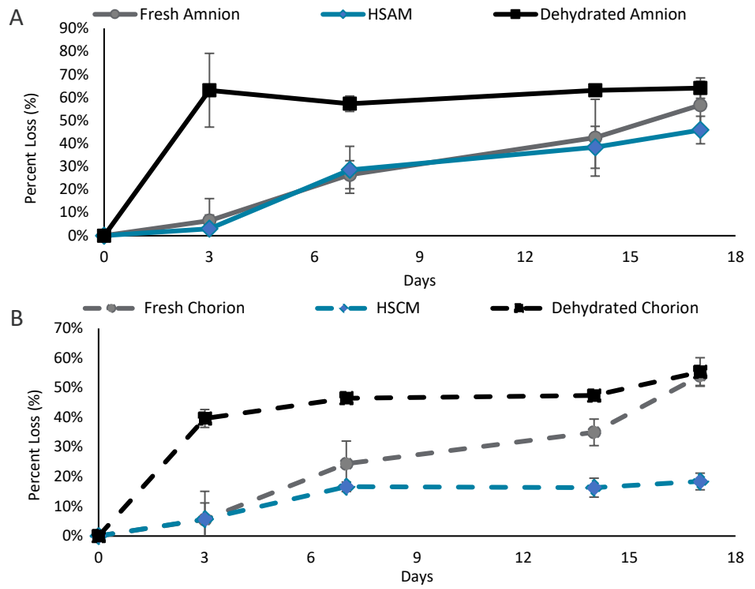

Figure 3: Percent loss of placental membranes after *in vitro* culture in SWF. (A) Amniotic membranes. **(B)** Chorion Membranes. Average ± standard deviation reported; n=3.

When evaluating degradation profiles, hypothermically stored tissues were more representative of fresh membranes, especially during early time points (days 0-7). In contrast, dehydrated membranes resulted in a more rapid degradation compared to fresh and hypothermically stored tissues.

Conclusions

- Both HSAM and HSCM demonstrated tissue architecture characteristic of native, unprocessed amnion and chorion tissues, respectively.
- Native amnion grafts and HSAM resulted in comparable graft thickness. As expected, due to the debridement step during HSCM processing, HSCM tissue thickness was reduced compared to native chorion, but all retained layers were comparable.
- Both fresh and hypothermically stored placental membranes were substantially thicker than dehydrated membranes.
- In an *in vitro*, model HSAM degraded at similar rates compared to fresh amnion.
- Both fresh and hypothermically stored placental membranes degraded at a slower rate compared to dehydrated tissues, resulting in greater product remaining after 17 days of culture.

Acknowledgements

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