Hypothermically Stored Chorion Membranes Inhibit Proteolytic Enzymes Highlighting the Retention of Key Components Within Fresh Chorion

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

[1]Organogenesis Discovery Center, Birmingham, AL

INTRODUCTION

Placental tissues have long been utilized in the treatment of chronic wounds such as diabetic foot ulcers and venous leg ulcers. To maintain the membrane integrity, several processing techniques have been employed, including cryopreservation, dehydration, and hypothermic storage. Fresh hypothermic storage (AlloFresh™) of placental membranes has been shown to maintain the structural characteristics of native chorion membranes (HSCM†). In this study, we have expanded on this work to assess the retention of native extracellular matrix (ECM) proteins and regulators, and whether retention of these factors can aid in the inhibition of proteases.

†Novachor, Organogenesis, Canton, MA

METHODS

- ECM structure and content was assessed using scanning electron microscopy (SEM), hematoxylin and eosin (H&E) and Masson's trichrome staining, and immunohistochemistry (IHC).
- Preservation of key regulatory proteins was assessed using a Quantibody array (RayBiotech).
- Proteolytic inhibition was determined using a *Clostridium histolyticum* type IV collagenase reporter system (EnzChek, Invitrogen).

SCANNING ELECTRON MICROSCOPY STRUCTURAL ASSESSMENT

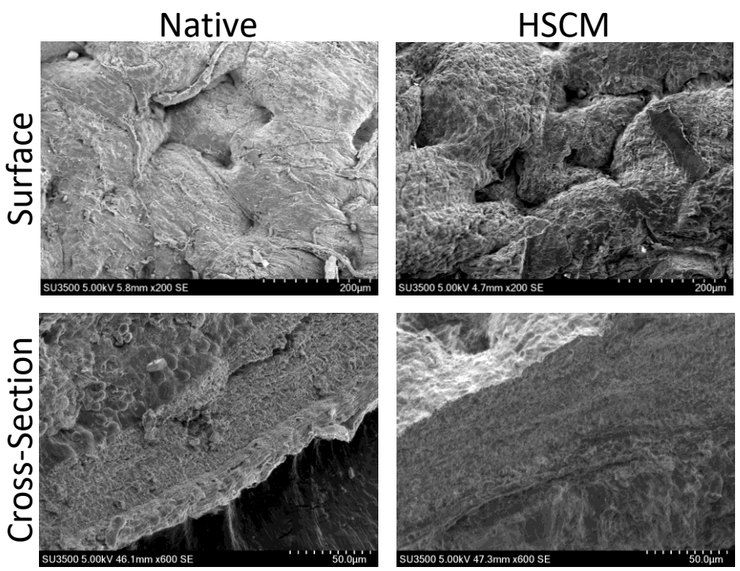

Figure 1. Cross-sectional assessment of native chorion and HSCMs. No surface sidedness was identifiable; porous layers of ECM scaffold are observable with cross-sectional views. Images captured at 200x and 600x using a Hitachi SU3500 SEM.

HISTOLOGICAL CHARACTERIZATION OF EXTRACELLULAR MATRIX

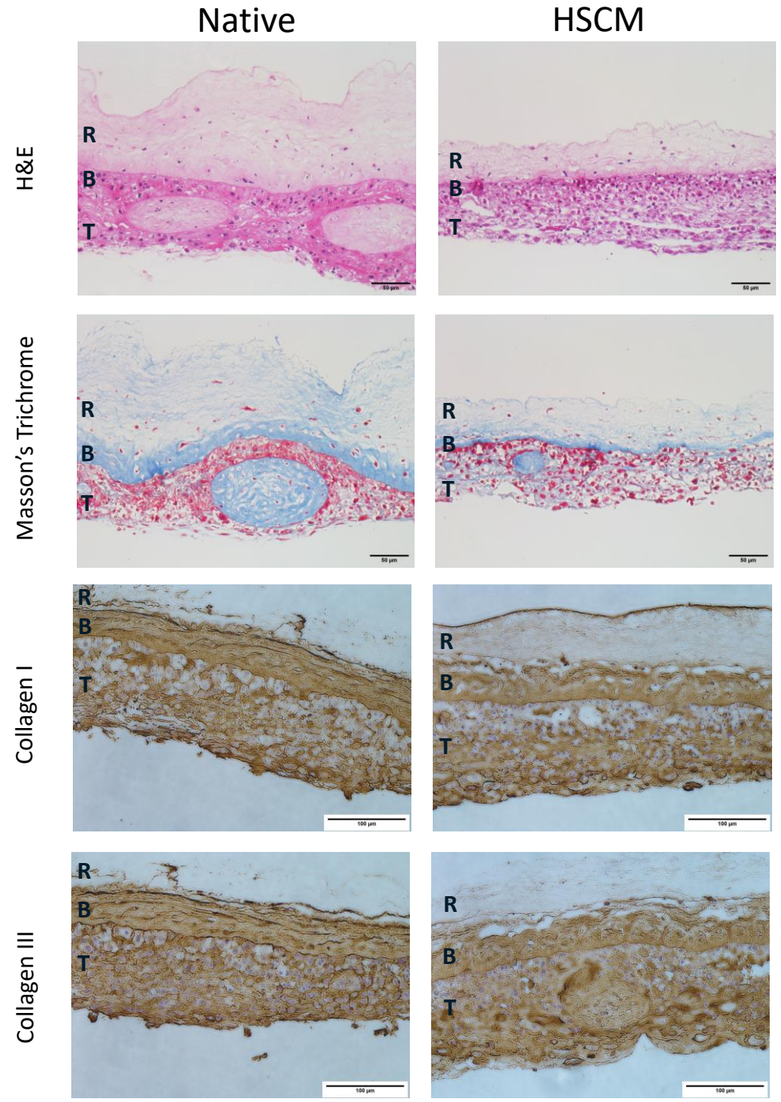

Figure 2. Histological assessment of scaffold components maintained in HSCMs. HSCMs maintained the open matrix structure and defined reticular, basement membrane, and trophoblast layers found within native chorion. Collagen I and collagen III are important components of the ECM scaffold. R = Reticular Layer, B = Basement Membrane, T = Trophoblast Layer. Scale bar indicates 50 µm for H&E/MT and 100 µm for IHC.

PROTEOMIC AND PROTEOLYTIC INHIBITORY ASSESSMENT

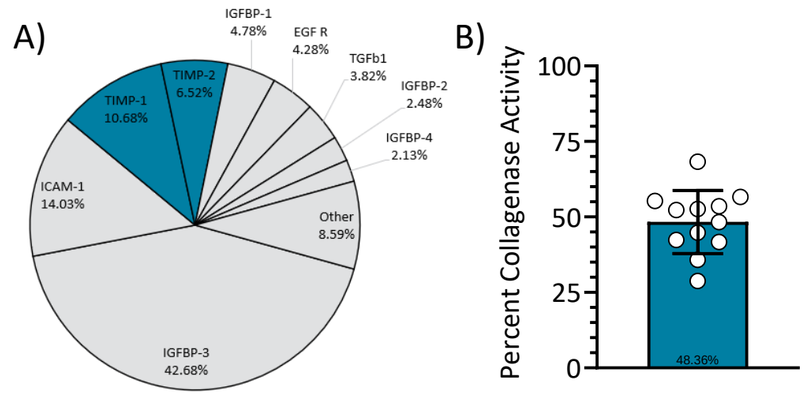

Figure 3. HSCM retains key proteins and retained ECM components inhibit collagenase. A) HSCMs retained 53 measured proteins at a concentration >10 pg/mL. Of these proteins, tissue inhibitors of matrix metalloproteinases-1 (TIMP-1) and TIMP-2 made up 10.68% and 6.52% of total protein, respectively. B) Inhibition of collagenase IV activity with HSCM resulted in a 52% reduction in activity compared to uninhibited control. Average ± std. dev reported.

CONCLUSIONS

- Structurally, HSCM maintained the reticular, basement membrane, and trophoblast layers of the chorion membrane. Layers were distinct and porosity was observable, highlighting the scaffold structure of HSCM.
- ECM composition as assessed by Masson's Trichrome and IHC showed a large amount of Collagen I and Collagen III present, which was preserved by HSCM processing.
- Protein retention assessment found not only did HSCMs maintain various key proteins expressed in native chorion, it also maintained TIMP-1 and TIMP-2.
- HSCM composition, with significant proportions of collagens and TIMPs, resulted in inhibition of approximately 52% of collagenase type IV activity using a *Clostridium histolyticum* reporter assay.
- AlloFresh™ processing maintains the native structure, layers, protein composition and functionality of native chorion.

ACKNOWLEDGEMENTS

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ORGANOGENESIS