Biological Evaluation of a Novel, Lyophilized Amniotic Membrane Allograft

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INTRODUCTION

The use of amniotic membranes in the treatment of wounds and soft tissue deficits has long been established. Previously, we have demonstrated that PURION-processed amniotic membrane allografts retain regulatory proteins inherent to amniotic tissue and preserve the bioactivity to stimulate cellular activities. A novel, patent-pending processing method was developed to produce a tri-layer lyophilized human amnion chorion membrane allograft (LHACM*), which will offer versatility to clinicians with improved handling while maintaining the natural tissue composition and intrinsic biological activities. This study characterizes LHACM's structural components, regulatory protein composition and biological properties that may support the healing cascade.

MATERIALS AND METHODS

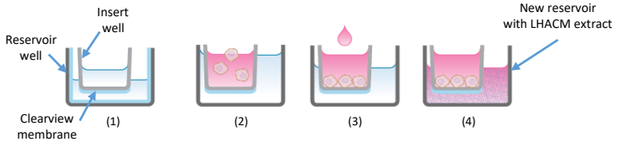
Histological Evaluation: Hematoxylin and Eosin (H&E) staining was performed on paraffin embedded 5 µm thick sections of hydrated LHACM. Immunofluorescence analysis was used to visualize type I and type IV collagens.

Scanned electron microscopy (SEM): SEM was performed on LHACM allografts at Particle Technology Labs (Downer Groves, IL) using JEOL NeoScope II. Both cross-sectional and surface SEM images were acquired.

Regulatory Protein Analysis: Samples from 5 LHACM donor lots were evaluated on a 640-target multiplex ELISA (RayBiotech, Norcross, GA).

Biological Activity Evaluation: Human dermal fibroblast (HDFs) were treated with basal medium supplemented with LHACM (N=5) extract at 10, 5, and 1 mg/mL. Following a 72-hour incubation period at 37ºC, the effect on cellular proliferation was determined using the CyQuant Cell Proliferation Assay (ThermoFisher Scientific, Carlsbad, CA).

Chemotactic Response Evaluation: The Incucyte Chemotaxis Cell Invasion Assay (Sartorius, Göttingen, Germany), which utilizes Clearview membranes with laser-etched pores to provide an optically transparent surface for label-free imaging, was employed.



(1) Clearview membrane was pre-primed in DPBS (20 minutes, 4ºC). (2) Overnight serum starved human microvascular endothelial cells (HMEC-1) suspended in a basement membrane extract were seeded in the insert plate and incubated for 45-60 minutes at 37ºC to allow for matrix polymerization. (3) Basal medium was added on top of the cell-matrix layer. (4) Insert plate was placed into a new reservoir plate that contained LHACM extract at 20, 10, 5, and 1 mg/mL.

Plate was incubated at 37ºC inside an Incucyte S3 to capture phase contrast images of both the top and bottom of the membrane every 6 hours up to 24 hours. Automated image processing was done using the Incucyte Chemotaxis Analysis Module (Essen, version 2019B REV2), which masked and differentiated between cells located on the top surface and those on the bottom surface of the membrane. The metric of "Total Phase Object Area Normalized to Initial Top Value" was calculated by normalizing the total area of the cells on the bottom surface of the membrane at each time point (bottom mask) to the initial area of the cells on the top surface at 0-hour time point (0-hour top mask).

RESULTS

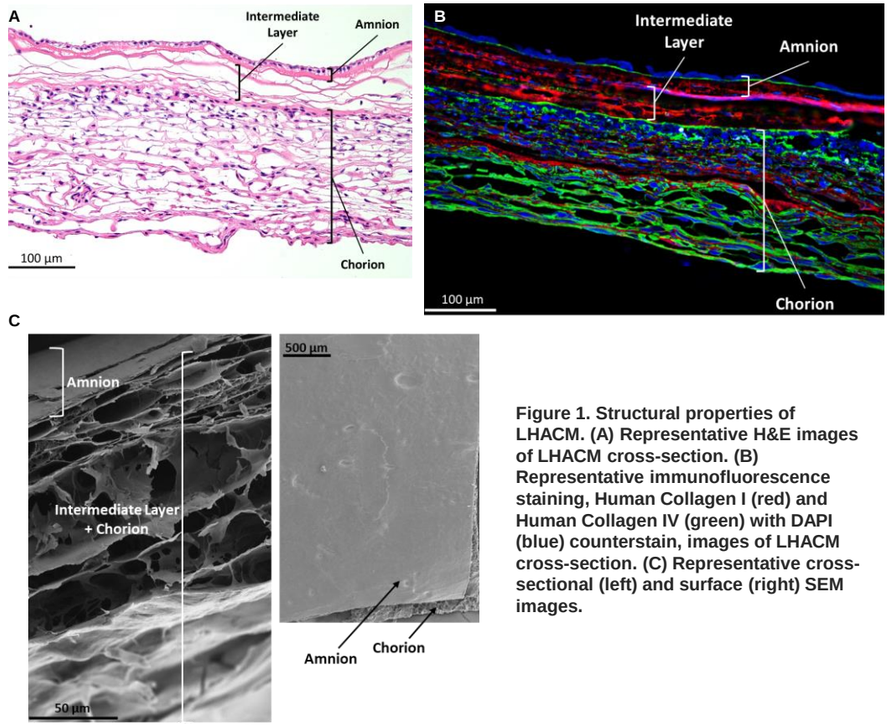


Figure 1. Structural properties of LHACM. (A) Representative H&E images of LHACM cross-section. (B) Representative immunofluorescence staining, Human Collagen I (red) and Human Collagen IV (green) with DAPI (blue) counterstain, images of LHACM cross-section. (C) Representative cross-sectional (left) and surface (right) SEM images.

300+ regulatory factors are identified in LHACM

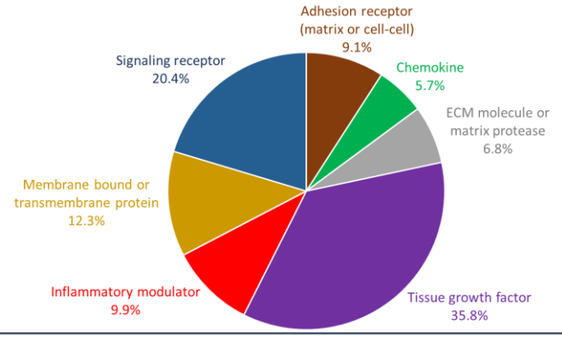


Figure 2. Protein composition of LHACM tissue lysates evaluated against an array of antibodies for 640 regulatory proteins. Over 300 different regulatory factors were identified in LHACM allografts and classified as shown in the pie chart.

RESULTS

LHACM promotes proliferation of HDFs in vitro

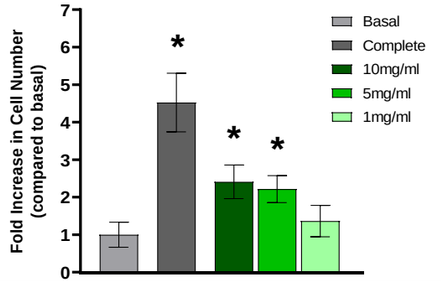


Figure 3. Proliferative response to LHACM. HDFs were incubated with LHACM extract for 72 hours. Data were normalized to the basal group and presented as the average fold increase in cell number. A twofold increase in cell number was observed at 10 mg/mL and 5 mg/mL treatments. * p<0.05 vs basal group. N=5.

LHACM promotes chemotaxis of HMEC-1 in vitro

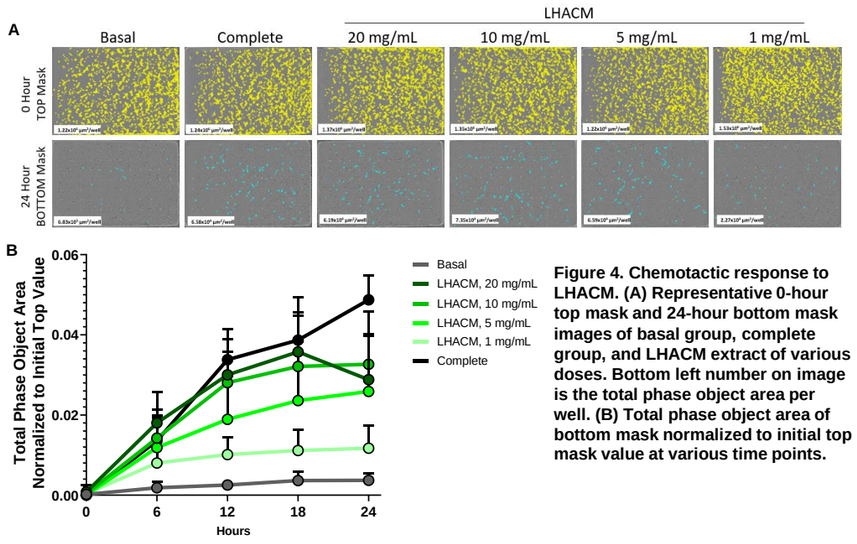


Figure 4. Chemotactic response to LHACM. (A) Representative 0-hour top mask and 24-hour bottom mask images of basal group, complete group, and LHACM extract of various doses. Bottom left number on image is the total phase object area per well. (B) Total phase object area of bottom mask normalized to initial top mask value at various time points.

CONCLUSION

The processing method of LHACM maintains the structural properties of amniotic membranes while retaining a natural mix of 300+ regulatory proteins which can stimulate cellular activities *in vitro*. LHACM represents a novel product that enhances the versatility of amniotic membrane allografts in various clinical and surgical applications.

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All authors are current or past employees of MIMEDX Group, Inc.