Characterization of a Novel, Lyophilized Amniotic Membrane Allograft

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Introduction

Amniotic membrane allografts have been gaining popularity as advanced options for managing wounds. Placental allografts differ in processing methods, resulting in various forms that lend themselves to specific applications. A novel, patent-pending processing method was developed to produce a tri-layer lyophilized human amnion chorion membrane (LHACM*). LHACM was designed to retain the natural thickness and architecture of amniotic membranes, resulting in a graft ideal for the management of deeper soft tissue deficits or surgical applications where fixation by suturing is desired. The structural and handling properties of LHACM are presented in this study to elucidate its potential applications.

MATERIALS AND METHODS

Histological Evaluation: Hematoxylin and Eosin (H&E) and Masson's Trichrome staining was performed on paraffin embedded sections of LHACM.

Thickness Measurements: A digital caliper was used to measure thickness of each LHACM graft. Three measurements were distributed across each graft. N=15 grafts.

Absorbency: Each graft was weighed following hydration in commercially available simulated wound fluid at 37°C for times indicated. The mass of each hydrated graft was recorded at indicated time points. The absorbency at each time point was calculated by subtracting the initial mass of the graft from the final recorded mass. N=3 grafts.

Suture testing: Ethicon 4-0 sutures were used to demonstrate the ability of the LHACM graft to be anchored in place on simulated skin. Three LHACM donors with five grafts per donor were evaluated. Suture pull-out testing was conducted using an INSTRON 5566 load frame at an extension rate of 5 mm/min. Prior to testing, LHACM was cut into 20 mm x 30 mm grafts and hydrated for 2 hours. Each sample was sutured (3-0 Vicryl suture) at the same position and suture ends were equidistant from the graft. The max load (Newtons) for each graft was used to calculate the average max load. N=7 grafts.

Barrier Properties: Grafts were hydrated in phosphate buffered saline (PBS) and placed in the dialysis chamber. A standard molecular weight marker or PBS was added to the cavity on each side of the dialysis chamber. The dialysis chamber was incubated at 4°C, for 3 days, allowing the passage the protein of the molecular weight marker with a diffusion dependent on the permeability of the barrier. Permeable proteins were evaluated using SDS-PAGE. N=3 grafts.

***In vivo* rat model:** LHACM (0.5 cm x 0.5 cm) grafts were implanted subcutaneously in 22 normal Sprague-Dawley rats using 3 unique processing batches of LHACM. The implant sites were evaluated at 1, 7, 14, 21, 42 and 90 days post implantation. At each time point each rat was euthanized, and the implant sites were harvested and fixed in neutral buffered formalin. Tissue samples were sectioned, mounted to glass slide, and stained with H&E.

*AMNIOEFFECT™, MIMEDX Group Inc. Marietta, GA

All authors are employees of MIMEDX Group, Inc.

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RESULTS

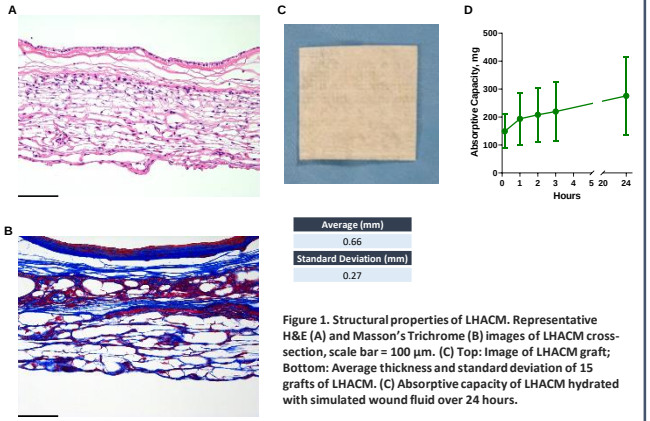

Figure 1. Structural properties of LHACM. Representative H&E (A) and Masson's Trichrome (B) images of LHACM cross-section, scale bar = 100 µm. (C) Top: Image of LHACM graft; Bottom: Average thickness and standard deviation of 15 grafts of LHACM. (C) Absorptive capacity of LHACM hydrated with simulated wound fluid over 24 hours.

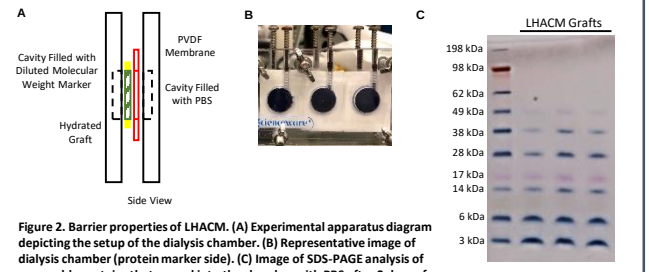

Figure 2. Barrier properties of LHACM. (A) Experimental apparatus diagram depicting the setup of the dialysis chamber. (B) Representative image of dialysis chamber (protein marker side). (C) Image of SDS-PAGE analysis of permeable proteins that moved into the chamber with PBS after 3 days of dialysis. Data shows that protein with >60 kDa did not diffuse through the membrane. Each lane represents a different LHACM graft.

RESULTS

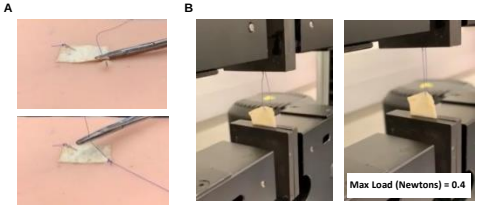

Figure 3. Handling properties of LHACM. (A) Demonstration of suturing LHACM onto simulated skin. (B) Images of suture pullout testing before testing (Left) and at load frame extension (Right).

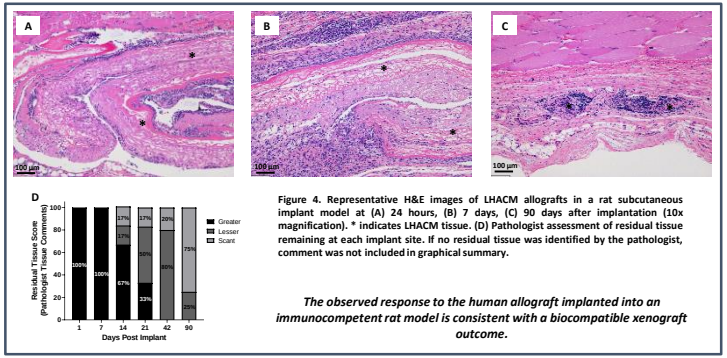

Figure 4. Representative H&E images of LHACM allografts in a rat subcutaneous implant model at (A) 24 hours, (B) 7 days, (C) 90 days after implantation (10x magnification). * indicates LHACM tissue. (D) Pathologist assessment of residual tissue remaining at each implant site. If no residual tissue was identified by the pathologist, comment was not included in graphical summary.

The observed response to the human allograft implanted into an immunocompetent rat model is consistent with a biocompatible xenograft outcome.

Conclusion

LHACM was developed to provide physicians with a graft comprised of the same amnion and chorion membranes that have been used extensively in clinical practice but with alternative handling properties relative to their dehydrated counterparts. LHACM is biocompatible and maintains the barrier function inherent to amniotic membranes. Additionally, the increased thickness of LHACM enables the tissue to hold a suture; however, data suggests it cannot support mechanical load. These properties may be ideal for indications which require increased manipulation and anchoring the graft to the application site.

Acknowledgements

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