Aseptically Processed Dehydrated Human Amnion/Chorion Allografts* Possess Anti-microbial, Anti-inflammatory and Non-Immunogenic Properties that Support Wound Healing

[1]Dasgupta, A; [1]Phipps, A; [1]Eng, S; [1]Huang, YC; [1]Chnari, E

[1]Musculoskeletal Transplant Foundation (MTF), Edison, NJ, USA

INTRODUCTION

Amnion/chorion membranes have key biological components that are important in the treatment of wounds. Apart from containing the rich matrix proteins and various growth factors, they maintain unique biological properties that are anti-microbial, anti-inflammatory, anti-oxidant and non-immunogenic1. We have previously shown that aseptically processed dehydrated human amniotic membrane allograft (dHAMA) without terminal sterilization retained beta-defensins (major group of anti-microbial peptides), and hyaluronic acid (aid in suppressing inflammatory cells), as well as the anti-inflammatory cytokines IL-6 and IL-10[1,2]. The objective of this study was to demonstrate the functionality and effect of these biological properties.

MATERIALS AND METHODS

Dehydrated amnion/chorion allografts were procured according to Good Tissue Practices and processed aseptically without terminal sterilization at Musculoskeletal Transplant Foundation (MTF, Edison, NJ, USA).

Anti-microbial properties were evaluated by investigating the inhibition of bacterial growth of both a Gram negative *(Pseudomonas aeruginosa)* and a Gram positive *(Streptococcus pyogenes)* organism in accordance with the AATCC Method 100 assay (WuXI Apptec). Initial bacterial levels (>1 x10^7 cells/0.1mL phosphate buffered water, PBW) were inoculated onto dHAMA with simulated wound fluid (10% FBS with PBW). Bacterial count was enumerated over time. The control was a denatured amnion/chorion membrane (treated with acid, base, and terminally sterilized).

Anti-inflammatory properties were examined by measuring exogenous matrix metalloproteinase-9 (MMP-9) (Enzo Life Sciences, NY) activity levels after exposure to dHAMA samples by incubation with a fluorogenic substrate (Enzo Life Sciences, NY). The resulting fluorescence is correlated to MMP-9 activity over time. The positive control was established with exogenous MMP-9 treated with an inhibitor, GM6001 (Enzo Life Sciences, NY).

Immunogenicity was evaluated by the mixed lymphocyte reaction (MLR) assay (Marin Biologics, LLC). Samples of dHAMA and dehydrated amnion (also processed aseptically) as a comparison were subjected to mixed lymphocytes from different blood donors to determine if any stimulation occurred due to the presence of the allograft.

RESULTS

The functional biological properties of aseptically processed dHAMA were examined. In particular, anti-microbial, anti-inflammatory and non-immunogenicity properties were investigated. Anti-microbial efficacy was assessed by examining the growth of *P. aeruginosa* and *S. pyogenes* upon exposure to dHAMA. They were separately inoculated onto dHAMA and monitored for 72 hours. Results revealed on average a greater than 4 log reduction from initial inoculum levels for both *P. aeruginosa* and *S. pyogenes.* Test group results were significantly greater ($p<0.05$) than control group at 24 and 72 hours for both organisms (one tailed T-test,$\alpha=0.05$). Anti-inflammatory studies revealed a decrease in the fluorescence which is associated with a decrease in MMP-9 activity over time. Immunogenicity testing through the MLR assay verified that dHAMA resulted in a low stimulation index (SI≤1.5) in comparison to the positive control (SI≥1.5). This confirmed that aseptically processed dHAMA is non-immunogenic.

AATCC Method 100 Assay Evaluated Functional Anti-microbial Properties Retained in Aseptically Processed dHAMA

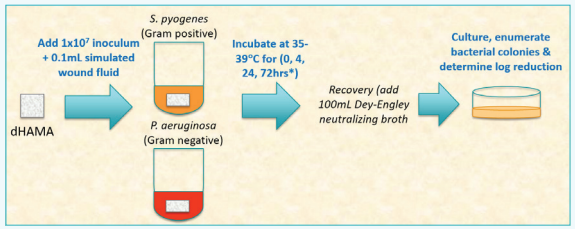

Figure 1: Samples of dHAMA were inoculated with S. pyogenes and P. aeruginosa and incubated at time zero for t=0, 4 and 24hrs. However for the 72 hour time-point, bacterial cultures were inoculated at t=72hrs. This was done to prevent bacterial death which can occur over 24hrs incubation. The control sample consisted of denatured amnion/chorion tissue harshly processed with chemicals (acid, bases) and terminally sterilized with E-Beam at 35kGY.

Sustained Inhibition of Bacterial Growth Over Time Upon Exposure to dHAMA

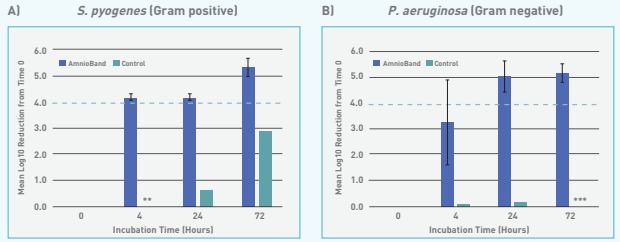

Figure 2: Bacterial growth was inhibited upon exposure to dHAMA (n=3 donors) in A) S. pyogenes and B) P. aeruginosa cultures. By 72hrs, a 5 log reduction was observed in both cultures. Having greater than a 4 log reduction is considered a marker for demonstrating anti-microbial ability[3,4].

Functional Anti-inflammatory Properties Quench Exogenous MMP-9

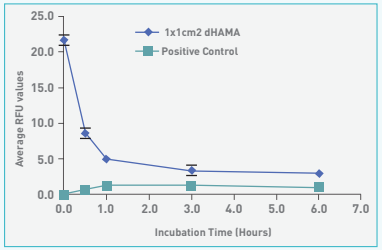

Figure 3: Samples of dHAMA exposed to exogenous MMP-9 were able to inhibit the activity of MMP-9 with a decrease of fluorescence observed over time. The positive control showcased inhibition with a GM6001 Inhibitor to the MMP-9 solution. Therefore, anti-inflammatory properties (IL-6, IL-10, hyaluronic acid– previously shown to be retained) are functional in aseptically processed dHAMA and able to quench/ inhibit MMP-9 activity over time.

dHAMA is Non-Immunogenic

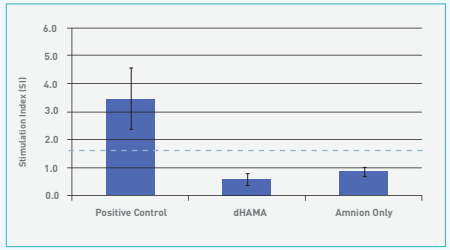

Figure 4: MLR assay was conducted on dHAMA and amnion only (n=3 donors). Low lymphocyte proliferation was observed after 5 days of incubation as compared to the positive control. Both dHAMA and amnion only exhibited a low stimulation index (SI)≤1.5. This confirmed that amnion and chorion membranes are non-immunogenic, which has been previously reported in literature[5].

CONCLUSION

Aseptically-processed dHAMA contains functional biological properties (anti-microbial, anti-inflammatory, non-immunogenic), which are critical in protecting wounds and reducing inflammation during the wound healing process.

REFERENCES

1. Niknejad H, *et al.* Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cell Mater.* 2008. 15:88-99.
2. Higa K, *et al.* Hyaluronic acid-CD44 interaction mediates the adhesion of lymphocytes by amniotic membrane stroma. *Cornea.* 2005. 24:206-212.
3. https://www.accessdata.fda.gov/cdrh_docs/pdf13/K130040.pdf
4. https://www.accessdata.fda.gov/cdrh_docs/pdf14/K140147.pdf
5. Szerekes-Bartho J. Immunological Relationship between the Mother and the Fetus. *Int Rev Immunol.* 2002.21:471-495.

*AmnioBand® Membrane is a registered trademark of the Musculoskeletal Transplant Foundation, Edison, NJ, USA