Aseptically Processed Dehydrated Human Amnion/Chorion Allografts* Promote Cell Attachment, Proliferation, New Matrix Deposition and In Vitro Angiogenesis That May Facilitate Wound Healing

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INTRODUCTION

Amniotic membranes have a long history of being employed in the treatment of wounds [1, 2]. Human amnion/chorion membranes derived from the placenta are rich in collagens and various growth factors that support the healing process to both improve wound closure and reduce scar formation [3-5]. Additionally, they have unique properties of being antimicrobial, anti-adhesive and lacking immunologic markers [3, 4, 6]. We have previously shown that aseptically processed dehydrated amnion/chorion allografts without terminal sterilization retained growth factors and matrix proteins that facilitated wound closure in a diabetic swine model [7].

The objectives of this study are to 1) quantify the presence of extracellular matrix components and an angiogenic growth factor in a dehydrated human amnion/chorion graft and 2) test the in vitro biocompatibility and angiogenic capacity based on the presence of these factors and demonstrate that the graft supports the wound healing process. Normal human dermal fibroblasts were seeded onto both sides of the graft and their attachment and proliferation were evaluated at various time points both qualitatively and quantitatively. In vitro angiogenesis was determined by assessing the ability of endothelial cells (HUVEC) to form three-dimensional structures (tube formation) on the dehydrated amniotic grafts [8].

MATERIALS AND METHODS

Dehydrated human amnion/chorion allograft membranes were processed aseptically without terminal sterilization at the Musculoskeletal Transplant Foundation (MTF, Edison NJ).

The amnion/chorion allograft was characterized for GAG (Biocolor), HA (Corgenix), and VEGF by Array Analysis (Raybiotech). Extracts of the amnion/chorion allograft were prepared by extracting for 24 hours and then blending via bullet blender (Next Advance). The angiogenic ability of HUVEC cells exposed to these extracts was investigated for 7 hours (Cultrex). HUVEC cells were also cultured on both amnion and chorion sides (0.3 million cells/7mm disc) for scanning electron microscopy (SEM).

Natural human dermal fibroblasts (NHDFs) were cultured (0.2 million cells/7mm disc) on amnion and chorion sides over time (day 0, 2, 5, 7, 10, 14 days). Cell viability was assessed via CCK-8 assay (Sigma), a colorimetric assay to elucidate cell proliferation. SEM and immunohistochemical (IHC) imaging examined cell attachment and matrix protein (laminin, fibronectin, collagen IV) deposition over time.

RESULTS

Quantitative microarray analysis confirmed that aseptically processed amnion/chorion allografts retained key biological components, VEGF, GAG and HA in similar levels compared to native unprocessed tissue. Extracts from dehydrated amnion/chorion allografts were presented to HUVEC cells to elucidate their angiogenic capacity. There was increased tube formation in cultures exposed to the amnion/chorion extracts compared to the control (basal medium). Culturing HUVECs on the amnion/chorion allografts highlighted the cell friendly matrix with cell attachment, matrix deposition and typical tubular configuration. NHDFs were also cultured on amnion/chorion allografts and found to adhere, proliferate readily and produce matrix proteins, as shown by SEM imaging. Furthermore, IHC analysis revealed cultured NHDFs produced different matrix proteins on top of the amnion layer, as is typical during granulation.

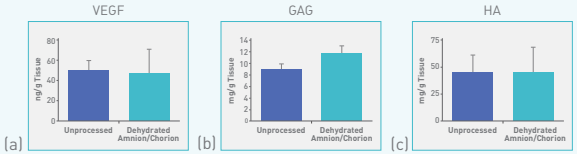

Figure 1: Quantitative analysis of (a) VEGF; b) GAG; c) Hyaluronic Acid (HA) present in dehydrated amnion/chorion allografts. Aseptic processing preserved extracellular matrix components and the angiogenic marker, VEGF, in similar levels compared to native unprocessed allografts. GAG and HA support cell migration, attachment and infiltration along with stimulating new matrix deposition. These are critical agents in granulation in normal wound healing [9,10].

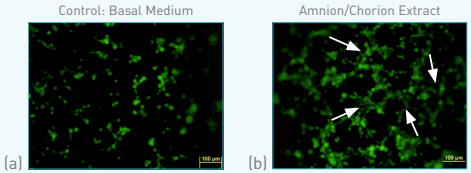

Figure 2: Angiogenic capacity of HUVECs cultured on Matrigel exposed to amnion/chorion extracts for 7 hours of incubation. Greater tube formation was observed upon exposure to amnion/chorion extracts compared to the negative control.

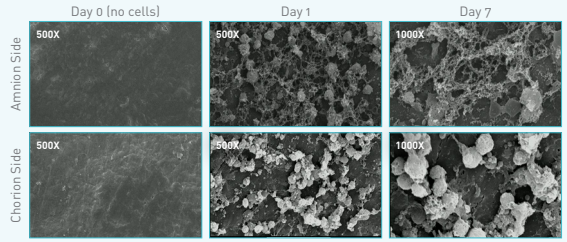

Figure 3. SEM images of HUVECs cultured on amnion and chorion sides of the allograft. Cells attached within 1 day and secreted matrix in a tubular configuration covering the amnion and chorion surfaces.

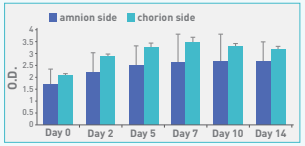

Figure 4: NHDFs proliferated on both amnion and chorion sides of the allograft, plateauing around day 7. Cell viability was monitored via the CCK-8 assay. The number of living cells is proportional to the amount of formazan dye converted from tetrazolium salts generated by the activity of dehydrogenases in cells.

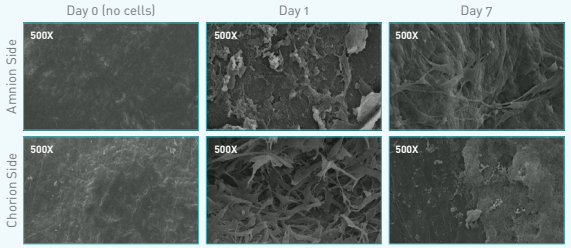

Figure 5. SEM images of NHDFs cultured on amnion side of allograft. Cells adhered and secreted matrix proteins over time covering the amnion and chorion surface.

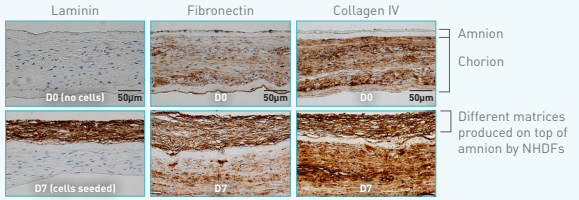

Figure 6. NHDFs readily adhered on the amnion side of allograft and secreted different matrix proteins by day 7. Similar observations were found on the chorion side, with these matrix proteins secreted in a similar fashion by day 7 (magnification 40X). These secreted proteins initiate the granulation process by establishing a new network of matrix proteins, anchoring new matrix to the native tissue, supporting/organizing cell attachment and migration and facilitating the normal wound healing cascade [9,10].

CONCLUSION

The data demonstrates that aseptically processed dehydrated human amnion/chorion allograft membranes support the cell proliferation and matrix deposition of two key wound healing cell participants (endothelial cells & dermal fibroblasts). These cells support angiogenesis and granulation, which are critical steps during normal wound healing.

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*AmnioBand™ Membrane: MTF, Edison, NJ