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 [5].

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 (HUEVC) to form three-dimensional structures (tube formation) on the dehydrated amnion 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 (Biscover), 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). Extracts of the amnion/chorion allografts were prepared by extracting for 24 hours and then blending via bullet blender (Next Advance). The angiogenic ability of HUEVC cells exposed to these extracts was investigated for 7 hours (Cultrex). HUEVC cells were cultured on both amnion and chorion sides (0.3 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 deposition 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.

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 HUEVC cells to elucidate their angiogenic 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 [6,10].

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.

REFERENCES

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*AmniBand™ Membrane: MTF, Edison, NJ