INTRODUCTION

Chronic diabetic foot ulcers (DFUs) are challenging wounds to heal clinically. Failure to heal DFUs can lead to lower limb amputation and significantly increased mortality rates. Impediments to healing such as hyperglycemia lead to abnormally elevated levels of advanced glycation end products (AGEs) and reactive oxygen species (ROS), which have deleterious effects on cells and the extracellular matrix (ECM). Recently, human amnion/chorion allografts have emerged as powerful tools for supporting wound healing. Their inherent cytokines, growth factors, and matrix proteins help stimulate wound healing. Numerous methods exist for processing and decontaminating amnion/chorion allografts for transplantation. These include chemical processing and sterilization via ionizing irradiation, which can negatively impact the resulting tissue, generating altered biological structures similar to those found in the diabetic microenvironment.

The goal of this study was to investigate the effects of three soft-tissue decontamination methods on the biological properties of dehydrated amnion/chorion allografts. Changes in the microstructure and matrix composition were evaluated via histology, enzymatic degradation biochemical assay, and differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Dehydrated human amnion allografts were procured according to Good Tissue Practices and processed at the Musculoskeletal Transplant Foundation (MTF, Edison, NJ).

The amnion/chorion membranes from each donated placenta were split into four processing groups: non-processed control, aseptic processing with PAA disinfection, and no terminal sterilization. After a partial decellularization in a hypertonic solution, terminally sterilized samples were dehydrated, while aseptic processing samples were treated with PAA and then dehydrated. E-beam and gamma irradiation occurred after dehydration for terminally sterilized samples.

RESULTS

Matrix Proteins Are Better Retained By Aseptic Processing with PAA

Figure 2: Histological imaging reveals the effects of PAA treatment and terminal sterilization by irradiation on select matrix proteins (Coll I and Coll III images at 5x magnification and Safranin O images at 10x magnification; representative images). IHC staining intensity was lower in the terminal sterilization treatment groups compared to both PAA treated and control.

Aseptic Processing with PAA Results in Tissue That is More Resistant to Enzymatic Degradation Than Terminal Sterilization via Irradiation

Figure 3: (A) Peptide release from samples of each processing group after one hour of enzymatic degradation. Data presented as Mean ± SE of one sample per group across three donors (n=3). Peptide release from gamma samples was significantly higher than from PAA samples (p<0.005). Post-hoc power was –99.9%. (B) Peptide release from E-beam samples was not significantly different from PAA samples (p<0.05). Post-hoc power was –70%, indicating sampling size was too small.

CONCLUSION

The presented data revealed that terminal sterilization by irradiation of dehydrated amnion/chorion results in a less stable ECM. Aseptic processing with PAA treatment, however, preserves the proteins of the ECM. Limitations of this study include: only terminal sterilization via irradiation was investigated, a limited number of donors was tested, and bacterial enzymes (not mammalian) were used for degradation testing.

The quality of the ECM provided to the wound is important as it provides a scaffold for host cells to infiltrate and regulates cell behavior; both of which play a significant role in wound healing. Future studies will aim to investigate the behavior of cells seeded onto tissue produced via aseptic processing with PAA or terminal sterilization to determine if the differences found in ECM also results in differences in cell interaction and function.

REFERENCES

1. Snyder, RJ and Hanft, JR, Ostomy Wound Manage. 2009;
2. Brantley, JN and Verla, TD, Advances in Wound Care. 2015;

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