Terminal Sterilization Negatively Affects Dehydrated Human Amnion/Chorion* Biological Properties While Aseptic Tissue Processing Preserves the Natural Matrix Structure and Composition

[1]Madans, A; [1]Phipps, A; [1]Dasgupta, A; [1]Chnari, E

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

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[1]. 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)[2]. 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 allograft 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 amniotic 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 chemical disinfection via peracetic acid (PAA) and ethanol, and terminal sterilization via electron beam (E-beam) irradiation at 17.5 kGy and via gamma irradiation at 17.5 kGy (Figure 1).

IHC: The processed and dehydrated amnion/chorion was characterized for matrix proteins through immunohistochemistry (IHC) (Figure 2) (IHC World, LLC and Premier Laboratory, LCC).

Enzyme Degradation: To investigate the stability of the matrix proteins in presence of enzymes, samples were subjected to enzymatic degradation via bacterial collagenase type I and thermolysin (Sigma-Aldrich, Co., St. Louis, MO) and assessed for amount of tissue remaining over time, as well as peptide released over time (Figure 3). Peptide release was evaluated by sampling supernatant of digested samples at various time points. Supernatants were tested for peptide concentration via L-Leucine absorbance assay. Absorbance at 570nm was read using a calibrated Synergy 2 plate reader with Gen 5 software (BioTek, Winooski, VT) and compared to an L-leucine (Sigma-Aldrich, Co., St. Louis, MO) standard.

DSC: The stability of the collagen in the tissue resulting from the three treatment methods versus control was investigated via DSC (Figures 4,5). DSC analysis was performed on prehydrated 4.5 mm diameter samples at a ramp rate of 3°C/min from 25-85°C using a calibrated Discovery Series DSC 25 (TA Instruments, New Castle, DE). Collagen denaturation onset temperature (Tm) and enthalpy (J/g) was measured from thermograms using TRIOS software (version 4.0).

Data Analysis: All data analysis was completed using Microsoft Excel and Minitab 17.0. Data was analyzed for statistical differences between pairs of processing groups using student's t-test. $p \leftarrow 0.05$ was considered significant. Power was analyzed post-hoc.

Schematic of Processing Steps for Each Group

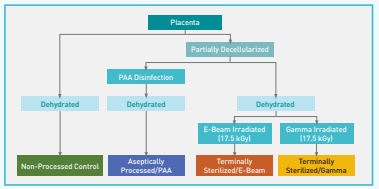

Figure 1: Schematic of processing groups. Non-processed control received no chemical processing and no terminal sterilization. After a partial decellularization in a hypertonic solution, terminally sterilized samples were dehydrated, while aseptic processing samples were treated with PAA and then dehydrated. E-beam and gamma irradiation occurred after dehydration for terminally sterilized samples.

RESULTS

Matrix Proteins Are Better Retained By Aseptic Processing with PAA

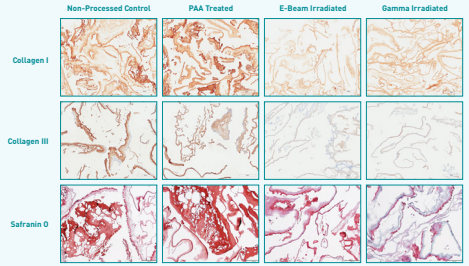

Figure 2: Histological imaging reveals the effects of PAA treatment and terminal sterilization by irradiation on select matrix proteins (Col I and Col III images at 5x magnification and Safranin O images at 10x magnification; representative images). IHC staining intensity was lower in the terminal sterilization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

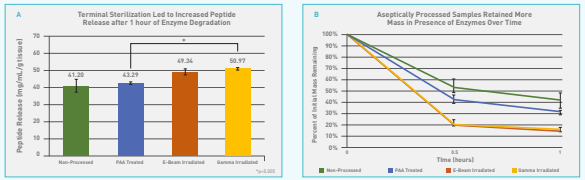

Figure 3. (A) Peptide released from samples of each processing group after one hour of enzymatic degradation. Data presented as Mean ± SE of one sample per group across three donors (n=3). Peptide release from gamma samples was significantly higher than from PAA samples ($p \leftarrow 0.005$). Post-hoc power was $\rightarrow$99.9%. Peptide release from E-beam samples was not significantly different from PAA samples ($p \leftarrow 0.052$). Post-hoc power was $\leftarrow$70%, indicating sampling size was too small. (B) Mass remaining from samples of each processing group over 1 hour of enzymatic degradation. Data presented as Mean ± SE of one sample per group at each time point across three donors (n=3). Mass remaining for PAA was significantly higher than both E-beam and gamma at both 0.5 hours and 1.0 hours ($p \leftarrow 0.05$ for E-beam and $p \leftarrow 0.01$ for gamma).

Terminal Sterilization via Irradiation Results in Decreased Collagen Stability

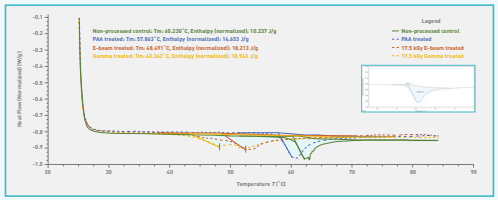

Figure 4. Representative DSC thermograms of different processing groups compared to non-processed control (n=1 per group). Decreased stability of matrix components (denaturation) was indicated by decreasing onset temperatures (Tm). Matrix alteration via crosslinking may be indicated by increasing enthalpy (J/g) values[3]. Widening of transition step curves are evident in both E-beam and Gamma treated samples, also indicating matrix structural changes[3].

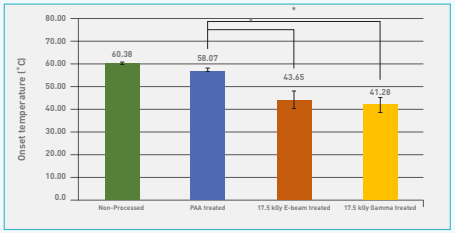

Figure 5. Denaturation onset temperatures of different processing groups compared to non-processed control. Data presented as Mean ± SD of 9 measurements per group from 3 donors, except for PAA group (n=8). Both E-beam and Gamma treated groups were significantly different from PAA treated onset temperature ($p \leftarrow 0.001$) at 95% confidence. Post-hoc power was $\rightarrow$99.9%.

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[4]. 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;
3. Gouk, SS, et al., J Biomed Mater Res B Appl Biomater.;
4. Maquart, FX and Monboisse, JC, Pathologie Biologie. 2014

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