Aseptically Processed Human Reticular Dermis* Promotes Cell Attachment, Proliferation and New Matrix Deposition that are Critical for Granulation Tissue Formation during Wound Healing[1]

[1]Chnari, E; [1]Huang, YC; [1]Imming, E; [1]Dasgupta, A; [1]Gidwani, M; [1]Jacobs, M
[1]Musculoskeletal Transplant Foundation (MTF), Edison, NJ

INTRODUCTION

The wound healing process can be subdivided in the consecutive and overlapping stages of inflammation, proliferation and remodeling [1, 2]. During the proliferative phase, fibroblasts begin to infiltrate the wound and contribute to granulation tissue formation, produce the cytokines that favor keratinocyte proliferation and migration, and differentiate into myofibroblasts to promote wound closure [3, 4]. Providing the appropriate matrix for cell attachment and migration accelerates the process of cell infiltration into the wound which may ideally speed up the healing process. Allogeneic dermal grafts are being used as wound coverings for burn and various wound applications. Aseptically processed grafts from the reticular dermis have uniform open architecture and preserved collagen structure to promote cell infiltration that is important for granulation tissue formation and wound closure.

A pre-hydrated human acellular dermis was used as a scaffold for cell attachment, infiltration and proliferation in vitro. Normal human dermal fibroblasts were seeded on both sides of the dermal graft and their attachment, proliferation and new matrix deposition were evaluated at various time points using a cell viability assay, confocal microscopy, scanning electron microscopy and histology.

MATERIALS AND METHODS

Dermal tissue was processed aseptically without terminal sterilization at the Musculoskeletal Transplant Foundation (MTF, Edison NJ).

Pre-hydrated human acellular dermis was characterized via immunohistochemical staining for matrix proteins (Collagen I, Collagen III, Collagen IV, Collagen VI, Elastin, Laminin, Fibronectin). Normal human dermal fibroblasts (NHDFs) were seeded (0.2 million cells/7mm disc) on both sides of the graft. Cell proliferation was monitored at day 0, 2, 4, 6, 8, 10, 12, 14 days by the CCK-8 kit (Sigma). Confocal microscopy, scanning electron microscopy (SEM) and histology were utilized to assess cell attachment and matrix production (collagen IV) at varying time-points (day 0, 1, 7, 14 days) on both sides of acellular dermal tissue (epidermal facing and dermal facing sides). To visualize cytoskeleton and confirm cell adhesion, samples were stained with DAPI and Fluorescein phalloidin (Life Technologies) for DNA and F-actin, respectively, and imaged with confocal microscopy.

RESULTS

Immunohistochemical (IHC) staining revealed the presence of various matrix proteins such as collagen I, III, IV, VI, VII. Trace levels of elastin, laminin, and fibronectin were also detected. Normal human dermal fibroblasts (NHDFs) readily attached to this matrix on both sides (epidermal facing side and dermis facing side). Cell proliferation proceeded over time and reached a plateau around day 12-14. Confocal imaging confirmed cell attachment and actin present in the cell cytoskeleton. SEM showcased cell attachment and infiltration within the open matrix structure and by day 7, an abundance of matrix covering the once open dermal structure was visualized. Histology verified cell attachment and infiltration along with matrix deposition via collagen IV production over time.

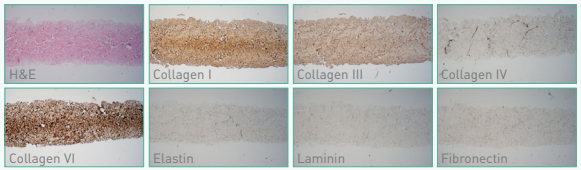

Figure 1: Immunohistochemical staining of pre-hydrated human acellular dermis for matrix proteins revealed preservation of collagens I, III, IV, VI, with trace levels of elastin, laminin, & fibronectin (magnification 2X).

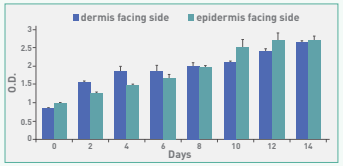

Figure 2: NHDFs adhered and proliferated on the pre-hydrated human acellular dermis readily. Cell proliferation plateaued around 12-14 days and were similar on both sides of acellular dermis tissue.

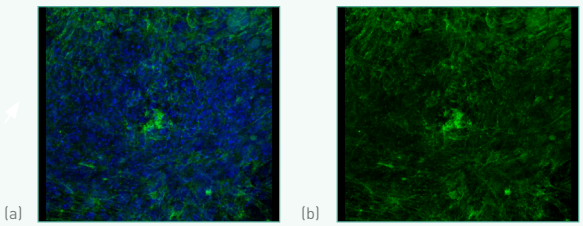

Figure 3: Confocal imaging showcased that (a) NHDFs attached readily to the acellular dermis (blue DAPI staining); (b) cell adhesion marker, actin (Fluorescein phalloidin) was stained at d14, revealing cell cytoskeleton stretching.

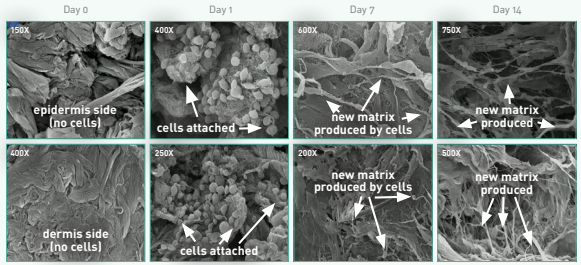

Figure 4: SEM imaging demonstrates acellular dermal tissue readily supported NHDF attachment and proliferation. Extensive newly formed matrix can be seen by day 7 covering the porous structure of the scaffold.

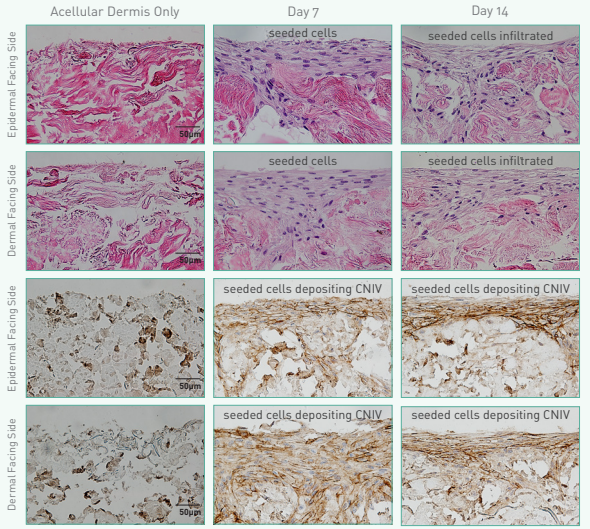

Figure 5. Histology analysis of NHDFs cultured on human acellular dermis highlights cell attachment, proliferation and infiltration along with collagen IV matrix deposition (magnification 40X).

CONCLUSION

The data demonstrates that aseptically processed reticular dermis grafts retain critical biological components that facilitate human dermal fibroblast attachment, proliferation and matrix production. These steps are critical for granulation tissue formation during wound healing. Future animal studies are needed to correlate these encouraging in vitro results with in vivo efficacy.

REFERENCES

1. Velnar T, *et al. J Int Med Res.* 2009
2. Reinke JM & Sorg H, *Eur Surg Res.* 2012
3. Mathieu P, *et al. Int J Clin Exp Pathol.* 2010
4. Gutner GC, *et al. Nature.* 2008

*AlloPatch® Pliable: MTF, Edison, NJ-1111 Rev. 0