Aseptically Processed Meshed Human Reticular Dermal Allografts* Help to Facilitate Surgical Wound Closure by Providing a Scaffold in Support of Angiogenesis and Granulation Activities

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ABSTRACT

According to the National Center for Health Statistics, in one year, there were 48 million inpatient procedures. Furthermore, the estimated compound annual growth for surgical wounds is expected to be 3.6% till 2024. Surgical procedures involving large excisions/incisions pose a great risk for complications, especially in patients with underlying co-morbidities such as obesity and smoking. Therefore, closing a surgical wound in a timely manner and ensuring that it remains closed is critical.

Aseptically processed human reticular dermal matrices (HR-ADMs) provide a natural scaffold with a favorable architecture for cell infiltration, which can aid toward natural wound closure. Utilizing a pre-meshed HR-ADM is beneficial as the graft can be expanded to cover large defects and facilitate drainage of exudate from the wound surface.

The aim of this study was to investigate cellular behavior of human dermal fibroblasts and endothelial cells in the presence of pre-meshed HR-ADMs. Cell attachment, infiltration kinetics and matrix deposition of cells cultured on pre-meshed HR-ADMs were assessed using histological and confocal imaging. Depth of infiltration was observed using 3-D Render series. Results from this study demonstrate that cells infiltrate into and throughout the meshed dermal scaffold due to the uniform open architecture of the matrix. The preserved, open 3-D architecture also supports the formation of endothelial tubes and neovessels, which are indicative of angiogenesis. Over time, these cells produce new matrix proteins that can assist host cell integration and surgical wound closure.

Therefore, aseptically processed pre-meshed HR-ADMs can provide the scaffold in support of angiogenesis and granulation activities in surgical wound sites. The structure and preserved components in the allografts can aid towards natural wound closure while being compatible with negative pressure wound therapy.

MATERIALS AND METHODS

Human dermis was recovered from donors with research consent, and the tissue was aseptically processed at the Musculoskeletal Transplant Foundation (Edison, NJ) according to Good Tissue Practices (Figure 1).

Cell Culture: Normal human dermal fibroblasts (NHDF) (Lonza, MD) were cultured in fibroblast growth media for this study. Normal human umbilical vein endothelial cells (HUVEC) (Lonza, MD) were cultured in endothelial growth media. Co-cultures of NHDFs and HUVECs at a 2:1 ratio were seeded per sample in non-treated tissue culture plates and in transwells (Corning, NY) for up to 14 days. Additional samples of just NHDFs and HUVECs alone were seeded as controls. Conditioned media was collected over time and an angiogenesis array (Raybiotech, GA) was ran to quantify several secreted growth factors, such as angiogenin, hepatocyte growth factor (HGF), and placental growth factor (PIGF). The tissue was kept stretched in culture with 3D-printed snap rings that were created using polylactic acid filament (PLA) and printed by Monoprice MP Select Mini 3D Printer (Figure 1C). The rings were sterilized via UV irradiation. A schematic of how the cell culture was performed is shown below (Figure 1D).

Assessment of Functional Activities and Migration into Pre-Meshed HR-ADM: Histological imaging (Premier Laboratories, LLC) was conducted to examine cell attachment and to identify secreted extracellular matrix proteins (vWF, laminin, fibronectin). Confocal imaging (Rutgers University, NJ) was used to visualize the expression of cytoskeletal actin (Phalloidin), tubular formation (CD31), matrix secretion (laminin) along with cell nuclei (DAPI). The confocal microscope (Zeiss, CA) was calibrated. All confocal and histological samples were blinded when being processed and imaged at Premier Laboratories and Rutgers University, respectively.

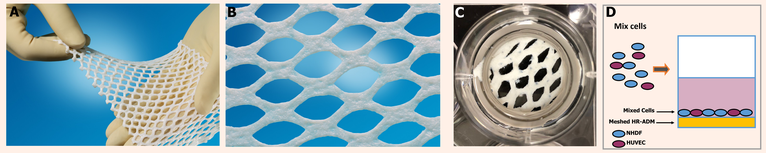

Figure 1: (A, B) Representative images of pre-meshed HR-ADM with a 3:1 meshing ratio. (C) Pre-meshed HR-ADM was held in place on tissue culture plates by 3D printed snap rings. (D) Schematic showing how cells were seeded on tissue.

RESULTS

Open Architecture of Pre-Meshed HR-ADM

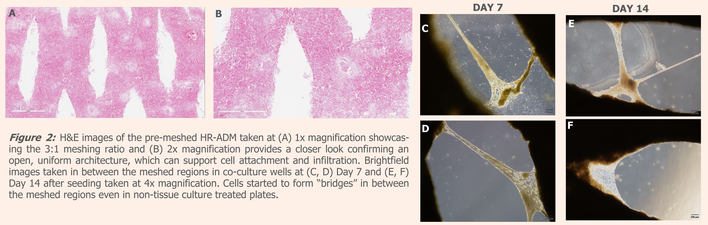

Figure 2: H&E images of the pre-meshed HR-ADM taken at (A) 1x magnification showcasing the 3:1 meshing ratio and (B) 2x magnification provides a closer look confirming an open, uniform architecture, which can support cell attachment and infiltration. Brightfield images taken in between the meshed regions in co-culture wells at (C, D) Day 7 and (E, F) Day 14 after seeding taken at 4x magnification. Cells started to form "bridges" in between the meshed regions even in non-tissue culture treated plates.

Fibroblasts and Endothelial Cells Adhere to Pre-Meshed HR-ADMs and Produce New ECM Proteins

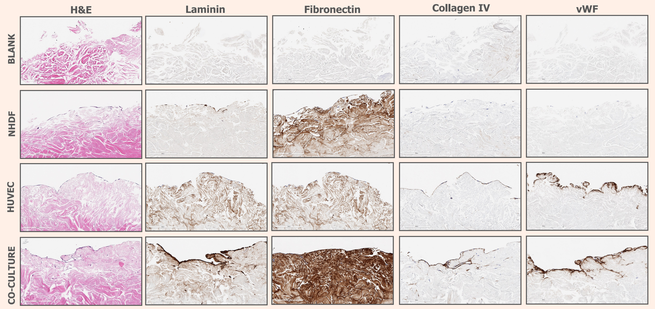

Figure 3: Cells attached and infiltrated into the pre-meshed HR-ADM at Day 7 as shown in the H&E images. Furthermore, they secreted new ECM proteins (laminin, fibronectin, Collagen IV) over time. These proteins play an important role during the granulation process of wound healing. The staining was more prominent in co-cultures, which are representative of a surgical wound environment. It is hypothesized that this effect would be enhanced in the presence of negative pressure wound therapy and more diverse cell types. Staining of vWF showed functionality of HUVECs that were seeded on the pre-meshed HR-ADM. Images were taken at 20x magnification.

Cells Secrete Angiogenic Growth Factors on Pre-Meshed HR-ADM

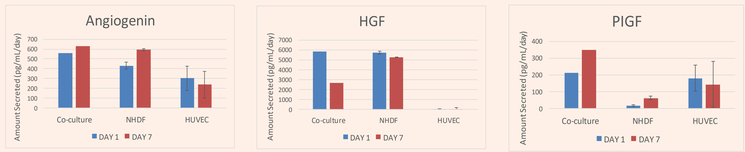

Figure 4: Angiogenic growth factor secretion is important for revascularization activities. The cells secreted various angiogenic factors (angiogenin, HGF, and PIGF) over time on pre-meshed HR-ADM. The data showed that fibroblasts and endothelial cells are instrumental in secreting angiogenic growth factors, but the secretion was enhanced in the co-culture condition, which is more representative of a wound bed. In a surgical wound environment, these angiogenic factors secreted within the pre-meshed HR-ADM can facilitate revascularization and wound closure activities.

Cells Secrete Functional Proteins on Pre-Meshed HR-ADM and Infiltrate Through Dermis

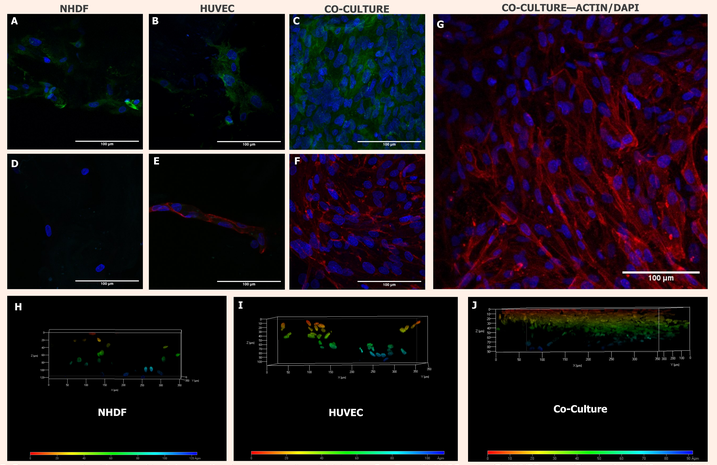

Figure 5: Fluorescent confocal images of laminin secretion (green) and cell nuclei (blue) on Day 7 by (A) NHDFs only, (B) HUVECs only, (C) co-cultures (40x magnification). Fluorescent confocal image of CD31 (red) and cell nuclei (blue) taken on Day 7 of (D) NHDFs only, (E) HUVECs only, (F) co-cultures. Increased CD31 staining was observed in the co-cultures. CD31 is a cell surface marker for endothelial cells and utilized to visualize tubular formation in functional HUVECs. (G) Fluorescent confocal image of cytoskeletal actin (red) and cell nuclei (blue) showed cell attachment on pre-meshed HR-ADM at Day 14 (40x magnification). Render series projection images of (H) NHDFs only, (I) HUVECs only, and (J) co-cultures demonstrate that cells infiltrated through the pre-meshed HR-ADM in different layers. In the color spectrum, red signifies the area on top of the pre-meshed HR-ADM where the cells were seeded, whereas blue indicates deeper cell infiltration into the reticular dermal layer. The thickness of pre-meshed HR-ADM is between 0.4-1.0mm. In this *in vitro* model, cells infiltrated from 60-160 um by day 7 (n=11). Clinically, negative pressure wound therapy would be used in conjunction with the tissue, so cells would be able to infiltrate faster and deeper into the graft. Future studies will examine cell infiltration over a longer time period.

CONCLUSION

Aseptically processed pre-meshed HR-ADMs have an open architecture which allowed human fibroblasts and endothelial cells to readily attach and proliferate. The cells were able to secrete new ECM proteins that support granulation activities. Render series imaging showed that the cells infiltrated through the dermis over time. Furthermore, day 14 data showed cells bridging between meshed gaps. This type of infiltration and integration can support surgical wound closure. In addition, the secretion of angiogenic growth factors by the cells can help facilitate revascularization in a surgical wound environment.

Limitations of this study include small sample size for the angiogenesis array testing and issues with processing tissue for histology. Also, more donors can be tested to account for donor to donor variability. Further studies will include *in vivo* studies to examine pre-meshed HR-ADM incorporation and host cell response in the presence of negative pressure wound therapy.

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