Aseptically Processed Dehydrated Human Amnion/Chorion Allografts* Possess Anti-microbial, Anti-inflammatory and Non-Immunogenic Properties that Support Wound Healing

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INTRODUCTION

An important goal in mediating the healing of chronic wounds is to shift the microenvironment to an acute setting where wound healing can occur in a timely manner. Amniotic membranes are known to facilitate the wound healing process as they contain collagens and various growth factors that can improve wound closure. Previously, we have shown that aseptically processed dehydrated amnion/chorion allograft membranes without terminal sterilization retained biological components compared to native unprocessed tissue, and these allografts helped promote angiogenic and granulation activities. The objective of this study was to examine how an amnion/chorion particulate version, which can be sprinkled over a large wound, can support cell attachment and matrix production to assist allograft bridging and aid in wound closure.

MATERIALS AND METHODS

Dehydrated human amniotic allografts were procured according to Good Tissue Practices and processed aseptically without terminal sterilization at the Musculoskeletal Transplant Foundation (MTF, Edison NJ).

Dehydrated human amniotic particulate was characterized for growth factors, cytokines and matrix proteins through immunohistochemistry (IHC), (IHC World, LLC, Ellicott, MD). To investigate cellular response, dehydrated amniotic particulate was sprinkled and immobilized onto hydrogels in 12-well insert wells *(Fig 1)*. Normal human dermal fibroblasts (NHDFs) were seeded (0.1 million cells/well) on these particulate-immobilized hydrogels. Cell attachment, proliferation and new matrix secretion were examined over time, through histology (HistoTox Labs, Boulder, CO), confocal microscopy performed at Rutgers University (Piscataway, NJ) using a calibrated Leica TCS SPII Confocal Microscopy Workstation (Leica, Allendale, NJ) and scanning electron microscopy (SEM) at Princeton University (Princeton, NJ) using a calibrated FEI XL30 FEG-SEM (FEI, Hillsboro, OR). Operators were blinded to samples.

RESULTS

IHC characterization of amniotic particulate revealed the retention of several key biological components such as VEGF, PDGF-AA, EGF, FGF-2, TIMP-1, HSP-90, SDF-1, collagens, hyaluronic acid and glycosaminoglycans *(Fig 2)*. Fibroblasts readily attached and proliferated to the amniotic particulate only, but not to the hydrogel, as observed through DAPI/ actin staining over time *(Fig 3)*. The data also demonstrated that fibroblasts are functional on amniotic particulate, by producing their own matrix proteins, which are associated with granulation activities *(Fig 4)*. The synthesis of new matrix proteins culminated in bridging and channel networks across neighboring amniotic particulate. Furthermore, extensive cell cytoskeletal stretching was observed, which confirmed cell-matrix interactions *(Fig 5)*, forming matrix bridges to connect neighboring amniotic particulate *(Fig 6)*.

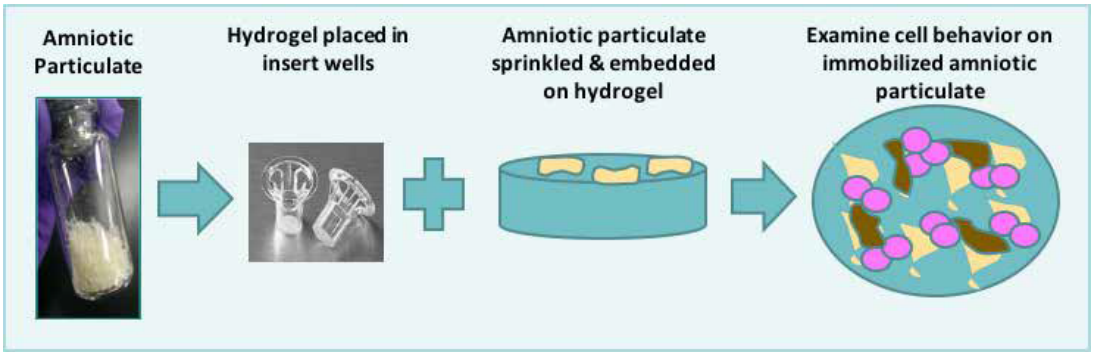

Figure 1: *Assessing cell behavior on immobilized amniotic particulate on hydrogel (agarose) gels (12-wells).*

Key Biological Components are Retained in Amniotic Particulate

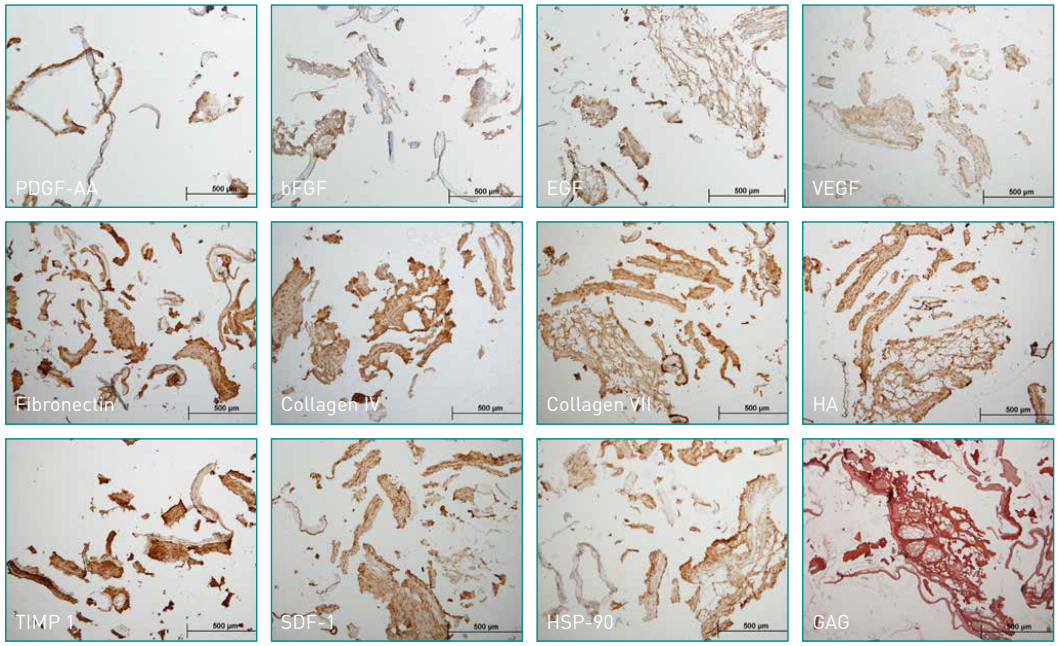

Figure 2: *IHC staining revealed the retention of several key growth factors, matrix proteins and otherb biological components through baseptic processing of amniotic particulate allografts (10x magnification). These components are critical in supporting wound healing activities [1, 2].*

NHDFs Readily Attached to Amniotic Particulate

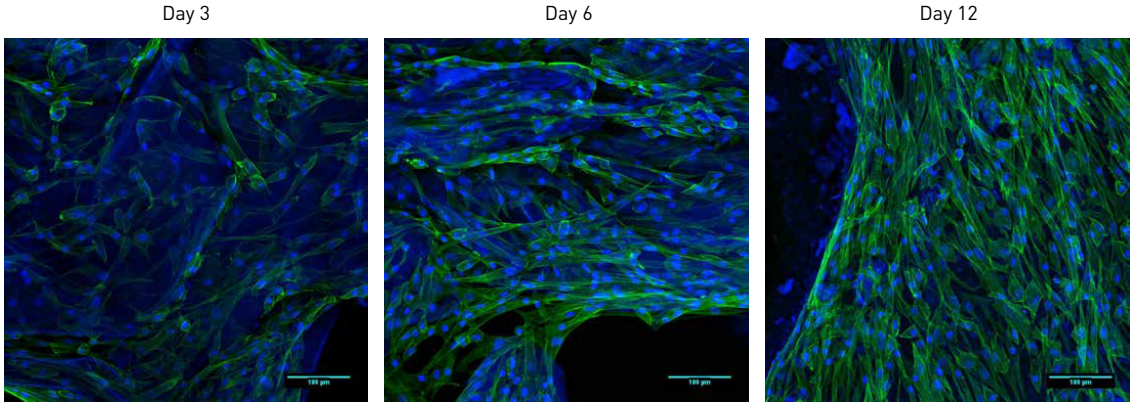

Figure 3: *Confocal imaging revealed that NHDFs adhered readily to the amniotic particulate allografts which are immobilized on agarose gels. DAPI staining (blue) visualizes the cell nuclei and Fluorescein Phalloidin staining highlights the cytoskeletal stretching of the cells on the amniotic particulate only over time (20x magnification). This demonstrates functional cell-matrix interactions.*

New Matrix Proteins are Secreted by NHDFs

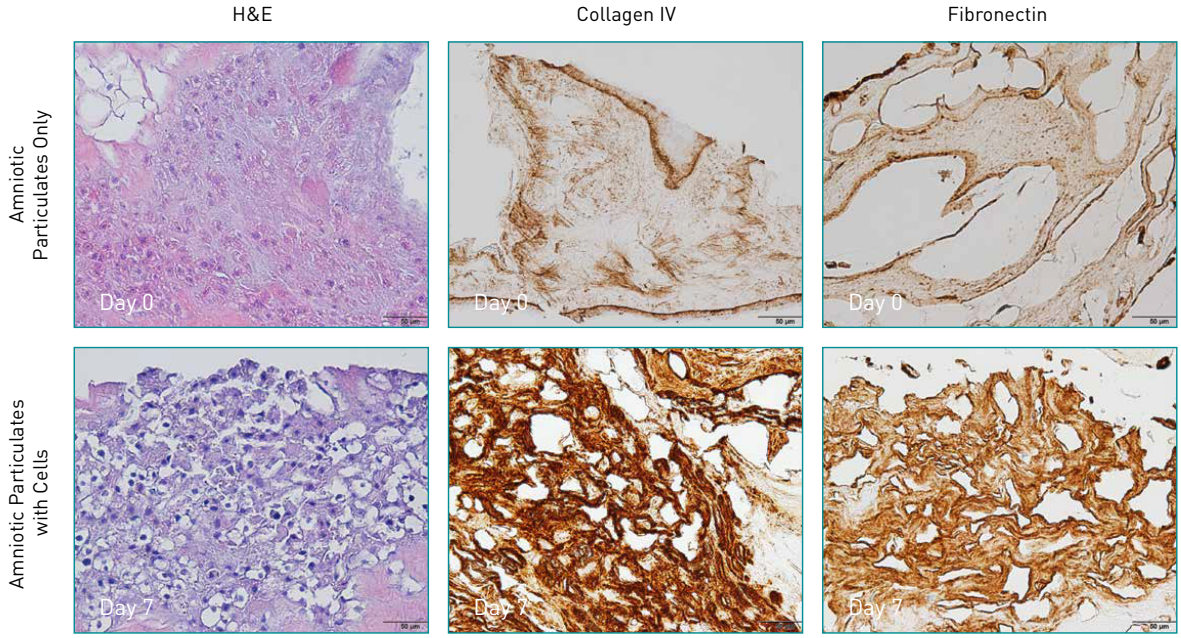

Figure 4: *NHDFs adhered readily (nuclei are visible through H&E staining) to the amniotic particulate and started secreting their own matrix proteins (collagen IV, fibronectin) that constitute the basal lamina and the extracellular matrix (40x magnification) by day 7. These components are known to support granulation activities [1, 2]. Some challenges arose in cutting across the 2-D immobilized amniotic particulate, especially the control samples (amniotic particulate only).*

NHDFs Proliferate & Secrete New Matrix to Bridge Neighboring Amniotic Particulate

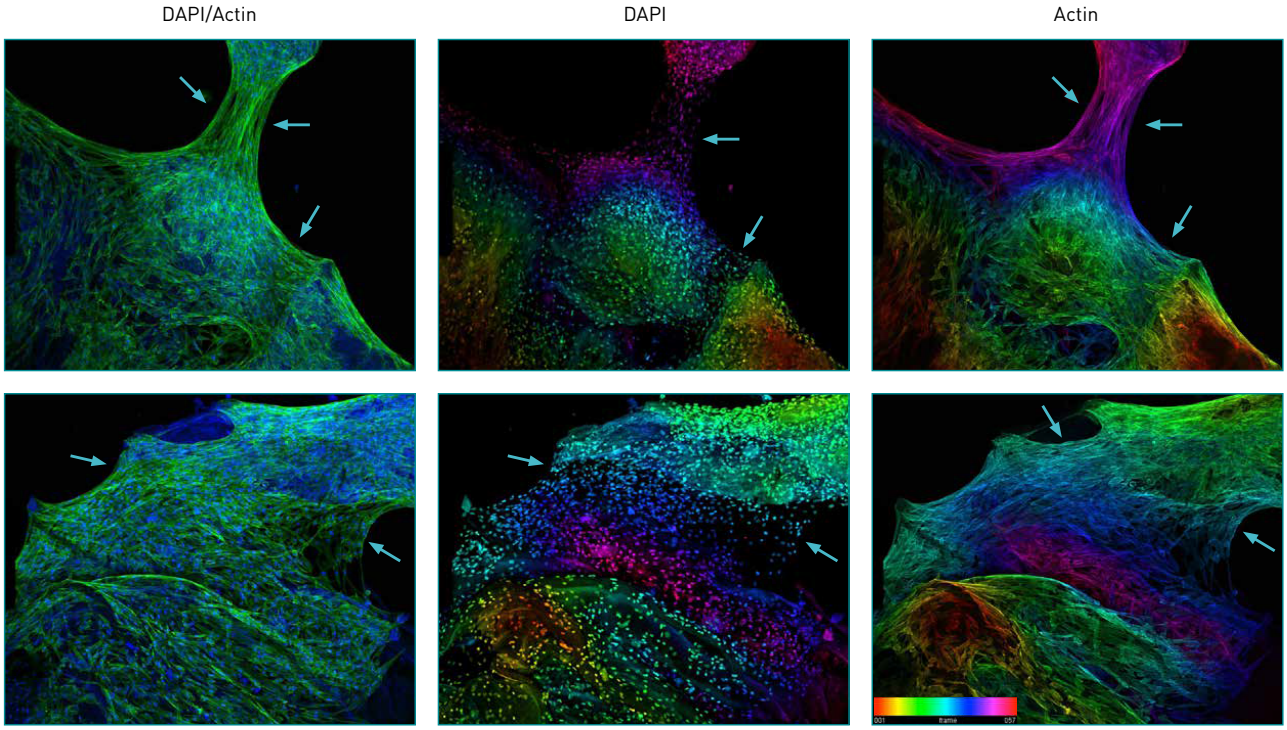

Figure 5: *NHDFs adhered and proliferated on amniotic particulate as observed through DAPI/Actin staining that culminated in bridging and network formation (blue arrows) across neighboring particles (10x magnification). This simulates the potential ability of functional cells to bridge neighboring amniotic particles across a large wound bed area.*

Bridging of New Matrix Between Amniotic Particulate

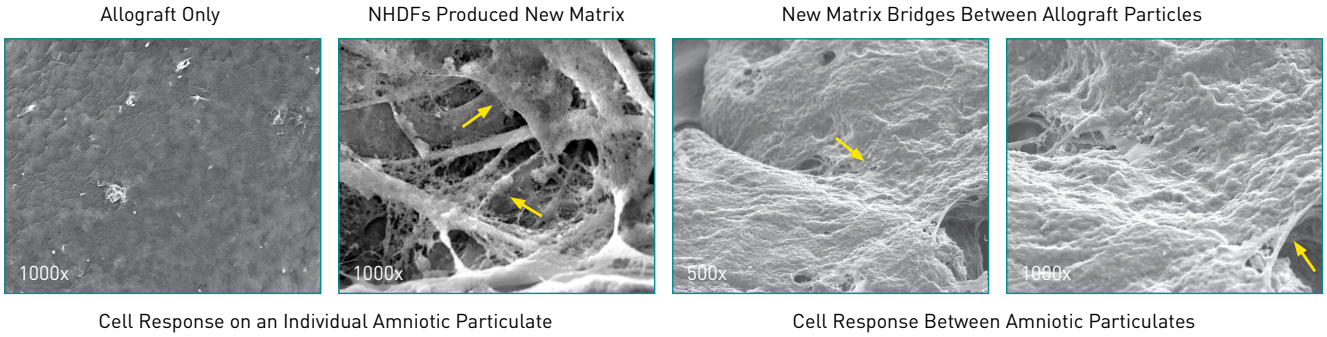

Figure 6: *SEM imaging visualizes NHDFs seeded on amniotic particulate sprinkled on agarose gels are producing an abundanc of new matrix proteins and bridging nearby particulate allografts by day 7. This mimics what can happen across a large wound bed where amniotic particulate can be sprinkled over to facilitate woun closure. A limitation of the study is that the sprinkled particulate on the cell seeding platform (agarose gels) may lead to heterogeneous distribution of particulate due to difference sizes of particulate at that scale (in the 12-well inserts). This may haveresulted in areas where bridging of new matrix formation may have been hindered.*

CONCLUSION

Aseptically-processed dehydrated human amniotic particulates retained many growth factors, matrix proteins and other key biological components involved in wound healing. These preserved components provide a quality allograft that supports granulation activities and ensures bridging networks between neighboring amniotic particulates. This can facilitate wound closure across large wounds. Future studies will investigate the kinetics of new matrix formation and coverage of wound area, along with assessing cellular behavior of other cell types (MSCs, endothelial cells, diabetic cells) and response under chronic conditions (exposed to high protease levels or pro-inflammatory cytokines).

REFERENCES

1. Vulnar T, et al. J Int Med Res. 2009;
2. Reinke JM & Sorg H, Eur Surg Res. 2012