Aseptically Processed Dehydrated Human Amnion/Chorion Allografts Promote Proliferation, Migration and New Matrix Deposition of Type II Diabetic Keratinocytes

INTRODUCTION

Normal wound healing involves a series of events in the wound bed microenvironment between the extracellular matrix, growth factors and cells that orchestrate the repair and regeneration process. Diabetic environments are compromised, thus altering the ability of cells to respond and progress towards healing in a timely manner [1]. Amniotic membranes are rich in matrix proteins, various growth factors and cytokines that support the healing process. We have previously shown that aseptically processed dehydrated human amniotic membrane allografts (dHAMA) support endothelial and fibroblast cell proliferation, angiogenic capacity and new matrix production, which are key activities during granulation.

The focus of this work is to investigate the responsiveness of diabetic cells to dHAMA, in vitro, and to evaluate whether their cellular activity and functionality can be enhanced to the extent comparable to those of normal cells. Cellular behavior of normal keratinocytes and type II diabetic keratinocytes that would represent the epithelialization process during wound healing were examined.

MATERIALS AND METHODS

Dehydrated amnion/chorion allografts were procured according to Good Tissue Practices and processed aseptically without terminal sterilization at Musculoskeletal Transplant Foundation (MTF, Edison, NJ, USA).

Normal human epidermal keratinocytes (normal) and diabetic Type II human epidermal keratinocytes (diabetic) (Lonza) were cultured (100,000 cells/7mm disc) on both sides of dHAMA. Cell viability was assessed via the CCK-8 assay (Sigma-Aldrich) at various time points along with matrix synthesis (laminin), which was evaluated histologically (Premier Laboratory, LLC). The chemotactic ability of dHAMA was explored qualitatively (scratch test) and quantitatively (transwell). The scratch test compared cell migration of both cell types (250,000 cells/well) upon exposure to dHAMA extracts (20mg/mL).

A confluent layer of cells was scratched (~1mm gap) and exposed to dHAMA. Transwells (Corning) in 24 well plates were coated with fibronectin at 4°C overnight, aspirated the next day and air-dried for 45 minutes. 5mm dHAMA discs were placed under the transwells in basal media alone (basal media) for both normal and diabetic keratinocytes at 4°C overnight.

RESULTS

The results indicate that the proliferation trends of diabetic keratinocytes was similar to normal (peaking around day 7) when cultured on dHAMA. In addition, the proliferation patterns were similar on the amnion and chorion facing sides (no siedness). Furthermore, diabetic cells secreted key extracellular matrix component (laminin) that constitute the basement membrane at a similar level to normal keratinocytes. The scratch studies revealed that normal and diabetic keratinocytes behaved similarly upon exposure to dHAMA extracts. At day 3, both cell types started migrating inward. However, a high seeding density and extensive matrix deposition may have prevented more effective migration in both cell types. A lower seeding density may be requisite to better examine the chemotactic effect of dHAMA, along with using mitomycin-C to inhibit cell growth. However, the quantitative assessment of cell migration through the transwell study demonstrated that both normal and diabetic keratinocytes were responsive to the presence of dHAMA and migrated towards it. Although diabetic keratinocytes exhibited slower overall migration than would be expected, both groups migrated significantly more in the presence of dHAMA when compared to the control (basal media).

CONCLUSION

Aseptically-processed dHAMA are ideal substrates for supporting diabetic cellular activities. Similar cell proliferation to normal keratinocytes was observed, along with significantly better quantitative assessment of normal and diabetic keratinocyte migration compared to the control. This demonstrated that dHAMA can help promote proliferation and re-epithelialization activities in diabetic keratinocytes in a similar manner to normal keratinocytes. This is critical in facilitating the wound healing processes in diabetic wound sites.

REFERENCES


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