Comparative Analysis of Amniotic Membrane Allografts Modulation of Keratinocyte Activity *In Vitro*

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INTRODUCTION

Re-epithelialization, the process of restoring a protective epithelial barrier over a wound, is driven by cellular mechanisms that depend on regulatory proteins to facilitate keratinocyte migration and proliferation [1]. This is the final phase in the healing cascade and for chronic wounds, wound closure is often facilitated by an advanced intervention like placental-based allografts. This study evaluates the ability of different placental allograft configurations to promote re-epithelialization *in vitro*. The products assessed included two multi-layer products: a lyophilized human amnion chorion membrane (LHACM*), a dehydrated human amnion chorion membrane (DHACM#), and two single-layer products: a lyophilized single-layer amnion (SA) and a lyophilized single-layer chorion (SC).

MATERIALS AND METHODS

Extract Preparation: Human amniotic tissues were processed using a proprietary and patent-pending cleansing process followed by lyophilization or dehydration and terminal sterilization. Soluble factors from LHACM, SC, SA and DHACM were extracted in assay-appropriate basal media at 4°C for 16 hours.

Luminex Assay: Soluble growth factors in all product extracts were assessed using bead-based Luminex multiplex assay (R&D systems). Elution was done as described above and extracts were assayed at 3 cm^2/mL. Extracts were incubated with beads coated in antibodies to specific growth factors of interest. Samples were then incubated with biotinylated detection antibodies followed by a streptavidin-phycoerythrin (PE) reporter. Analytes of interest were quantified using Luminex instrument (Luminex FLEXMAP 3D, R&D Systems).

Proliferation: HaCaT cells were treated with basal media supplemented with LHACM, SC, SA or DHACM extract at final concentrations of 1.5, 0.75, 0.375 and 0.1875 cm^2/mL. Basal DMEM (with 0% FBS) and complete media (DMEM with 10% FBS) media served as controls. Following a 120 hour incubation at 37˚C, cellular proliferation was determined by CyQuant Assay.

Migration: HaCaT cells were plated at confluence on ImageLock plates (Sartorius) and incubated for 3 hours at 37°C. Cells were treated with mitomycin C at 8 µg/mL for 1 hour. Monolayers were then scratched using the WoundMaker (Sartorius) and treatments applied at final concentrations of 1.5, 0.75 and 0.15 cm^2/mL LHACM, SC, SA or DHACM extract. Basal and basal media supplemented with 10 ng/mL recombinant human epidermal growth factor (EGF) media served as controls. Cellular migration was determined by live cell imaging for 24 hours with automated image processing to determine % Wound Confluence at each time point (S3 IncuCyte, Sartorius).

Histology: Hematoxylin and Eosin (H&E) staining was performed on paraffin embedded sections of LHACM, SC, SA and DHACM . Immunofluorescence analysis was used to visualize type I and type IV collagens.

RESULTS

Histological evaluation of placental allografts

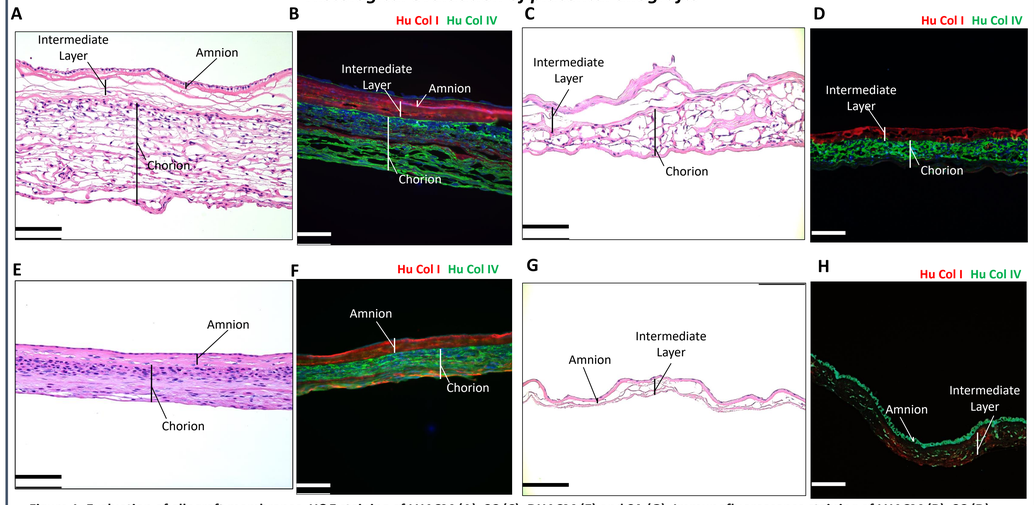

Figure 1. Evaluation of allograft membranes. H&E staining of LHACM (A), SC (C), DHACM (E) and SA (G). Immunofluorescence staining of LHACM (B), SC (D), DHACM (F) and SA (H). with type I collagen (red), type IV collagen (green) and DAPI (blue). Scale bar = 100 µm.

RESULTS

Soluble fraction of amniotic membrane allografts contains growth factors that promote re-epithelialization

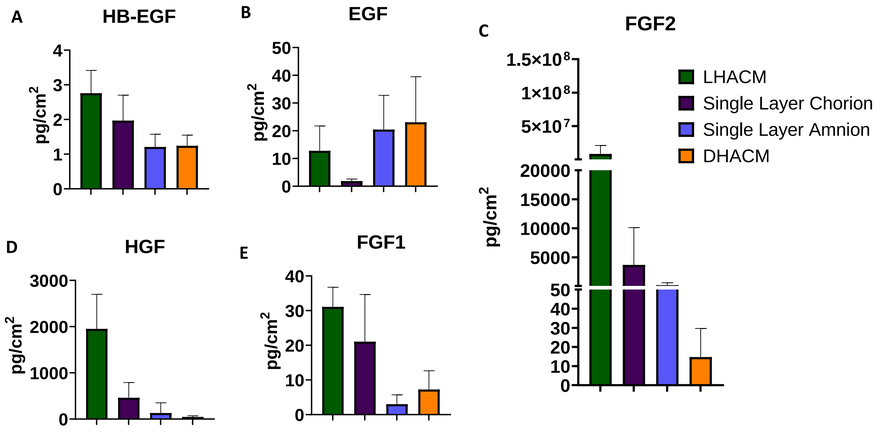

Figure 2. Quantitative analysis of extracted soluble growth factors in LHACM, Single Layer Chorion, Single Layer Amnion and DHACM. (A) HB-EGF (B) EGF (C) FGF2 (D) HGF (E) FGF1

Allograft membranes impact in vitro cellular proliferation of HaCaT cells

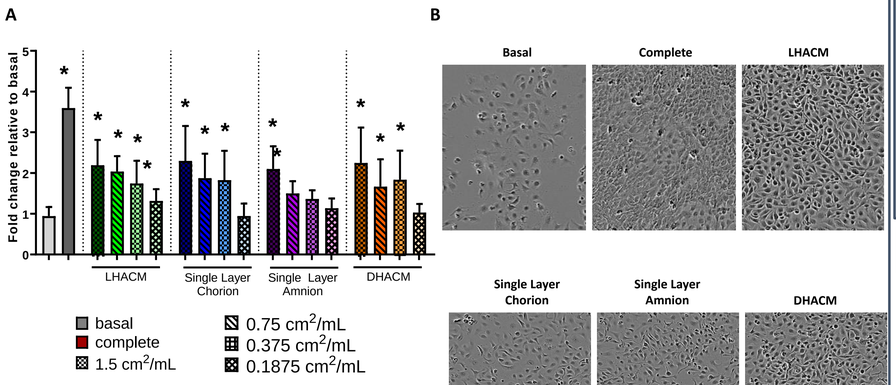

Figure 3. *In vitro* proliferation of HaCaTs. (A) Proliferative response to LHACM (green), single layer chorion (blue), single layer amnion (purple), and DHACM (orange) when HaCaTs were incubated with extract for 120 hours. *p<0.05 relative to the basal group. (B) Cell images showing confluence of cells after 120 hours treatment with basal media, complete media and 1.5cm^2/mL LHACM, single layer chorion, single layer amnion or DHACM.

RESULTS

Allograft membranes impact in vitro cellular migration of HaCaT cells

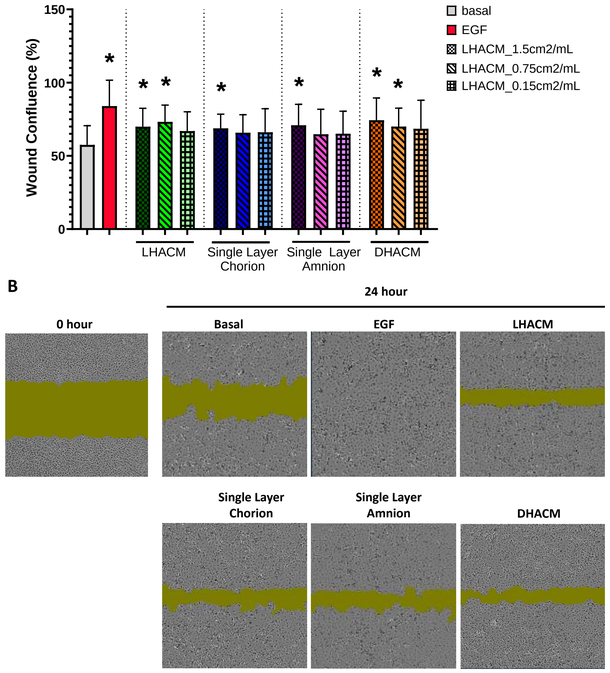

Figure 4. *In vitro* migration in HaCaTs. (A) Migration of HaCaT cells in a 2D scratch wound assay. Live cell label-free imaging of HaCaTs over 24 hours under exposure of LHACM, single layer chorion, single layer amnion or DHACM extracts or controls. *p<0.05 relative to the basal group. (B) Representative cell images showing scratch wound (yellow) in basal media, EGF, LHACM, single layer chorion, single layer amnion or DHACM (1.5 cm^2/mL) at 24hour time point.

CONCLUSION

The findings from this study suggest that amniotic allografts can activate keratinocytes, promoting both proliferation and migration *in vitro*. However, the configuration of the tissue allograft does appear to affect the efficacy in this model. This research provides valuable insights into the potential uses of different configurations of amniotic membrane products to support an optimal wound healing environment. By fostering keratinocyte function, these products may play a critical role in supporting re-epithelialization during wound repair.

REFERENCES

1. Pastar, I., et al., Epithelialization in Wound Healing: A Comprehensive Review. Advances in Wound Care, 2014. 3(7): p. 445-464.

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