Dehydrated Human Amnion/Chorion Membrane Regulates Stem Cell Activity In Vitro

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Background

- Adult stem cells are important for the normal maintenance and repair of wounded tissues through their ability to differentiate, remodel extracellular matrix (ECM), modulate immune responses, and secrete growth factors and cytokines that stimulate cell migration and neovascularization.
- Bone marrow mesenchymal stem cells (BM-MSCs) and adipose-derived stem cells (ADSCs), and hematopoietic stem cells (HSCs) are recruited to healing wounds, where they support healing in a variety of ways, including paracrine signaling of immunomodulatory cytokines.
- Human-derived placental tissues have been shown in randomized controlled clinical trials to be effective for healing of chronic wounds.
- Dehydrated human amnion/chorion membrane (dHACM) is a dehydrated human placental tissue allograft comprised of laminated amnion and chorion membranes.
- dHACM allografts have demonstrated the ability to recruit stem cells, including MSCs and HSCs, to wound sites in vitro and in vivo.
- A specific configuration of dHACM was used in this study; therefore, these results apply only to dHACM.

Methods

- ADSCs and BM-MSCs were plated at 15,000 and 10,000 cells/well, respectively, in an Oris™ Pro Cell Migration Assay (Figures 1 and 2). A specific configuration of dHACM was used in this study; therefore, these results apply only to dHACM.‡
- Epifix® amniotic membrane allograft; PURION® Process was used in this study.
- dHACM was minced into ~1x1 mm pieces, and allowed to extract overnight at 4 °C at 5 mg/mL in basal media. Tissue residue was removed by centrifugation, and extracts were sterile filtered.
- dHACM extracts also caused accelerated closure of the acellular zone (Table 1).
- Migration of ADSCs and BM-MSCs was scored using an Ori®-TM Pro Cell Migration Assay (Figures 2 and 3).
- Cytokine production by ADSCs, BM-MSCs, and HSCs was measured using Multiplex ELSA Human Growth Factor and Inflammation Arrays (RayBiotech).

Results

- dHACM Promotes Proliferation of ADSCs, BM-MSCs, and HSCs
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Conclusions

- dHACM treatment promoted proliferation and migration of ADSCs, BM-MSCs, and HSCs, along with modulation of secreted proteins from these cells in vitro, including regulators of inflammation, mitogenesis, and wound healing. Stem cell secreted immunomodulatory proteins may be crucial in chronic wounds to transition the wound out of a state of sustained inflammation and into a normal acute healing response. Therefore, dHACM may impact wound healing by amplifying host stem cell populations and modulating paracrine stem cell responses in treated wounds.

References

5. MiMedx Group, Inc., Marietta, GA

Table 1: Media formulations

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<tr>
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<th>ADSCs</th>
<th>BM-MSCs</th>
<th>HSCs</th>
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<tr>
<td>Complete media</td>
<td>&lt;50% Cell proliferation</td>
<td>&lt;50% Cell proliferation</td>
<td>&lt;50% Cell proliferation</td>
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<tr>
<td>Basal media</td>
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<td>&gt;0% Cell proliferation</td>
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<tr>
<td>Complete + dHACM extract</td>
<td>&gt;100% Cell proliferation</td>
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Figure 1. Cellular proliferation of (A) ADSCs, (B) BM-MSCs, and (C) HSCs over 24 hours in response to treatment with dHACM extracts. Treatment with soluble extracts of dHACM tissue stimulated ADSCs, BM-MSCs, and HSCs to grow/proliferate with a significant increase in cell number after 24 hr, over their respective negative controls with growth supplements (basal media). dHACM extracts also caused cell number to approach or exceed those of positive controls containing growth supplements (complete media, * indicates p < 0.05).

Figure 2. In vitro cellular closure responses by ADSCs and BM-MSCs following treatment with dHACM extract over 72 hours. (A) Representative calcium Alizarin stained images of BM-MSCs grown in complete media at each time point evaluated in the closure assays. (B) ADSC and (C) BM-MSC migration represent as percent closure of the cell-free area in response to treatment with 5, 1, and 0.5 mg/mL of dHACM extract, compared to basal and complete media. dHACM treatment significantly accelerated closure of the wound area by ADSCs and BM-MSCs, with up to 85% closure after 72 hours of treatment, compared to only 46% and 65% closure of ADSCs and MSCs, respectively, in basal media. * indicates p < 0.05 compared to basal control after 72 hr (B).

Figure 3. Regulation of protein secretion by ADSCs, BM-MSCs, and HSCs in response to 72 hours of dHACM treatment. (A) Heat map with upregulation of cytokine secretion in response to dHACM treatment. Factors that were either up or down regulated by greater than tenfold were grouped into general functional categories such as Broad Mitogenesis, Pro-inflammatory, Immunomodulatory, Stem cell, and Wound healing.

* Fold Change, Relative to Basal Treatment

(C) BM-MSCs

(D) HSCs

(E) Negative Control

(F) Complete Media

(G) Basal Media

(H) dHACM Extract