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.[1]
- Bone marrow mesenchymal stem cells (BM-MSCs), 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.[1,2]
- Human-derived placental tissues have been shown in randomized controlled clinical trials to be effective for healing of chronic wounds.[3-6]
- 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*.[7,8]
- A specific configuration of dHACM[†] was used in this study; therefore, these results apply only to dHACM.[‡]

Purpose

The purpose of this study was to examine the ability of dHACM allografts to modulate activity of various types of reparative stem cells *in vitro*.

References

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[†] EpiFix® amniotic membrane allograft; [‡] PURION® Process

Methods

dHACM Extracts

- Amnion and chorion layers were isolated from donated placentas, processed using a proprietary gentle cleansing process,[‡] laminated, and dehydrated under controlled conditions.
- dHACM was minced into ~1x1 mm pieces, and allowed to extract overnight at 4 °C at 5 mg tissue/mL in basal media.
- Tissue residue was removed by centrifugation, and extracts were sterile filtered.

Proliferation

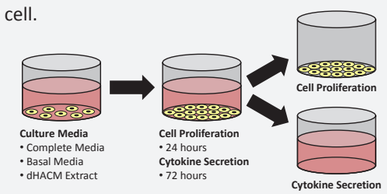
- MSCs and ADSCs were seeded at 2,500 cells/well of a 96-well plate and allowed to adhere overnight.
- HSCs, which are non-adherent cells, were seeded in suspension at 10,000 cells/well.
- Cells were rinsed with PBS, and cultured for 24 hr in complete media, basal media, or dHACM extract at 5, 1, or 0.5 mg/mL (Table 1).
- Cell number was determined using CyQUANT® Cell Proliferation Assay (Thermo Fisher) for adherent MSCs and ADSCS and Calcein AM (Thermo Fisher) for HSCs, according to a standard curve of known cell quantities.

Cytokine Secretion

- Following 72 hr of culture in 5 mg/mL dHACM extract, the supernatant was recovered and tested for the presence of 80 cytokines using Quantibody® Multiplex ELISA Human Growth Factor and Inflammation Arrays (RayBiotech).
- Growth factor content in the starting treatments was subtracted from that in the culture media after 72 hr, and values were normalized to cell number to determine the amount of additional growth factor produced per cell.

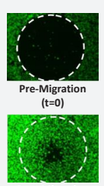
Table 1. Media formulations

	Formulation
Complete Media	Cell specific media WITH serum supplement
Basal Media	Cell specific media WITHOUT serum supplement
dHACM Extract	dHACM extracted overnight into basal media (without serum)



Migration

- ADSCs and BM-MSCs were plated at 15,000 and 10,000 cells/well, respectively, in an Oris™ Pro Cell Migration Assay (Platypus Technologies).
- A circular gel stopper at the center of each well dissolved after 20 min, producing a 2 mm diameter cell-free zone.
- Cells were rinsed with PBS, and cultured for 72 hr in complete media, basal media, or dHACM extract at 5, 1, or 0.5 mg/mL.
- Closure of the acellular zone was measured over 72 hr using a microscope.



Results

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

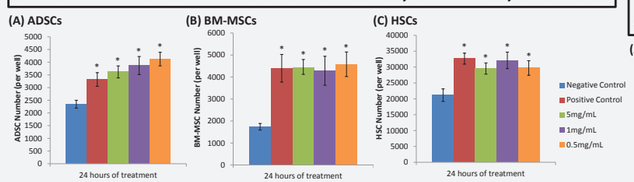


Figure 1. Cellular proliferation of (A) ADSCs, (B) BM-MSCs, and (C) HSCs over 24 hours in response to treatment with dHACM extracts. Treatment with soluble extracts of dHACM tissue stimulated ADSCs, BM-MSCs, and HSCs to ***proliferate with a significant increase in cell number*** after 24 hr, over their respective negative controls without growth supplements (basal media). dHACM extracts also caused ***cell numbers to approach or exceed those of positive controls*** containing growth supplements (complete media). * indicates $p \leq 0.05$ compared to negative controls (ADSCs and BM-MSCs: n=4, HSCs: n=5).

dHACM Stimulates Migration of ADSCs and BM-MSCs

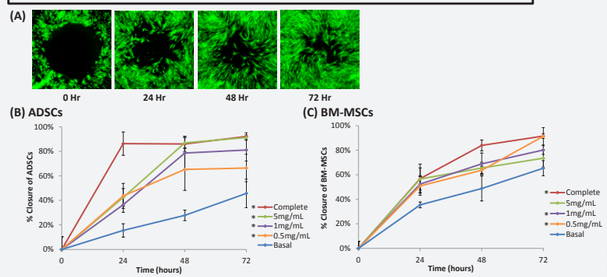


Figure 2. *In vitro* cellular closure responses by ADSCs and BM-MSCs following treatment with dHACM extract over 72 hours. (A) Representative calcein AM stained images of BM-MSCs (green) in complete media at each time point evaluated in the closure assay. (B) ADSC and (C) BM-MSC migration expressed as percent closure of the cell-free zone 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 acellular zone*** by ADSCs and BM-MSCs with up to ***91% closure after 3 days of treatment***, compared to only 46% and 65% closure of ADSCs and MSCs, respectively, in basal medium. * indicates $p \leq 0.05$ compared to basal control after 72 hr (n=5).

dHACM Modulates Cytokine Secretion by BM-MSCs, ADSCs, and HSCs

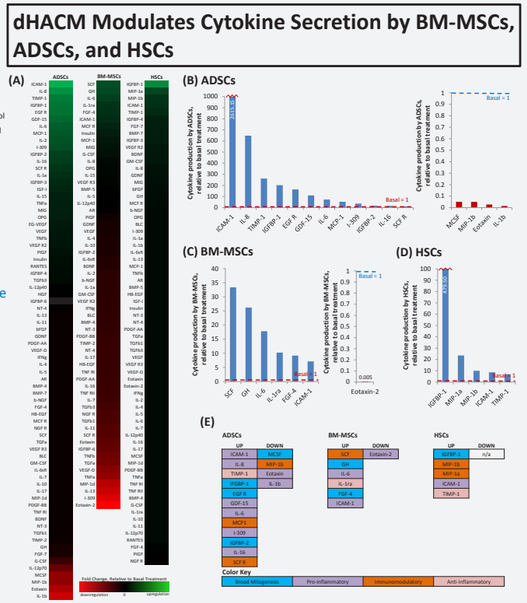


Figure 3. Regulation of protein secretion by ADSCs, BM-MSCs, and HSCs in response to 72 hours of dHACM treatment. (A) Heat map with upregulation of cytokine secretion represented in varying intensities of green coloration and downregulation represented in varying intensity of red coloration (5 pooled wells per sample group). ADSCs, BM-MSCs, and HSCs ***modulated endogenous production of a number of various soluble signals*** in response to dHACM. Factors that were either up or down regulated by greater than tenfold over the basal controls are further reported for (B) ADSCs, (C) BM-MSCs, and (D) HSCs. (E) These growth factors and cytokines can be grouped into general functional categories such as ***broad mitogenic, pro-inflammatory, immunomodulatory, stem cell maintenance, or anti-inflammatory factors*** to identify the processes being regulated by dHACM treatment.

Conclusions

dHACM treatment promoted proliferation and migration of ADSCs, BM-MSCs, and HSCs, along with modulation of secreted proteins from those cells *in vitro*, including regulators of inflammation, mitogenesis, and wound healing. Stem cell secreted immunoregulatory 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 wound tissues.