Acellular Matrices Derived from Embryonic Stem Cells for Wound Healing Applications

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Statement of Purpose: Embryonic stem cells (ESCs) are capable of differentiating into all somatic cell types, thereby providing a robust cell source for a variety of regenerative cell-based therapies. ESCs can also produce factors locally *in vivo* that direct tissue morphogenesis [1], suggesting that the biomolecules ESCs produce may be as important for regenerative therapies as the functional cell types they can become. ESCs are typically induced to differentiate *in vitro* via cell aggregates referred to as embryoid bodies (EBs). EBs recapitulate most aspects of embryonic tissue development, including molecular signaling that regulates early cell fate decisions. The objective of this project is to develop a novel acellular ESC-derived extracellular matrix (ECM) from differentiating EBs, using methods similar to those that have previously yielded acellular matrices from somatic tissue sources that retain structural and biochemical properties of the native tissue ECM [2]. Thus, acellular ESC-derived matrices may provide a complex assembly of embryonic molecular cues capable of mediating tissue regeneration in adult organisms.

Methods: Mouse ES cells (D3) were differentiated for 4, 7, or 10 days in rotary suspension culture to form EBs. The EBs were harvested and subjected to acellularization regimens involving successive 1% Triton X-100 and 1 mg/mL DNase treatments. Hematoxylin and eosin (H&E) staining was used to assess cell and EB morphology and scanning electron microscopy was performed to examine the ultrastructure of the EBs and acellular matrix. Reduction of cell viability was assessed quantitatively using alamarBlue (Biosource) and visually using Live/Dead staining (Molecular Probes), while DNA content was analyzed with PicoGreen (Molecular Probes) and DAPI staining. Total protein content was quantified using the bicinchoninic acid (BCA, Pierce) assay, and mass retention (lyophilized dry weight measurements) was analyzed. Additionally, expression of extracellular matrix (ECM) proteins (fibronectin, collagen IV, laminin-1), glycosaminoglycans, (hyaluronan) and proteoglycans (versican) by EBs were detected by quantitative PCR and visualized using immunostaining, as well as retention of such molecules in the final acellular product. *In vivo* dermal wound healing models to assess the functional regenerative potential of the acellular matrix are being conducted by applying the acellular matrix to excisional wounds on the backs of mice using fibrin glue as a delivery vehicle. Macro- and microscopic assessment of wound healing parameters, such as wound closure rate, re-epithelialization, and vascularization are being done.

Results / Discussion: DAPI- and H&E-stained samples, as well as scanning electron micrographs, demonstrated that the acellularization process caused the EBs to agglomerate to form a compacted mass of tissue. Cell viability data (Fig. 1) and Live/Dead staining showed a decrease in viability for EBs of different maturation, indicating that final matrices were rendered "acellular." In general, 55-90% of DNA content was efficiently removed, whereas 35-55% of the original protein content was retained, constituting a large portion of the final mass retained (Fig. 1). Gene and protein analysis indicated that fibronectin, collagen IV, laminin-1, hyaluronan, and versican were produced by EBs during the course of differentiation and retained after the acellular procedure. Preliminary *in vivo* dermal wound healing studies have suggested that wound closure is delayed in mice treated with the acellular matrix, with histological differences evident between treated wounds and fibrin controls.

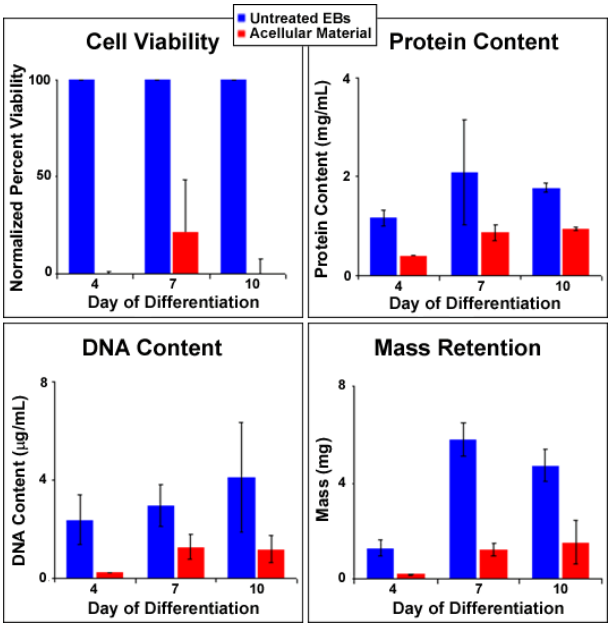

Figure 1. Cell viability (alamarBlue) and DNA content (PicoGreen) were reduced in the acellular material compared to untreated EBs. Total protein content (BCA) and mass were retained over the course of differentiation.

Conclusions: EBs acellularized by detergent extraction methods yield novel ESC-derived matrices. PicoGreen and DAPI staining results suggest a significant decrease in DNA content, while BCA, PCR and immunostaining studies indicate that native ECM is retained. *In vivo* wound healing experiments currently underway are expected to elucidate the effects that acellular embryonic matrices derived from ESCs have on tissue regeneration.

References:

1. Fraidenraich D. Science. 2004; **306**:247-52.
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