Development of an Acellular Stem Cell-Derived Biomaterial

Rekha Nair[1], Todd C. McDevitt[1,2].
[1]The Wallace H. Coulter Department of Biomedical Engineering,
[2]The Parker H. Petit Institute for Bioengineering and Bioscience,
Georgia Institute of Technology / Emory University, Atlanta, GA, USA.

Statement of Purpose: Embryonic stem cells (ESCs) can differentiate into all somatic cell types, thereby providing a robust cell source for a variety of cell transplantation therapies. Preliminary experimental evidence has suggested that ES cells can also produce paracrine factors that promote wound healing and facilitate tissue repair [1]. Thus, the biomolecules ESCs produce may be as important for regenerative therapies as the cell types they can become. ESCs are typically induced to differentiate via cell aggregates referred to as embryoid bodies (EBs). EBs recapitulate many aspects of embryonic tissue development and contain molecular cues that influence early cell fate decisions and direct tissue morphogenesis. The objective of this project is to develop a novel ESC-derived biomaterial via the acellularization of differentiating EBs. Previously, homeostatic tissues have been successfully acellularized to yield natural biomaterials that retain structural and biochemical properties of the original tissue[2]. Acellularized ESC-derived biomaterial could provide a unique repertoire of regenerative signals capable of mediating repair of adult tissues.

Methods: Mouse ES cells (D3) were differentiated for four days in suspension culture to form EBs. The EBs were then harvested and subjected to acellularization regimens involving different concentrations of Triton X-100 and DNase, as well as variations on treatment duration and frequency. Sample structure was assessed using DAPI staining to image nuclear material and H&E histological staining to note changes in cell and EB shape. In order to quantify the extent of the remaining nuclear and cytoskeletal content of the acellularized material, fluorescent measurements of incorporated Hoechst and Phalloidin-FITC dyes, respectively, were taken. Acellularized material was solubilized using 6M Guanidine-HCl and fluorescent intensity readings were taken on a microplate reader. Measurements from the acellularized samples were compared to the untreated EB values to determine the relative decrease in cellular content and efficiency of extraction.

Results / Discussion: The acellularization process caused the EBs to conglomerate and form a viscous mass of tissue. Fluorescent (DAPI-stained) and histological (H&E-stained) images taken after 0.1% Triton X-100 treatment showed that while material was still present, the structure had changed from showing clear, distinct nuclei and an overall circular shape (Figure 1a) to evidencing a fibrous, distorted morphology (Figure 1b). Nuclei were indistinct after Triton X-100 treatment, indicating the ability of the solvent to disrupt cell nuclei. Weight measurements pre- and post-treatment indicated very little mass loss after Triton X-100 treatment suggesting that a significant portion of the material was retained after the solvent extraction process. Samples treated with Triton X-100 showed that significant cytoskeletal material was removed, indicated by a 45% reduction in Phalloidin intensity (Figure 1c). Combining Triton X-100 extraction with DNase treatment further reduced remaining cellular content based on Hoechst measurements, while DNase alone had little effect.

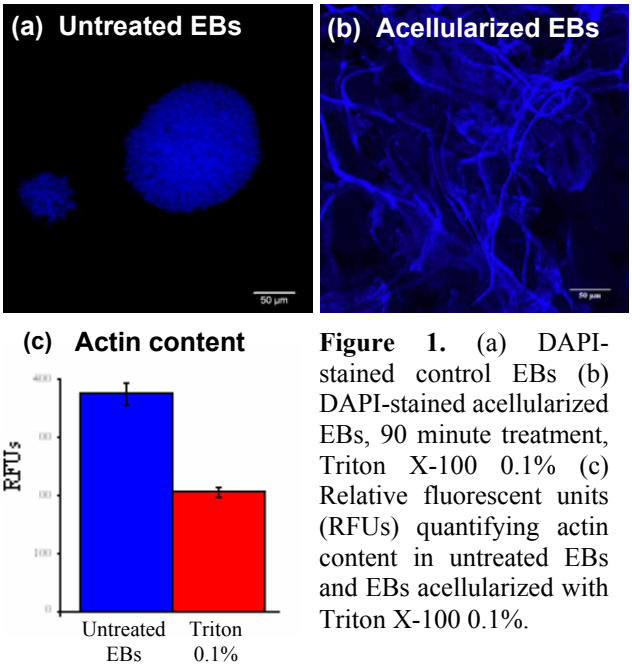

Figure 1. (a) DAPI-stained control EBs (b) DAPI-stained acellularized EBs, 90 minute treatment, Triton X-100 0.1% (c) Relative fluorescent units (RFUs) quantifying actin content in untreated EBs and EBs acellularized with Triton X-100 0.1%.

Conclusions: EBs can be acellularized by detergent extraction methods to yield novel biomaterials. Fluorescent intensity readings suggest a significant decrease in cellular and cytoskeletal content while histological and fluorescent imaging suggests that ECM material remains. Acellularized EBs are also being analyzed by immunohistochemistry and RT-PCR to identify specific extracellular matrix components produced by the cells. Histological analysis using matrix-specific stains is also being performed to evaluate the presence of collagen, elastin, and glycosaminoglycans in both untreated and acellularized EBs. After optimization of the acellularization protocol, future work will include *in vivo* experiments to assess the efficacy of the acellularized EBs as a novel biomaterial to facilitate tissue repair.

References:

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