Derivation of Embryonic Acellular Matrices from Stem Cells via Mechanical Acellularization Techniques

Alyssa V. Ngangan[1], James Waring[3], Todd C. McDevitt[1, 2]
[1]The Wallace H. Coulter Department of Biomedical Engineering,
Georgia Institute of Technology & Emory University, Atlanta, GA, USA
[2]The Parker H. Petit Institute for Bioengineering and Bioscience,
[3]The George W. Woodruff School of Mechanical Engineering,
Georgia Institute of Technology, Atlanta, GA, USA

Introduction: The extracellular matrix (ECM) is a complex reservoir of secreted biomolecules that are critical to regulating cellular activity and can be dynamically modulated by cells undergoing morphogenic processes. During development, extracellular matrix environmental cues stimulate embryonic cells to proliferate, migrate, and differentiate. Despite the lack of significant cell differentiation and repopulation of ESCs used in cell transplantation therapies for tissue regeneration, ESC-secreted molecules appear to play an integral part in regenerating various tissues [1]. Thus, acellularization of early differentiating ESC aggregates, referred to as embryoid bodies (EBs), may yield stem cell-derived ECM that retains biomolecules secreted by stem cells undergoing differentiation that could provide instructive cues to facilitate regeneration of wounded tissues. The objective of this project is to derive a novel acellular stem cell-derived matrix using mechanical means in order to retain secreted growth factors found in the ECM.

Methods: D3 mouse ESCs (4 x 10^5 cells/ml; 10 ml per 100 mm plate) were cultured without LIF in rotary suspension culture (40 rpm) to induce EB differentiation. Gene expression analysis was performed via qRT-PCR by extracting mRNA from EBs cultured for 4, 7, and 10 days of differentiation to compare to undifferentiated ESCs. Using an RT^2 Profiler PCR Array (SuperArray), 84 genes for growth factors were assessed simultaneously. In order to examine specific growth factor protein production and secretion, EB-conditioned media (EB-CM) was collected and analyzed by ELISA. EB-CM (serum-free, 0.1% bovine serum albumin) was collected following 2 days conditioning by EBs formed for 4, 7, and 10 days with serum. Investigation of acellularization techniques, including lyophilization and repeated freeze-thaw cycles (3 cycles) utilized day 7 EBs. Mechanical acellularization techniques were combined with DNase treatment (1 mg/mL for 15 minutes) to extract cellular DNA. Efficacy of acellularization was analyzed for viability (AlamarBlue™), protein (bicinchoninic acid (BCA) assay) and DNA (PicoGreen™) content, and histological examination (hemotoxylin and eosin (H&E), Hoechst). Following acellularization, EB matrices were incubated in PBS (+0.1% BSA) for 6, 12, and 24 hours at 4°C to extract soluble proteins and collected protein fractions were analyzed by BCA assay and protein electrophoresis (SDS-PAGE).

Results: Over the progressive time course of EB culture, growth factor gene expression was differentially regulated by ESCs undergoing differentiation (figure 1A). In general, growth factor protein production increased as EB differentiation progressed (Figure 1B). Lyophilized EBs exhibited non-spherical shaped EBs with intact nuclei, while freeze-thawing of EBs yielded a cohesive mass of acellular tissue with disrupted nuclei. Following DNase treatment, both mechanical methods produced a cohesive pellet with little DNA content as indicated by Hoechst staining; however, the composition of the acellular mass, exhibited by H&E staining, differed between the freeze-thaw and lyophilized samples (figure 1C). Freeze-thaw and lyophilization of EBs completely inhibited cell viability, while retaining similar amounts of protein as untreated EBs. Subsequent DNase treatment retained lower amounts of protein and significantly reduced cellular DNA content. Protein extracts exhibited similar amounts of protein from lyophilized EBs compared to untreated EBs, with a large molecular weight range of proteins present in all extracts.

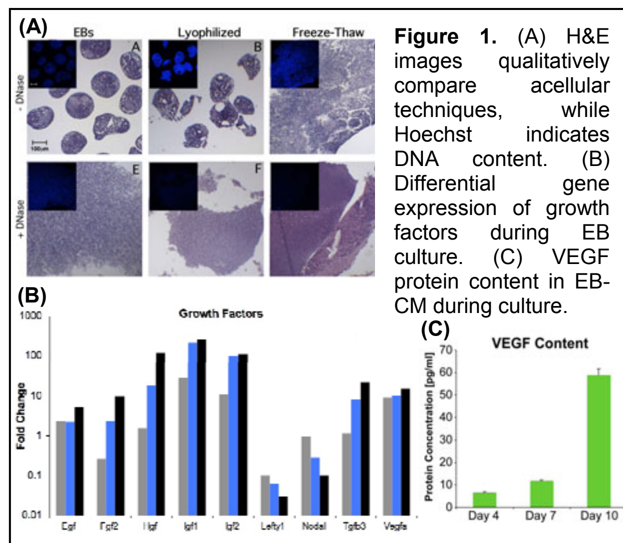

Figure 1. (A) H&E images qualitatively compare acellular techniques, while Hoechst indicates DNA content. (B) Differential gene expression of growth factors during EB culture. (C) VEGF protein content in EB-CM during culture.

Conclusion: These studies demonstrate that EBs modulate the levels of gene and protein expression of different growth factors during the course of differentiation. Mechanical means of acellularization are capable of removing cellular material and permit the extraction of a variety of proteins to be analyzed for specific protein content and bioactivity. The derivation of acellular EB-matrices containing bioactive growth factors could yield novel tissue regeneration therapies.

References

1. Fraidenraich D, et al. Science, 306, 247, 2004.