Hydrodynamic Mixing Regulates Differentiation of Embryonic Stem Cells within Size-Controlled Embryoid Bodies

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Statement of Purpose: Embryonic stem cells (ESCs) possess the unique capacity to differentiate into all somatic cell types, making them a potential cell source for regenerative therapies. However, for the therapeutic promise of ESCs to be fully realized, scalable directed differentiation methods must be developed. Suspension culture is amenable to high throughput production of embryoid bodies (EBs) through the use of bioreactors. However, large-scale suspension culture often imparts hydrodynamic forces on the cell aggregates due to fluid mixing conditions. Using rotary orbital suspension culture, our lab has demonstrated the impact of hydrodynamic conditions on EB formation and differentiation [1, 2]. However, variation in initial EB formation kinetics and size may alter differentiation through modulation of endogenous signaling [3]; thus, the influence of hydrodynamic forces on ESC differentiation within EBs still remains largely unknown. The objective of this study was to examine the effects of hydrodynamic environments created by rotary orbital culture on the differentiation of uniformly sized populations of EBs.

Methods: Murine ESCs (D3 line) were forced to aggregate by centrifugation into 400µm diameter PDMS micro-wells, with approximately 1000 cells/well. The resultant population of EBs was transferred to rotary orbital suspension culture (approximately 1500 EBs per 100 mm dish) after 24 hours of formation and maintained at speeds ranging from 25rpm to 65rpm. Size and yield of EBs were monitored using morphometric analyses. Histological examination of EB sections was performed by staining with hematoxylin and eosin (H&E) to assess cell organization within the aggregates. Markers for pluripotent (Oct-4, Nanog) and differentiated (Nkx 2.5, AFP, Pax-6) cell phenotypes were analyzed using real time PCR. Cardiomyocyte differentiation was examined by the appearance of contractile foci after plating of EBs onto gelatin-coated dishes. Whole mount in situ immunofluorescence was performed using similar markers for pluripotency and germ layer formation.

Results: The forced aggregation method of EB formation produced a uniform initial population of cell aggregates (Figure 1 A-C). When transferred to variable speed rotary culture, the slowest speed, 25rpm, resulted in agglomeration of individual EBs (Figure 1 D-F). However, when maintained at 45rpm and 65rpm, the size of EBs at the different rotary speeds was not statistically different. This was verified by maintenance of similar EB yield in both rotary conditions (1217 ± 161 EBs at 45rpm, 1328 ± 342 EBs at 65rpm after 4 days of differentiation), demonstrating that the hydrodynamic conditions imposed at 45rpm and 65rpm prevent agglomeration of pre-formed

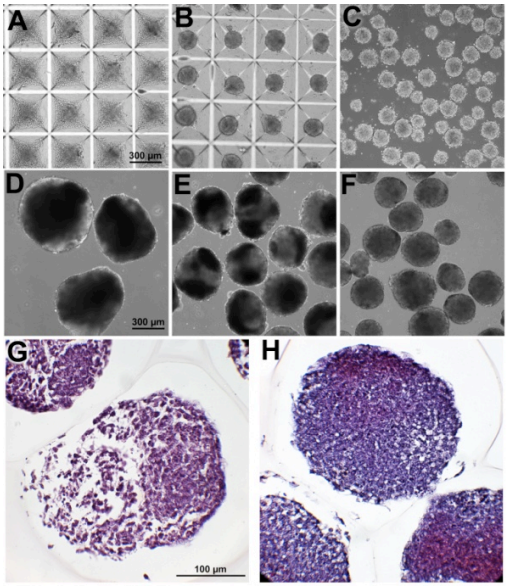

Figure 1. Forced aggregation and differentiation of EBs. (A) EBs were centrifuged into PDMS micro-wells, (B) forced to aggregate for 24 hours, and (C) transferred to rotary culture. Rotary speeds of (D) 25rpm (E) 40rpm, and (F) 65rpm modulated EB size. EBs at (G) 45rpm and (H) 65rpm exhibited histological differences after 10 days of differentiation.

EBs. Although EB size remained consistent, morphological differences were apparent by H&E staining of EB sections, which revealed distinct regions of cell compaction and dispersion in EBs cultured at 45rpm, whereas 65rpm EBs maintained a population of densely packed nuclei throughout the interior of the EB (Figure 1 G-H). Gene expression of the pluripotency markers Oct-4 and Nanog persisted through the course of EB culture in all populations; however, the expression of differentiation markers was altered by rotary speed. This was corroborated by the delayed appearance of contractile foci in 65rpm EBs compared to those maintained at 45rpm. Characterization of the relative populations of pluripotent and differentiated cells within EBs at different rotary speeds is being quantified by ongoing whole mount immunofluorescence staining analysis.

Conclusions: These results demonstrate that modulation of the hydrodynamic environment created by rotary orbital suspension culture can impact the phenotype of cells within differentiating EBs, independent of the size of ESC aggregates. This work highlights important principles for scalable bioprocessing and may serve as a novel method to modulate differentiation.

References:

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