Fluid Shear Stress Pre-conditioning Affects Endothelial Morphogenesis of Embryonic Stem Cells within Embryoid Bodies

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Introduction Fluid shear stress enhances the differentiation of embryonic stem cells (ESCs) to vascular cell types such as endothelial cells, hematopoietic cells, and smooth muscle cells [1, 2]. ESCs can be cultured in vitro within 3D aggregates termed embryoid bodies (EBs), which recapitulate many aspects of embryogenesis, including angiogenesis with the presence of immature hematopoietic and endothelial cells [3, 4]. Although endothelial and hematopoietic differentiation can occur spontaneously in EBs, the percentage of endothelial and hematopoietic cells within EBs varies greatly and is often less than 10% [4]. Preconditioning ESCs with fluid shear stress prior to EB formation may enable increased vascular cell differentiation within EBs. Thus, the objective of this study was to examine how fluid shear stress pre-conditioning affects endothelial differentiation and morphogenesis of ESCs within EBs.

Materials and Methods D3 mouse ESCs were seeded on collagen IV coated glass slides at a density of ~20,000 cells/cm² and subjected to either 0 (static) or 5 dynes/cm² (shear) for 48hrs in a parallel plate flow chamber. EBs were formed on a rotary orbital shaker at 40RPM from a single-cell suspension of 2x10⁶ pre-conditioned ESCs in 100mm² Petri dishes and cultured for 10 days. Endothelial and hematopoietic gene expression profiles were assessed through examination of endothelial genes Flk-1, Flt-1, VE-Cadherin, and PECAM and hematopoietic genes Tal1 and Runx1. Expression of endothelial proteins VE-Cadherin, PECAM and vWF was determined through immunofluorescence. EB morphology and morphometry was examined through phase microscopy.

Results Shear pre-conditioning significantly increased the expression of Flk-1, VE-Cadherin, PECAM, and Runx1 in ESCs. EBs formed from static and shear pre-conditioned ESCs were similar in size and displayed no apparent differences in formation kinetics. However, by day 7 of differentiation, ~70% of EBs formed from shear pre-conditioned ESCs contained dark regions centrally located within the EB (Fig. 1A,F). Additionally, Flt-1, VE-Cadherin, PECAM, and Runx1 genes were significantly higher in EBs containing shear pre-conditioned ESCs on day 7. EBs formed from shear pre-conditioned ESCs contained clusters of VE-Cadherin positive cells centrally located within EBs at all time points examined throughout differentiation (Fig. 1B-E). However, EBs containing ESCs pre-conditioned at 0 dyn/cm² did not appear to contain VE-Cadherin positive cells on days 2, 4, 7, and 10 (Fig. 1G-J). Dark regions observed by phase microscopy in day 7 shear pre-conditioned EBs, overlapped with the location and size of the VE-Cadherin positive cell clusters (Fig. 1K-L) On day 7, EBs containing shear pre-conditioned ESCs were negative for PECAM but positive for vWF, a more mature endothelial marker, which co-localized with VE-Cadherin suggesting that these cells were differentiating towards a more mature endothelial phenotype (Fig. 1M-O).

Discussion and Conclusions The expression of more mature endothelial proteins several days after fluid shear pre-conditioning suggests that fluid shear stress pre-conditioning exerts prolonged effects on endothelial morphogenesis, cellular organization, and differentiation of ESCs within EBs. This study illustrates that mechanical simulation not only has immediate effects on cell phenotype but also has long term affects on many cellular processes. Thus, fluid shear pre-conditioning may have significant implications in the field of stem cell bioprocessing and in in vitro models of vascular developmental biology.

References
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