Engineering the Homogeneity of Embryonic Stem Cell Microenvironments to Direct Cellular Differentiation

Richard L. Carpenedo, Carolyn Y. Sargent, Jonathan Kent, Todd C. McDevitt.
The Wallace H. Coulter Department of Biomedical Engineering,
The Parker H. Petit Institute for Bioengineering and Bioscience,
Georgia Institute of Technology & Emory University, Atlanta, GA.

Statement of Purpose: Embryonic stem (ES) cells may serve as a robust cell source to treat a variety of degenerative diseases because of their inherent pluripotentiality. However, directing the differentiation of ES cells to a particular cell or tissue lineage has hindered the translation of stem cells into clinically viable regenerative therapies. The heterogeneity of ES cell differentiation is largely due to a lack of well-defined, reproducible methods to control the differentiation of the cells in a manner feasible for commercial scale-up. Thus, engineering control of the microenvironment of embryonic stem cells may provide a new route to directing more homogeneous and reproducible differentiation of the cells. The objective of this study was to assess differences in the homogeneity of ES cell differentiation resulting from different initial differentiation methods.

Methods: Mouse ES cells (D3) were cultured and induced to form embryoid bodies (EBs) either in hanging drops (HD) or in static or rotary suspension cultures. Hanging drops were formed by suspending 500-600 ES cells in 15 µl drops on inverted petri dish lids and suspension cultures were inoculated with 1-4x10^5 cells/ml in 10 ml of culture media. Morphometric analysis of the size (cross-sectional area) and shape (shape factor) of the EBs were assessed during the first 7 days of differentiation using image analysis software (ImageJ); shape factor was defined as the elliptical ratio of the minor axis to the major axis. After 7 days in suspension or hanging drops, the EBs were plated onto gelatin-coated substrates and allowed to continue to differentiate for up to 14 days. Gene expression analysis was performed at 4, 7, 10, and 14 days of differentiation by real-time PCR to detect markers of undifferentiated and differentiated cell types of the three germ lineages (ectoderm, mesoderm and endoderm). Changes in gene expression were expressed as the fold change compared to undifferentiated ES cells normalized to a housekeeping gene, such as GAPDH.

Results / Discussion: EBs formed by all three of the culture methods gave rise to differentiated progeny, including rhythmically contracting cell foci indicative of cardiomyogenesis. After 1-2 days of EB formation, differences in the size and shape of EBs produced by the different methods were clearly observed and persisted during the period of suspension culture (up to 7 days). Hanging drops exhibited greater uniformity in EB size and circularity than static suspension cultures which contained a heterogeneous mix of EBs of varying size and shape. In contrast, rotary suspension cultures produced EBs with similar spatial characteristics to hanging drop

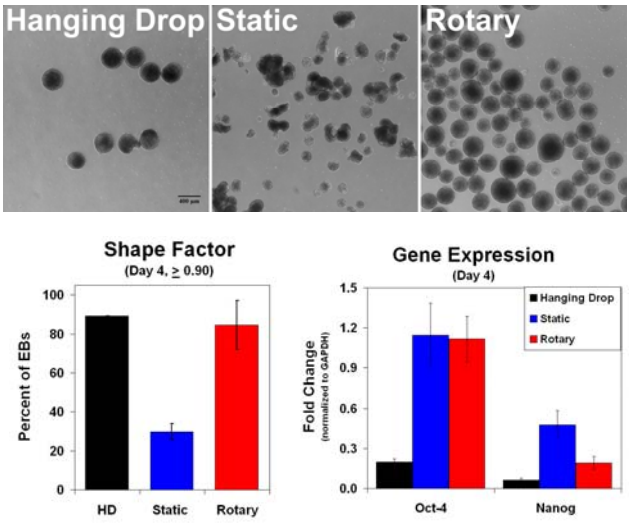

Figure 1. (Top) Phase contrast images of EBs formed by hanging drop, static suspension or rotary suspension at day 4 of differentiation. (Bottom) Shape factor analysis of EBs (left) and real-time PCR analysis of pluripotent stem cell markers (right) at day 4 of differentiation.

cultures, but yielded a significantly greater number of EBs than either hanging drop or static suspension cultures. In addition, fewer of the EBs agglomerated with one another in the rotary suspension cultures compared to static suspension, thus preserving their size and shape. Real-time PCR analysis indicated a decrease in the expression of pluripotent stem cell markers, such as Oct4 and Nanog, with increasing differentiation time in all of the cultures. However Nanog expression decreased more significantly and sooner occurred sooner in the more homogeneous EB populations (i.e. hanging drop and rotary) than in the static suspension cultures.

Conclusions: Significant differences in the size and shape of differentiating EBs were observed between hanging drop, static suspension and rotary suspension methods. Rotary suspension EBs were more uniform in size and shape, similar to hanging drop EBs, than static suspensions and also produced a greater yield of EBs than static suspension cultures. The apparent correlation between the spatial homogeneity of EBs and cell differentiation supports the notion that improving the homogeneity of the stem cell microenvironment can direct the fate and function of stem cells.