Efficient Generation of Hematopoietic Stem Cells from Mouse ES cells: A Bioreactor-based Approach

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Statement of Purpose: Although there have been significant increases in the study of embryonic stem (ES) cell differentiation, most ES cell work has focused on differentiation in static culture systems. Recently attempts have been made to differentiate ES cells in polymer scaffolds as well as various dynamic culture systems. ES cells provide a potentially unlimited cell source for cell therapies; however, reliable methods must be developed to provide clinically-relevant numbers of homogenous therapeutic cell populations. We hypothesize that employing dynamic cultures would encourage ES cell differentiation and have the ability to achieve clinically relevant scale-up. We have previously demonstrated that stirred-bioreactor based dynamic culture conditions could improve hematopoietic differentiation of ES cells (1). Our goal was to directly compare stirred tank type bioreactors with rotary type microgravity bioreactors and optimize a variety of dynamic culture parameters (speed, cell seeding density, etc) and maximize the spontaneous differentiation of hematopoietic progenitor cells (HPCs) from ES cells. Our results indicate that varying cell seeding density and speed in bioreactor systems can significantly effect embryoid body formation and increase the differentiation efficiency of ES cells into HPCs. In addition, extensive gene expression analysis indicates that dynamic, bioreactor-based cultures directly alters the global gene expression profile of ES cells and affects hematopoietic as well as mesoderm related genes.

Methods: Mouse R1 embryonic stem cells were maintained in an undifferentiated state by culture on an inactivated MEF cell layer (ATCC). ES cells were then resuspended in differentiation medium and cultured as a suspension in a low attachment plate (Static), a spinner flask system (Corning), or a microgravity rotating vessel (Synthecon Inc.) to initiate embryoid body (EB) formation. To study the effect of initial cell seeding density in bioreactor systems, cells were seeded at 50,000, 100,000, 500,000, and 750,000 cells/ml for both bioreactor systems. Rotation speed was held constant; spinner flasks were cultured at 100 rpm, and Synthecon vessels were cultured at 20 rpm. The influence of bioreactor speed on EB formation was examined with cell seeding density held constant (750,000 cells/ml). Cell seeding density in the Static system was 10,000 cells/ml, which was based on previously published methods used for static differentiation. On day 7 of differentiation, flow cytometry was performed to determine the percentage of cells positive for two hematopoietic stem cell markers, c-Kit and sca-1. In addition, RNA was isolated from cells differentiated in various culture systems, and cDNA microarray analyses (4-plex format, 72K, Roche Nimblegen) were performed. Statistical analysis was performed using an ANOVA.

Results: To evaluate EB characteristics, concentration and diameter of EBs were calculated (data not shown). The range of average diameters was relatively similar in the different systems. EB concentration in the spinner flask was higher than the Synthecon and static systems; however, the concentration decreased in the spinner flask noticeably with time. Increase in initial cell density showed increased generation of HPCs in the spinner flask bioreactor system with the highest percentage of c-Kit+, sca-1+ cells in the culture initiated with 750,000 cells/ml (Figure 1). HPC generation did not show any differences with varying rotation speed in the spinner flask (Figure 1). The rotary bioreactor system showed an increase in the percentage of HPCs with increasing cell seeding density with maximal differentiation at 500,000 cells/ml (Figure 1). The variation in speed appears more important in the Synthecon system, where higher speeds reduced hematopoietic differentiation. The Synthecon system at 20 rpm produced significantly greater differentiation of ES cells into HPCs (Figure 1). Under the most efficient

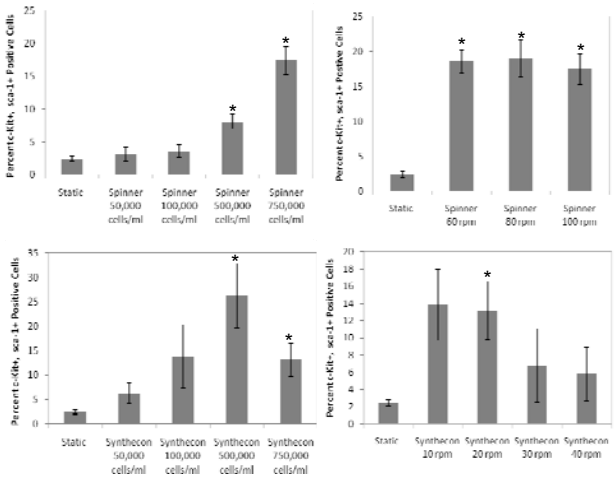

Figure 1. Effect of cell seeding density and rotation speed on HPC generation from ESCs in spinner flask (top panels) and Synthecon Rotary Cell Culture System (bottom panels). * represents $p<0.05$ compared to static culture

differentiation conditions, spinner flask culture (with 750,000 cells/ml at 100 rpm) generated 17.4 ± 2.2% compared to 26.2 ± 6.6% in the Synthecon (with 500,000 cells/ml at 20 rpm). Using cDNA microarray analysis, the gene expression profile of ES cells differentiated in static and dynamic cultures was evaluated with focus on the expression level differences of genes involved in pluripotency, germ layers formation, and hematopoietic differentiation. Results show that after 7 days in culture, the expression of specific hematopoietic genes was up-regulated in the three systems compared to undifferentiated cells (data not shown).

Conclusions: Culture parameters significantly affect hematopoiesis of ES cells in bioreactors. Our current work focuses on evaluating the functionality and clinical applicability of ES cell generated HPCs.

References:

1. Liu H. Tissue Eng. 2005; 11: 319-330.

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