**Hematopoietic Differentiation of Mouse ES cells in Bioreactor Culture Systems**

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**Statement of Purpose:** Embryonic Stem (ES) cells provide an unlimited cell source for cellular therapies; however, reliable methods must be developed to provide clinically relevant numbers of therapeutic cell populations. Current therapeutic efforts for hematopoietic cells involve transplanting adult cells from the bone marrow or blood. However, disadvantages with current methods include the difficulties and efficiency of isolation as well as problems with expansion of adult hematopoietic cells *in vitro*. Dynamic cultures may encourage ES cell differentiation and are amenable to large-scale cell production. Our goal is to optimize dynamic culture parameters (bioreactor type, speed, cell seeding density, conditioned medium, hypoxia, etc) to maximize the generation of hematopoietic stem and progenitor cells (HSPCs) from ES cells and also to investigate the ability of dynamic culture-derived HSPCs to generate terminally differentiated hematopoietic cells. Our results indicate that varying cell seeding density and speed in two different bioreactors significantly affects embryoid body (EB) formation and ES cell differentiation efficiency into HSPCs. Combining bioreactor cultures with directed differentiation strategies via conditioned medium and hypoxic culture could further encourage hematopoietic differentiation. Whether these ES cell-derived HSPCs would be potentially suitable for transplantation was evaluated by examining the terminal differentiation into dendritic cells (DCs) and comparing their gene expression to native *in vivo* derived HSPCs from the bone marrow (BM) and fetal liver (FL) of mice.

**Methods:** Mouse R1 ES cells were cultured as a suspension in a static low attachment plate, a spinner flask system (Corning), or a rotating vessel (Synthecon Inc.). To examine the effects of initial cell seeding density, rotation speed, OP9 stromal cell conditioned medium, and hypoxia, EB diameters and concentrations were determined, and the percentage of cells positive for two HSPC markers, c-kit and sca-1, was examined using flow cytometry. Differentiation of bioreactor-derived HSPCs into dendritic cells was studied using flow cytometry. RNA was isolated from differentiated cells, and cDNA microarray analyses were performed. The gene expression profile of native HSPCs from mouse BM and FL were compared to c-kit$^+$sca-1$^+$ cells generated from ES cells. Statistical analyses were performed using an ANOVA.

**Results:** The range of average EB diameters was relatively similar in the different systems; EB concentration in the spinner flask was higher than the Synthecon and static systems [1]. In general, increased cell seeding density generated higher percentages of HSPCs [1]. Synthecon rotary bioreactors produced more sca-1$^+$ progenitors, and spinner flasks generated more c-kit$^+$ progenitors, demonstrating their unique differentiation profiles [1]. cDNA microarray analysis of genes involved in pluripotency, germ layer formation, and hematopoietic differentiation showed unique gene expression profiles in the two bioreactors with the expression of hematopoietic genes more up regulated in the Synthecon cultures [1]. Conditioned medium and hypoxic cultures also alter the EB formation (data not shown) and generation of c-kit$^+$sca-1$^+$ cells in static and spinner flask systems (Figure 1), and combined conditioned medium and hypoxia could have a synergistic effect to further increase the percentage of c-kit$^+$sca-1$^+$ cells. Dynamically cultured ES cell-derived HSPCs were further differentiated into a phenotype typical of DCs (Figure 2) which had the ability to process antigen (data not shown). Additionally, microarray analysis of isolated c-kit$^+$sca-1$^+$ cells demonstrated differences in the gene expression from native HSPCs isolated from the bone marrow or fetal liver of mice. Greater differences where seen when comparing to BM than FL derived HSPCs, and the ES cells differentiated in static cultures showed the highest similarity to the native HSPCs (Figure 3). The expression level of specific genes important for hematopoiesis, as well as other non-hematopoietic lineages was also compared (data not shown).

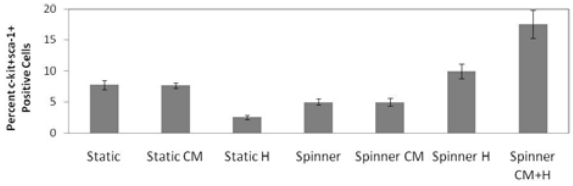


**Figure 1**. Percent cells positive for HSPC markers c-kit and sca-1. CM- conditioned medium, H- hypoxia

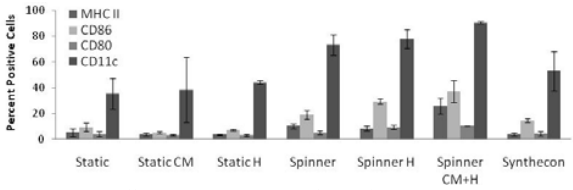


**Figure 2**. Cells positive for markers for DC maturation and activation. CM- conditioned medium, H- hypoxia

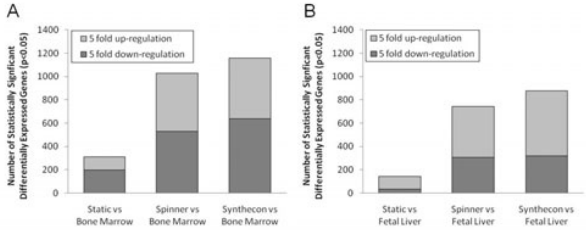


**Figure 3.** Number of differentially expressed genes ($p<0.05$) of to c-kit$^+$sca-1$^+$ cells from ES cells differentiated in static, spinner flask, and Synthecon rotary culture conditions compared to HSPCs from bone marrow (A) and fetal liver (B).

**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 HSPCs.

**References:**

1. Fridley et al. Tissue Eng. 2010, 16(11): 3285-98.

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