Effect of Scaffold Architecture and Culture Conditions on Hematopoietic Differentiation of Embryonic Stem Cells

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Statement of Purpose:

Use of embryonic stem (ES) cells and their lineage-specific derivatives for cell therapy could provide new opportunities in variety of complex disorders. However, the ultimate clinical applicability of stem cell-based therapies requires the development of practical technologies for efficient, scalable and high-throughput production of therapeutic cells suitable for on-demand transplantation. Current efforts to provide therapeutic HPCs involve isolation from patient's (or matched donor) marrow and subsequent expansion. Technologies leading to efficient generation and expansion of therapeutic HPCs from ES cells in a synthetic, controlled environment could provide a renewable, on-demand and readily available cell source for a variety of disease applications. Existing methods for ES cell hematopoiesis fail to provide the three dimensional framework and the spatial cues native to the intrinsic microenvironment in hematopoiesis. Our approach is to systematically understand how the physical properties of the 3D cellular microenvironment created by different biomaterials and various culture parameters affect ES cells and their lineage-specific differentiation. Here we report the significant effect of "tunable" scaffold properties, i.e. pore size, pore shape and polymer concentration (i.e. mechanical properties), on hematopoietic differentiation efficiency of ES cells.

Methods:

Undifferentiated mouse R1 ES cells were expanded on LIF (leukemia inhibiting factor)-producing irradiation-inactivated embryonic fibroblast cells for 10 days. At this stage, $2x10^5$ cells were seeded on PLA scaffolds. The seeded cells were cultured in 24 well tissue culture plates as static culture for 7 or 14 days**.** PLA scaffolds were synthesized using 5%, 7.5%, 10% and 20% w/v poly(DL-lactic acid) with <150 µm, 150-425 µm, >425 µm pore sizes through standard salt leaching procedures. On days 7 or 14, cells were double stained for sca-1 and c-Kit and analyzed using the FACS. Mechanical properties of scaffolds were characterized by testing four specimens from each scaffold type under compression between parallel plates using an Instron and In-SpecTM 2200 PDA Emulator software.

Results / Discussion:

As shown in Figure 1A, our results indicate that decreasing scaffold pore size (< 150 µm versus 150-425 µm and > 425 µm pores) could significantly increase hematopoietic differentiation of ES cells. In addition, increasing polymer concentration (and hence scaffold compression modulus, as shown in Figure 1B) might provide significantly enhanced hematopoiesis ($p < 0.05$, 2 sided Students' T test). We have also demonstrated (data not shown), that co-culture with marrow derived stromal cells (OP9 cells) can have a significant effect on ES cell differentiation. A large increase in hematopoietic progenitors was observed in the presence of paracrine factors from OP9 cells. Our current work focuses on further optimization of scaffold properties and evaluation of bioreactor-based co-culture conditions along with optimization of cell seeding density and ECM composition on ES cell hematopoiesis.

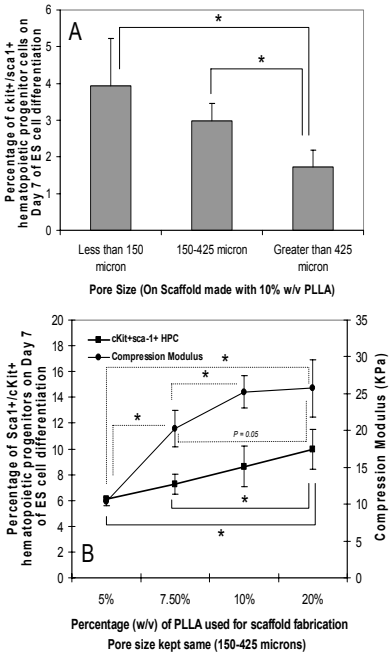

Figure 1: *Effect of scaffold pore size and polymer concentration on ES cell hematopoiesis. (A) Generation of dual-positive (ckit+/sca-1+) HPCs is impaired at higher pore sizes. (B) Higher polymer concentration could provide higher efficiency of HPC generation from ES cells. * indicates groups with significant difference in HPC generation ($p < 0.05$, T test)*

Conclusions:

We have demonstrated the significant role scaffold microarchitecture plays in hematopoietic stem cell differentiation by examining the pore size, polymer concentration and compression modulus of biodegradable polymer scaffolds. Hematopoiesis of ES cells, in various 3D scaffolds could provide significant new understanding on how ES cells behave under dynamic 3D culture conditions, how biomaterial environments modulate their phenotype and ultimately how the cellular microenvironment influences hematopoiesis in general. The fundamental understanding of ES cell-material microenvironment interactions could provide new understanding of stem cell behavior and provide novel methods for high throughput generation of transplantable cells.

References:

1. Liu, H. & Roy, K. *Tissue Eng* 11, 319-30 (2005).

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