Ex-vivo Expansion of Human Hematopoietic Stem/Progenitor Cells in a Human Stromal-based Culture System

Cláudia Lobato da Silva
Institute for Biotechnology and Bioengineering
Centre for Biological and Chemical Engineering
Instituto Superior Técnico, Lisboa, Portugal
claudia_lobato@ist.utl.pt

Abstract

One of the major focuses in Experimental Hematology is the *in vitro* manipulation of hematopoietic stem/progenitor cells (HSPC) with the goal of generating clinically relevant cell numbers from the limited number of stem/progenitor cells present in available samples for multiple settings such bone marrow transplantation, somatic cell gene therapy and production of mature blood cell types.

We had recently demonstrated that a non-transformed, donor-independent human bone marrow (BM) mesenchymal stem cells (MSC)-derived stromal feeder layer system allows an efficient expansion/maintenance of HSPC from BM and umbilical cord blood (UCB) [1, 2]. Significant expansion of BM and UCB cells occurred in this stromal-based system, whereas in stroma-free cultures BM and UCB cells showed a significantly reduced expansion.

A simple kinetic modeling study using two parameters (expansion rate, μ, and death rate, k_k) was established [3]. Parameter estimation was carried out by non-linear least-squares regression. UCB cells were only efficiently expanded in the presence of a human feeder layer, for which k_e ranged from 0.14-0.24 day^{-1} and k_k ranged from 0.004-0.035 day^{-1}. For BM cells, k_e ranged from 0.11-0.36 day^{-1} in both stroma-containing and stroma-free cultures. On the other hand, k_k ranged from 0.005-0.024 day^{-1} in stroma-containing conditions whereas when a feeder layer was not present, k_k was always much higher (0.057 day^{-1}). This simple two-parameter model gives a global measure of culture performance and addresses the dependency of cell death on the environment in which cell are cultured (stroma versus stroma-free). Overall, UCB and BM cells expanded at approximately the same rate, whereas cell death rate was increased in the absence of stroma *ex-vivo*.

A first-order kinetic predictive model was also developed accounting for hematopoietic cell expansion, differentiation, and death, which estimated adequately total cell numbers and relative amounts of the different phenotypes (*e.g.* $CD34^+$, $CD34^+CD38^-$ cells), allowing the prediction of expansion/differentiation pathways and identification of key steps in the hematopoiesis scheme [4]. The model indicates that the presence of the stromal layer is useful for the *ex-vivo* expansion of HSPC since: (i) enhances the expansion of the majority of the more mature cells; (ii) reduces the death rate constant for the more primitive cells; (iii) reduces the differentiation for the more mature cells.

Finally, we decided to combine PKH67 cell membrane labeling with CD34/CD38 immunostaining to compare cell division kinetics between expanded human BM and UCB HSPC and correlated CD34 and CD38 expression with the cell divisional history. Data were analyzed using the Proliferation Wizard module of the *ModFit* software (Becton Dickinson) since it allows the determination of the number of cellular generations in culture. This program is based on histograms of fluorescence intensity and applies deconvolution algorithms to determine the proportion of proliferating cells at each cell division.

UCB cells began dividing 24h earlier than BM cells, and significantly higher numbers underwent mitosis during the time in culture. By day 10, over 55% of the UCB-cells reached the 9^{th} generation, whereas BM cells were mostly distributed between the 5^{th} and 7^{th} generation. By day 14, all UCB cells had undergone multiple cell divisions, while 0.7-3.8% of BM $CD34^+$ cells remained quiescent. Furthermore, the percentage of BM cells expressing CD34 decreased from 60.8±6.3 to 30.6±6.7% prior to initiating division, suggesting that down modulation of this antigen occurred before commencement of proliferation. Moreover, with BM, all primitive $CD34^+CD38^-$ cells present at the end of culture arose from proliferating $CD34^+CD38^+$ cells that down-regulated CD38 expression, while in UCB, a $CD34^+CD38^-$ population was maintained throughout culture. This study show that BM and UCB cells differ significantly in cell division kinetics and expression of CD34 and CD38, and that the inherent modulation of these antigens during *ex vivo* expansion may lead to erroneous quantification of the stem cell content of the expanded graft.

Keywords: hematopoietic stem/progenitor cells, *ex-vivo* expansion, stroma, kinetic modeling, cellular division

1. da Silva, C. L. Gonçalves, R., Cabral, J. M., Zanjani, E. D. & Almeida-Porada, G. A. A human stromal-based serum-free culture system supports the ex vivo expansion/maintenance of bone marrow and cord blood hematopoietic stem/progenitor cells. Exp Hematol 33, 828-35 (2005).

2. Gonçalves, R., da Silva, C. L., Cabral, J. M., Zanjani, E. D. & Almeida-Porada, G. A Stro 1+ Universal Stromal Feeder Layer to Expand/Maintain Human Bone Marrow Hematopoietic Stem/Progenitor Cells
In a Serum-Free Culture System. Experimental Hematology 34, 1353-1359 (2006).
3. Gonçalves, R., da Silva, C.L., Ferreira, B.S.., Lemos, M.A.N.D.A., Lemos, F., Almeida Porada, G., Cabral, J.M.S. Kinetic analysis of the ex-vivo expansion of human hematopoietic stem/progenitor cells. Biotechnology Letters 28, 335-340 (2006).
4. da Silva, C. L., Gonçalves, R., da Silva, C.L., Lemos, M.A.N.D.A., Lemos, F., Almeida-Porada, G., Cabral, J.M.S. Modelling of ex vivo expansion/maintenance of hematopoietic stem cells. Bioprocess Biosyst Eng 25, 365-9 (2003).