MATHEMATICAL MODELING OF PRECURSOR HEMATOPOIETIC STEM CELL MOBILIZATION AND APHERESIS

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Abstract

Hematopoietic stem cells (HSC) travel from bone marrow to peripheral blood and vice versa. We believe that this travel process is crucial for the cells to maintain their stem characteristics and for allowing the homogeneous distribution of the cells in the bone marrow. A mathematical model was developed to describe the mechanism proposed, where stem cells leave the bone marrow upon differentiation to the peripheral blood, and subsequently return to the bone marrow. The experimental data used in this work was obtained from the process of apheresis, where stem cells ($CD34^+$) are collected from peripheral blood. Experimental data from 14 healthy volunteers was used to estimate parameters required for the model and determine ranges for all the variables that characterize the apheresis process. The model describes the apheresis process and provides an estimate for the net transport rate of the cell from the bone marrow. This is the initial theoretical step for characterizing the mobilization process and determining the feasibility and conditions required to experimentally test the proposed mechanism, in order to improve the stem cell collection process and provide new insights for HSC *ex vivo* amplification.

Keywords

Dynamic models, Hematopoietic stem cells, $CD34^+$, Apheresis.

INTRODUCTION

Hematopoietic stem cells (HSC) are the precursor for all the mature blood lineages. These elements are produced through a process of proliferation and differentiation. The amplification process for HSC has not been successfully reproduced *in vitro*, since HSC mature and loose their stem cell characteristics. Thus the perpetual amplification of HSC has not yet been achieved (Jiang, Jahagirdar et al. 2002). This is one of the most sought after goals of cellular therapy, due to the large and increasing number of clinical applications associated with these cells, such as hematopoietic reconstitution after cytotoxic therapies, culture of hematopoietic cells and potential for nonhematopoietic tissue engineering (Collins, Nielsen et al. 1998; Lagasse, Connors et al. 2000).

In adults, the proliferation and differentiation processes occur in the bone marrow. Even though the mechanisms mediating proliferation, differentiation and mobilization of these cells are not fully understood, we know for a fact that HSC migrate from the bone marrow to the peripheral blood and back, thus providing the system with ubiquity and uniformity of HSC distribution in the marrow of the organism (Kronenwett, Martin et al. 2000). It has also been shown that there are significant phenotypic differences between $CD34^+$ cells obtained from bone marrow *vs*. cells obtained from peripheral blood, particularly in genes involved in cell cycle and DNA synthesis (Steidl, Kronenwett et al. 2002). Furthermore, this mobilization and homing processes

appears to be crucial for the cells to retain their stem characteristics, both *in vivo* and *in vitro*.

In the process of apheresis, mononuclear cells are collected from peripheral blood. It has been reported in that this process exerts a stimulatory effect on the release of $CD34^+$ cells into peripheral blood, also known as stem cell recruitment (Cull, Ivey et al. 1997; Fontana, Groebli et al. 2006; Hillyer, Tiegerman et al. 1991). A better understanding of the mobilization and recruitment processes can help improve peripheral blood stem cell collection and transplantation.

In this work we propose a mathematical model to describe the mobilization and return processes of stem cells during apheresis. Data collected from 14 human volunteers was used to estimate parameters required for the model and determine ranges for all the variables that characterize the apheresis processes. The model accurately describes the apheresis process and provides an estimate for the net transport rate of the cell from the bone marrow. This is the initial theoretical step to determine the feasibility and conditions required to experimentally test the proposed mechanism and improve the stem cell collection process and to provide new insights for HSC *ex vivo* amplification.

Cellular Mobilization Model

Two complementary theories help us to understand how the HSC perform their intrinsic function. HSC need to proliferate in order to produce the differentiated cells lineages of the blood; however it is crucial for cells to maintain their stem function for the continuity of the system. According to the niche theory, there is a specific place in the bone marrow where cells can retain their stem characteristics. The mobilization process is initiated by stress-induced activation due to stimulation with granulocyte colony-stimulating factor (G-CSF), which results in the release of stem cell factor, proliferation of progenitor cells, as well as changes in adhesion molecules (Lapidot and Petit 2002). When cells mobilize from the bone marrow and proliferate, a stem cell can return to its niche and maintain its multipotent characteristics. The stochastic theory indicates that stem cells stochastically decide either to differentiate into a committed blood cell lineage or maintain their stem condition (Hoffman, Benz et al. 2004). It is possible that cells randomly commit to differentiation into a blood lineage but need a niche for their differentiation. None of the two models fully explains the phenomenon, since the fact that HSC are able to migrate to peripheral blood and return to bone marrow is not considered in either of them. These mobilization and return processes have been widely described, and are taken advantage of for HSC collection from peripheral blood, and for autologous and allogenic stem cell transplants.

We propose a model that is complementary to the two theories mentioned above. Experimental data has shown that there is a difference between mobilized stem cells and bone marrow cells. We believe mobilization to be essential for cells to maintain their stem cell characteristics. The end of the cellular cycle coincides with the ability of cells to migrate, so cells can mobilize from bone marrow to peripheral blood. Daughter cells that remain in the marrow most likely enter apoptosis. When mobilized, cells undergo changes that allow them to retain their multipotency and self-renewal characteristics. When cells that migrated to peripheral blood return to the niche, they recover their quiescent stem condition, duplicate or differentiate, therefore maintaining a homogeneous hematopoietic distribution in the bone marrow (see Figure 1).

In summary, passing to peripheral blood appears to be a requirement for cells to maintain their stem condition, to ensure functional migration, homing, and repopulation potential (Cottler-Fox, Lapidot et al. 2003; Voermans, Kooi et al. 2001). Mathematical modeling of this process dynamics is essential to understand HSC $CD34^+$ residence time in peripheral blood and determine the main variables involved in this process.

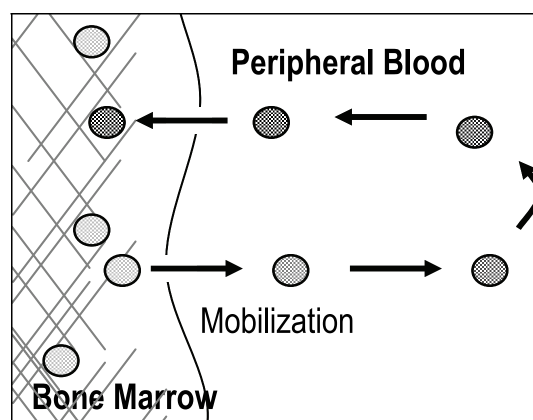

Figure 1: Diagram for cell mobilization.

MATERIALS AND METHODS

Cell Collection

Human peripheral blood mononuclear cells were collected from 72 volunteers who donated hematopoietic stem cells for either autologous or allogenic transplant. Three distinct volunteer data groups were distinguished: patients with multiple myeloma, patients with lymphoma and healthy donors. The volunteers received 300 μg/day of human

Table 1: Experimental data (average values) pre and post apheresis for healthy donors.

Bone Marrow Volume	[ml]	1.27E+03 ± 5.37E+02
Total Blood Volume (TBV)	[ml]	4.59E+03 ± 9.49E+02
Pre Apheresis CD34+	[cells/ml]	4.73E+07 ± 5.86E+07
Post Apheresis CD34+	[cells/ml]	8.15E+03 ± 3.26E+03
Leuco Pre aferesis	[cells/ml]	4.46E+07 ± 1.14E+07
Leuco Post aferesis	[cells/ml]	3.45E+07 ± 2.62E+06
Apheresis Time	[min]	2.13E+02 ± 2.44E+01
Apheresis Volume	[ml]	3.43E+02 ± 4.17E+01

recombinant G-CSF for three days and then 600 μg/day until cell collection was completed. Collection began on day 5. Candidates for autologous transplant received chemotherapy in addition to G-CSF and cells were collected in the recovery period. Healthy donors received only G-CSF. Cells were collected by large volume leukapheresis using a COBE-Spectra cell separator Apheresis System v5.1 (*Gambro BCT, Lakewood, USA*). Four total peripheral blood volumes were processed for each patient. $CD34^+$ cell concentration was determined by flow cytometry quantization according to International Society for Cell Therapy (ISCT) protocols (Sutherland, Anderson et al. 1996). $CD34^+$ cell concentration was monitored, previous to cell collection (pre apheresis), after cell collection (post apheresis) and in the collection process (apheresis).

RESULTS AND DISCUSSION

Healthy donors have in circulation an average of 4.7×10^7 $CD34^+$ cells/lt pre-apheresis. Differences in mobilization were observed between the three patients group, with a higher $CD34^+$ number in circulation for chemotherapy treated patients (2.7×10^8 $CD34^+$ cells/lt for lymphoma patients and 3.7×10^8 $CD34^+$ cells/lt for myeloma patients).

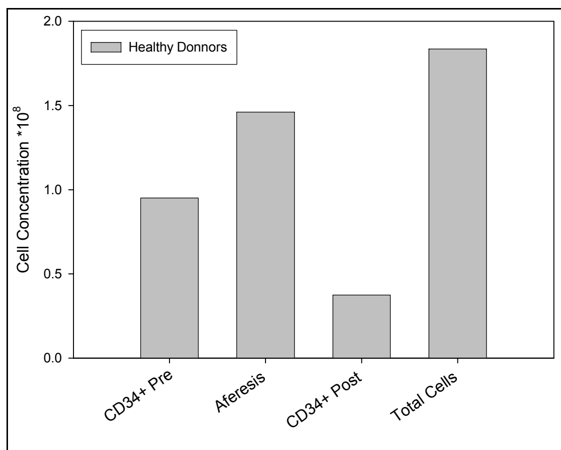

Figure 2: CD34+ recruiting. CD34+ cells in PB pre apheresis, collected and post apheresis. The total cells column corresponds to CD34+ cells in PB plus collected cells.

As shown in Figure 2, we observed that for healthy donors, pre-apheresis $CD34^+$ cell number in PB is over 50% lower than the total cell number post apheresis (calculated as the sum of $CD34^+$ post apheresis and cells collected in the apheresis process). This reveals a recruiting phenomenon during the apheresis process since the total number of $CD34^+$ cells after the apheresis process is 90% higher than pre-apheresis. This phenomenon was observed in all three patient groups (data not shown), and has been reported to occur as a result of apheresis (Fontana, Groebli et al. 2006; Moller, Dickmeiss et al. 2001).

Mathematical Model Proposed

To represent the apheresis process, we developed a compartmentalized dynamic model based on mass balances for blood mononuclear cells ($CD34^+$, leucocytes). This model considers three separate CSTR compartments: bone marrow (V_M), peripheral blood (V_P), and the apheresis bag (V_A). All three compartments were assumed as continuous stirred tank reactors. Three fictitious elements were included: A reactor tank T, to account for changes and difference between bone marrow HSC (C) and peripheral blood HSC (C^*); a separator S for the returning of undifferentiated HSC to the bone marrow; and a separator S' to represent the selectivity of the apheresis process. Details for the process representation are shown in Figure 3. Data including PB volume, apheresis volume, apheresis time, pre and post apheresis cell counts, and collected cell number collected from 14 healthy volunteers, was used to estimate parameters required for the model and determine ranges for all the variables that characterize the apheresis processes. Average values used for simulation are listed on Table 1. An average bone marrow volume for human adults reported in literature was considered (Zingsem, Zeiler et al. 1993).

HSC cells (C) and leucocytes (L) are produced in the bone marrow at a rate r_0. These cells travel to the peripheral blood at a rate r_M. Once mobilized, HSC

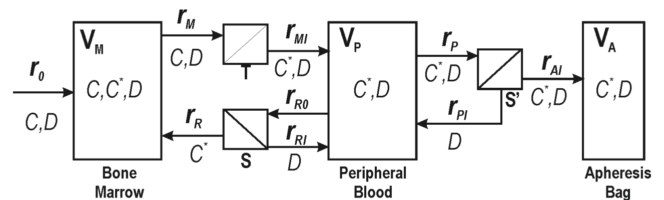

Figure 3: Proposed representation of the apheresis process.

undergo changes (C*) that differentiate them from bone marrow cells (C). Modified cells go to the peripheral blood. Some of these cells are able to return to the bone marrow, at a rate r_R. HSC from peripheral blood are selectively collected during the apheresis process at a rate

r_A. The initial bone marrow production rate was estimated asuming than in 45 days 80% of an individuals bone marrow can be replenished, as reported in literature (Van Zant, Chen et al. 1991).

After simplification, the set of equations derived for this system is the following:

$$\frac{dC_{V_M}}{dt} = \frac{r_0}{V_M} C_0 - \frac{r_M}{V_M} C_{V_M} \quad (1)$$

$$\frac{dC^*_{V_M}}{dt} = \frac{r_R}{V_M} C^*_P \quad (2)$$

$$\frac{dD_{V_M}}{dt} = \frac{r_0}{V_M} D_0 - \frac{r_M}{V_M} D_{V_M} \quad (3)$$

$$\frac{dC^*_P}{dt} = \frac{r_{MI}}{V_P} C_{V_{MI}} - \frac{r_{R0}}{V_P} C^*_P - \frac{r_P}{V_P} C^*_P \quad (4)$$

$$\frac{dD_P}{dt} = \frac{r_{MI}}{V_P} D_{VMI} - \frac{r_{R0}}{V_P} D_P + \frac{r_{RI}}{V_P} D_{RI} - \frac{r_P}{V_P} D_P + \frac{r_{PI}}{V_P} D_{PI} \quad (5)$$

$$\frac{dC^*_A}{dt} = \frac{r_{AI}}{V_A} C^*_{AI} - \frac{r_{AI}}{V_A} C^*_A \quad (6)$$

$$\frac{dD_A}{dt} = \frac{r_{AI}}{V_A} D_{AI} - \frac{r_{AI}}{V_A} D_A \quad (7)$$

$$\frac{dV_A}{dt} = r_{AI} \quad (8)$$

Subject to the following restrictions derived from the steady state conditions imposed for all fictitious elements:

$$r_M C_M = r_{MI} C^*_{MI} \quad (9)$$

$$r_P C^*_P = r_{AI} C^*_{AI} \quad (10)$$

$$r_P D_P = r_{AI} D_{AI} + r_{PI} D_{PI} \quad (11)$$

$$r_{R0} C^*_P = r_R C^*_R \quad (12)$$

$$r_{R0} D_{R0} = r_{RI} D_{RI} \quad (13)$$

The model, shown in Eq. 1-6, was implemented in Matlab© (Mathworks Inc., R2006a), using average preapheresis concentrations for leucocytes, and $CD34^+$ measured experimentally as initial conditions (see Table 1). We assumed fast dynamics for the intermediate fictitious elements, and enforced a steady state condition on them. The generation of bone marrow cells was estimated from reported data indicating that in 45 days 80% of bone marrow is replenished. Volume and pressure considered as constant for all compartments, except for the apheresis bag whose volume increases during the apheresis process.

The model proposed allowed us to estimate the dynamics of the apheresis process. Simulation results in Figure 4 show how the concentration of $CD34^+$ and leucocytes decreases in PB as the apheresis process progresses, with a larger reduction in $CD34^+$ concentration in PB (Figures 4-A, 4-B, respectively). The total amount of $CD34^+$ cells, shown in Figure 4-C, increases with a smaller increase slope as the process progresses, due to the reduction of $CD34^+$ cell concentration in PB, while the volume increases at a constant rate (Figure 4-D). In the apheresis bag we see an reduction in concentration, since, as the process progresses, there are less cells to be collected from PB, for both $CD34^+$ cells and leucocytes, (Figure 4-E and F). This implies that initially, more cells are collected per unit of processed volume. Total cell concentration in the apheresis bag reach similar values as obtained experimentally, of the order of $2 * 10^7$ cells, with a small overestimation by the model in the amount of cells collected, since all separation processes are considered as ideal. The model, along

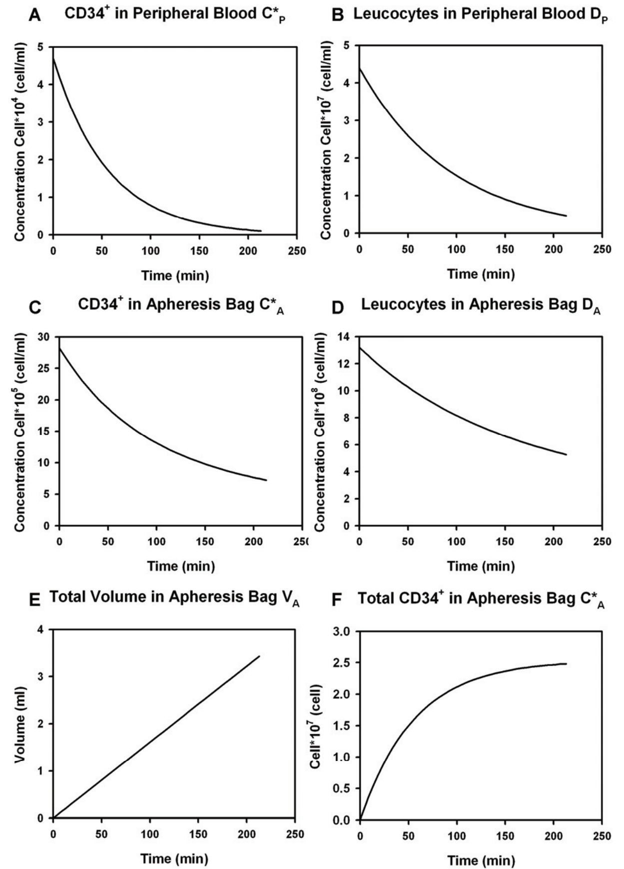

Figure 4: Simulation results for the apheresis process.

with our experimental data allowed us to estimate a net mobilization rate from the bone marrow, which corresponds to the difference between the forward and reverse transport rates ($r_M - r_R$). This net rate can not be measured in vivo and its determination is crucial for further understanding the influence of the apheresis process in the mobilization of cells, and the mobilization process itself.

With the mathematical model developed, presented in this work, we are able to describe and predict the dynamics for cell concentration in all the compartments relevant to the apheresis process and obtain an estimate for the net mobilization rate of hematopoietic stem cells with and without apheresis. This can be used as a starting point to determine other parameters associated to the process and characterize the non-iedalities associated with it. We expect this will lead us towards a better understanding on how the mobilization process occurs, and if we can take advantage of it to improve stem cell collection and for *ex-vivo* amplification of HSC.

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