A DETERMINISTIC MODEL OF BONE MARROW WITH HOMEOSTATIC PROPERTIES AND WITH STEADY PRODUCTION OF DIFFERENTIATED CELLS

Manish P. Kurhekar and Umesh A. Deshpande
Department of Computer Science and Engineering, Visvesvaraya National Institute of Technology, Nagpur, India

Keywords: Stem cell modeling, Agent based simulation.

Abstract: There is a significant interest in studying stem cells, to learn about the biological functions during development and adulthood as well as to learn how to utilize them as new sources of specialized cells for tissue repair. Modeling of stem cells not only describes, but also predicts, how a stem cell's environment can control its fate. The first stem cell populations discovered were Hematopoietic Stem Cells (HSCs). In this paper, we present a biologically feasible deterministic model of bone marrow that hosts HSCs. Our model demonstrates that a single HSC can populate the entire bone marrow. It almost always produces sufficient number of differentiated cells (RBCs, WBCs, etc.). It also overcomes the biological feasibility limitations of previously reported models.

We have performed agent-based simulation of the model of bone marrow system proposed in this paper. We have included the details and the results of this validation using simulation in the Appendix. The simulation also demonstrates that a large fraction of stem cells do remain in the quiescent state. The program of the agent-based simulation of the proposed model is made available on a public website.

1 INTRODUCTION

Stem cells and their descendents are the building blocks of life. How stem cell populations guarantee their maintenance and self-renewal, and how individual stem cells decide to transit from one cell stage to another to generate different types of mature differentiated cells are long standing and fascinating questions (Roeder and Radtke, 2009). There is a significant interest in studying stem cells, both to elucidate their basic biological functions as well as to learn how to utilize them as new sources of specialized cells for tissue repair (O'Neill and Schaffer, 2004). There are several major challenges within the field, such as the identification of new signals and conditions that regulate and influence cell function, and application of this information towards the design of stem-cell bioprocesses and therapies. Both of these efforts can significantly benefit from the synthesis of biological data into quantitative and increasingly mechanistic models that describe and predict how stem cell can control its fate.

Blood is the life preserving fluid, whose major functions are supply of nutrients and oxygen to the tissues, self-immunity and defense against pathogens. In order to carry out these tasks, human blood contains a variety of cells, each precisely adapted to its specific objective. All the different blood cells develop from a kind of a master cell, called the Hematopoietic (blood forming) Stem Cell (HSC). HSCs are stem cells that give rise to all the differentiated blood cell types including White Blood Cells (WBC), Red Blood Cells (RBC) and Platelets. HSCs are primarily present in the bone marrow. Fully mature differentiated cells migrate into the blood stream leaving back an empty space in the bone marrow. The transition of HSCs from quiescence (not undergoing any cell cycle) into proliferation, or differentiation, is governed by their cell-cycling status and by hormones secreted by neighboring cells in their immediate microenvironment.

It is believed that one HSC is sufficient to reconstitute the entire blood system (De Haan, Dontje and Nijhof, 1996). This extraordinary regenerative ability of the bone marrow is not surprising, considering that it has a vital role that

P. Kurhekar M. and A. Deshpande U..
A DETERMINISTIC MODEL OF BONE MARROW WITH HOMEOSTATIC PROPERTIES AND WITH STEADY PRODUCTION OF DIFFERENTIATED CELLS.
DOI: 10.5220/0003706200150023
In *Proceedings of the International Conference on Bioinformatics Models, Methods and Algorithms* (BIOINFORMATICS-2012), pages 15-23
ISBN: 978-989-8425-90-4

must remain unaffected by stem cells depletion, e.g. as a result of chemotherapy, radiation or disease. It should be emphasized that though the supply of blood cells in the periphery is steady, the bone marrow, considered as a physical entity is not static. It is dynamic in the sense that it constantly changes in its constitution and arrangement, and these changes occur at varying rates. The bone marrow is in the state of homeostasis that can be considered as a dynamic equilibrium between its constituents.

Theise and Harris (2006) in their paper describe how stem cells and their lineages are examples of complex adaptive systems. Profound understanding of a complex adaptive system can be gathered by generating computer models using computational techniques. Agent based modeling is a way to represent such complex adaptive systems in software. An agent is a high-level software abstraction that provides a convenient and powerful way to describe a complex software entity in terms of its behavior within a contextual computational environment. Agents are flexible problem-solving computational entities that are reactive (respond to the environment), autonomous (not externally controlled) and interact with other such entities.

To understand the behavior of the blood system, modeling of HSCs and their behavior in different circumstances is an area of active research. One of the significant contributions to stem cell modeling was a paper by Agur, Daniel and Ginosar (2002). The main aim of their paper was to provide a mathematical basis for the bone marrow homeostasis. More precisely, they wanted to define simple properties that enabled the bone marrow to rapidly return to a steady supply of blood cells after relatively large perturbations in stem-cell numbers. Their model is represented as a family of cellular automata on a connected, locally finite undirected graph. Their model can be briefly described as follows. It has three types of cells, stem cells, differentiated cells and null cells. Each cell has an internal counter. Stem cells differentiate when their immediate neighborhood is saturated with stem cells and their internal counter reaches a certain threshold. A differentiated cell converts to a null cell after its internal counter crosses the required threshold – a process that denotes the passing of a differentiated cell to blood stream leaving empty the place it had earlier occupied in the bone marrow. A null cell, with a stem cell neighbor, is converted to a stem cell when its internal counter reaches a particular threshold.

d'Inverno and Saunders (2005) have listed the following drawbacks of Agur et al.'s (2002) model.

1. The specification of Agur et al's model reveals that the null cells must have counters. In a sense, an empty space has to do some computational work. This lacks biological feasibility and is against what the authors state about modeling cells, rather than empty locations, having counters.

2. Stem cell division is not explicitly represented; instead, stem cells are brought into existence in empty spaces.

3. A stem cell appears when a null cell has been surrounded by at least one stem cell for a particular period. However, the location of the neighboring stem cell can vary at each step.

4. In the model, if a stem cell is next to an empty space long enough then it divides so that its descendent occupies this space. However, an empty cell might be a neighbor of more than one stem cell. The rule does not state that a particular neighboring stem cell must be present for every tick of the counter. Biologically it would be more intuitive to have the same stem cell next to a null cell for the threshold length of time in order for division to occur into the null cell space but the model lacks any directional component.

5. The state of a stem cell after division is not defined. Nothing is said about what happens to a stem cell after a new stem cell appears in the null cell space. For example, should the counter of the stem cell be reset after division? Neither does it give any preconditions on the particular neighboring stem cell S that was responsible for converting the null cell space to a stem cell. For example, should S's local counter have reached an appropriate point in its cycling phase for this to happen?

In order to overcome the limitations, d'Inverno and Saunders (2005) introduced the concept of a controlling microenvironment that links a null cell that has reached a threshold with a stem cell that can differentiate. All the cells send and receive signals from the microenvironment and act on its suggestions. They also performed an agent based implementation with the incorporation of Agur et al.'s model in two dimensions. However, the improvement suggested by them does not have any biological basis. Moreover, there are additional limitations of the model described by Agur et al., which have not been considered by d'Inverno and Saunders (2005). The additional limitations are discussed below.

1. There are no intermediate cells or transitive cells in the model proposed in Agur et al. (2002). Transitive cells are intermediate cells that have limited stem cell like properties and they are

eventually converted to mature differentiated cells. For Hematopoietic system, common lymphoid progenitor (CLP) and common myeloid progenitor (CMP) are transitive cells (Gordon, 2007).

2. As an effect of the fourth drawback mentioned above, a stem cell can potentially differentiate more than once in the same time instant since it might be surrounded by more than one empty cell. Hence, it can potentially convert more than one of its neighboring empty cells into stem cells. Clearly, this lacks biological feasibility.

In this paper, we have addressed all the limitations by augmenting the model proposed by Agur et al. (2002), thereby making the model closer to biological reality. The model we present is aimed to simulate a situation in which a cell's behavior is determined only by a combination of the types and states of cells in its proximity and its own cell cycle represented by its internal counter. The main assumptions of our model are:

- Cell behavior is determined by the number and type of its neighbors. This assumption is aimed at describing the fact that cytokines, secreted by cells into the microenvironment are capable of activating cells into changing their types (De Haan et al., 1996) (Roeder and Radtke, 2009).
- Each cell has internal counters, which determine the time required by the cells to change its type, as well as the transit time of a differentiated cell before it migrates to the blood stream.

To validate the model, we have performed an agent-based simulation of the model of bone marrow stem cell system proposed in this paper. The program for the same is available on the website: http://sites.google.com/site/stemcell
model. We have included the results of validation of the proposed model using agent-based simulation in the Appendix.

The paper is organized as follows. In the next section, we describe our model and the rules that govern it. Later we show how a single stem cell can populate the bone marrow. In section 3, we show that the model almost always provides a steady supply of differentiated cells to the blood stream. In section 4, we show the steady states and death and we conclude the paper in section 5. The results of the agent-based simulations are included in the Appendix.

2 DESCRIPTION OF THE MODEL

Our model contains three basic types of cells and a notation for an empty space:

- **Stem** cell (S), either can proliferate generating new stem cells or can convert to a transitive cell.
- **Transitive** cell (T), either can convert to a differentiated cell or can convert back to a stem cell when there are no stem cells in its near neighborhood.
- **Differentiated** cell (D), is the final product of a stem cell. After maturation, these cells leave the bone marrow and circulate in the blood, leaving back empty space.
- **Empty** space (E), represents vacant space in the bone marrow.

In our model, the bone marrow is represented as a connected, locally finite undirected graph. This describes the neighborhoods of bone marrow cells.

Let $G = (V, L)$ be a connected, locally finite undirected graph that denotes the bone marrow. Its vertex set V denotes the cells and the set of edges L describes the neighboring cells to which a cell is connected to in the bone marrow (Figure 1).

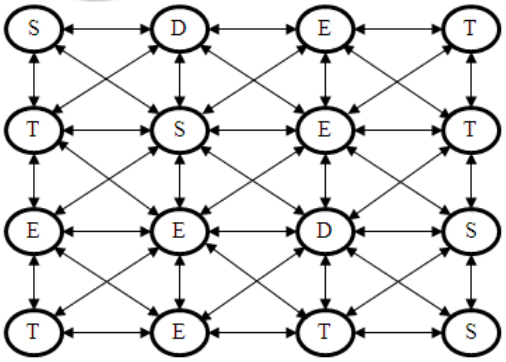

Figure 1: Example graph showing part of bone marrow system in 2-Dimension. Each vertex is a cell and it has eight neighbors. The label of vertex denotes its type.

Diagrammatically, the transitions of different types of cells in Agur et al.'s (2002) model and our proposed model are depicted in Figure 2 (N denotes a null cell in Agur et al.'s model).

For every $u, v \in V$ we denote by $\rho(u, v)$ the distance between these vertices in the shortest-path metric induced by G. $N(v) = \{u \in V \mid \rho(u, v) = 1\}$ denotes the *immediate neighborhood* of a vertex $v \in V$, i.e. the set of vertices joined to v by an edge. $B(v, n)$ denotes the *ball of radius n* centered in $v \in V$. It is

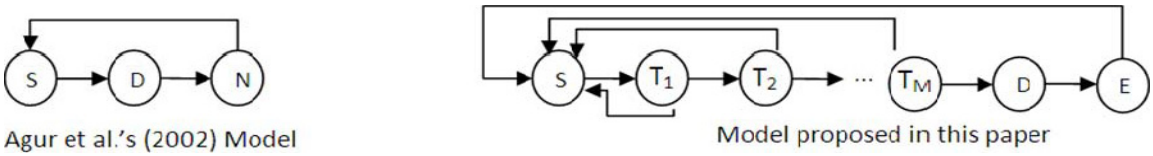

Figure 2: Comparison of Agur et al.'s (2002) model and the model proposed in this paper.

the set of all vertices such that their distances from v do not exceed n. We write $B(v, n) = \{u \in V \mid \rho(u, v) \leq n\}$. $B(v, n)$ defines the *near neighborhood* of size n of vertex v.

If $U \subseteq V$ is a nonempty subset of vertices then for every $v \in V$ let $\rho_U(v) = \min_{u \in U} \rho(u, v)$ be the minimum distance between v and another vertex u contained in set U.

A *state* of a vertex is a 1-tuple, a 2-tuple or a 3-tuple depending on the cell type. The first coordinate denotes the cell's type (S, *T, D* or *E* denoting a stem cell, a transitive cell, a differentiated cell or an empty space respectively). For a stem cell, the second coordinate denotes the direction of proliferation and the third coordinate denotes the simulated time τ as an internal counter. For a transition cell, the second coordinate denotes its generation (progeny) while the third coordinate denotes the simulated time. A differentiated cell has only two coordinates and the second coordinate denotes the simulated time. Finally, an empty space does not have any counter or any other property, thus it has a single coordinate that denotes the type.

Let μ be the maximum number of immediate neighbors possible for any cell. μ also denotes the number of directions for a stem cell to proliferate. A stem cell, when it proliferates, can occupy an empty space, if available, in its immediate neighborhood.

A transitive cell can go through several generations (progeny) before it converts to a differentiated cell. A transitive cell moves from one generation to another after its internal counter reaches a certain threshold. There are M generations for a transitive cell, where M is greater than or equal to 1. When a transitive cell has moved into its last generation (i.e. M^{th} generation) and when its internal counter reaches a threshold, it converts to a differentiated cell. In circumstances when there is not even a single stem cell in the near neighborhood of a transitive cell, a transitive cell converts back to a stem cell. The rules given below also capture the fact that a transitive cell's ability to convert back to a stem cell diminishes with each subsequent generation. Let η denote the distance multiple for a transitive cell to convert back to a stem cell. Thus, the conversion from a transitive cell to a stem cell depends on the distance multiple η and its current generation.

Let Ω be the set of states of a vertex.

A map x: $V \rightarrow \Omega$ is the state of the entire graph. The set of all the states of the bone marrow graph G is denoted by Ω^V. A state $x \in \Omega^V$ of the bone marrow graph G at time t is denoted by x^t. The state of a vertex v at time t is denoted by $x^t(v)$.

With the above definitions, we are now ready to define the rules of an iterative operator on all states Ω^V. It depends on three positive nonzero integers Φ, Ψ, and Θ. The rules for the state changes can be regarded as describing a family of cellular automata.

The first sub-rule of rule (1) states that a stem cell converts to a transitive cell, if its internal counter representing its cycling phase has reached a threshold Ψ and its immediate neighborhood consists only of stem cells. This corresponds to receiving a signal that the microenvironment is saturated with stem cells. The evidence for such a feedback is provided by De Haan et al. (1996), where the authors show that Hemopoietic cell amplification in vivo is regulated by various mechanisms that appear to be under control of many Hemopoietic growth factors, including the activation and deactivation of the quiescent stem cells into the cell cycle. The second sub-rule within this rule specifies that if a stem cell's internal counter has reached a threshold Ψ but its immediate neighborhood is not saturated by stem cells, then the stem cell enters into a quiescent state, i.e. it retains the same state. The third sub-rule states that when a stem cell's internal counter reaches a threshold Ψ and there exists an empty space in its neighborhood, then it proliferates such that one of its descendants occupies the empty space and the other remains in the original location. The sub-rule also defines that the new stem cell as well as the stem cell at original location receive the renewed biological time. With this sub-rule, we also denote a systematic way of choosing the empty space for proliferation. The method we propose is by adding a directional component d in the state of every stem cell and by arranging all the possible directions μ in a circular (round-robin) order. A stem cell proliferates in the empty space in the direction of the directional component d of its state. If the direction given by the directional component d of

$$x^t(v) = (S,d,\tau) \Longrightarrow$$
$$x^{t+1}(v) = \begin{cases} (T,1,0) & \text{if } \forall u \in N(v), x^t(u) = (S,*,*) \wedge \tau = \Psi \\ (S,d,\Psi) & \text{if } \exists u \in N(v), x^t(u) \neq (S,*,*) \wedge \tau = \Psi \\ \{x^t(v) = (S,(d+1) mod\ \mu,0), x^t(u) = (S,0,0)\} & \text{if } \exists u \in N(v), x^t(v) = (S,d,\Psi) \wedge x^t(u) = E \\ (S,d,\tau+1) & otherwise \end{cases} \quad (1)$$

$$x^t(v) = (T,g,\tau) \Longrightarrow x^{t+1}(v) = \begin{cases} (D,0) & \text{if } \exists u \in B(v,\eta * g), x^t(u) = (S,*,*) \wedge \tau = \Theta \wedge g = M \\ (T,g+1,0) & \text{if } \exists u \in B(v,\eta * g), x^t(u) = (S,*,*) \wedge \tau = \Theta \wedge g \neq M \\ (S,0,0) & \text{if } \forall u \in B(v,\eta * g), x^t(u) \neq (S,*,*) \wedge \tau = \Theta \\ (T,g,\tau+1) & otherwise \end{cases} \quad (2)$$

$$x^t(v) = (D,\tau) \Longrightarrow x^{t+1}(v) = \begin{cases} (E) & \text{if } \tau = \Phi \\ (D,\tau+1) & otherwise \end{cases} \quad (3)$$

$$x^t(v) = (E) \Longrightarrow x^{t+1}(v) = (E) \quad (4)$$

the state is occupied by any cell, then the stem cell continues to choose the next direction, in the round-robin order, for availability of the empty space. After proliferation, the directional component of stem cell is incremented to point to the next direction. The last sub-rule states that if the internal counter of a stem cell has not reached a threshold Ψ then it is just incremented.

Transitive cells are intermediate cells that can convert back to stem cells if there are not enough stem cells in their near neighborhood, a situation that can occur following radiation or organ damage. Theise and Harris (2006) detail the dedifferentiation, i.e., reversion of an intermediate cell into a stem cell. Rule (2) states that when a transitive cell's internal counter reaches a threshold Θ it moves on to the next generation unless it is not in its last (M^{th}) generation. If a transition cell's counter has reached the threshold Θ and it is in its last generation then it gets converted to a differentiated cell. In certain circumstances when a transitive cell does not have any stem cell in its near neighborhood then it gets converted back to a stem cell. The near neighborhood is governed by a constant η and the generation of the transitive cell. The stem cell like property of a transitive cell goes on decreasing with subsequent generations. The near neighborhood size to find a stem cell keeps on increasing with each subsequent generation of a transitive cell, implying its reduced capacity to regenerate and the requirement of an even stronger signal to convert back to a stem cell.

Rule (3) states that when a differentiated cell's internal counter reaches a threshold time Φ, it maturates. After maturation, the cell migrates to the blood stream leaving the original space occupied by the differentiated cell as empty space.

Rule (4) specifies that an empty space does not change by itself. It does not have any internal counter nor is involved in any computation.

We show next that the proposed model has strong homeostatic properties, similar to Agur et al.'s model.

3 HOMEOSTASIS PROPERTY OF THE BONE MARROW MODEL

We begin by investigating the property of stem cells to expand throughout the bone marrow. The following lemma shows that any point in the bone marrow graph gets occupied by a stem cell, given that initially there is at least one stem cell in the bone marrow graph.

Lemma 1. *For any Φ, Ψ, Θ if there exist two vertices $u, v \in V$ such that at some time t, vertex v is not occupied by a stem cell and u is, then there exists an $s > 0$ such that v will be occupied by a stem cell at time $t + s$.*

Proof: From rule (1), we conclude that if u and v are neighbors then u remains a stem cell as long as v is not a stem cell. The vertex v itself turns into a stem cell in no more than $\Phi+\mu\Psi$ time steps. This is the maximum time required including the time required for cell at vertex v to migrate to the blood stream (in case it was a differentiated cell), turn into an empty space and as it is a neighbor of a stem cell, become a stem cell after a maximum $\mu\Psi$ time steps. We can use induction on the distance $\rho(u, v)$ to obtain a bound on the time that is needed for v to turn into a stem cell:

$$s \leq \Phi + \mu\, \rho(u, v)\, \Psi \quad (5)$$

The proof above conveys that the distance $\rho_{U(t)}(v)$ between a vertex v, which is not occupied by a stem cell at time t, to the subset $U(t) \subseteq V$ of vertices which includes a stem cell vertex at time t is a non-increasing function. Furthermore, there exists $s \leq \Phi + \mu\rho_{U(t)}(v)\Psi$ such that $\rho_{U(t+s)}(v) = 0$.

We now show that if $r \geq t + s$ then $\rho_{U(r)}(v) \leq M\eta$ in any two consecutive time slots. This means that from the time $t + s$ onwards there always is a stem

cell not farther than $M\eta$ edges from v in any two consecutive time slots.

Lemma 2. *Suppose that a vertex v becomes a stem cell at time t_0, then for every $t \geq t_0$ there is a vertex $u \in B(v, M\eta)$ which is occupied by a stem cell at time t or t+1.*

Proof: A necessary condition for the production of a stem cell at a vertex v at time t_0 is that $\exists v' \in N(v)$, $x^{t_0-1}(v') = (S,*, \Psi)$. Now, the cell at vertex v remains a stem cell until last three conditions of rule (1) hold. Therefore, if the cell at vertex v becomes a transitive cell at time $t_1 > t_0$, either it still has a stem cell neighbor at time t_1 or all of its neighbors become transitive cells simultaneously with v. If it is the first scenario then we are done. The second scenario can happen only if all the stem cells have their internal counters synchronized and reach the threshold Ψ simultaneously at time t_1. In such a case, either there is a stem cell in the near neighborhood of size $M\eta$ or the vertex v will again convert from a transitive cell to a stem cell at time $t_1 + 1$ as all its near neighbors are not stem cells. Thus if v is not a stem cell, there is a stem cell in $B(v, M\eta)$ at time t_1 or t_1+1. Applying Lemma (1) ensures that until the next time the vertex v is occupied by a stem cell, the distance from v to the closest stem cell will not exceed $M\eta$ in any two given consecutive time instances.

A direct conclusion from Lemma (2) is the estimation for the density of stem cells in bounded vicinity. We state the same in the following lemma for graphs with bounded degree. The bone marrow can be described as a graph of bounded degree with each vertex connected only to its adjacent vertices.

We need two more notations:

If the graph G has the property that there exists Δ such that $|N(v)| \leq \Delta$, $\forall v \in V$, we say that G has *bounded degree*, and write $deg(G) \leq \Delta$.

The *density* of stem cells in a given finite subset of vertices $U \subset V$ at time t is the proportion at time t of the number of stem cells S in U and the total number of vertices in U. It is denoted by $\delta_t(U)$.

Lemma 3. *Let G be a graph of bounded degree. Suppose that at some time t_0 a vertex v is occupied by a stem cell, then for every ball $B = B(v, M\eta) \subset G$,* $\lim_{t\to\infty} \delta_t(B) \geq 1/(2*(\Delta^{M\eta} + 1))$.

Proof: By Lemma (1) and Lemma (2), any ball of radius $M\eta$ admits a stem cell from a certain moment on for any two consecutive time slots. The size of such a ball contains less than or equal to $\Delta^{M\eta} + 1$ vertices.

In essence Lemma (1), Lemma (2) and Lemma (3) show that not only is it true that one stem cell is sufficient to bring back the bone marrow system homeostasis, it is also true that the bone marrow has a built-in mechanism guaranteeing that stem cells do not become too scattered. Every ball of radius $M\eta$ is occupied by at least one stem cell at any two consecutive time steps from the moment it was occupied by a first stem cell.

4 STEADY PRODUCTION OF DIFFERENTIATED CELLS

We have seen that stem cells do fill the bone marrow graph nicely. In this subsection, we show that the system almost always generates enough mature differentiated blood cells. Before proving the same, we mention some observations:

- When a transitive cell is created and if it has a stem cell neighbor, then it would always proceed to create a differentiated cell. The stem cell neighbor will remain a stem cell at least till the time a transitive cell becomes a differentiated cell, the differentiated cell becomes an empty space and the empty space is occupied by another stem cell.
- Starting from non-saturated, if the complete available space is to be saturated with stem cells then every stem cell should divide into two stem cells and any stem cell should not convert to a transitive cell. If any stem cell converts to a transitive cell, then the condition above will ensure that it becomes a differentiated cell.

An extreme situation can occur, when the system contains only stem cells at a time t and the internal counters of all stem cells are synchronized. In such a case, all the stem cells will convert to transitive cells on or before $t + \Psi$. At the next time instant, all these transitive cells will convert back to stem cells, as there will not be a single stem cell in their near neighborhood. This system would not produce any differentiated cells, but will also not die out. We can call such a state as *resonant state* as the cells will resonate between stems cells and transitive cells without producing any differentiated cells.

A resonant state can occur for a block of holding capacity of 2^{μ} cells if it is occupied completely by stem cells starting from a single stem cell in $\mu\Psi$ number of time steps. The physical occupancy of stem cells in a given block depends largely on the round-robin way of choosing the directions and

initial stem cell population. If the manner in which the round-robin arrangement of directions is clockwise or counter-clockwise then the resonant state would not occur, if starting with a single cell in two dimensional space as $2^{\mu}/\mu^2$ is greater than 1 when μ is greater than 4. For example, with $\mu = 8$ in a two dimensional space, $2^8 = 512$ thus in 8Ψ time steps 512 cells would be generated, but the ball of radius 8 from the vertex v can hold only $(8+1+8)^2 = 289$ number of cells. Thus, some stem cell would be surrounded by stem cells within 8Ψ time steps and it would convert to a transitive cell. The possibility of reaching a resonant state drops further after considering the co-ordination of a similar event in neighboring blocks.

A resonant state would occur if all the cell positions are occupied by stem cells and their internal time is also synchronized. This is an extreme case. Thus, there are very few resonant states out of the total number of states and hence, there is a very low possibility that the model will be in a resonant state.

Lemma 4. *Suppose that a vertex $v \in V$ is occupied by a stem cell or a transitive cell at time t. Then either v or one of its near neighbors in B(v, Mη) will be occupied by a differentiated cell within $(\mu+1)\Psi + (M+1)\Theta + 1$ iterations unless the system is in a resonant state.*

Proof: Assume that at vertex v there is a stem cell that has no differentiated neighbors, otherwise we are done. $N(v)$ will consist only of stem cells by at most $\mu\Psi$ time steps. Then v or one of its neighbors will convert to a transitive cell after Ψ time steps. Such a transitive cell will always have stem cells in near neighborhood. Then after M generations of a transitive cell, it would convert to a differentiated cell, i.e. after $(M+1)\Theta$ time steps.

If v is a transitive cell and if v has a stem cell in its near neighborhood then after $(M+1)\Theta$ time steps it becomes a differentiated cell. If v is a transitive cell and if v does not have any stem cell in its near neighborhood, it becomes a stem cell in the next time instance and the argument above follows.

Thus, except in the case of a resonant state, there is a differentiated cell every $(\mu+1)\Psi + (M+1)\Theta + 1$ iterations in $B(v, M\eta)$.

Lemma (4) shows that in an eventuality of a severe perturbation, a transitive cell will convert back to a stem cell and bring back the entire system to a steady state as shown in Lemma (3).

Note that in this model, one cannot guarantee that a particular stem cell will eventually be converted to a differentiated cell. The lemma above does guarantee that in the close vicinity of any stem cell some cell differentiates during a fixed bounded time interval unless the system is not in a resonant state. An immediate consequence of this is a lower bound on the supply of differentiated cells to the blood stream.

Corollary 5. *Suppose that at some time t_0 a vertex v is occupied by a stem cell, then every ball of radius $2M\eta$ eventually supplies at least one mature cell every $(\mu+1)\Psi + (M+1)\Theta + 1 + \Phi$ time steps unless the system is in a resonant state.*

Proof: By Lemma (3), every ball of radius $M\eta$ admits a stem cell from a certain moment onwards in any two consecutive time instances. Lemma (4) says that either this cell or one of its near neighbors (and so we argue about balls of radius $2M\eta$) converts to a differentiated cell within $(\mu+1)\Psi + (M+1)\Theta + 1$ time steps and migrate from the bone marrow as mature cells after Φ additional time steps. Thus, every ball of radius $2M\eta$ eventually supplies at least one mature cell every $(\mu+1)\Psi + (M+1)\Theta + 1 + \Phi$ time steps.

5 STEADY STATES AND DYING OUT STATES OF THE BONE MARROW MODEL

We consider the unique state satisfying $\forall v \in V$, $x(v) = (E) \vee x(v) = (D,*)$ as the *death state* of the system. A state x^t for which there exists a $k \in Z^+$ such that x^{t+k} is the death state, will be called a *dying out state*. Thus, a state not consisting of a single stem cell or a transitive cell is a dying out state. We claim that there is no other dying out state.

Lemma 6. *The dying out states are only those consisting of no stem cells or no transitive cells.*

Proof: Let $x^t \in \Omega$ be a state, which is not one of the dying out states. If there exists $v \in V$ which is not a stem cell at time t and since there exists a stem cell at time t, v turns to a stem cell by Lemma (1). So by Lemma (2) there is always a stem cell in $B(v, M\eta)$ in any two consecutive time instants from the time v has converted to a stem cell. The system does not die out. Even if v is a transitive cell then it will become a stem cell if there is no stem cell in its near neighborhood.

Assume, therefore, that V admits only stem cells at time t. If the counters are not synchronized they do

not convert to transitive cells at the same time instance and the system does not die out. If the counters are synchronized they enter into resonant state and again the system does not die out.

Thus, we have proved that the model representing the bone marrow is dynamic in the sense that it continuously changes in its constitution and arrangement, and these changes occur at varying rates depending on the constants Φ, Ψ, and Θ. We have also seen that except for the death state, the system never dies out. The bone marrow is in the state of a dynamic equilibrium that can be considered as if it is in homeostasis.

If there exist states $x \in \Omega$ in which for all $k \in Z^+$, $x^{t+k} = x^t$, then these are the *steady states* of the system.

Lemma 7. *For every Φ, Ψ, Θ the model does not have steady states other than the death state and the resonant state.*

Proof: The fact that each differentiated cell matures and leaves the bone marrow eventually, combined with Lemma (1) and Lemma (6) implies the above.

6 DISCUSSION

In this paper, we have proposed a biologically feasible model of bone marrow by extending the model of Agur et al. (2002). The proposed model adds the ability to recover from severe perturbations of the bone marrow by adding rules that can convert a transitive cell back to a stem cell and bring back the system homeostasis.

The main properties of our model are achieved from the feedback demand of rule (1), namely that a stem cell does not convert to a transitive cell unless its immediate microenvironment is saturated with stem cells. The feedback demand in rule (2) is also significant in the sense that a transitive cell can convert back to a stem cell in cases of severe perturbations resulting into loss of several stem cells. We obtain the results that stem cells are eventually dense (Lemma 2 and Lemma 3) and that, let alone the case when there is no stem cell or transitive cell, the system never dies out (Lemma 6). Even though our extension of the Agur et al.'s model is simple, the properties that emerge are general, and hold for more complex descriptions. It is a step ahead in the direction to model the immensely complex bone marrow system.

Our extension of Agur et al.'s model removes all the drawbacks associated with it. To summarize:

1. Our model has empty spaces but they no longer need any counters.
2. In the model, a stem cell division is explicitly represented.
3. The model has incorporated a directional component for the division of stem cells.
4. A stem cell's internal counter comes back to its initial state after division; i.e. it becomes a true daughter stem cell. Thus, a stem cell divides into two identical daughter stem cells.
5. Transitive cells that have limited ability to convert back to stem cells are represented in our model. Their ability to regenerate to a stem cell diminishes with subsequent generations.
6. One stem cell divides into a single empty space. For another division, the stem cell has to wait for its internal counter to reach a threshold as its internal counter gets reset after division.

Our model overcomes all the drawbacks of Agur et al.'s (2002) model. It also does not require message passing between cells and the controlling microenvironment, as required by the model of d'Inverno and Saunders (2005). Hence, it is closer to biological reality. The validation of this fact is underscored by the agent-based simulations that we have carried out. The results of these simulations are included in the Appendix. These simulations also demonstrate that, as predicted, large fractions of stem cells do remain in the quiescent state (Gordon, 2007).

There are several other options of bringing the model even more closer to biologically observed complexity. There are two extensions that we would like to work on in the future. The first is to make a provision of apoptosis (cell death) for all types of cells. Secondly, we can provide a stochastic behavior for the stem cell proliferation to capture variations in the human hematopoietic system. Addition of the stochastic behavior will also enable the introduction of a randomized directional component. We would also like to increase the scale of the simulation of the bone marrow system and to perform the same in three-dimensional space, typically with simulated bone marrow size that can hold 10^8 to 10^{12} number of cells and with the model constants matching observed parameters (Michor, Hughes, Iwasa, Branford, Shah, Sawyers and Nowak, 2005).

REFERENCES

Agur Z., Daniel Y. and Ginosar Y. (2002). The Universal

Properties of Stem Cells as pinpointed by a Simple Discrete Model. In *Journal of Mathematical Biology*, 44(1):79-86.

De Haan G., Dontje B. and Nijhof W. (1996). Concepts of Hemopoietic Cell Amplification. Synergy, Redundancy, and Pleiotropy of Cytokines affecting the Regulation of Erythropoiesis. In *Leukemia Lymphoma*, 22(5-6):385-94.

Gordon M. (2007). Stem Cells and Haemopoiesis. In Hoffbrand A., Catovsky D. and Tuddenhan E. (Eds.), *Postgraduate Haematology*, pp. 1-12. Blackwell Oxford, 5th edition.

d'Inverno M. and Saunders R. (2005). Agent-based Modeling of Stem Cell self-organization in a Niche, Engineering Self- Organizing Systems: Methodologies and Applications. In Brueckner S., Di Marzo G., Serugendo, Karageorgos A. and Nagpal R. (Eds.), *LNAI*, 3464. Springer.

Michor F., Hughes T., Iwasa Y., Branford S., Shah N., Sawyers C. and Nowak M. (2005). Dynamics of chronic myeloid leukaemia. In *Nature* 435(7046):1267-70.

O'Neill A. and Schaffer D. (2004). The biology and engineering of stem-cell control. In *Biotechnology and Applied Biochemistry* 40(Pt 1):5-16.

Roeder I. and Radtke F. (2009). Stem Cell Biology meets Systems Biology. In *Development* 136(21):3525-30.

Theise N. and Harris R. (2006). Postmodern Biology: (Adult) (Stem) Cells Are Plastic, Stochastic, Complex, and Uncertain. In *Handbook of Experimental Pharmacology* (174): 389-408.

APPENDIX

An agent-based model was developed for the bone marrow system proposed in this paper. We have implemented the program in the C programming language and it is available at the following website: http://sites.google.com/site/stemcellmodel.

The results given below are on a two dimensional grid of size 30 x 30 for the following constants:

$\Phi = 4$ Constant for differentiated cells.

$\Psi = 1$ Constant for stem cells.

$\Theta = 1$ Constant for transitive cells.

$\eta = 2$ Constant for distance measure for near neighborhood from a particular transitive cell.

$\mu = 8$ Number of directions in a 2-D space.

$M = 3$ Number of generations of transition cells.

We specified colors like Silver (S), Titanium Yellow (T), and Dark Red (D) to stem cells, transitive cells, and differentiated cells respectively. Also note that the directional component moves in the clockwise manner.

Simulation 1: Starting with 10% stem cells

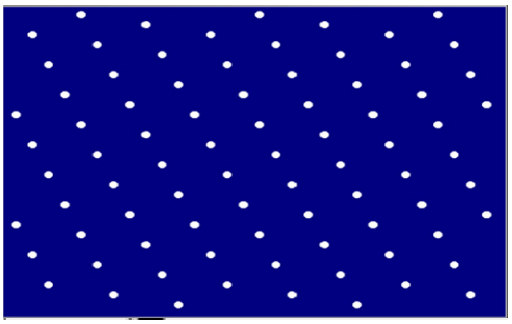

Figure 3: Initial Screen (with 10% stem cells).

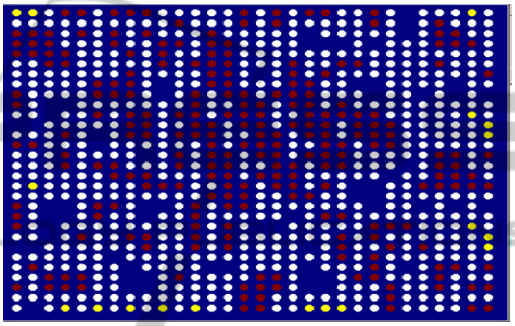

Figure 4: After 100 time steps, 93.3% stem cells quiescent.

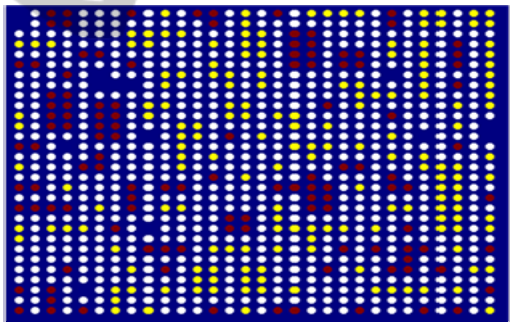

Figure 5: After 3000 time steps, 88% stem cells quiescent.

Simulation 2: Starting with single stem cell at top left.

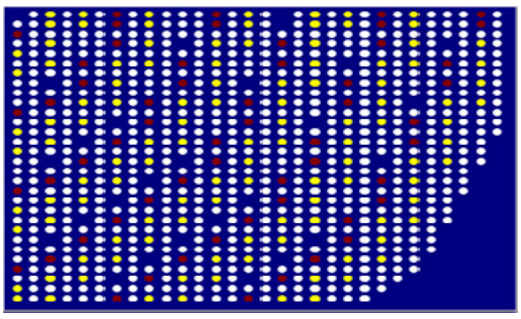

Figure 6: After 100 time steps, 91.9% stem cells quiescent.