# Agent-Based Modeling of Osteogenic Differentiation of Mesenchymal Stem Cells in Porous Biomaterials

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***Abstract*— Mesenchymal stem cells (MSC) have shown promise in tissue engineering applications due to their potential for differentiating into mesenchymal tissues such as osteocytes, chondrocytes, and adipocytes and releasing proteins to promote tissue regeneration. One application involves seeding MSCs in biomaterial scaffolds to promote osteogenesis in the repair of bone defects following implantation. However, predicting *in vivo* survival and differentiation of MSCs in biomaterials is challenging. Rapid and stable vascularization of scaffolds is required to supply nutrients and oxygen that MSCs need to survive as well as to go through osteogenic differentiation. The objective of this study is to develop an agent-based model and simulator that can be used to investigate the effects of using gradient growth factors on survival and differentiation of MSCs seeded in scaffolds. An agent-based model is developed to simulate the MSC behavior. The effect of vascular endothelial growth factor (VEGF) and bone morphogenic protein-2 (BMP-2) on both survival and osteogenic differentiation is studied. Results showed that the survival ratio of MSCs can be enhanced by increasing VEGF concentration. BMP-2 caused a slight increase on survival ratio. Osteogenesis strongly depends on the VEGF concentration as well because of its effect on vascularization. BMP-2 increased the osteogenic differentiation of MSCs.**

## I. Introduction

Mesenchymal stem cells (MSCs) are the focus of attention in cell-based tissue regeneration techniques. MSCs have been widely used in bone regeneration applications due to their high potential to differentiate into bone cells [1-5]. Use of synthetic or natural biomaterials to repair large bone defects is a promising alternative, however the success of these biomaterials is limited due to their lack the osteogenic and osteoinductive properties of bone autografts [6]. Planting the cells with osteogenic potential in these biomaterials can compensate this deficiency and promote bone formation. *In vivo* survival and osteogenic differentiation of MSCs strongly depend on the sustained presence of local growth factors (GF) and oxygen supply by the vascular structure. Bone morphogenetic protein 2 (BMP-2) is considered the gold standard of GF treatment to promote osteogenesis. BMP-2 is an expensive protein with a relatively short half life and maintaining the effective dose of this protein *in vivo* is required for differentiation of MSCs. Angiogenesis, the formation of new blood vessels from pre-existing ones, is the major mechanism for scaffolds vascularization and has an impact on the survival, proliferation and differentiation of cells seeded in scaffolds.

Agent-based modeling (ABM) provides a powerful simulation environment, naturally suitable for modeling of multi-cellular biomedical systems where cells can be represented as autonomous, interacting agents. The ABM framework has been used to develop various models to simulate cancer progression [7-10], vascular development [11-14], bone tissue [15], wound healing [16,17]. The main focus of this research is to develop a multi -agent system to simulate stem cell behavior and osteogenic differentiation based on the diffusible biological factors and vascular structure. Angiogenesis in porous media stimulated by GF gradient has been modeled in our group [14,18,19]. This model extends that model to investigate the combined effects of angiogenesis and bone stimulating GF on the osteogenic differentiation of MSCs.

## II. Methods

### A. Agent Based Model of Mesenchymal Stem Cells

An ABM is developed to simulate the differentiation of multi-potent stem cells into osteoblasts in 3D porous polymeric scaffolds. The ABM is implemented in Java using Repast (REcursive Porous Agent Simulation Toolkit), which is a Java-based open source agent modeling toolkit. The angiogenesis model developed previously focused on the behavior of individual endothelial cells (ECs) to form vessel sections in response the GF gradient in the environment. EC agents mimic the actions that ECs perform during angiogenesis, including elongation, proliferation, tip cell migration, and anastomosis, as governed by the rule base. The behavior of EC agents was determined by their internal state based on a non-adaptive set of rules that control their actions and stochastic variations [14]. The scaffold in this work is assumed to be non-degrading and has been modified to provide dynamic gradient of vascular endothelial growth

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factor VEGF and BMP-2 by incorporating release data into the model. It is described in detail in section C.

The MSC model developed describes the behavior of these cells in response to the local environment and governs the differentiation to osteoblast (OB) cells. The MSC agent designed as one leading cube-shaped agent surrounded by other dummy agents (cell part agents having cube shapes) randomly distributed in the immediate neighborhood of the leading agent. The leading agent is the decision maker and the parts are following while randomly changing shapes every time the cell moves in the continuous space. This design approach provides the cell a random shape and volume in the space. Growth is implemented by changing the number of cell part agents as time progresses while a cell is going through the cell cycle (Fig. 1). This agent design is also open to sub-cellular level extension considering the cell as comprised of interdependent entities.

A rule-base regulates the behavior of MSC agent and based on the environmental conditions updating the parameters of the rules governing the actions such as migration, proliferation as a committed MSC, and finally changing the type of MSC agent to an OB. The OB agent will inherit most of the basic actions from MSC agents but with significantly different parameters. It is abstracted as having the same irregular shape with MSC agents. The OB agent is a final state and cannot change the type from an OB.

*B. MSC Rule Base and Parameters*

A rule base is derived for the tissue layer and its agents (MSCs, OBs) based on a comprehensive literature survey. The MSC agent is capable of sensing the GF concentration in its neighborhood, migrating towards the BMP-2 gradient, going through cell cycle, growing in size, contributing to the VEGF concentration in the environment and committing and then differentiating to a bone cell (OB agent) in a continuous space. There are two levels of rule set governing the behavior of MSC agent. The first level is the base level rule set to regulate the cell cycle and survival of cells. A higher-level rule set is introduced to the model to update the parameters of a cell going through the differentiation.

In the cycle rule set, each agent first calculates the distance to the closest stable blood vessel. Only the stable (anastomosed) blood vessels are considered as having blood flow and a source for oxygen and nutrition. If there is no blood vessel in the close vicinity (0-100µm), cell will

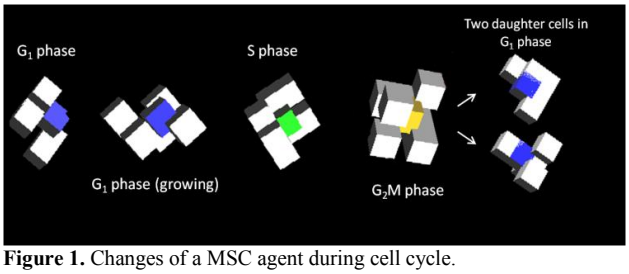


**Figure 1.** Changes of a MSC agent during cell cycle.

change its state to quiescent phase ($G_0$). Hypoxic cell state (H) will be assigned to the cell if there is no blood vessel in a further distance (100-200µm) and the hypoxia clock will be started. The time that cells can spend under hypoxia is limited [20,21]. If no blood vessel reaches the close vicinity of a hypoxic cell, the cell will go through apoptosis. The oxygen distribution is not explicitly modeled, however oxygen diffusion from the blood vessel is assumed to be a few hundred microns [22]. If the cell is in the vicinity of a stable blood vessel, then it will go through the cell cycle and spend the required time at each state and complete the actions before it could change the state in the cycle. Each cycle phase has an embedded clock to track the time. Cells can only grow in size at $G_1$ state until they reach the maximum volume or contact inhibition occurs. $G_2$ and M phases have been combined to one state called $G_2M$ for convenience. Cells can only proliferate at the end of the $G_2M$ phase if they reach the maximum volume and are not surrounded by more than two cells. If there is no available location for the new daughter cell then contact inhibition occurs and cell will be on $G_2M$ hold. Both MSCs and OBs migration is governed by chemotactic random migration through BMP-2 gradient. Chemotactic index (CI) values for MSC and OB are found from the literature [23,24] in the presence of BMP-2 and converted to migration probabilities. Migration speed of the cells also depends on the crowding effect. While the leading cells are relatively more motile, the cells located behind them and surrounded with other cells move more slowly [25].

Cells can differentiate only in $G_1$ state and this is when the higher-level rule set is called. The "Differentiation" rule set (Fig. 2) is responsible for changing the parameters in the "Cycle" rule set based on the environmental condition (BMP-2 concentration).

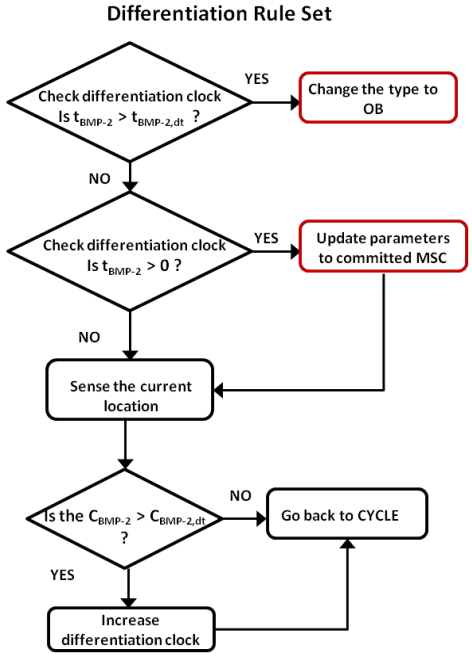


**Figure 2.** Rule set governing the MSC cell differentiation into OBs.

Before a MSC agent differentiates to an active OB, it is required to expose it to a certain level of BMP-2 for a certain time. A differentiation clock is embedded in the agent's rule

set to keep track of the time spent under the required BMP-2 dose. As soon as MSCs are exposed to BMP-2 concentration higher than the threshold value they will become committed MSCs and update migration and proliferation parameters because undifferentiated cells are observed to move more rapidly *in vitro*. Another requirement that a MSC agent should satisfy to differentiate is being in the close vicinity of a stable blood vessel since stem cells tend to differentiate toward osteoblasts when they are located closer to blood vessels and exposed to higher oxygen pressure [22].

### C. Growth Factor Releasing scaffolds

Porous scaffolds with a constant pore size, 150 µm, and constant pore connectivity were generated. Scaffolds are assumed to have a layer at the top with degradable microspheres filled with GF. Dynamic gradient was created by assuming that GF is released from the microspheres and diffuses through the scaffold (Fig. 3). The gradient, formed by the transport of GF through the hydrogel, was modeled following Fick's second law, which assumes that diffusion is the only mechanism of transport and that the proteins do not interact (react) with the porous structure as they diffuse.

This diffusion model was implemented in our ABM to provide dynamic gradients of growth factors. Different doses of BMP-2 and VEGF have been studied to investigate their effects on the differentiation and survival of MSCs.

### D. Initial Model Set-up

3D rectangular (8 x 3.5 x 2 mm) porous scaffold is placed on the host tissue with 20 initial host blood vessels to be in contact with the scaffold. Cells were randomly seeded in the pores of the scaffold. Four different cases are studied to see the effect of BMP-2 and VEGF on the osteogenesis and survival of MSCs (Table 1).

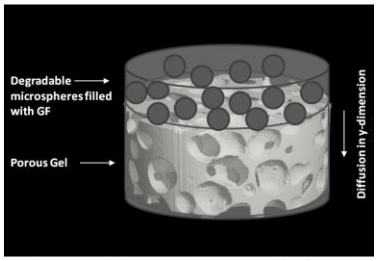


**Figure 3.** Dynamic growth factor release profile from the distal layer

TABLE I. GF CONCENTRATIONVALUES FOR DIFFERENT CASES

| Cases | VEGF (µm) | BMP-2 (ng) |
|---|---|---|
| Low VEGF Low BMP-2 | 10 | 40 |
| Low VEGF High BMP-2 | 10 | 500 |
| High VEGF Low BMP-2 | 100 | 40 |
| High VEGF High BMP-2 | 100 | 500 |

## III. RESULTS

The effect of different BMP-2 concentrations on osteogenic differentiation and survival of MSC's were studied. Model runs were performed to simulate 4-week growth of MSCs in porous scaffold. Survival of MSC calculated as defined in (1):

$$Survival\ Ratio = 1 - \frac{Number\ of\ Apoptosed\ Cells\ from\ Initial\ Seeding}{Number\ of\ Initially\ Seeded\ Cells} \quad (1)$$

Differentiation is evaluated using the ratio of osteoblasts to total number cells in (2):

$$OB\ Ratio = \frac{Number\ of\ OBs}{Total\ Number\ of\ Cells} \quad (2)$$

The survival rate is calculated using (1) for four different cases. Results were reported as an average of 3 simulation runs. The highest survival rate was observed in the case with high VEGF and high BMP-2 and it was followed with high VEGF, low BMP-2 case (Fig. 4). This is due to a strong relationship between angiogenesis and survival of seeded cells. High VEGF concentration stimulates the ECs and resulted in faster angiogenesis. Hence, the cells reached by the blood vessels while they were hypoxic could survive. Higher BMP-2 with the constant VEGF slightly increased the survival ratio due to its angiogenic effects [26,27].

Differentiation of MSCs into osteoblasts was reported for the same cases (Fig. 5) Osteogenesis of MSCs depends on the oxygen supplied by the blood vessels. Because the cases with low VEGF concentration resulted with less angiogenesis the differentiation ratio was low. BMP-2 increased the differentiation ratio for the same concentration of VEGF.

The results were consistent with the findings from the literature. VEGF loading improves the scaffold vascularization [28] and it has an impact on cell survival. Jeon et al. reported BMP-2 releasing porous scaffolds seeded with undifferentiated adipose-derived stromal cells (ADSC) can stimulate osteogenic differentiation and bone formation [29].

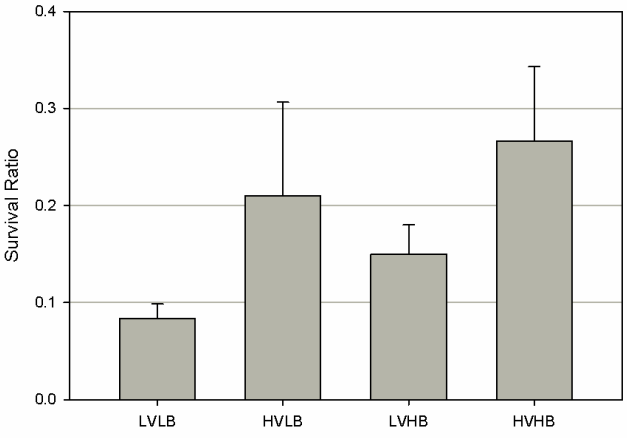


**Figure 4.** Survival ratio of homogeneously seeded MSCs at the end of 4 weeks.( LVLB: Low VEGF low BMP-2, HVLB: High VEGF low BMP-2, LVHB: Low VEGF high BMP-2, HVLV: High VEGF high BMP-2)

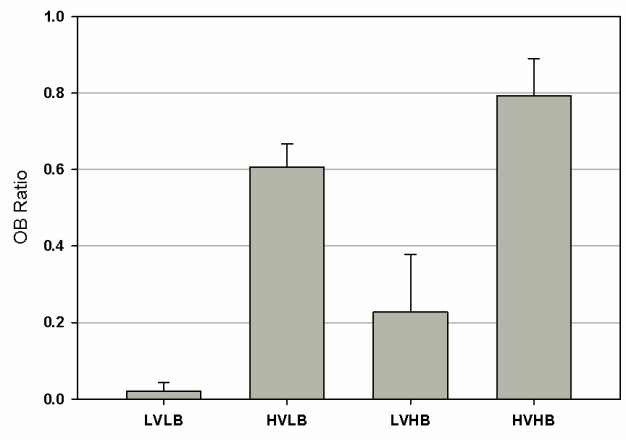


**Figure 5.** Osteoblast ratio to the total number of cells at the end of 4 weeks. ( LVLB: Low VEGF low BMP-2, HVLB: High VEGF low BMP-2, LVHB: Low VEGF high BMP-2, HVLV: High VEGF high BMP-2)

The results also suggested that even though higher VEGF can be used in scaffolds to stimulate more vascularization and increase the survival of seeded cells, the ratio of survival is still limited.

## IV. Conclusions

The agent-based model and software developed can be used to investigate the combined influences of growth factors and biomaterials on MSC differentiation and cell survival. Different strategies to increase cell survival can be also investigated by using this software.

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