Agent-Based Modeling of Vascularization in Gradient Tissue Engineering Constructs

Elif Seyma Bayrak¹, Banu Akar², Nan Xiao¹, Hamidreza Mehdizadeh¹, Sami I. Somo², Eric M. Brey² Ali Cinar^{1,2}

¹Illinois Institute of Technology, Department of Chemical and Biological Engineering, USA (e-mail: ebayrak@hawk.iit.edu, nxiao1@hawk.iit.edu, hamidreza.mehdizadeh@pfizer.com, cinar@iit.edu). ²Illinois Institute of Technology, Department of Biomedical Engineering, USA (e-mail: bakar@hawk.iit.edu, ssomo@hawk.iit.edu, brey@iit.edu).}

Abstract: An agent-based model is developed to simulate the vascular growth in engineered biomaterials. This study investigates the influence of growth factor release rate on rapid and stable vascularization. Different growth factor release profiles are generated and tested using the agent-based model. The simulation results are verified with experimental studies. Effective release policies are identified; microsphere properties for promoting angiogenesis are suggested.

Keywords: Agent-based modeling, angiogenesis, gradient biomaterials, growth factor release, vascularization

1. INTRODUCTION

Tissue engineering approaches to replace, repair and regenerate severely damaged or missing tissue have been recognized as a superior alternative to current techniques such as organ, tissue transplants and reconstruction surgeries (Fuchs et al., 2001). Engineering of living tissue replacements is a complex system where biomaterial, transplant cells, blood vessels and host organism are continuously interacting.

Synthetic or natural biomaterial scaffolds are the common tools to repair large tissue defects, when they are combined with living precursor cells. Survival of these transplant cells are often limited in large constructs due to oxygen and nutrient deficiency caused by insufficient vascularization. Angiogenesis, new capillary growth from already developed blood vessels, is a natural mechanism for scaffolds vascularization and is still considered as a milestone keeping tissue engineering from ultimate success. Hence, many researchers have focused on developing strategies to enhance angiogenesis in tissue-engineered constructs including scaffold architecture design, delivery of angiogenic factors, in vivo and in vitro pre-vascularization (Rouwkema et al., 2008, Novosel et al., 2011).

Delivery of angiogenic growth factors to stimulate endothelial cell (cells lining the walls of blood vessels and leads to angiogenesis) activation, migration, proliferation and survival can enhance the vascularization and speed up the process (Riley et al., 2006, Patel et al., 2008, Kim et al., 2013, Xie et al., 2013). Many research results underlined that vascular endothelial growth factor (VEGF) is essential for the survival of endothelial cells (Lee et al., 2011) and sustained presence of local VEGF delivery can up regulate angiogenesis (Rocha et al., 2008). VEGF has relatively short half-life and its administration by uncontrolled methods can cause fast degradation resulting with capillary regression, while large doses can cause harmful side effects (Elcin et al., 2001). For more sustained release, VEGF can be physically encapsulated within the scaffolding material and in micro particles (Reed and Wu, 2014).

The sustained, controlled delivery of VEGF to maximize angiogenesis and avoid regression of immature capillaries is a challenging but an interesting optimization problem for mathematical modelers. Computational studies aiming at understanding and predicting of various aspects of the angiogenesis process have been reported (Peirce, 2008), including models that consider the angiogenesis within porous tissue engineering scaffolds.

Agent-based modeling (ABM) allows the system to be partitioned into its main actors who do not have a global view of the system but are only affected with the dynamics of their immediate vicinity. These main actors are called agents whose decision impacts the global behavior of the system. An agent can sense its environment, process this information and make an independent decision. The behaviors of agents are governed with a pre-determined rule set, however the decisions are dynamic and made during execution time. The rules are often simple, addressing the actions of the agent while the emergence as a result of dynamic decision-making often explains the most complex system-level behaviors.

ABM is one of the advanced simulation methods with applications in many diverse fields. Due to its behavioral focus, not surprisingly it was first used in social sciences (Macy and Willer, 2002). Some interesting applications include but are not limited to modeling of human systems (Bonabeau, 2002), finance (LeBaron, 2000), and supply chain (Kaihara, 2003).

ABM is a natural choice for modeling biological systems in which the building blocks may be cells, tissues or organ systems, which act and interact. Some examples of biological ABMs focusing on individual cell behavior include modeling of angiogenesis (Mehdizadeh et al., 2013, Qutub et al., 2009, Bentley et al., 2008), osteogenesis of stem cells (Bayrak et al., 2014), brain tumors (Zhang et al., 2009), and arterial adaptation in hypertension (Thorne et al., 2011), bone tissue (Ausk et al., 2006), and wound healing (Walker et al., 2004b, Walker et al., 2004a).

An ABM is developed in our group previously to investigate the effect of biomaterial physical structure on angiogenesis, where a static growth factor profile was artificially introduced. In this work, we extended the existing model to investigate different growth factor release profiles of biomaterials on angiogenesis.

2. METHODS

2.1 Agent-Based Model and Simulation Environment

An agent-based model is developed to simulate sprouting angiogenesis focusing on individual endothelial cell (EC) behavior. The model is constructed in java using Repast Simphony that provides libraries to built simulation environment, user interface and to schedule time or event driven actions with a double precision scheduler (North et al., 2013).

A three-dimensional rectangular grid layer is used to store the environmental information, including concentrations of soluble factors and the details of the biomaterial structure at each grid points. Agents actions are governed by the environmental conditions in their Moore neighborhood on this grid layer, however, agents are not restricted on a grid, can act, and move on a continuous space layer (Mehdizadeh et al., 2011).

2.2 Angiogenesis and EC Agent

Endothelial cells (ECs) are the main agent of angiogenesis simulation model. Literature derived rule-base governs the behavior of each individual EC their actions together forms the capillary structure. An EC agent consists of a leading tip cell (decision maker) followed by a stalk cell. Angiogenesis starts from host vasculature when an EC is activated by growth factor (GF) stimuli. Tip cell senses its neighborhood and migrates towards the highest gradient of GF. The migration process of the tip cell results in elongation of the stalk cell attached to it. When the EC reaches a maximum size, it proliferates and makes a new EC with half of its size. The parent EC is fixed and newly created one becomes the tip cell continues to perform the migration-elongationproliferation actions. ECs can also make new branches depending on the local GF concentration (Ausprunk and Folkman, 1977). If the tip cell meets with another capillary segment it can connect and anastomose. In Fig.1, a snap shot from simulation environment is illustrated. Anastomosed capillaries are assumed to have blood flow and marked as stable (green labeled ECs). EC agent structure and governing

rule base were explained in detail elsewhere (Mehdizadeh et al., 2013).



Fig. 1 A snapshot from agent-based simulation interface

Not only migration, proliferation and branching, but also survival of ECs and newly formed capillaries depend on the local GF concentration. Each EC holds a GF threshold to be activated. The threshold is also used to implement capillary regression into the model. Endothelial cells apoptose due to GF deficiency, resulting in capillary regression (Tang et al., 2004). For EC apoptosis, cells should stay under this concentration for a certain time. The flow chart of EC actions depending on GF concentration is illustrated in Fig. 2. Parameter values are reported in Table A.1.

Blood vessels are only allowed to move in the pores of the scaffold. Pores are assumed to be filled with ligand, and higher ligand density speeds up the migration of EC (Maheshwari et al., 2000). The scaffold region is not allowed for vessel invasion. When the tip cell encounters a part of the scaffold it will change its direction and try to find an available location before it becomes deactivated.



Fig. 2 Agent actions depending on local VEGF concentration

2.3 VEGF releasing scaffolds

Computer models of scaffolds with rectangular shaped pores were generated to study growth factor release. First, the centers of all pore locations were determined based on predetermined size of pores ($300-500 \mu m$). A pore is placed within a three dimensional matrix. Its rotation within that matrix is randomly chosen. The addition of subsequent pores

is allowed as long as there is some degree of overlap between neighboring pores. The matrix is filled until a desired porosity is achieved.

In an experimental study in our group, a salt leached method was used to prepare porous Poly (ethylene glycol) diacrylate (PEG-DA) hydrogels (Chiu et al., 2013). Top part (distal layer) of the porous hydrogels was modified with Poly (lactic-co-glycolic acid) (PLGA) microspheres. Growth factor was encapsulated into PLGA microspheres by using double emulsion method(Jiang et al., 2013). Growth factor released due to degradation of microspheres and diffusion of growth factor through the porous hydrogel system was modeled based on Fick's second law (Fig.3) assuming onedimensional diffusion along the y-axis of the scaffold. In the model, the boundary condition at the distal layer was adapted from the experimental release kinetics data and tissue interface was assumed as an infinite sink. Diffusion coefficients within the hydrogels were estimated by fitting data to the experimental release results in MATLAB. Two assumptions were made in the development of the model: diffusion is the only mechanism of transport and the growth factors do not bind to the hydrogel.



Fig. 3 GF release profile, modeled using the experimental data.

Using the experimentally verified release profile, four additional concentration profiles were generated in order to investigate the effects of various release rates on angiogenesis. The control case indicates the concentration profile of the current experimental system. A mathematical algorithm is developed to manipulate the current release profile where GF at a time point and location is shared in further time points based on the condition of keeping the total released GF constant. Using this algorithm release was slowed down, %25 (case 1), %50 (case 2), %75 (case 3), %100 (case 4) (Fig. 4).

3. RESULTS

First, simulation runs were performed using the real release profiles obtained from experimental data (control case) Normalized vessel invasion and anastomosed vessel invasion depth of blood vessels into scaffold is reported (Fig. 5 A and



Fig. 4 Different release profiles case 1 (A), case 2(B), case 3(C), case (D)

5 B). Fig. 5 B shows that at week 4 blood vessels invaded around 60% of the scaffolds but due to the VEGF withdrawal, non-perfused vessels disappeared and final invasion was around 40%. Total blood vessel and density results revealed that capillary regression started after week 2 and kept increasing up until only anastomosed vessels exist in the scaffold.



Fig. 5 Simulation runs of control case, normalized anastomosed depth (A), normalized invasion depth (B), total blood vessel length (μ) (C), density (μ^{-2}) (D)

Simulation results were compared with experimental studies performed under similar conditions in our group to validate the ABM. Vessel invasion data were collected from a rodent subcutaneous implantation model (Jiang et al., 2014) *in vivo* at 3 and 6 weeks. The experimental study also showed that there is significant capillary regression after week 3. Simulation and experimental results were consistent and the ABM accurately predicts the final vessel invasion at the end of week 6 (Fig. 6).

Slower cases are generated by using experimentally verified control case release profiles. The comparison between experimental (control) case and slower release cases are illustrated in Fig. 7. Case 1, where release was slowed down



Fig. 6 ABM simulation comparison with in vivo experiments

by 25% showed similar results to control case, capillary regression is observed after week 4 due to insufficient local VEGF concentration. However, further slowing resulted in significant improvement and finally release speed slowed by 75% (case 3) showed no reduction in vessel depth and highest blood vessel length is obtained. Density is only calculated 150 µm above the interface, therefore slow release resulted with significant low interface density. Further decrease in release speed resulted in steady-state like profile (case 4 Fig. 7 D). No regression was observed and there was no significant difference from case 3. Sustained slower release promotes sprouting angiogenesis, resulting in deeper vessel invasion, longer total blood vessel length, and higher anastomosed depth. The slower the growth factor release, the more significant is the influence on angiogenesis. A crossover occurred in the profiles of each case, suggesting that slower release cases have lower growth rate initially and catch up later, which agrees with the concentration distribution profile that slower releasing model have mitigated initial concentration burst. Capillary regression varied significantly with the speed of release, sustained release avoided capillary regression for cases 3 and 4.

4. DISCUSSION

A simulation based on a computational model for complex tissue engineering systems can provide a tool for rapidly screening alternatives, determining the most promising experimental search space optimal angiogenesis and investigating ways of intervening during the process for functional tissue formation in biomaterials.

Researchers in computational biology and biomedical engineering have started using ABM techniques to study complex systems with many interacting elements. The objective of this study was to develop an ABM for studying the effects of a strategy to enhance biomaterial vascularization that is currently being investigated experimentally.

We analyzed the effects of different growth factor gradients by adjusting release rates. Our motivation was to investigate if the capillary regression can be avoided with slower and sustained VEGF release. Gradient delivery of GF resulted in



Fig. 7 Comparison of different release speed with control case; normalized invasion depth (A), normalized vessel depth (B), total blood vessel length (μ) (C), density (μ^{-2}) (D)

week 4

week 3

week 5

week 6

week 2

more directed and controlled angiogenesis and showed higher vessel invasion. Slower release cases, had a lower initial vascular growth rate that speeded up later, which agrees with the concentration distribution profile that slower releasing model have mitigated initial concentration burst. Capillary regression varied significantly with the speed of release, sustained release avoided capillary regression for cases 3 and 4. In this case study, total dose was kept the same to solely focus on release speed. Since release of the growth factor depends on the degradation rate of the microspheres, this approach is feasible when growth factor is encapsulated with a polymer that has slower degradation rate than what was used in current experiments.

Simulation findings were consistent with literature findings as well. Gradient release can cause more directed EC migration where as in homogeneous concentration migration can be in a more random fashion. Endothelial cells tend to migrate in response to the gradient of VEGF (Gerhardt et al., 2003). Sustaining the spatially controlled growth factor release is essential for tissue-engineered constructs. VEGF is a potent angiogenic stimulator, and many in vivo experiments have demonstrated that if VEGF is released in a controlled manner it can enhance and guide scaffold vascularization (Borselli et al., 2010, Rocha et al., 2008, Golub et al., 2010, Sacchi et al., 2014).

5. CONCLUSIONS

An agent-based modeling was used to optimize growth factor releasing scaffolds. Simulation findings verified with experimental studies suggested that ABMs provide a powerful alternative to simulate biological systems. The success in tissue regeneration is inherently linked with combination of different strategies with an engineering approach. The model developed in this study allows biomedical engineers to try different hypothesis rapidly and explore alternatives. Simulation results were supported with experimental findings and suggested optimal designs for biomaterials to be used in promoting angiogenesis and tissue growth.

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Appendix A.

Table A1 Parameters of EC Agent

Definition	Value
VEGF threshold	1-5 ng/ml
Time to regress under threshold	5 days
Unit elongation speed of EC	10 µm/tick
Maximum EC length	100 µm
EC diameter	10 µm
Branch delay time	8 time steps
Persistency time	2 time steps
Sensing distance	20 µm