

CELLULAR AUTOMATA MODEL OF MICROVASCULAR REMODELING

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ABSTRACT

Microvascular growth and remodeling in the adult animal involves angiogenesis, arteriogenesis, and regression. Many cellular behaviors, such as proliferation, differentiation, migration, and apoptosis contribute to these processes, and coordination of these events is achieved via combinations of biochemical and biomechanical signals. This study examines patterning changes in subcutaneous microvascular networks induced by focal applications of exogenous vascular endothelial growth factor (VEGF) and models the spatial and temporal growth response using a novel cellular automata (CA) computer simulation. The CA model predicts experimentally-verified changes in spatial vascularity and vessel maturation, or perivascular cell recruitment, in response to the growth factor stimulus. The model also parametrically analyzes how growth factor sequestration by the extracellular matrix (ECM), a phenomenon hypothesized to regulate diffusible growth factor levels, affects network remodeling.

PREVIOUS COMPUTATIONAL MODELS VS. THE CELLULAR AUTOMATA APPROACH

The development and use of mathematical and computer models for the study of pattern formation in biology has a history over twenty years long [1, 2], and computational models based on a system of field equations have been used to study biological patterning phenomenon ranging from pigmentation patterns in the shells of mollusks [3] to neuronal connections in the developing brain [4]. Recently, computational models of whole cells, such as ECELL [5], have been developed to understand complex metabolic signaling pathways in individual cells.

In the field of microvascular remodeling, computational models have been developed and employed to study network pattern changes, and in general, these models consist of systems of equations that describe functional characteristics of the system, such as pressure distributions across a network or growth factor concentrations within an extracellular matrix environment, which are then solved in space and time using analytical and numerical techniques [6-8]. Arterial tree formation has also been modeled using constrained constructive

optimization (CCO), in which new terminal segments are successively added at randomly selected locations in the tree while the geometric location of each connection is optimized with respect to intravascular volume [9]. In contrast to probabilistic models, fractal analysis [10] has given rise to diffusion limited aggregation (DLA) models of vascular growth.

Here we report on a novel computational model based on cellular automata (CA), an approach which allows pattern evolution through the independent behavior of discrete cells and growth factors. Global boundary conditions and parameters obtained from the literature are applied to the complex system, and the ensuing pattern is a result of individual cell behaviors. We demonstrate the utility of this approach in studying microvascular remodeling, a complex biological patterning phenomenon generated by autonomous cells responding to a state-altering stimulus of exogenous growth factor. By comparing measurements of angiogenesis and vessel maturation obtained experimentally with those predicted by the CA model, we report graphical and numerical agreement between *in vivo* and *in silico* remodeling events. We also analyze the role of extracellular matrix material in concentrating growth factor in focal tissue regions and the resultant effect on vascular patterning.

METHODS

Twelve 250 gram Fischer 344 male rats were implanted with dorsal skin window chambers. Five days later, two 150 μm -diameter alginate microbeads containing VEGF₁₆₄ (10 $\mu\text{g}/\text{ml}$) and two containing vehicle (PBS) were implanted, one per quadrant, into opposing quadrants of subcutaneous tissue. Light microscope images of stimulated tissue quadrants were acquired prior to bead implantation and 4 and 14 days later. Images were used to obtain functional length measurements of microvascular networks, reflecting the amount of angiogenesis in response to the state-altering stimulus. At each analysis time-point, three animals were sacrificed and their window chamber tissues were harvested, fixed in 4% paraformaldehyde, and immunostained with 1A4-Cy3 smooth muscle α -actin mouse monoclonal antibody. Total lengths of vessels containing perivascular

cells expressing smooth muscle α -actin were measured to determine the amount of vessel maturation in response to the exogenous stimuli.

Using JAVA-based modeling software Netlogo version 1.1, we graphically mapped three digitized *in vivo* networks onto the *in silico* tissue space. Over 1,000 cells were arranged into vessels according to the network maps and assigned the appropriate cell types, including smooth muscle α -actin-expressing perivascular cells and endothelial cells. Interstitial precursor cells were also randomly placed in the tissue space. Relative cell sizes were scaled to the dimensions of the *in silico* tissue space according to published values. Exogenous point sources of VEGF₁₆₄ were input into the simulated tissues in locations corresponding to the *in vivo* bead placement. Endogenous growth factors, PDGF-B and TGF- β , were also included in the model based on literature descriptions of their production and effects on cell types involved in the simulation.

The simulated remodeling events were defined by a set of 50 free parameters that were varied simultaneously and independently according to prescribed rules obtained from the literature. These parameters controlled cellular behaviors such as proliferation, differentiation, migration, cell-to-cell contact recognition, and apoptosis. Ten simulations per network were performed, and network remodeling, characterized by total functional length and smooth muscle α -actin-positive cell extension were recorded and averaged at time points before and after state-altering interventions.

RESULTS

Remodeled *in vivo* networks were graphically consistent with remodeled *in silico* networks (Figure 1). Fourteen days after VEGF₁₆₄-containing micro delivery devices were inserted (* designates location in Figure 1), *in vivo* and *in silico* microvascular networks showed visible localized angiogenesis in regions surrounding the micro-delivery beads. The average total lengths of vessels less than 25 microns in diameter was statistically similar between the experimental and simulated cases, and values were significantly elevated above control levels in both cases.

The pattern of vessel maturation within the networks was also comparable in experimental and CA-modeled networks. Localized vessel maturation was seen in tissue regions within 1 mm of the VEGF₁₆₄ source after 14 days of stimulation (arrow in Figure 1). The lengths of vessels containing a smooth muscle α -actin-expressing perivascular cell coating *in vivo* (0.24 ± 0.05 mm/mm³) were increased above both steady state (0.13 ± 0.04 mm/mm³) and sham lengths at this time point (0.14 ± 0.04 mm/mm³). Furthermore, the growth response in remodeled experimental networks after 14 days was statistically similar ($p < 0.05$) to that predicted by the CA model (0.21 ± 0.02 mm/mm³).

When the ECM in the CA model was simulated as a sink for 25% of the VEGF₁₆₄ entering a particular matrix element in the tissue space, the remodeling pattern was visibly changed. Total vessel density increased by 10% and the new vessels were aligned more closely with the ECM fibers. However, the length of smooth muscle α -actin-expressing cells did not change with varying levels of ECM growth factor sequestration. This result was expected due to the fact that VEGF₁₆₄, principally responsible for total vessel density, was sequestered, while TGF- β and PDGF, which are responsible for perivascular cell recruitment, were not sequestered by the ECM in these simulations.

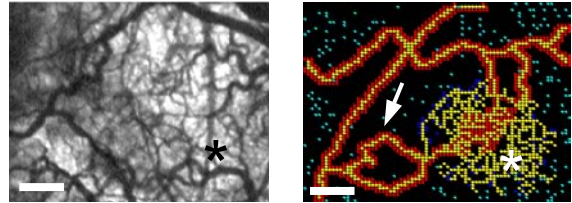


FIGURE 1. *In vivo* and *In silico* microvascular remodeling after 14 days of focal VEGF stimulus (scale bar = 100 μ m)

DISCUSSION

In conclusion, this CA model examines a complex biological system in which independent and discrete cell behaviors are controlled by multiple variables changing in space and time. The first cell-based computer simulation of vascular network remodeling to incorporate simultaneous changes in both perivascular cell coverage and capillary density, this model integrates biochemical signaling events and cellular behaviors within assemblies of thousands of interacting cells. The model predicted with relative accuracy aspects of *in vivo* remodeling, while providing insight into a possible role for the ECM in the sequestration of growth factors and guidance of new vessels.

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