Empirical and computational study of vasculogenesis

Ph.D. Thesis

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Introduction

Self-organized behavior in biological physics is studied from length scales of bacterial colonies to huge bird flocks. In such systems several individuals interact with each other to create a large-scale structure. During multicellular organism development, the architecture of the whole body and its details, such as tissues and organs, are constructed through the interaction of cells. Although our knowledge of individual cell behavior is rapidly expanding, we are still far from understanding the mechanisms behind the several processes of embryogenesis.

Vasculogenesis, the formation of the primary vascular network, is one of the earliest processes that involves the formation of a large scale structure. This self-organizing process in the development of warm-blooded animals has been explained by two main mechanisms. The chemo-mechanical approach describes cells pulling on the elastic extracellular matter (matrix), and creating a pre-pattern this way. Cells are migrating towards higher concentrations of matrix, or along oriented fibers, and cluster into a network. The chemotaxis approach incorporates diffusing molecules (chemoattractants) that trigger cell motility in the direction of their concentration gradients.

Although these models are indeed likely to correctly describe some aspects of the process, their mechanisms or dynamics are not always biologically feasible. Our experiments reveal that linear multicellular structures and networks form under conditions under which these models cannot be applied.

In this study we set out to suggest a new type of cell behavior to explain the emergence of vascular structures. Experimental studies reveal the importance of multicellular sprouting during vasculogenesis, therefore we aim to construct theoretical models that describe the emergent patterns by multicellular sprout formation.

Methods

Our hypotheses have been studied through two kinds of computational simulations, each resolving individual cells: an interacting particle model and the cellular Potts model. In the particle model cells are represented only by the position

of their cell center and their velocity. We use an Ornsetin-Uhlenbeck dynamics augmented with an interaction term between the cells.

In the cellular Potts model, cells are represented as simply connected domains on a lattice. Integer values, or spins, at the grid-points define the cell number and interact with each other. Cell movement is resulted from elementary steps, in which an attempt is made to copy a spin value onto one of its neighbor locations. A target function is assigned to each elementary step and the probability to accept the step is defined in terms of this function.

We compare the behavior of the simulated systems to our time-lapse experiments of cell cultures, as well as re-analyzed published data.

Analysis of cell configurations was carried out with standard and novel methods as well. Local anisotropy of cell configurations was determined by a new, diffusion-based procedure. The characteristic pattern size of emerging networks is derived from the structure factor of cell configurations.

Cell trajectories were extracted from the time-lapse images using manual or automatic tracking techniques, including cross-correlation analysis of consequent images (particle image velocimetry). Cell velocities, displacements and neighbor separation functions were calculated from tracked cell trajectories. To better describe collective streaming observed in high density cell cultures, we have determined the average flow fields around moving cells.

Results

1. Multicellular linear structures and networks form through preferential attachment to elongated cells

   (a) Empirical findings

   - Network formation is not a specific ability of vascular cells: several cell types in culture give rise to multicellular sprouts and connect into networks.
   - Linear structures form on rigid substrates and constantly convecting medium on top of the cells.

- Cell motility is enhanced upon close contact with elongated structures.

(b) Computational analysis of the preferential attachment hypothesis with the particle model

- Cells preferentially attracted to elongated structures in the model produce networks similar to empirically observed ones.
- Simulated networks are quasi-stationary and have a non-trivial characteristic pattern size.
- Percolation threshold of the system is at 0.2 relative volume fraction. The emerging characteristic pattern size depends only slightly on particle density above this threshold, in good agreement with previous experimental findings.

(c) Computational analysis of the preferential attachment hypothesis with the cellular Potts model

- Cells with preferential attachment to elongated structures in the model produce networks similar to empirically observed ones.
- Simulated networks are quasi-stationary and have a non-trivial characteristic pattern size.
- The characteristic pattern size depends only slightly on the cell density above 0.3 relative volume fraction.
- The hypothesized interaction term is inherently asymmetric.
- Sprout formation is counteracted by the smoothening effect of surface tension.

2. Cell polarity linked self-propulsion is a possible explanation of streaming behavior of cells cultured in high density.

(a) Empirical findings

- Motion of high density cell cultures reveals non-trivial velocity patterns: cells form 200-300 μm long and 100μm wide, temporary streams.

- Although cell movement is correlated in the streams, cells exhibit a fluid-like behavior on longer time-scales.

(b) A new self-propulsion model of cell locomotion

- A new model for cell motion is constructed that incorporates the interaction of cell polarization and cell displacement. Polarization is enhanced with successful cell displacements and decays in the lack of motility; cell motion is enhanced in the direction of the polarity vector, therefore the system constitutes a feed-back loop.
- Simulations of individual cells correctly reproduce the experimentally observed persistent random walk performed by individual cells.
- In high density, collective motion of self-propelled cells give rise to streams similar to the experimental ones.
- Cell speed and stream width are controlled by the propulsion strength and memory length.
- Stronger cell-cell adhesion results in wider streams.

3. Active motion of leader and stalk cells is necessary during sprout formation.

(a) Cellular Potts model for multicellular sprout growth

- A model of leader cell initiated sprouting is constructed based on the assumption that the only distinguishing feature of leader cells is their long persistence time.
- Sprouting without leader cells occurs spontaneously, where the speed of sprout growth is decreasing with time.
- Cell-cell adhesion alone is insufficient to maintain sprout growth.
- Cells that preferentially attach to elongated neighbors are able to provide the necessary amount of cells for the maintenance of extended sprout growth.
- Growth speed of leader cell initiated sprouts is constant, in good agreement with experimental findings.

Conclusion

The emergence of the vascular network has been studied for more than a century. Models explaining vasculogenesis are evolving as new experimental results provide more details about the process. In this study, network formation is described as a general ability of cells, even under experimental conditions that fall outside the applicability of previous vasculogenesis models.

Our hypothesis, that cells preferentially adhere to elongated structures, represents a new mechanism that is capable of explaining our new findings and thus provides a new alternative to explain vasculogenesis. The cell biological basis for the hypothesis, however, is not yet known. The micro-mechanical properties of the cell cytoskeleton are altered during elongation. As cells were shown to detect mechanical properties of the underlying substrate, they also might be able to detect the change in the stiffness of their neighbor cells.

An important mechanism observed during vasculogenesis in bird embryos is the formation of multicellular branches through sprouting. To successfully model the linear growth profile and extended length of sprouts, cellular self-propulsion is addressed. Although several self-propulsion models exists, they have not been reported to reproduce the streaming behavior observed in high density cell cultures. We present a transparent model including a feed-back mechanism between cell polarity and locomotion that reproduces individual cell migration as well as the observed streaming behavior. Using this self-propulsion, we show that sprout formation is only possible with actively following cells, in contrast with the widely accepted model of passively gliding followers.

Related publications

- **A. Szabo**, E.D. Perryn, and A. Czirok. Network formation of tissue cells via preferential attraction to elongated structures. *Phys Rev Lett*, 98(3): 038102, 2007.

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- **A. Szabo**, E. Mehes, E. Kosa, and A. Czirok. Multicellular sprouting in vitro. *Biophys J*, 95(6):2702–2710, 2008. doi: 10.1529/biophysj.108.129668.

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- **A. Szabo**, R. Unnep, E. Mehes, W. O. Twal, W. S. Argraves, Y. Cao, and A. Czirok. Collective cell motion in endothelial monolayers. *Phys Biol*, 7 (4):046007, 2010. doi: 10.1088/1478-3975/7/4/046007.