Sprouting and lumen formation during angiogenesis

A cell-based computational model

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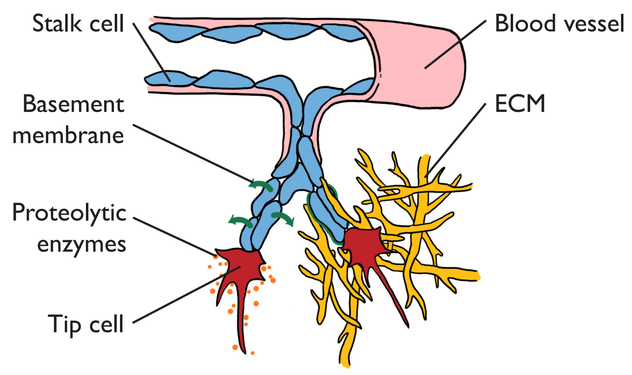
Introduction

Angiogenesis is the formation of new blood vessels from existing vessels. It is a complex process that involves many interconnected mechanisms that are poorly understood experimentally. Computational models are needed to comprehend the interplay, functioning and significance of these mechanisms. We developed a computational model, based on the *in vitro* model of Koolwijk et al. (1996), to unravel the mechanisms that drive sprouting and lumen formation. This systems biology approach links conceptual, experimental and computational models to gain insight in angiogenesis.

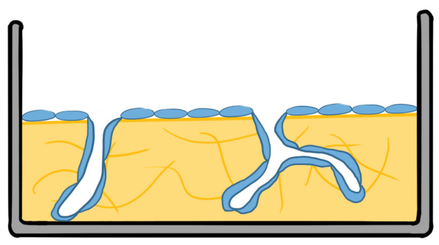
Conceptual model

During sprouting, the tip cell leads migration and stalk cells follow. The extracellular matrix (ECM) is remodeled and a lumen (hollow space) is formed inside the sprout.



Experimental model

A monolayer of endothelial cells is seeded on a 3D fibrin matrix. Upon stimulation with TNFα and VEGF and/or bFGF, sprouts grow into the fibrin matrix and form capillary-like tubular structures (Koolwijk et al., 1996).

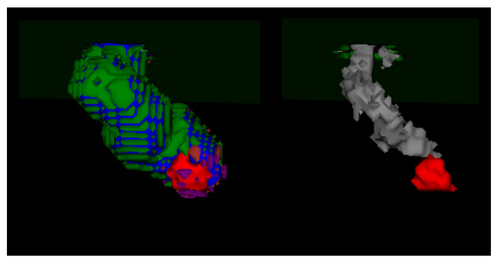


Validation

Predictions

Computational model

A cell-based model, based on the cellular Potts model, represents the experimental model of Koolwijk et al. (1996). It allows individual cells to move, depending on biological relevant constraints as cell size and adhesion.



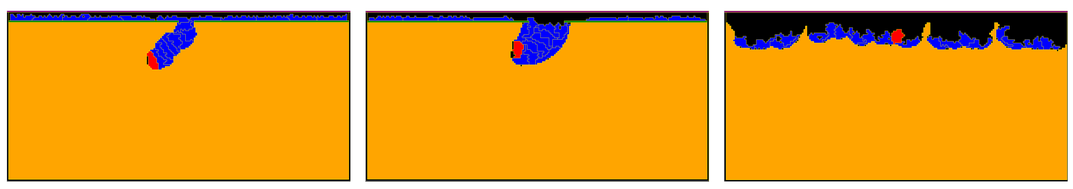
Sprouting

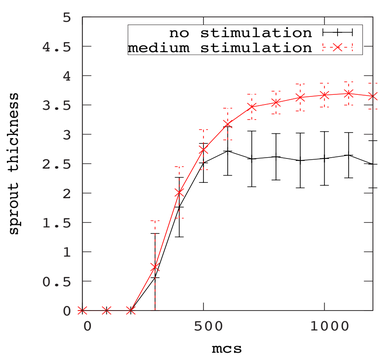
The computational model can be used to find key factors in angiogenesis and to give predictions of their effect on angiogenesis.

Hypothesis

Experimentalists have observed blob formation when cells are mildly stimulated in the production of proteolytic enzymes, lowering of the entire mono-layer when strongly stimulated and sprouting when not stimulated. We suggest that only the tip cell normally secretes proteolytic enzymes to degrade the extracellular matrix, resulting in sprouting. Upon stimulation, stalk cells start to secrete some proteolytic enzymes as well and a blob is formed. When strongly stimulated, stalk cells secrete as much as tip cells and the entire mono-layer lowers.

Stimulation: **none**	Stimulation: **medium**	Stimulation: **high**
Secretion tip cell: **high**	Secretion tip cell: **high**	Secretion tip cell: **high**
Secretion stalk cells: **none**	Secretion stalk cells: **medium**	Secretion stalk cells: **high**





Measuring Sprout Thickness

We developed a measure to express how thick a sprout is by calculating the average number of neighbors of each cell in the sprout. A sprout is unicellular when the average number of neighbors in a sprout is close to two and multicellular when it is larger. The graph shows that the sprout is much thinner when only the tip cell secretes proteolytic enzymes and the sprout is thicker (or blob like) when stimulated.

Conclusion and suggestions for experiments

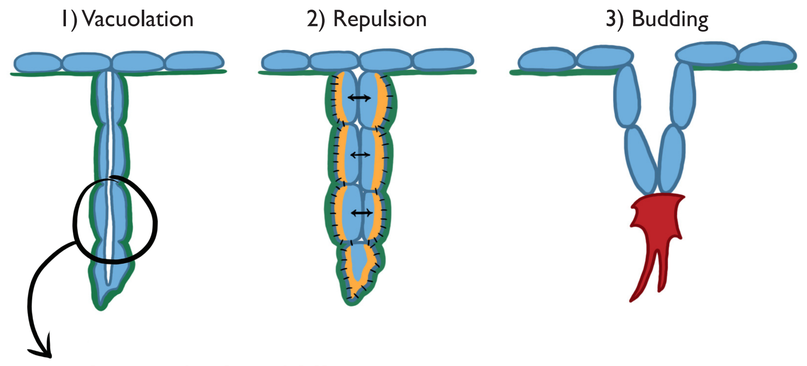
Important parameters in angiogenesis can be identified and examined in the computational model to do predictions. We predict that the shape of the sprout is strongly dependent on the distribution of secretion of proteolytic enzymes over tip and stalk cells. Experiments should test if stalk cells are indeed sensitive to stimulation of proteolytic enzyme production.

Lumen formation

The computational model can be used to test new hypotheses for logical consistency.

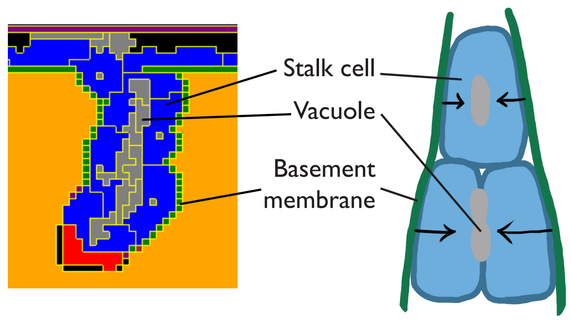
How is a lumen formed in a sprout?

Three alternative hypotheses explain lumen formation (Lubarsky et al., 2003). 1) Vacuoles can be formed in the endothelial cells and they can grow and fuse with vacuoles of neighboring cells to create a tube. 2) Endothelial cells in the sprout polarize and the apical sides subsequently lose adhesion or even repulse each other to create a space. 3) Endothelial cells bud from the vessel and keep the lumen continuous with the lumen of the original vessel.



How do the vacuoles stay in the middle of the sprout?

Integrins have been implicated in regulating formation and maintenance of vacuoles (Bayless et al., 2000). We propose that the basement membrane (BM) regulates the formation and even localization of vacuoles, through integrin signaling. By keeping the vacuoles away from the BM, the vacuoles will stay in the middle of the sprout in sprouts that are one cell as well as multiple cells thick.



Conclusion and suggestions for experiments

The computational model of angiogenesis can be used to test hypotheses for logical consistency. By implementing the theory that the BM regulates the formation and localization of vacuoles, we were able to develop a working model of lumen formation. Experimentally, we would like to visualize the temporal order of BM deposition and the initiation of vacuolation to validate this theory. Integrin blockage can give insights in the signaling mechanisms.

Conclusion: Cooperate with computational biologists

Systems biology is an effective approach to gain understanding in the functioning and interplay of mechanisms in angiogenesis. Computational models can be used to test conceptual hypotheses and to identify and examine key factors in angiogenesis. Predictions based on the computational model lead to hypothesis-based experiments in the laboratory, which can be used to improve and validate the computational model. Thus, a close cooperation between computational and experimental biologists is needed to ultimately reconstruct the process of angiogenesis.

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