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Developmental Biology 289 (2006) 44 – 54

DEVELOPMENTAL BIOLOGY

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Cell elongation is key to in silico replication of in vitro vasculogenesis and subsequent remodeling

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Received for publication 1 April 2005, revised 28 September 2005, accepted 4 October 2005

Abstract

Vasculogenesis, the de novo growth of the primary vascular network from initially dispersed endothelial cells, is the first step in the development of the circulatory system in vertebrates. In the first stages of vasculogenesis, endothelial cells elongate and form a network-like structure, called the *primary capillary plexus*, which subsequently remodels, with the size of the vacancies between ribbons of endothelial cells coarsening over time. To isolate such *intrinsic* morphogenetic ability of endothelial cells from its regulation by long-range guidance cues and additional cell types, we use an in vitro model of human umbilical vein endothelial cells (HUVEC) in Matrigel. This quasi-two-dimensional endothelial cell culture model would most closely correspond to vasculogenesis in flat areas of the embryo like the yolk sac. Several studies have used continuum mathematical models to explore in vitro vasculogenesis: such models describe cell ensembles but ignore the endothelial cells' shapes and active surface fluctuations. While these models initially reproduce vascular-like morphologies, they eventually stabilize into a disconnected pattern of vascular "islands." Also, they fail to reproduce temporally correct network coarsening. Using a cell-centered computational model, we show that the endothelial cells' elongated shape is key to correct spatiotemporal in silico replication of stable vascular network growth. We validate our simulation results against HUVEC cultures using time-resolved image analysis and find that our simulations quantitatively reproduce in vitro vasculogenesis and subsequent in vitro remodeling.
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Keywords: Vasculogenesis; Vascular development; Angiogenesis; Vascular remodeling; Cell shape changes; Computational modeling; Cellular Potts model; Human-umbilical-cord endothelial-cells; HUVEC; Vascular endothelial growth factor; Endothelial cells; Image analysis

Introduction

Vasculogenesis—de novo formation of a primary vascular network from initially dispersed endothelial cells—is the first step in the development of the circulatory system in vertebrates. Under control of long-range guidance cues, including VEGFs and FGFs, the initial network, called the *primary vascular plexus*, expands through angiogenic sprouting. Later, association of the primary vascular plexus with additional cell types, including pericytes and smooth muscle cells, and regression of underused vessels transform the primary network into a vascular tree. Hence, vascular development results from the interplay between the endothelial cells' *intrinsic* ability to self-organize and external regulation by guidance cues and additional cell types.

Here, we ask which aspects of vascular development result from such self-organization of endothelial cells and which aspects require additional cell types and guidance cues. Thus, experimentally, we must distinguish the endothelial cells' intrinsic ability to form vascular-like patterns from those mechanisms requiring guidance and regulation by external tissues. To do so, we use a cell culture model, human umbilical vein endothelial cells (HUVEC) in Matrigel, which is a popular experimental model of capillary development (see, e.g., Chen et al., 2001; Kim et al., 2002; Mezentzev et al., 2005; Segura et

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0012-1606/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2005.10.003

al., 2002; Serini et al., 2003). Matrigel, which is obtained from mouse tumors, contains most of the growth factors the endothelial cells would normally encounter in vivo, while the cell culture model excludes interactions with additional cell types and the influence of remote guidance cues. The extracellular macromolecules and growth factors in the Matrigel stimulate HUVEC cells to elongate and form networks resembling vascular networks in vivo (Fig. 1), where cords of endothelial cells surround empty lacunae. The HUVEC cells do not penetrate into the Matrigel, forming instead a quasi-two-dimensional vascular-like pattern. Thus, our in vitro model compares best to in vivo quasi-two-dimensional vasculogenesis, e.g., in the avian or murine yolk sac (Gory-Fauré et al., 1999; LaRue et al., 2003).

Developmental biology classically aims to understand how gene regulation leads to the development and morphogenesis of multicellular organisms. Tissue mechanics is an essential intermediary between the genome and the organism: it translates patterned gene expression into three-dimensional shapes (Brouzés and Farge, 2004; Forgacs and Newman, 2005). We aim to understand how genetically controlled cell behaviors structure tissues. What cell behaviors are essential? How do cell shape changes structure the tissue? After identifying these key mechanical cell-level properties, we can separate genetic from mechanical questions. Which genes or gene modules influence the cells' essential behaviors and shapes? How do genetic knock-outs modify cells' behaviors? How do these modifications affect tissue mechanics, producing knock-out phenotypes?

Cell shape is an important determiner of tissue mechanics. Cells can change shape *passively*, due to mechanical strain, differential adhesion, or as a result of chemotactic migration by other cells, or *actively* by cytoskeletal remodeling. Such active, genetically controlled cell shape changes are ubiquitous in development, as Leptin and Wieschaus first demonstrated in the early nineties for *Drosophila*. Apical constriction of epithelial cells in early *Drosophila* embryos drives epithelial folding during ventral furrow formation. Numerous genes control these shape changes, including *twist* and *snail* (Leptin and Grunewald, 1990) and *concertina* (Parks and Wieschaus, 1991). In this example, active cell shape changes control morphogenesis by inducing stresses and strains in the ventral furrow. Cell shape can bias chemotactic cell migration by setting a preferred direction of motility. This synergy occurs, for example, during convergent extension in zebrafish, where *slb/wnt11*-controlled, oriented, bipolar shape changes aid persistent, directional cell migration (Ulrich et al., 2003).

Endothelial cells dramatically change shape during angiogenesis and vasculogenesis; in response to growth factors including VEGF-A and VEGF-C, intracellular-store-based calcium entry remodels the cells' actin cytoskeletons, changing their shape from rounded to elongated and bipolar (Cao et al., 1998; Drake et al., 2000; Moore et al., 1998). These calcium-induced shape changes drive the formation of intracellular gaps in confluent rat pulmonary arterial endothelial cell (RPAEC) cultures (Moore et al., 1998), gaps which are similar to the lacunae in HUVEC cultures and in the yolk sac (Gory-Fauré et al., 1999; LaRue et al., 2003).

Computational models of cell aggregates provide important insights into the self-assembly of cells into tissues. They show how relatively simple cell-like behaviors, including cell shape changes, chemotaxis, haptotaxis, cell adhesion, differentiation or induction can produce biological patterns and shapes. Explanatory models of biological morphogenesis address situations ranging from the formation of bacterial (e.g., Budrene and Berg, 1995) and mesenchymal cell (Kiskowski et al., 2004) aggregation patterns and *Dictyostelium* morphogenesis (e.g., Vasiev and Weijer, 1999) to avascular tumor growth (Drasdo and Hohme, 2003), limb patterning (Hentschel et al., 2004) and gastrulation (Drasdo and Forgacs, 2000; Peirce et al., 2004). Many of these models treat cell aggregates as continua or treat cells as points or rigid spherical particles, thus ignoring the role of cell morphology in tissue shape changes. Glazier and Graner's (1993) Cellular Potts Model (CPM) is a simulation technique which describes cell surfaces mesoscopically, allowing for detailed, yet computationally efficient tissue modeling. Glazier and Graner's model has provided useful insights into a range of developmental mechanisms (reviewed in Merks and Glazier, 2005a), including *Dictyostelium* morphogenesis (Marée and Hogeweg, 2001), convergent extension during gastrulation (Zajac et al., 2003), epidermal patterning (Savill and Sherratt, 2003) and tumor invasion (Turner and Sherratt, 2002), but no studies have used the CPM explicitly to assess the role of cell shape changes in tissue morphogenesis. Ultimately, the utility of these models depends on their ability not only to reproduce but to predict experimental phenotypes, e.g., due to gene knock-outs.

Several in silico models have reproduced in vitro vessel-like patterns (Ambrosi et al., 2004; Gamba et al., 2003; Manoussaki et al., 1996; Murray, 2003; Namy et al., 2004; Serini et al., 2003). These models generate static patterns resembling those of the initial stages of the vascular network, but the papers did not compare the development of the in silico network to experimental results. Additionally, patterning in these models is *transient*:

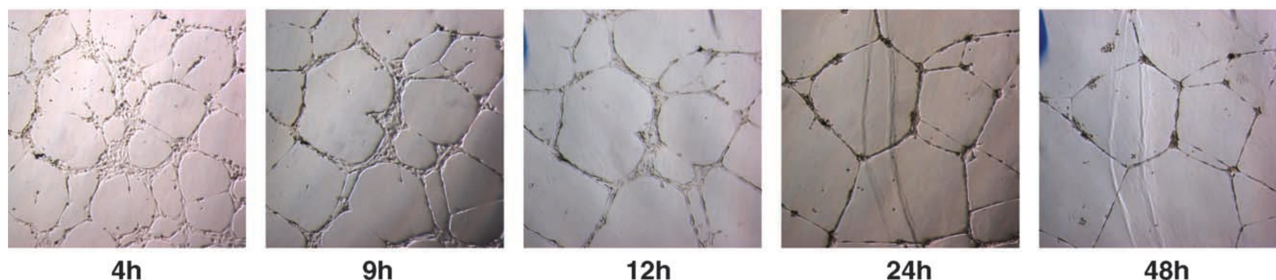

Fig. 1. Typical time sequence of in vitro vasculogenesis at 4 h (h), 9 h, 12 h, 24 h and 48 h after incubation. Scale bar is 500 μm.

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