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Mechanical regulation of vascular network formation in engineered matrices☆

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ABSTRACT

Generation of vessel networks within engineered tissues is critical for integration and perfusion of the implanted tissue in vivo. The effect of mechanical cues in guiding and stabilizing the vessels has begun to attract marked interest. This review surveys the impact of mechanical cues on formation of vascular networks in 2D and 3D gel matrices. We give less emphasis to regulation of endothelial monolayers and single endothelial cells. Several vascularization models have consistently found that the stress generated in the gel, and encountered by embedded cells, control various aspects of vascular network formation, including sprouting, branching, alignment, and vessel maturation. This internal stress is generated by cell contractile forces, and is balanced by gel stiffness and boundary constrains imposed on the gel. Actin and myosin II are key molecular players in controlling initiation of vessel sprouting and branching morphogenesis. Additionally, the impact of external mechanical cues on tissue vascularization, and studies supporting the notion that mechanical forces regulate vascularization in the live animal are reviewed.

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1. Introduction

Insufficient tissue vascularity can result in ischemia, initiating local tissue injury, which can escalate to organ damage. Thus, when designing thick and metabolically demanding tissue constructs, functional blood vessels should be incorporated to supply oxygen and essential nutrients. Incorporation of such vascular networks constitutes a pivotal step toward successful implantation of tissues in vivo. Vascular networks can be formed via vasculogenesis, a process in which endothelial cells (ECs) assemble into blood vessels, and via angiogenesis, formation of new capillaries from preexisting blood vessels.

ECs lining the inner layer of blood vessels are constantly exposed to mechanical signals originating from both fluid shear stress and tension conditions due to tissue remodeling events and cell contractile forces. Elucidation of the biophysical cues controlling endothelial morphogenesis

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into organized networks bears significant therapeutic potential in vascular diseases and cancer and can be of value in guiding and optimizing the creation of vascularized tissue, and its subsequent successful integration and perfusion in vivo [1].

Using various cellular models of angiogenesis and biomaterials, studies have revealed that vascular networks respond to mechanical stimuli, in the form of internal stresses generated within the gel due to cell contractile forces, changes in stiffness, or induced by external forces. For example, internal tension generated by contractile cell forces, can guide endothelial sprouting with the direction of fiber alignment [2]. Alternatively, alteration of the physical properties of gels, either in a 2D polyacrylamide (PAA) system or in fibrin/collagen systems, was found to be a critical factor in controlling vascular organization. In general and as consistently shown with different models of vascularization, formation of vessel networks was more profound in compliant environments, possibly due to increased cell–cell interaction versus cell–matrix interactions. Similar results were obtained when the gels were constrained by rigid boundary conditions, which altered the effective stiffness of the gels. All of these approaches revealed a strong mechanical regulatory effect on vessel network formation, which is discussed below in more detail.

2. Biomaterials

Fibrin mesh formed during wound healing, supports the establishment of new angiogenic sprouting into the wound, while collagen is the most abundant extracellular protein in tissues. Therefore, both fibrin and collagen are extensively used as materials for induction of vascular networks in vitro. Experiments using fibrin and collagen gels have enabled culture of ECs in various ways, as discussed in more detail in Section 3. Both extracellular materials are made of 3D interconnected fibrous structure, with unique physical properties [3], which can be highly remolded by mechanical conditions. For example, anisotropic alignment of fibers due to cell-mediated forces, or external stretching, provided guidance cues that controlled the orientation of endothelial sprouts [2]. Alternatively, synthetic materials, such as PAA and polyethylene glycol (PEG) hydrogels, are useful for creation of mechanically and chemically-controlled cell culture environments. Experiments with PAA gels have demonstrated that compliant or stiff environments can determine whether ECs will form monolayers or organize into connected networks [4,5]. PEG hydrogels provide an inert environment, into which selective peptides and proteins of interest can be covalently attached. As such, it creates a controlled environment to which cells are exposed [6]. Another advantage of PEG hydrogels (for example vs. PAA gels) is that they are generated via a photo crosslinking process that permits encapsulation of viable cells within the 3D material. Experiments with PEG hydrogels functionalized with various extracellular matrix (ECM) biomolecules demonstrated the importance of key ECM proteins in interaction with endothelial vessels. For example, PEG hydrogels incorporated with laminin peptides [7] or heparin [8] showed to boost the creation of vascular networks.

3. Cellular models

A number of cellular models have been developed to study formation of vessel networks both in vitro and in vivo. These include generation of endothelial spheroids, which consists of carboxymethylcellulose-embedded EC (spheroids of 500–1000 cells). The EC form stable tubes sprouting away from the spheroid [9]. Alternatively, the use of EC-coated beads was also shown to be effective in forming endothelial sprouting [10]. In both cases, the spheroids and beads serve as starting points for EC sprouting and network formation, which closely mimic their behavior during angiogenesis in vivo. Our group and others have focused on in-vitro prevascularization of engineered tissues by co-culturing ECs and stromal cells to generate extensive vessel networks [11,12]. The stromal cells act as pericytes that provide physical cues and secrete angiogenic factors that promote vessel formation, maturation and stabilization [13]. We have demonstrated the effectiveness of the co-culturing technique in vascularization of pancreatic [14], cardiac [15,16] and skeletal muscle tissues [17]. Another effective cellular model of endothelial sprouting is seeding an endothelial monolayer on the surface of collagen gels in the presence of pro-angiogenic factors which allows endothelial-sprouting response downward [18]. Other vascularization models use isolated vessel explants [19] or microvessels [20] cultured in native gels. Cells migrating out of the explants form endothelial tube structures, similar to angiogenic vessels in vivo [19]. Models consisting of ECs cultured on synthetic materials such as PAA gels have also been employed. The PAA gels enable tuning of the stiffness of the underlying substrate, independently of the ECM ligand coating. Formation of endothelial networks exhibiting stiffness-dependent stability was reported in this system [5]. Lastly, 3D printing [21], and microfabrication techniques [22] have been utilized to construct well-defined endothelial networks that integrated and perfused following implantation.

4. Cell-induced forces

An inherent property of most living cells, including ECs, is their ability to exert mechanical forces on their surrounding environment. These actomyosin-driven forces are important in a variety of cellular processes, including differentiation [23], migration [24], and division [25]. Such forces can modify the mechanical and structural properties of surrounding fibrillar ECM matrices, which can subsequently trigger feedback responses which regulate cell function. Measurement of EC-induced traction forces has been performed on endothelial monolayers, via traction force microscopy that measures gel displacement by tracking fluorescent particles [26,27]. Other studies of vessel-network formation in 3D fibrous matrices have inferred cell tractions by observing and quantifying matrix fiber remodeling.

Bayless and colleagues developed a system in which an endothelial monolayer is cultured on top of a collagen gel, and vessel sprouting occur downward [18]. Using this system, in conjunction with non-linear optical microscopy, Lee et al. [28] found that the collagen matrix is highly remodeled around endothelial lumens. The collagen remodeling (reflected by altered collagen intensity) was elevated at the periphery of the lumens and branching points, while less remodeled at advancing tips, and was regulated by cell contractile forces [28]. In a different study, using a microrheology technique, local ECM stiffening was measured near the tip of sprouting capillaries [29], suggesting that the endothelial sprouts exert mechanical forces that reorganize the matrix; these matrix alterations may affect subsequent tube formation.

Another indication of the role of cell-induced forces in vascular organization was reported by Korff and colleagues [2]. They observed that the outgrowth of endothelial sprouts in collagen gels was directed toward sprouts of a nearby EC spheroid (600–800 μm apart) (Fig. 1). The sprouts followed the direction of tension-aligned fibers generated by the ECs [2]. When the gels were stretched by an external device, the sprouts aligned in the direction of the external force. This suggests that the forces induced by the sprouting vessels themselves were sufficient to induce long-range deformations of the gel that resulted in directional sprouting. The effect of cell-generated deformation of the matrix was also reflected in a model system in which ECs were cultured on top of PAA gels of varying stiffnesses [30]. The model suggested that ECs communicated through substrate deformations caused by the traction stresses of neighboring cells. In a different study, it was shown that the sprouting occur along cell-induced alignment of the fibers, which also resulted in longer sprouts [31] which further support the importance of cell-generated forces in vascular growth.