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Collagen I hydrogel microstructure and composition conjointly regulate vascular network formation

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ABSTRACT

Neovascularization is a hallmark of physiological and pathological tissue remodeling that is regulated in part by the extracellular matrix (ECM). Collagen I hydrogels or Matrigel are frequently used to study vascular network formation; however, in isolation these materials do not typically mimic the integrated effects of ECM structure and composition that may influence endothelial cells *in vivo*. Here, we have utilized microfabricated 3D culture models to control collagen I microstructure in the presence and absence of Matrigel and tested the effect of these variations on vascular network formation by human cerebral microvascular endothelial cells (hCMECs). Varied collagen microarchitecture was achieved by adjusting the gelation temperature and subsequently confirmed by structural analysis. Casting at colder temperature increased collagen fiber thickness and length, and inclusion of Matrigel further pronounced these differences. Interestingly, the presence of Matrigel affected vascular network formation by modulating hCMEC growth, whereas altered collagen fiber structure impacted the morphology and maturity of the developed vascular network. These differences were related to substrate-dependent changes in interleukin-8 (IL-8) secretion and were functionally relevant as vascular networks preformed in more fibrillar, Matrigel-containing hydrogels promoted angiogenic sprouting. Our studies indicate that collagen hydrogel microstructure and composition conjointly regulate vascular network formation with implications for translational and basic science approaches.

Statement of Significance

Neovascularization is a hallmark of both tissue homeostasis and disease and is in part regulated by cell remodeling that occurs in the extracellular matrix (ECM). The use of bio-mimetic hydrogel cell culture systems has been used to study the effects of the ECM on cell behavior. Here, we employ a hydrogel system that enables control over both the structure and composition of the ECM and subsequently investigated the effects that these have on blood vessel dynamics. Finally, we linked these differences to changes in protein secretion and the implications that this may play in scientific translation.

1. Introduction

Hydrogel-based 3D cultures are widely used to study the formation of new blood vessels from naïve endothelial cells (vasculogenesis) or the preexisting vasculature (angiogenesis) in health and disease [1–3]. Much focus has been placed on utilizing endothelial cell-embedded hydrogels to delineate the role of soluble factors including growth factors and cytokines as well as oxygen and nutrient gradients in microvascular network assembly [4,5]. Yet the extracellular matrix (ECM) plays a similarly important role in regulating endothelial cell behavior, but our understanding of the underlying cellular and molecular mechanisms remains relatively limited. Indeed, ECM composition and structure have been independently shown to influence vascularization in the native microenvironment [6]. However, few hydrogel-based model systems currently exist that allow systematic investigations into the combined and individual effects of ECM composition and structure on microvascular network assembly.

Instructive signals from the ECM can both drive and suppress vascular development and remodeling [7,8]. The basement membrane, a highly porous and organized network of proteins,

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surrounds the endothelium *in vivo* and typically contributes to vessel stabilization [9]. Loss of contact with the basement membrane either through tissue insult or proteolytic degradation initiates endothelial cell migration and sprouting [10]. Induction of endothelial tip cells defines the path of neovascularization and the following stalk cells establish a nascent lumen and remodel basement membrane [11,12]. In addition to directly regulating endothelial cell polarization, migration, adhesion, and function, the basement membrane also physically anchors the endothelium to the interstitial matrix, typically collagen I [13].

Collagen I is the primary ECM component of most connective tissues, easily remodeled by cells, and also represents one of the main structural proteins of blood vessels [14]. The importance of collagen I in angiogenesis is evident as inhibition of collagen crosslinking decreases angiogenesis, while collagen degradation releases sequestered proangiogenic morphogens such as growth factors and ECM fragments [15,16]. Consequently, collagen I is an attractive material for mimicking ECM-related aspects of the microenvironment. However, most current studies disregard that the microscale structure of collagen I can vary significantly, and these structural differences may significantly affect microvascular network assembly [17]. For example, fibrotic ECM remodeling during obesity is associated with both differences in collagen fiber length and thickness and abnormal vasculature, but whether these two phenomena are functionally linked remains unclear [18].

Many current hydrogel-based approaches to investigate microvascular network assembly as a function of the above-described ECM-mediated phenomena rely on the use of either Matrigel or collagen I [19,20]. However, Matrigel, a tumor-derived ECM cocktail, differs from the native basement membrane by lacking physiologically relevant matrix microstructure [21,22]. Inversely, collagen I hydrogels can be fabricated reliably with controlled microstructure depending on the utilized isolation and gelation protocols but lacks key compositional elements of the ECM that influence endothelial cell behavior *in vivo* [23,24]. Hence, exclusive use of collagen I or Matrigel is likely insufficient in mimicking the complex cell-ECM and ECM-ECM interactions that influence endothelial cell behavior during vascularization.

Here, we describe a method for evaluating 3D microvascular network formation as a function of ECM composition and microstructure. We have created hydrogels composed of both collagen I and Matrigel and adjusted their microstructure through varying the hydrogel casting temperature. Subsequently, we tested whether the resulting differences in hydrogel microarchitecture and composition altered endothelial cell vasculogenic and angiogenic potential. Lastly, we examined matrix-dependent changes of endothelial cell-secreted interleukin-8 (IL-8) as a potential molecular mechanism underlying the detected changes in endothelial cell response.

2. Materials and methods

2.1. Cell culture

Immortalized human cerebral microvascular endothelial cells (hCMECs) were provided by Dr. Babette Weksler (Weill Cornell Medical College, New York, NY [25]). Cells were routinely cultured in Endothelial Growth Media-2 (EGM-2, Lonza) containing 2% FBS, growth factors, and 1% penicillin/streptomycin on bovine collagen I-coated (26.35 mg/mL, BD Biosciences) cell culture flasks, at 37 °C, and 5% CO_2. For experiments, hCMECs were used between passages p13 and 19. Fluorescently labeled hCMECs were generated by transfecting the cells with a lentiviral vector containing the mCherry gene. Prior to experiments, mCherry labeled hCMECs were FACS-sorted using a FASCAria III (BD Biosciences) to enrich for highly fluorescent mCherry cells. Human umbilical vein endothelial cells (HUVECs, p2-6) (Lonza) were cultured in Bio-Whittaker medium 199 (M199) supplemented with 20% FBS, endothelial cell growth supplement (ECGS, Millipore), 1% penicillin/streptomycin, 2 mM Glutamax, and 5 units/mL heparin.

2.2. Microwell and collagen hydrogel fabrication

Poly(dimethylsiloxane) (PDMS, Dow Corning) microwells (4 mm in diameter, 250 μm in depth) were fabricated as described previously [26]. Briefly, PDMS was cast onto a silicon master coated with a SU-8 negative photoresist pattern and demolded after

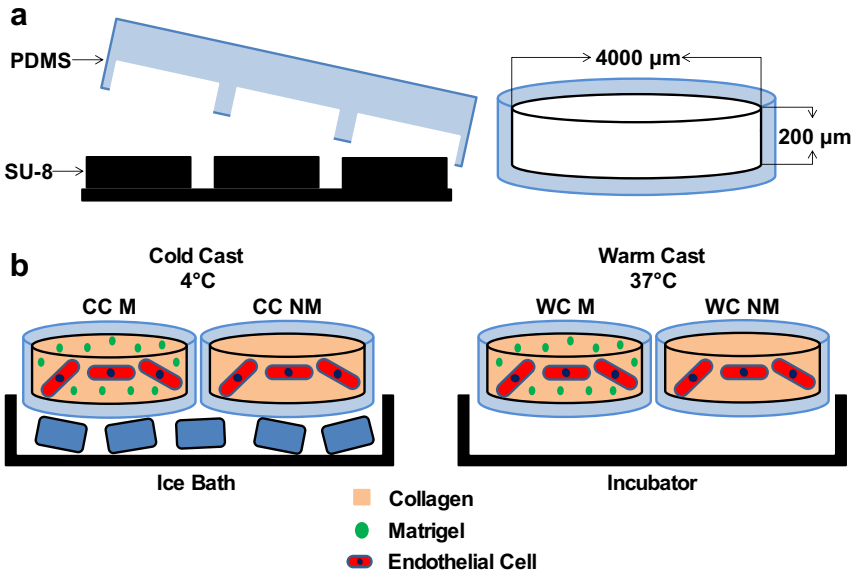

Fig. 1. Experimental design. (a) Microwells were fabricated by polymerizing poly(dimethylsiloxane) (PDMS) on photolithographically etched wafers and were individually punched out and transferred into conventional 24-well culture plates. (b) Type-1 collagen was cast into the microwells with (M) and without (NM) Matrigel and allowed to crosslink either in an ice bath (cold cast [CC], 4 °C) or in an incubator (warm cast [WC], 37 °C). For all cell experiments, human endothelial cells were suspended in the different hydrogels prior to gelation, while characterization experiments were performed with cell-free gels.