CONFIGURABLE GEL GEOMETRY VIA FLOW PATTERNING FOR ANGIOGENISIS ASSAYS

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ABSTRACT

Here we introduce a versatile flow patterning technique (Fig1b) for pillar-less patterning of straight (Fig3) and highly curved (Fig2) gel geometries to test a wide range of in vivo-like vessel sprouting conditions. Much current research focuses on implementing angiogenesis on Organ-on-Chip (OOC) platforms for investigation of the role of signaling proteins[1] and the role of angiogenesis in cancer treatment[2]. Our technique avoids using pillars to confine the gel (Fig 1a) and thereby reduces the contact area between the growing spouts and the unnaturally stiff chip material which is known to affect migration rate of adherent cell types[4].

KEYWORDS: Organ-On-Chip, Laminar Flow Patterning, Angiogenisis

INTRODUCTION

Recent work where excess sprouting is seen in sharp corners suggests that vessel curvature plays a significant role in the induction of vessel sprouting, [6] but this phenomenon has yet to be thoroughly explored. By patterning meandering channel geometries, our technique also allows facile realization of gel geometries with continuous and variable curvature (Fig 2) as opposed to pillar-based patterning where the interface is locked to the lowest energy path between adjacent pillars. In addition, patterning by flow focusing enables fine tuning of gel geometries without the need for total redesign of chips.

With our devices we will investigate the role of signaling protein gradient steepness during angiogenesis [1], as well as the role of curvature on the induction of vessel sprouting.[6]

METHODS

To define our gel geometry we deposit a local hydrophilic coating of molecular collagen in an otherwise hydrophobic polydimethylsiloxane (PDMS) microfluidic channel by hydrodynamic focusing[5]. The non-coated areas remain hydrophobic due to an amino silanization and subsequent glutaraldehyde pre-treatment. A 4mg/mL collagen gel is then introduced, filling the hydrophilic region by capillary forces, and left to set overnight at 37ºC. Finally, human umbilical vein endothelial cells (hUVECs) are cultured in-chip (Fig2). The geometry of the patterned region can be controlled by directly changing the PDMS device geometry or simply by adjusting the relative flow rates used during patterning(Fig2,3).

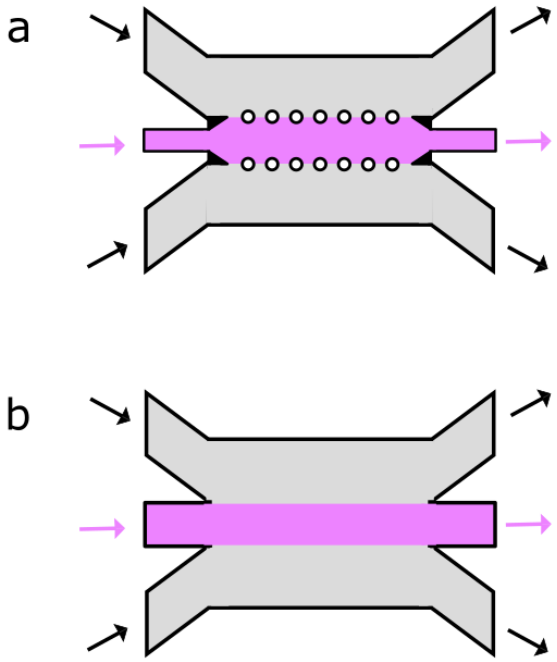

***Figure 1**: Schematic top view representations of angiogenesis-on-chip devices. The gray areas are microfluidic channels, arrows indicate fluidic inlets and outlets and purple regions indicate collagen-filled volumes. (a) Conventional devices contain micropillars for patterning gels[1]. (b) The same gel geometry can be created by surface coating during hydrodynamic focusing.*

RESULTS

We demonstrate the configurability of the patterned region by flow patterning in various ways(Fig2,3). The width of the gel plug can be tuned to taste by applying different flow rate ratios during patterning, reproducing gel geometries common for studying angiogenesis driven by artificially applied growth factor gradients[3](Fig3 Top). In tapered channels (Fig3 Bottom) we show that both the width of the gel and the degree of tapering can be fine-tuned. Highly curved geometries are also realized(Fig2 Bottom) by patterning the gel in a meandering channel. In

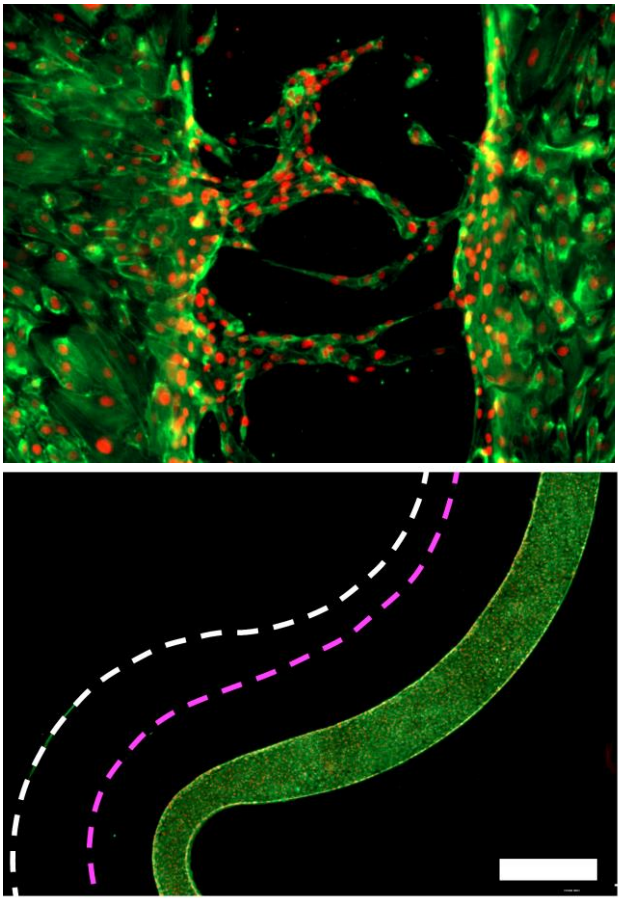

***Figure 2**: Fluorescent stains of hUVECs in our device. (top) Sprouts across the gel (black) as a response to a growth factor cocktail added to the right channel. (bottom) confluent layer in a meandering device. The white line indicates PDMS geometry while the pink line indicates gel geometry. F-actin is stained green using ActinGreen™ 488 while the nuclei of the cells are stained red with NucRed™ Live 647. Scale bars are 500µm.*

spite of long channel lengths(2cm) and highly curved gels our gel patterns remain attached and leak-free even after long term (1week) culture of endothelial cells.

We found that our devices can be used for both the culture of cell layers on the gel/media interface and for the growth of cells into the gel from layers of endothelial cells. hUVECSs were cultured to full confluence in one channel(Fig2 Bot) and could be induced to migrate into the gel via application of growth factor gradients over the gel(Fig2 Top).

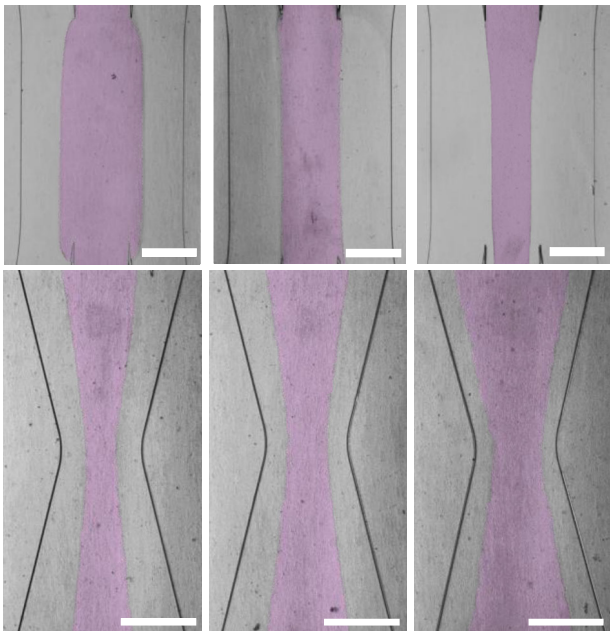

***Figure 3**: Patterned devices with collagen gel (artificially colored pink). Gel is cured and fluidic access channels are filled with 1xPBS solution. (top) straight devices (bottom) tapered devices. Different flow ratios during patterning result in the different gel widths. Flow ratios are 1:2:1 (left) 1:1:1 (middle) and 1:0.5:1*

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