MICROFLUIDIC PLATFORM TO EXAMINE TUMOR ANGIOGENESIS AND METASTASIS AT HIGH SPATIOTEMPORAL RESOLUTION

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ABSTRACT
The process of cancer metastasis follows a complex cascade of events. Animal models, the conventional model of choice, have improved our understanding of metastasis, but they provide limited spatial and temporal resolution of cellular events, and are thus not suitable to understand microenvironmental stimuli. Using microtechnology and tissue engineering, we have developed a microfluidic platform that supports growth of tumor spheroids spatially separated, but in communication through controlled diffusion and convection, from vascular networks. In this study we have characterized interstitial flow patterns and morphogen distribution within the platform, and then demonstrated its utility by assessing the effect of interstitial flow and vascular endothelial growth factor (VEGF) gradients on angiogenesis. We found that vessel sprouting is independently influenced by interstitial flow and the VEGF spatial gradient.

KEYWORDS: Organ-on-a-chip, cancer technologies, tumor microenvironment, angiogenesis.

INTRODUCTION
Metastasis, the most devastating consequence of cancer, remains challenging to treat, and better understanding of the metastatic process is required to devise improved treatments. Simple 2D cell culture systems are clearly inadequate as they lack the intrinsic 3D complexity of in vivo tissues. Animal models have improved our understanding of metastasis, but they provide limited spatial and temporal resolution of cellular and molecular events. Thus, animal models are inadequate to control and isolate effects of microenvironmental stimuli, which includes a host of chemokines, fluid forces, ECM, and infiltrating white blood cells. Therefore, our lab is developing organ-on-a-chip platforms using microfabrication and microfluidic technology together with tissue engineering to better understand the cancer microenvironment, and potentially improve therapies [1-3].

The vasculature plays a critical role in the dissemination of cancer cells from a primary tumor. A tumor can secrete numerous soluble and diffusible factors such as VEGF that can lead to angiogenesis, increased vascular leakiness, and enhanced branching, all of which can facilitate metastasis. The distribution of morphogens in tissues is affected by interstitial fluid flow, which also exerts forces in the tissue microenvironment. Numerous cells in the tumor microenvironment can sense and respond to force, yet our understanding of the independent effect of morphogen gradients and interstitial flow in tumor angiogenesis is limited. This is due, in part, to inadequate experimental models that cannot decouple spatial distribution of morphogens and interstitial flow. We have developed a platform that supports growth of multicellular tissues, including a vascular network and microtumors that are spatially separated, but can communicate by diffusion and convection. In addition, the interstitial flow and exogenous concentration gradients can be precisely controlled in the device. Using this platform, we assess the independent effect of interstitial flow and morphogen gradients on angiogenesis.

EXPERIMENTAL
The microfluidic platform is constructed of polydimethylsiloxane (PDMS) polymer and is comprised of three parallel microporous tissue chambers (< 0.1 mm³ total volume) that are media-fed with microfluidic channels (Fig. 1A). Each microfluidic channel is connected to a sink and a source reservoir. The level of cell culture media in these reservoirs can be adjusted to create a hydrostatic pressure drop and thus the desired interstitial fluid flow across the chamber. A gradient of exogenous morphogens can be created by simply maintaining the fluidic lines (and associated reservoirs) at different concentrations of the morphogen of interest. To estimate the interstitial flow and concentration gradients under various conditions,
a mathematical model of mass and momentum transport was developed using COMSOL Multiphysics finite element analysis software. The model was then experimentally validated using fluorescently labelled dextran as surrogate for VEGF.

In other experiments, endothelial colony forming cell-derived endothelial cells (ECFC-EC) and stromal fibroblasts in extracellular matrix (ECM) proteins (e.g., fibrin and collagen) were seeded in the central tissue chamber. The side tissue chambers were filled either with ECM or with ECM mixed with tumor cells and/or stromal cells. The ECFC-ECs form interconnected microvascular networks with lumens.

Figure 1. (A) Schematic of the microfluidic device, showing three tissue chambers (blue) and the fluidic lines (red). (B) The microfluidic device supports growth of SW620 colon cancer tumors (green) in the vicinity of interconnected ECFC-EC microvasculature (red) when seeded with Fibroblasts (blue) in fibrin gel.

Figure 2. Concentration profiles under diffusion (no interstitial flow; top panel) and at average interstitial flow velocity of 15 m/s (bottom panel) for a morphogen (VEGF), which is added to the top microfluidic channel.

Figure 3. VEGF gradient and direction of interstitial flow independently affect angiogenesis. ECFC-ECs (green) and fibroblasts in fibrin gel were seeded in the central tissue chamber, and acellular fibrin tissues was created in the two side chambers. The devices were stained endothelial cell specific CD31 mAb (green) and DAPI (blue). A) The vessels sprouted in the direction of positive VEGF gradient under minimal interstitial flow conditions (similar to Fig 2, top panel). (B) The vessels sprouted against the direction of interstitial flow.
RESULTS AND DISCUSSIONS

The tissue chamber design of the device is such that it retains the ECM gel and cells in the intended tissue chamber without leakage into adjoining compartments, and yet the gel forms a continuous tissue without any air gaps. This arrangement allows spatial separation for growing microtissues in the same microdevice. The micropores of the tissue chamber allow fluid exchanges between tissue chambers and between a tissue chamber and a microfluidic line. Thus, the device allows growth of 3D heterogeneous microtissues, which is demonstrated by growth of spatially separated microvascular tissue and tumor tissue (Fig. 1B). For some studies, microvascular tissue was created in the central compartment, and acellular fibrin tissue was created in the adjacent or outside compartments. This arrangement allows creation of positive and negative concentration gradients (with respect to the central tissue chamber) in the same device.

Variety of flow and concentration profiles can be generated in these devices by simply changing the pressure drop (level of the media in the reservoirs) or by changing the concentration of the morphogens (Fig. 2). To characterize the flow and morphogen distribution in the device, mathematical simulations and experimental studies were performed on the device with acellular microtissue in all the tissue chambers. Mathematical simulations yielded conditions to create average interstitial flow velocities in the range of interstitial flow velocities of physiological tissues (0.5 m/s to 15 m/s; [4]). The concentration profiles were found for various concentrations of exogenously added VEGF under minimal and maximum interstitial flow velocities. Some of the mathematical simulations were experimentally validated by imaging fluorescent dextran in epifluorescence microscopy. These data demonstrate that the device design allows precise control over interstitial flow and concentration gradients, and a variety of velocity and concentration profiles can be generated using the device.

To demonstrate the utility of platform for deciphering angiogenesis, microvascular tissue was created in the central compartment, and acellular fibrin tissue was created in the side compartment. Under minimal interstitial flow conditions, angiogenesis was biased towards a positive concentration gradient of exogenously added VEGF (Fig. 3A). In the absence of a VEGF gradient, microvessels demonstrated a striking bias for angiogenesis against the direction of the interstitial flow (Fig. 3B). Furthermore, the extent of migration against the flow increased with increasing interstitial flow.

CONCLUSION

In conclusion, we have developed a microfluidic platform that can decouple the effects of interstitial flow and concentration gradients, and using this platform we show that angiogenesis is independently influenced by interstitial flow and concentration gradients of tumor secreted morphogens such as VEGF.

ACKNOWLEDGEMENTS

We thank funding sources National Institutes of Health (CA170879-01 and TR000481; both to SCG). We thank Mr. Vinson Tran for technical assistance.

REFERENCES


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