A HIGH THROUGHPUT MICROFLUIDIC DEVICE FOR GENERATING
MULTIPLE HUMAN MICROTISSUES WITH PERFUSED CAPILLARIES

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ABSTRACT

This paper reports a model system that can generate multiple human microtissues with perfused human capillaries on a single microfluidic platform. Inspired by the understanding of vasculogenesis process, a high throughput microfluidic platform that can provide a three-dimensional biologically and anatomically correct environment was developed. A physiological environment with controlled interstitial flow was generated in each cell construct, seeded with stromal cells and endothelial cells, and the vasculogenic process was initiated to develop vascular network. Perfused capillaries in developed human microtissues were evidenced within 18-day culture by flowing 1 µm fluorescent polystyrene beads and confocal imaging.

KEYWORDS: Microtissue, Perfused capillaries, Vasculogenesis, Interstitial flow

INTRODUCTION

In the last decade, microfluidic devices have been used to mimic physiological conditions for studying cell behavior in 3-D microenvironments [1,]. For example, endothelial cell migration, angiogenic process, and capillary growth through a collagen gel were investigated by introducing angiogenic stimuli through 3-D collagen gel constructs [2, 3]. Also, angiogenic capillary formation was successfully stimulated and analyzed using cocultures of hepatocytes and endothelial cells on two sides of a gel scaffold in a 3-D microfluidic system [4]. These pioneer works successfully replicated living tissue environment and provided a controlled means to investigate cellular behaviors in 3-D.

The development of microvascular network was based on two fundamental mechanisms, one is angiogenesis and the other is vasculogenesis. The new capillaries sprouting from pre-existing blood vessel are angiogenic process [5]. Vasculogenesis, on the other hand, is responsible for the early vascular formation during the embryo development [5]. Recent study also found that circulating bone marrow-derived endothelial progenitor cells can be recruited by ischemic tissue and initiated vasculogenic process. Identified inducers of angiogenic and vasculogenic processes include mechanical (interstitial flow) and chemical (hypoxia and VEGF) stimuli [4-6]. In this paper, we report a model system that can generate multiple human microtissues with perfused human capillaries based on vasculogenic process. By introducing interstitial flow and controlling the nutrition and oxygen supply in each cell construct, microvascular network with perfused capillaries was found after 18 days culture. This preliminary result demonstrated that multiple metabolically active microtissues could be developed on one single microfluidic platform, where nutrients and waste exchange through perfused capillaries – a fundamental feature of all living tissues. By simulating in vivo-like human microtissues, this platform has the potential to revolutionize drug screening, toxicity screening, and biologic mechanisms that depend on a dynamic microcirculation such as tumor metastasis, all in a high throughput manner.

THEORY

To develop multiple microtissues with perfused capillaries based on vasculogenic process, a simple method that can create a controlled physiological environment in multiple cell constructs was developed [Fig. 1]. The key design features can be separated into five parts. First, the multiple gel constructs was created by loading a fibrin gel seeded with normal human lung fibroblasts (LFs) and endothelial progenitor cell-derived endothelial cells (ECs) in a series of mm-sized and diamond-shaped microchannels [Fig. 1(A) and 1(C)]. These microchannels were connected and separated by 50 µm wide necking microchannels. These necking microchannels provided a means to load all the cell constructs with one single loading, and it also served to minimize the influence of vasculogenic stimuli between adjacent chambers. Second, the gel constructs were connected to a media filled side channel through a single pore on each side. These pores and the mm-sized diamond-shaped microchannel controlled the amount and distribution of nutrients introduced into each microchannel, where metabolic deficit environment could be induced. The diamond-shaped chamber also provided a symmetrical geometry for balancing the contractile forces generated by the microtissue while not compromising the vasculogenic process. Furthermore, the concept of capillary burst valve was applied to the design of the connecting pores, where they had smooth curvatures toward the side channel. This method altered the direction of surface tension and prevented leakage of cell suspension during loading. Third, the physiological environment was achieved by generating interstitial flow in each chamber via a high pressure gradient through two connecting pores. The cell constructs responded to the chemical and mechanical stimuli and initiate vasculogenesis within each chamber. The interstitial flow was controlled between 1–5 µm/s, consistent with the range that had been previously shown to generate multiple branches and lumen-containing capillaries [7]. Fourth, to generate identical interstitial flow in each microchannel, the pores of each chamber were connected to a long side channel with a fixed distance between them [Fig. 1(C)]. The pressure gradient was maintained near constant using the high fluidic resistance from the long
side channel and the large media reservoirs [Fig. 1(B)]. This effect was verified by finite element simulation as shown in Fig. 1(D) and 1(E). The velocity profile of interstitial flow is also shown in Fig. 1(F).

Fifth, to facilitate post investigating on the developed microtissues, a microfluidic “jumper” was added in the the path of the long side channel and separated it into two long channels on each side of microchambers. By installing microfluidic jumper, a controlled interstitial flow could be simultaneously introduced in all the microchambers during development of microvascular network. Once microvascular networks developed, this jumper can be removed and different pressure and nutrition compositions can be applied separately in each channel, where one can simulate arteriole and the other can simulate venule. Furthermore, this offers an additional freedom for using the developed microtissues for drug and toxicity screening since the side channels on each side are decoupled.

**EXPERIMENTAL**

Since the physiological environment can be achieved by using (1) connecting pores, (2) mm-sized microchamber, (3) long side channels, and (4) reservoirs with large cross-section, active microfluidic components (valves and pumps) were eliminated. A single mask SU-8 photolithography process was sufficient to create the master for creating polydimethylsiloxane (PDMS) microfluidic platform. After silanization process, a 10-to-1 ratio PDMS mixture was casted on the SU-8 mold to create a thick PDMS layer, and molded 100 μm in depth microchannels was plasma bonded to a 1mm thick PDMS plate after curing. This step created multiple PDMS microchambers for fibrin gel constructs, where we had experimentally found that PDMS surface had a strong adhesion with fibrin gel. Another 3” by 2” glass slide was also plasma bonded to the other side of the PDMS plate to block oxygen access and also provide mechanical support. The thickness of the top PDMS layer was made to be more than 6 mm to create a long path for oxygen diffuse into microchannels, where studies had found oxygen would need more than 10 hours to diffuse through 1 cm thick PDMS. Thus, the main oxygen and nutrition supplies were delivered from the long side channels and connecting pores through the controlled interstitial flow.

The sequence of the experimental procedure is the following. A 30 µl of cell mixture of LFs (5 million cells/ml) and ECs (2.5 million cells/ml) in DPBS were first mixed with 3 µl thrombin in DI water (1 unit/ml). This cell suspension was flowed into microchambers connected by necking microchannels. After 30 min gelation process in a 37 °C incubator, EGM-2 media was flowed into the side channels, and jumper was then installed to complete connection. An interstitial flow less than 0.5 µm/s was maintained for first 12 hours to allow cells settling down in a 20% O2 incubator. Then, the media in the side channel and media reservoir were replaced with vasculogenic growth factors free EGM-2 media (e.g., vascular endothelial growth factor, VEGF). The levels of this EGM-2 media in two media reservoirs were also leveled to maintain a constant interstitial flow in a range from 1 to 5 μm/s in each microchannel. Then, the loaded microfluidic platform were put in 5% O2 incubator to allow the media in side channel had same oxygen tension in normal tissue. Culture media were changed every other day during 14-day culture. Vasculogenic growth factors were introduced back to the system on day 15 and maintained until day 18 for allowing developed capillaries connect to the side channels through connecting pores. The microfluidic jumper was then removed and 1 μm in diameter polystyrene beads were flown through to verify the completion of capillary network.
RESULTS AND DISCUSSION

Fig. 2 shows experimental results of developed microtissues with perfused capillaries. Figure 2(A) and 2(B) are fluorescent images of two of the microtissues fixed after an 18-day culture. Fig 2(C) shows a confocal image of a capillary connecting to the side channel (left). By disconnecting jumper and maintaining a 1.5 mmH2O pressure difference across microtissues, flowing microbeads from high to low pressure were evidenced. Figures 2(E) and 2(F) are the time-lapse of microbeads flowing in the perfused capillary. The confocal images also verified that the microbeads were inside the capillary [Fig. 2(D)]. These results demonstrated that the presented microfluidic platform has the ability to develop multiple microtissues with perfused human capillaries and can serve as a new model for tissue engineering studies.

CONCLUSION

Multiple human microtissues were successfully developed on one microfluidic platform. By maintaining physiological environment during vascular development, including interstitial flow and nutrition supply, a perfused capillary network was evidenced after 18-day culture. The proposed methods could also be generalized to create a host of physiological environments for tissue engineering applications.

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