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Novel Microfluidic Perfusion Bioreactor for Vascularized Organ-on-a-Chip

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Abstract— **By combining advanced microfluidic techniques, the perfusion-based bioreactor plays a critical role in 3D cell/tissue culture, especially for the construction of organ-on-a-chip models. This paper reports a novel microfluidic perfusion bioreactor design by separating microfluidic channels and tissue culture chambers into two separate layers, which can be applied to different applications by designing arbitrary structure with versatile flow profiles. As a proof of concept we have created a novel bioreactor design with more uniform line-to-line flow profiles and higher interstitial flow velocity compared to the conventional communication pore design with point-to-point flow profiles on the same layer. In addition, the microfluidic channel design perpendicular to the gel loading direction was implemented to realize either independent or interconnected horizontal flow pattern rather than the vertical one appeared in most conventional designs. The effectiveness of this novel bioreactor design is validated by using an *in vitro* vascularized organ-on-a-chip model.**

I. Introduction

The circulatory system plays a vital role to maintain homeostasis in human body. It comprises a closed network of arteries, veins, and capillaries that allow blood to circulate throughout the body, not only for waste product removal, but also for gas exchange and nutrient transportation, all of which are essential for organ viability [1]. Recently, the concept of "organ-on-a-chip" has been proposed to establish *in vitro* models that can mimic the microphysiological function and three-dimensional (3D) microstructure of human organ more accurately and specifically compared to the traditional two-dimensional (2D) cultures and animal models [2]. To better mimic the characteristics and functions of specific human organs *in vitro*, it is necessary to integrate a perfusable and functional 3D microvasculature to different organ-on-a-chip systems.

Microfluidic technologies have emerged as useful tools for the development of organ-on-a-chip, which can offer precise control over various aspects of the cellular microenvironment such as a different profile of fluid flow, gradient of various growth factors, and mechanical properties of versatile biomaterials. In order to provide an avenue for cells to access nutrients and oxygen, development of perfusion-based bioreactor with microphysiological flow through tissue interstitial space has become critical [3-5]. The most commonly used method is to establish fluid flow by setting up two microfluidic channels adjacent to a tissue chamber with cell seeded extracellular matrices (ECM) suspension [6-8]. In this geometry, the microfluidic channels need to connect with the tissue chambers through different microstructures or micropillar arrays on the same layer, and the direction of medium flow inside microfluidic channel has to be aligned parallel to the gel loading direction inside tissue chamber, which would greatly limit the chip design and its applications.

Here, we present a novel and flexible microfluidic perfusion bioreactor design by separating the microfluidic channel and tissue chambers into two different layers. Compared with the conventional design, our proposed bioreactor exhibits multiple beneficial features. First, arbitrary structure design of both microfluidic channels and tissue chambers can be implemented based on specific applications, like multi-tissue co-culture, tumor angiogenesis, etc. Second, versatile flow profiles can be simply realized by adjusting the size and shape of the contact area between the microfluidic channel and tissue chambers, like line to line, curve to curve or multi-point to multi-point, etc. Third, either horizontal or vertical flow direction relative to the gel loading direction can be flexibly realized with either independent or interconnected flow pattern. As a model application that takes advantages of these capabilities, the comparison experiment was conducted based on the conventional communication pore design with point-to-point flow profiles and the novel design with more uniform line-to-line flow profiles. Furthermore, to demonstrate the effectiveness and flexibility of this novel microfluidic perfusion bioreactor, the vascularized organ-on-a-chip model was performed inside tissue chambers with vertical and horizontal flow profiles, respectively.

II. Materials and Methods

A. Microfluidic Bioreactor Design and Fabrication

Figure 1 shows the schematic diagram of both conventional and novel microfluidic perfusion-based bioreactor design. For the conventional design, the entire device structure consisted of three central millimeter-sized diamond tissue chambers (1 × 2 mm) and two side square cross-sectional microfluidic channels (100 × 100 μm) that connected to the tissue chambers through a series of communication pores with the width of 50μm as capillary burst valves[9]. The microfluidic channels are coupled in an asymmetrical design with one medium inlet and outlet, which can generate the hydrostatic pressure drop across the tissue chambers. In this design, the middle tissue culture chambers and side microfluidic channels are on the same layer, as shown in Fig. 1(a). However, for the novel design, the microfluidic channels are located on the top layer, while the tissue culture chambers lie on the bottom layer, as shown in Fig. 1(b). In order to prevent gel bursting upwards, an obstacle with the width of 40μm is positioned in the middle of microfluidic channel to divide it into two narrow microfluidic channels with the width of 30μm. With this design, the microfluidic

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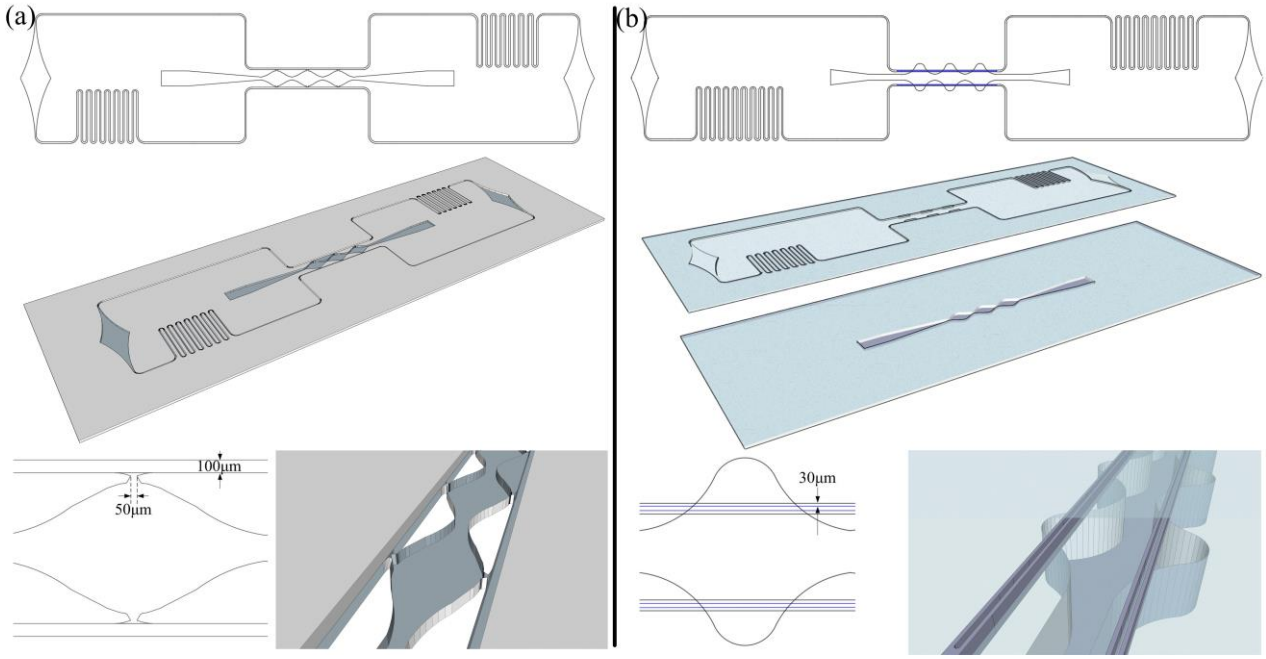

Figure 1. Schematic of conventional and novel microfluidic perfusion-based bioreactor design. (a) Conventional design: the microfluidic channel and tissue culture chamber interconnected through communication pores as capillary burst valve on the same layer; (b) Novel design: the microfluidic channel and tissue culture chamber separated on two different layers.

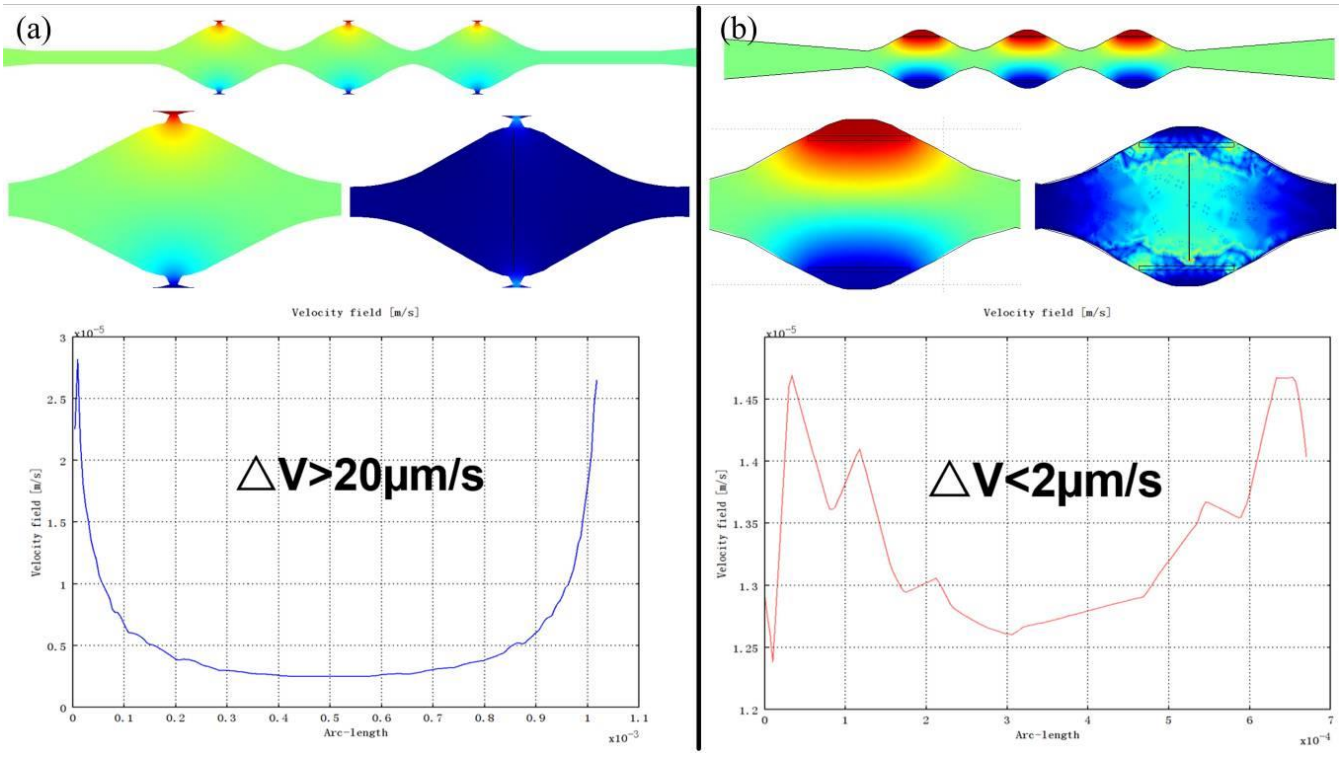

Figure 2. Finite element simulation results on flow profile across tissue chamber inside the conventional and novel designs. (a) Point-to-point pattern with relative low average interstitial flow velocity with large variation; (b) Line-to-line pattern with more uniform and higher interstitial flow velocity with little difference.

channel can be flexibly placed above the tissue chambers regardless of the gel loading direction.

The microfluidic chip was made of polydimethylsiloxane (PDMS) (Sylgard-184, Dow Corning) by micro-molding from SU-8 patterned silicon wafers using standard soft lithography techniques. The height of microfluidic channels and tissue culture chambers were all 100 μm. After the plasma bonding between the microfluidic chip and the PDMS-coated glass slide, two bottomless plastic vials were glued at the inlet and outlet ports using the precured PDMS mixed with curing agent. Fabricated devices were autoclaved at 121 °C for 30 minutes before being used for experiments.

B. *Finite Element Simulation*

Finite element simulations for pressure distribution and interstitial flow through ECM embedded in the tissue culture chamber was performed using COMSOL Multiphysics 5.0 (Comsol Inc., Burlington, MA, USA), as described in our previous work [10, 11]. In the 3D microfluidic model, the Brinkman's equation was employed for momentum transportation through a porous fibrin gel with low permeability (1.5×10^{-13} m^2). Under the driven of hydrostatic pressure drop, only small volumes of media flowed into the tissue chamber due to the high hydraulic resistance of confined gel, which could generate the interstitial flow to stimuli the cultured cells/tissues.

C. *Cell Culture and Flow Control*

For cell culture, human endothelial colony forming cellderived endothelial cells (hECFC-ECs) were obtained from cord blood as previously described [5, 6, 9]. hECFC-ECs were selected and expanded on flasks coated with 10 μg mL^{-1} fibronectin (Sigma Aldrich) in EGM-2 (Lonza). Normal human lung fibroblasts (NHLF) were purchased from Lonza and expanded in 10% FBS DMEM (Corning). hECFC-ECs and NHLFs were used for experiments between passage 4 and 9 and cultured in a 37 °C, 5% CO_2, and 20% O_2 incubator in 100% humidified air. Due to the coupled microfluidic design, the hydrostatic pressure drop between medium inlet and outlet will reduce over time. To solve the concern of insufficient stimuli to the cultured cells/tissues inside tissue chamber, the predefined hydrostatic pressure drop is reset every 24 hours, based on the limits we previously established [5]. Experimental set up for microtissue culture in microfluidic platform was performed as described in our previous work [9-12]. All experimental procedures were performed inside a Biosafety Level 2 laminar flow hood with sterile techniques.

III. Experimental Results

A. *Finite Element Simulation on Flow Profile Across Tissue Chamber*

Figure 2 shows the finite element simulation results of flow profile across tissue chamber inside the conventional and novel bioreactor design under the same hydrostatic pressure drop of 5mm H_2O. In the conventional design, due to the fully symmetrical configuration of communication pores along both side microfluidic channels, the pressure drop across each tissue chamber from the top communication pore to the bottom one was almost the same, which induced the quite similar interstitial flow profile inside each tissue chamber. For the point-to-point pattern in the conventional design, the interstitial flow velocity across the tissue chamber along the vertical direction varies in a wide range (>20 μm/s), as shown in Fig. 2a. However, for the novel bioreactor design, the more uniform interstitial flow profile with little difference (<2 μm/s) was generated inside tissue culture chamber due to the line-to-line pattern. In addition, under the same hydrostatic pressure drop, more liquid volume would perfuse into the ECM inside the novel bioreactor to generate the higher average interstitial flow velocity (~13 μm/s) compared to that (~2.5 μm/s) inside the conventional bioreactor, which can better stimuli the cultured cells/tissues inside tissue chambers under the optimal conditions.

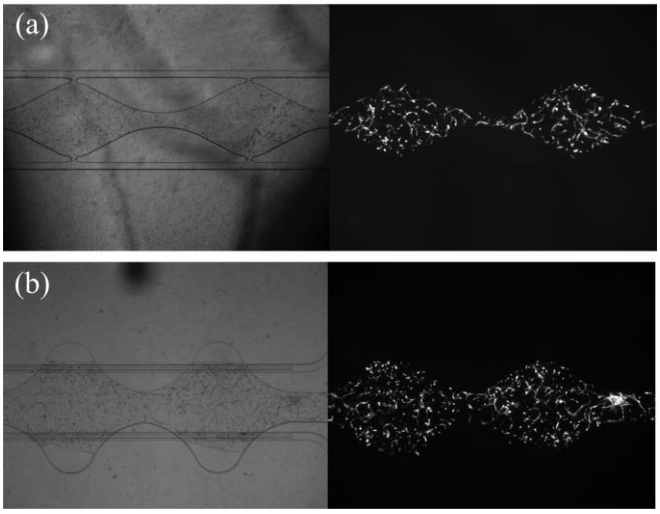

Figure 3. Generation of vascular fragments inside tissue chamber on Day 2. (a) Conventional bioreactor design; (b) Novel bioreactor design.

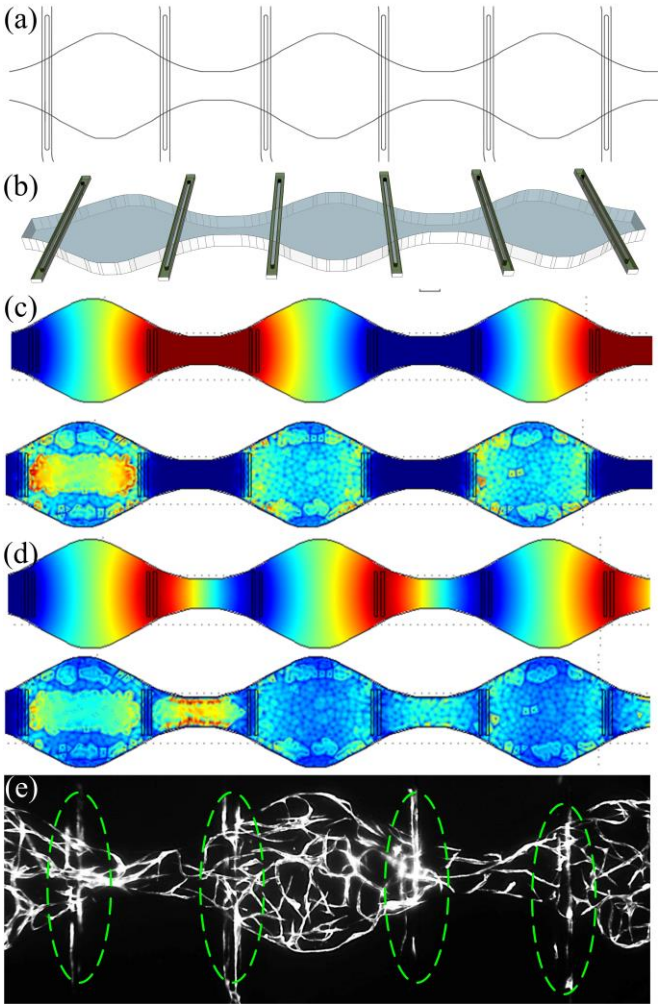

Figure 4. Microfluidic channel located perpendicular to the gel loading direction. (a) 2D schematic; (b) 3D schematic; (c) Independent flow pattern inside tissue chambers; (d) Interconnected flow pattern inside tissue chambers; (e) Microvascular network formation within tissue chambers with interconnected flow profiles and anastomosis formation by migrating upward to the top microfluidic channel on Day 6.

B. Gel Loading and Generation of Vascularized Microtissues Inside Tissue Culture Chamber

The cell-matrix mixture was prepared by suspending ECs (5×10^6 cells per mL) and NHLFs (5×10^6 cells per mL) in fibrinogen solution (10 mg mL^{-1}, Sigma-Aldrich). The suspension was then mixed with 50 U mL^{-1} thrombin (Sigma-Aldrich) to a final concentration of 3 U mL^{-1}, and quickly injected into tissue culture chambers using a micropipettor. By optimizing both the structure and dimension of tissue chamber and microfluidic channel, the loaded gel can be well-confined inside tissue chamber without bursting into the microfluidic channels. For example, the communication pores in the conventional design can function as the capillary burst valve that could precisely control the gel interface at a predefined position aligned with the side microfluidic channel wall. However, for the novel bioreactor design, the narrow channel on the top microfluidic channel layer could confine the gel along the guiding tracks inside bottom tissue chamber without bursting as well. The gel was allowed to polymerize in the incubator at 37 °C for 15 minutes.

The initial pressure drop of 5 mm H_2O to maintain the physiological level of mechanical stimuli on vasculogenesis was established by filling the reservoirs to different culture medium heights. After establishing interstitial flow across the tissue culture chamber, ECFC-ECs formed vascular fragments as early as day 2 in both designs as shown in Fig. 3. Different from the conventional design, more vascular fragments appeared inside the tissue chambers of novel design due to the higher interstitial flow velocity and more uniform flow profile.

C. Independent or Interconnected Flow Pattern Along Gel Loading Direction

Due to the advantages of novel bioreactor design, the relative location between the microfluidic channel and tissue chamber can be flexibly adjusted. Figure 4 shows the microfluidic channel alignment perpendicular to the gel loading direction. By adjusting the relative value of hydrostatic pressure between the adjacent microfluidic channels, both independent and interconnected flow pattern inside tissue culture chambers can be generated, as shown in Fig.4c and 4d, respectively. For example, for the independent flow pattern, the hydrostatic pressures inside microfluidic channels from left to right were set to be 0mm H_2O, 5mm H_2O, 5mm H_2O, 0mm H_2O, 0mm H_2O, and 5mm H_2O, respectively. However, for the interconnected flow pattern, the corresponding hydrostatic pressures from left to right were set to be 0mm H_2O, 5mm H_2O, 0mm H_2O, 5mm H_2O, 0mm H_2O, and 5mm H_2O, respectively. No matter in which flow pattern, the interstitial flow profile along the gel loading direction inside tissue chamber is highly uniform due to the line-to-line pattern. By day 6, the capillary network was lumenized and interconnected between the adjacent tissue chambers based on the interconnected flow pattern, as shown in Fig. 4e. Interestingly, the microvascular would migrate upward to the top microfluidic channel to form the anastomosis (labeled with the dashed ellipse) [9]. In addition, since angiogenesis is more active in the reverse direction of interstitial flow [13], the orientation of microvascular network was horizontal along the gel loading direction.

IV. Conclusion

In this paper, we present a novel microfluidic perfusion bioreactor design by separating microfluidic channel and tissue culture chamber into two separate layers. Compared to the conventional design, versatile flow profiles like line-to-line pattern can be generated inside tissue chamber to provide higher interstitial flow velocity and more uniform flow profiles. In addition, the microfluidic channel design perpendicular to the gel loading direction was implemented to realize the horizontal flow profile relative to the gel loading direction rather than the vertical one existed in most conventional designs. Furthermore, by adjusting the relative hydrostatic pressure between the adjacent microfluidic channels, both independent and interconnected flow pattern along gel loading direction can be realized. Experiments on vascularized organ-on-a-chip model were performed to demonstrate the effectiveness of this novel bioreactor design, which could be easily and flexibly extended to the other organ-on-a-chip applications.

Acknowledgment

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References

[1] R. K. Jain, "Molecular regulation of vessel maturation," *Nat Med*, vol. 9, pp. 685-693, 2003.

[2] S. N. Bhatia and D. E. Ingber, "Microfluidic organs-on-chips," *Nat Biotechnol*, vol. 32, pp. 760-772, 2014.

[3] S. Kim, H. Lee, M. Chung and N. L. Jeon, "Engineering of functional, perfusable 3D microvascular networks on a chip," *Lab Chip*, vol.13, pp. 1489-1500, 2013.

[4] Y. Zheng, J. Chen, M. Craven, N. W. Choi, S. Totorica, A. Diaz-Santana, P. Kermani, B. Hempstead, C. Fischbach-Teschl, J. A. Lopez and A. D. Stroock, "In vitro microvessels for the study of angiogenesis and thrombosis," *Proc Natl Acad Sci U S A*, vol. 109, pp. 9342-9347, 2012.

[5] Y. H. Hsu, M. L. Moya, P. Abiri, C. C. Hughes, S. C. George and A. P. Lee, "Full range physiological mass transport control in 3D tissue cultures," *Lab Chip*, vol. 13, pp. 81-89, 2013.

[6] Y. H. Hsu, M. L. Moya, C. C. Hughes, S. C. George and A. P. Lee, "A microfluidic platform for generating large-scale nearly identical human microphysiological vascularized tissue arrays," *Lab Chip*, vol. 13, pp. 2990-2998, 2013.

[7] J. H. Yeon, H. R. Ryu, M. Chung, Q. P. Hu and N. L. Jeon, "In vitro formation and characterization of a perfusable three-dimensional tubular capillary network in microfluidic devices," *Lab Chip*, vol. 12, , pp. 2815-2822, 2012.

[8] M. B. Chen, J. A. Whisler, J. S. Jeon and R. D. Kamm, "Mechanisms of tumor cell extravasation in an in vitro microvascular network platform," *Integr Biol (Camb)*, vol. 5, pp. 1262-1271, 2013.

[9] X. Wang, D. T. Phan, A. Sobrino, S. C. George, C. C. Hughes and A. P. Lee, "Engineering anastomosis between living capillary networks and endothelial cell-lined microfluidic channels," *Lab Chip*, vol. 16, pp. 282-290, 2016.

[10] X. Wang, D. Phan, D. Zhao, S. C. George, C. Hughes and A. P. Lee, "An on-chip microfluidic pressure regulator that facilitates reproducible loading of cells and hydrogels into microphysiological system platforms," *Lab Chip*, vol. 16 , pp. 868-876, 2016.

[11] D. Phan, X. Wang, B. M. Craver, A. Sobrino, D. Zhao, J. C. Chen, L. Lee, S. C. George, A. P. Lee and C. Hughes, "A vascularized and

perfused organ-on-a-chip platform for large-scale drug screening applications," *Lab Chip*, vol. 17, pp. 511-520, 2017.

[12] X. Wang, D. Zhao, D. Phan, J. Liu, X. Chen, B. Yang, C. Hughes, W. Zhang and A. P. Lee, "A hydrostatic pressure-driven passive micropump enhanced with siphon-based autofill function," *Lab Chip*, vol. 18, pp. 2167-2177, 2018.

[13] X. Wang and Q. Sun and J. Pei, "Microfluidic-Based 3D Engineered Microvascular Networks and Their Applications in Vascularized Microtumor Models," *Micromachines (Basel)*, vol. 9, pp.493, 2018.