Fabrication of Microchip Devices for Organ-on-a-Chip and Lab-on-a-Chip

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Abstract:

Organ-on-a-chip is a microfluidic cell culture platform, integrated circuit (chip) that simulates the activities, mechanics, and physiological response of an entire organ or an organ system. Our lab aims to create organ-on-a-chip devices to study the mechanism of various diseases. In the past year, we mainly focused on two projects: glaucoma-on-a-chip study and Micropatterned traction force microscopy for blood and lymphatic endothelial monolayer in pancreatic ductal adenocarcinoma (PDAC).

Summary of Research, Project 1: Glaucoma-on-a-Chip Model

Fluid homeostasis in the human eyes is maintained by the eye-specific fluid drainage system comprised of Trabecular meshwork (TM) and Schlemm's canal (SC). Structural and functional defects in TM and SC may impede ocular fluid drainage, elevating intraocular pressure (IOP) and damaging retinal ganglion cells, which initiates glaucoma pathogenesis. Here, we developed a dual-layer microfluidic chip system that allows coculture of TM and SC cells, recreating a three-dimensional (3D) SC channel surrounded by TM cells, mimicking the eye-specific fluid drainage system.

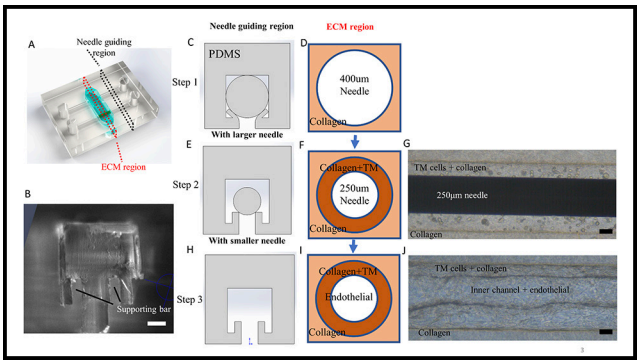

Figure 1: Fabrication of the glaucoma-on-a-chip model. (A) Schematic image of the microfluidic device. (B) The cross-section of the needle guiding region. (C-G) Three steps for fabricating the dual layer structure of TM cells and SC endothelial cells. Scale bar: (B, G, J) 50 μm.

The glaucoma-on-a-chip device design is shown in Figure 1A. In order to achieve the bilayer structure, we designed the cross-section of the needle guiding region as Figure 1B, and the function of this needle guiding region is shown in Figure 1C-J. In the first step, the larger needle was inserted into the needle guiding region, bending the supporting bar, and creating a larger cylinder channel in the ECM region (Figure 1C, D). In the second step, we filled the channel with TM cells suspended in collagen solution and inserted a smaller needle. The supporting bar kept standing to keep the smaller needle in the center of the larger channel (Figure 1E-G). In the last step, the smaller needle was withdrawn and a monolayer of endothelial cells was seeded onto the inner channel, forming a dual layer of TM and SC cells (Figure 1H-J).

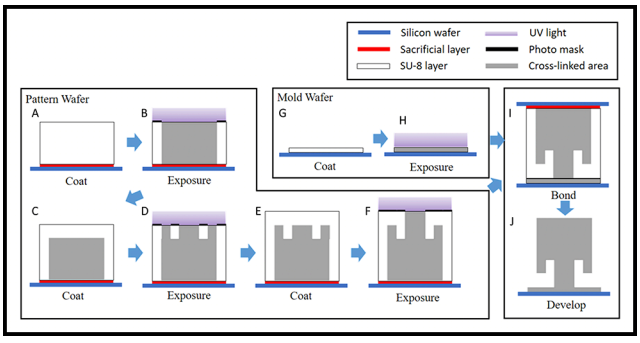

Figure 2: Modified "sandwich" method for fabricating the SU-8 needle guiding region. (A-F) One layer of OmniCoat and three layers of SU-8 were coated and exposed on the pattern wafer. (G-H) One single layer of SU-8 50 was coated and exposed on the model wafer. (I-J) The two wafers were joined before the final PEB, and the final needle guiding layer with two supporting bars was fabricated as designed.

In order to fabricate the needle guiding region with a supporting bar as shown in Figure 1B, we modified the "sandwich" fabrication method which was reported previously [1]. The microfabrication photolithography for the PDMS mold needs to combine two 100 mm

silicon wafer substrates, one was fabricated as a 'pattern wafer' (Figures 2A-F), and another was fabricated as a 'mold wafer' (Figure 2G, H).

Based on the dual layer glaucoma-on-a-chip model, we found that the coculture of SC and TM could better model the development of glaucoma compared with the monoculture of TM or SC Further, Based on NicheNet and angiogenesis reverse western arrays, we further identified VEGF-C and TGFβ as potential targets that may play a role in glaucoma development. Our finding suggests targeting VEGF-C and TGFβ pathways for the development of future glaucoma therapeutic methods.

Summary of Research, Project 2: Micropatterned TFM for Blood and Lymphatic Endothelial Monolayer in PDAC

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and one of the deadliest neoplastic malignancies in humans. It is characterized by desmoplastic stroma, paucity of tumor blood vessels, colonization of tumor-associated immunosuppressive cells, dysfunctional lymphatic vessels, and highly elevated solid stress and interstitial fluid pressure (IFP) [2]. The efficacy of immunotherapy relies on the infiltration of T cells in the tumor microenvironment (TME), but T cell infiltration is difficult under high IFP and with dysfunctional vasculature [3,4].

By combining anti-angiogenic therapy to normalize blood vessels with checkpoint blockade therapy targeting PD-L1, many mechanisms have been described for blood vascular normalization enhancement of immunotherapy in mouse tumor models [5-7]. More recent reports indicate that lymphatic vessels may play an equally significant role in the efficacy of cancer immunotherapies, previously overshadowed by harmful metastasis [8,9].

As a result of these recent findings, lymphatic vessels are now being investigated as targets to alter the TME and response to immunotherapy.

In order to understand the correlation among the intratumoral IFP, blood vessels, and lymphatic vessels, we need to investigate the relationship between vascular endothelial cadherin (VE-cad) junction and biophysical properties (i.e., traction force, intercellular force, and permeability) of endothelium with or without tumor. By creating a patterned endothelial cell monolayer on a force sensing platform, we were able to quantify and characterize the traction and intercellular force of endothelial cells in a control or tumor condition.

A microstamp mimicking the cannula shape of a vessel was fabricated using the soft lithography method (Figure 3A). The surface of a polyacrylamide gel (PAG) based substrate was preprocessed and stamped with cell adhesion molecules (e.g., fibronectin) for cell attachment (Figure 3B). Traction maps were then generated by measuring the displacement of the microbeads embedded in the gel substrate using fluorescent microscopy and Matlab processing (Figure 3C). Results were shown in Figure 4.

References:

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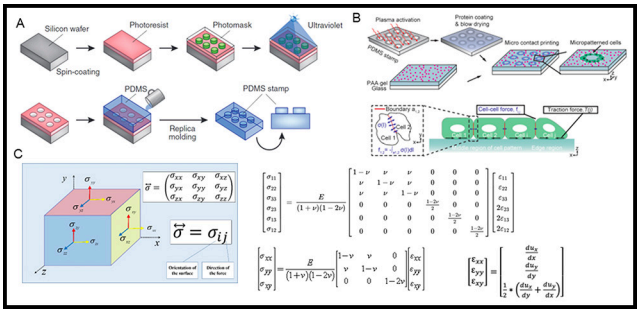

Figure 3: Schematic for micropatterned TFM. (A) Microfabrication process of PDMS stamps for micro-contact printing. Adapted from Bhatia et al., Nat. Biotech., 2014. (B) Fabrication of polyacrylamide gel based substrates with embedded fluorescent microbeads for force detection. (C) Computational modeling of traction force between the cell and the force detection substrate. (B) and (C) are both adapted from Cui, et al., Biophysical Jour., 2020.

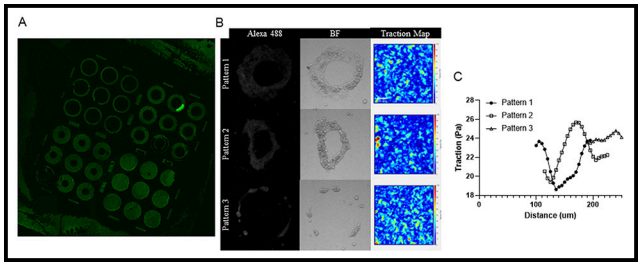

Figure 4: Micropatterned TFM for cancer cell mechanophenotyping. (A) Micropatterned surface of the force detection substrate. (B) Traction map of patterned cancer cells generated from the force detection substrate. (C) Quantification of the traction force in (B).