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

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Principal Investigator(s): Esak (Isaac) Lee, Ph.D.

User(s): Renhao Lu

Affiliation(s): Meining School of Biomedical Engineering, Cornell University

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Contact(s): el767@cornell.edu, rl839@cornell.edu

Primary CNF Tools Used: Heidelberg mask writer - DWL2000, ABM contact aligner, MVD 100

Abstract:

Triple-negative breast cancer (TNBC) is one of the most insidious forms of breast cancer. Among multiple metastatic processes, extravasation determines the final site of the metastasis. We developed a rapid multilayer microfabrication method of transferring a three-dimensional (3D) overhang pattern to a substrate with a sacrificial layer to reconstitute a 3D blood vessel surrounded by the extracellular matrix containing organ-specific parenchymal cells. Based on our *in vitro* model, we found that bone-like microenviroment with osteoblasts and mesenchymal stem cells promoted extravasation of the bone-tropic TNBC cells, whereas the lung-like microenviroment promoted extravasation of the lung-tropic TNBC cells.

Summary of Research:

Despite numerous research efforts, triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer and still the leading cause of cancer deaths in breast cancer patients. Physicians have discovered that breast cancer highly prefers to certain organs for metastases [1], referring as organotropic cancer metastasis. To better understand and treat TNBC metastasis, we need to look into TNBC interactions with blood vessels through bio-mimic models.

Needle-based casting method has been used as a conventional tool for fabricating engineered vessels with an ease of obtaining a cylindrical shape in 3D matrices [2]. However, a needle buffer layer has to be prepared, and additionally mix of photo resist is required to produce a blocking layer [2], which significantly harms the purity of the materials and weakens the bonding strength between the layers, thereby deteriorating the durability of the mold.

Here, we developed a novel microfabrication method allows the fabrication of a vascular channel with only single casting mold by transferring a 3D overhang two-layer SU-8 pattern to a substrate with a sacrificial layer. The casting mold with a 3D overhang pattern provides a more reproducible, reliable vascular conduit structure than previous methods described above.

The microfluidic vascular channel device design is shown in Figure 1(a). The device contains a circular inlet and outlet, an extracellular matrix (ECM) hydrogel cavity located in the center, and two ports to access the vessel lumens. The design is made using L-edit computer-aided design software, and the ultraviolet (UV) photomasks for three layers are engraved on 5-inch-chromium masks by a laser pattern generator (Heidelberg mask writer - DWL2000).

Our PDMS microfluidic chip was fabricated using a conventional polydimethylsiloxane (PDMS) casting process. The microfabrication photolithography for PDMS mold began with two 100 mm silicon wafer substrates, one was used as a 'pattern wafer' (Figures 1(b) and (c)), and another was used as a 'mold wafer' (Figure 1(d)). For the pattern layer, firstly, a layer of OmniCoat™ was spin-coated on the whole surface. Next, a 450 μm thick layer of SU-8 2150 for a 100 μm thick gel-top layer and a 350 μm thick needle guide layer was spin-coated and soft-baked. This layer was exposed by two different masks (needle guide layer mask and gel-top layer mask) using ABM manual mask aligner.

After post-exposure baking (PEB) and cooling down, a 100 μm thick layer of SU-8 100 for the needle buffer layer was spin-coated, soft-baked and exposed. Simultaneously, on the mold wafer, a 50 μm thick layer of SU-8 50 for the bonding layer was spin-coated, soft-baked, and flood-exposed with 450 mJ cm^{-2} of UV exposure without a mask (Figure 1(d)).

Consequently, the two wafers were joined together facing each other, and we performed PEB and developed them together (Figure 1(e)). After the development, the wafers were further developed in an alkaline developer MF319 to remove the OmniCoat™ sacrificial layer, completing

the transferring process. The surface of the fabricated mold wafer was treated by a monolayer of FOTS through the molecular vapor deposition system (MVD 100).

Based on our PDMS microfluidic chip, we investigated breast tumor extravasation in distinct organs to recapitulate the critical step in the organotropic metastasis. Our study showed that bone-like microenviroment with osteoblasts and mesenchymal stem cells promoted extravasation of the bone-tropic TNBC cells, whereas the lung-like microenviroment promoted extravasation of the lung-tropic TNBC cells (Figure 2). Given that these organ-specific parenchymal cells do not impact vascular permeability, our results suggest that the parenchymal cells dictate selective extravasation of the bone-tropic or lung-tropic TNBC cells in our system.

Conclusions and Future Steps:

By transferring multilayer pattern to another wafer, we have achieved building SU-8 overhanging structure without any blocking layers. Furthermore, we found the tissue specific TNBS extravasation phenomenon based on our microfluidic chips.

In the future, we plan to dig into the biological mechanisms behind our findings. Also, we hope to expand our microfabrication chip into other biological applications, including lymphatic and glaucoma organ-on-a-chip models.

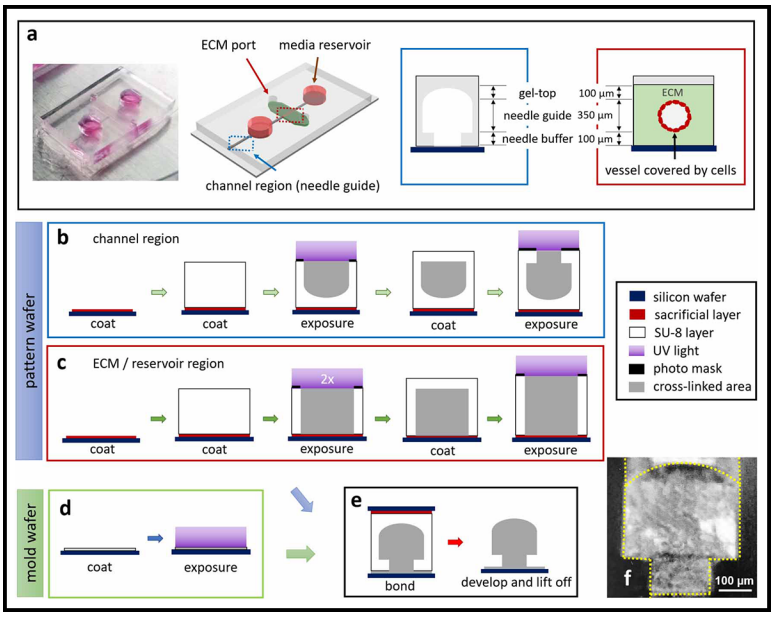

Figure 1: A schematic illustration of the rapid multilayer microfabrication method. (a) A real image of the device and an isometric illustration of the device (left); and in the right, there are cross-section views of the channel region (blue box) and the ECM hydrogel region (red box). (b)-(e) A multi-layer mold fabrication process for the channel region (b) and ECM /reservoir region (c) on the pattern wafer. (d) A flood-exposure is used to form a SU-8 bonding layer on the mold wafer. (e) The two wafers are bonded together by facing during a post-exposure bake (PEB) process and are developed to remove non-crosslinked SU-8 and the sacrificial layer. (f) A cross-section image of a multi-layered overhang structure in the channel region.

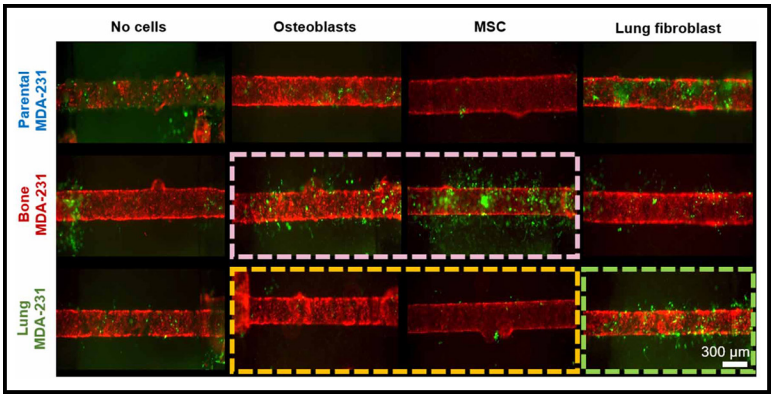

Figure 2: Organ-specific tumor metastasis 3D in vitro. *Parental, bone-tropic, and lung-tropic MDA-MB-231 cells were introduced into blood vessels surrounded by collagen I with no cells, osteoblasts, bone-marrow derived MSC, and lung fibroblasts. The experiment was maintained for six days. Representative images at Day 6 are presented. The highlighted boxes (pink, orange, green) indicate the organ-specific extravasations.*

References:

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[2] Polacheck, William J., et al. "Microfabricated blood vessels for modeling the vascular transport barrier." Nature protocols 14.5 (2019): 1425-1454.

[3] Kwak, Tae Joon, and Esak Lee. "Rapid multilayer microfabrication for modeling organotropic metastasis in breast cancer." Biofabrication 13.1 (2020): 015002.