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Integration of Highly Porous Membranes with Microfluidic Body-on-a-Chip Devices

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Abstract:

Body-on-a-chip devices are devices that contain *in vitro* tissues of multiple organs of the human body. The devices mimic part of the human body in a scaled down fashion on a silicon or polymer chip. They are used to carry out experiments in which the efficacy and safety of new drugs is tested in an inexpensive way and without the need of animal experiments. Here, we aimed to combine a barrier tissue, the gastrointestinal (GI) tract, with liver tissue within one system. Barrier tissues are important because they allow us to simulate the uptake and bioavailability of drugs. Here we microfabricated highly porous membranes that we inserted into body-on-a-chip devices for the purpose of growing GI tract epithelial cells. We also fabricated polymer chips and the corresponding housing for the devices. Finally, we carried out cell culture tests with Caco-2 cells (gastrointestinal epithelial cells) and evaluated the suitability of the devices to support the culture of these cells, providing physical stimulation through fluidic flow and enough oxygen to support cell function. The developed model will be used to test the bioavailability of drugs and nano-scale drug carriers.

Introduction:

Microfluidic body-on-a-chip systems that contain barrier tissues can be used to study the travel of drugs across such tissues and their bioavailability at the target organ. Barrier tissues consist of epithelial cells that grow on a basement membrane. The basement membrane consists of extracellular matrix components such as collagen and laminin. It is difficult to construct such natural membranes *in vitro*, making it necessary to fabricate an equivalent membrane using nanotechnology. The growth of epithelial cell on such an artificial, porous membrane allows us to access both sides of the barrier tissue during drug uptake studies.

At present, commercially available products only provide a porous area of 10% or lower. We have previously developed a fabrication protocol that allows us to microfabricate membranes that are 2-3 μm thick and up to 40% porous [1]. Here, we developed this protocol further, creating a frame around the membrane that allows us to handle it with tweezers to place the membrane into any cell culture system.

In this study, we fabricated highly porous membranes, integrated them into a GI tract/liver system, and cultured Caco-2 cells in the system to validate the proposed device.

Experiment Procedure:

Membrane Fabrication. Figure 1 shows the fabrication process of the membrane. This membrane was made of SU-8 50. SU-8 is a negative photoresist that provides biocompatibility. In this fabrication process, we first spun an SU-8 layer on Si substrate using a spin-coater. We then exposed the frame pattern. After a post exposure bake (PEB), the membrane pattern was exposed. The thickness of the membrane was about 2 to 3 μm. After the sacrificial SU-8 was removed, we released the membrane from the Si substrate. The geometry of the membrane was round with the porous area being of a diameter of 6 mm. The frame extended beyond the membrane by 3 mm. The pores were square holes of 4 μm width. The thickness of the frame was about 40 μm. The total diameter of the membrane, including the frame, was 12 mm.

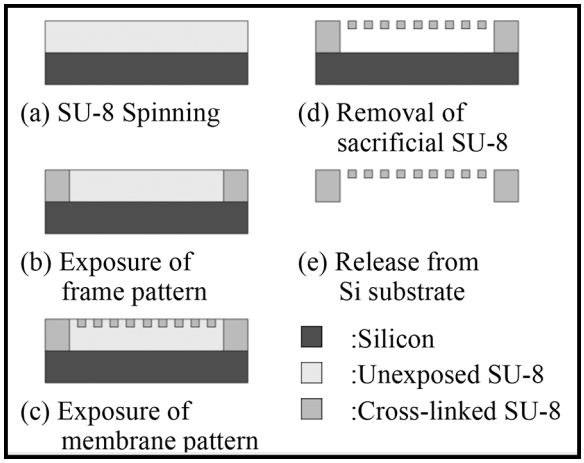

Figure 1: Fabrication process.

Chip and Housing Construction. This cell culture device consisted of two polymer chips with 6 mm holes between

which we sandwiched the membrane. The chip was placed into a polymer housing that contained an inlet and outlet for each of the two microfluidic circuits, which allowed us to access the top and the bottom side of the membrane. The device was 3D printed using an OBJET30 Pro (ALTECH, Israel) and cleaned before adding cells for cell culture. To culture cells, we seeded them into the devices at a concentration of 100,000 cells per cm^2. We then placed the device on a rocker platform that tilted back and forth by 12°, creating gravity-driven fluidic flow. We constructed the devices so that we would achieve the equivalent fluid residence time as seen in the GI tract and liver in *vivo*.

To achieve this, we constructed the microfluidic circuits so that we would achieve a flow rate which of 12 μL/min. We also constructed electrodes that were set into the hole of the top and the bottom parts. We used these electrodes to evaluate the condition of the Caco-2 cell layer.

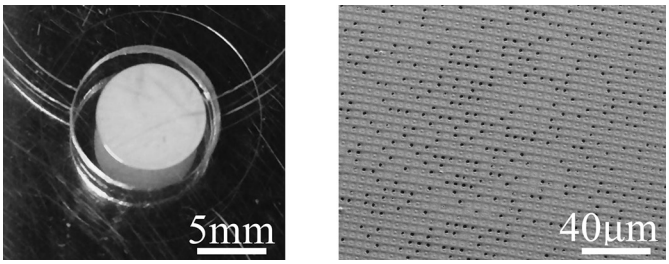

Figure 2: Fabricated membrane. (a) Photograph. (b) SEM image.

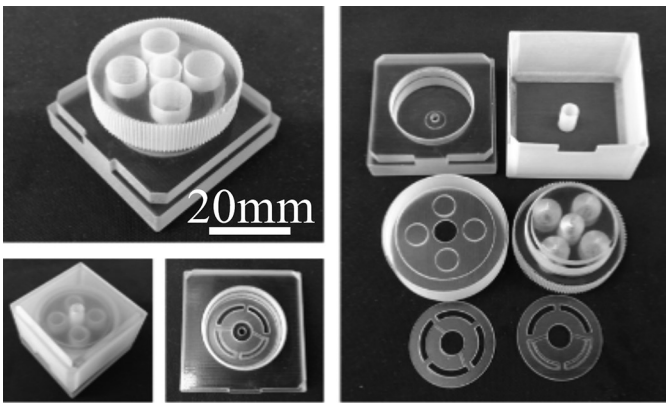

Figure 3: Fabricated device.

Results and Discussion:

Figure 2 shows a photograph and an SEM image of one of the fabricated membranes. The membrane consisted of a frame and a porous inner circle. We were able to pick up the membrane and place it in between the two polymer chips we constructed with 3D printing. The pores of the membrane, however, were only partially open, so that the overall porous area was about 10%. To achieve a greater number of open pores, we need to further optimize the fabrication protocol, balancing the exposure time, so that the membranes are thick enough to be handled, but at the same time, keeping the exposure time low to that a larger number of pores stay open.

We also printed the described polymer chips and the cell culture housing (Figure 3). We tested the volume flow rate in top and bottom fluidic circuit, finding that it was 13.55 μL/min in the top circuit and 23.56 μL/min in the bottom circuit.

We also seeded Caco-2 cells onto the porous membranes, cultured them for 14 days in the incubator, and then inserted the chip with membrane and cells into the cell culture device.

Figure 4 shows Caco-2 cells after the 14-day culture on the membrane, showing that the entire membrane was covered with cells.

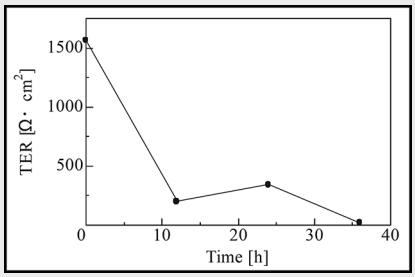

Figure 4: Time response of the resistance.

After inserting the membrane into the cell culture device, we measured the transepithelial resistance (TER). Right after the device was assembled, we measured a high resistance, confirming that the cells had established the tight junctions necessary to reliably test the uptake of drugs. However, the resistance dropped on the following day to values that indicated a damaged cell layer. Further experiments need to be done to determine the cause of this drop in resistance and to enable us to culture Caco-2 cells in the devices for more than one day.

Conclusion:

We have fabricated porous membranes with a frame that makes it possible for us to handle the membrane with tweezers and place it between two 3D printed polymer chips. The membrane supported the culture of Caco-2 cells in a Petri® dish for 14 days. Culture inside the microfluidic device was only successful for one day, as indicated by a high TER on the first day, but a subsequent drop to lower values, indicating the loss of barrier function. To extend the cell culture period of Caco-2 cells inside the devices we will conduct further experiments.

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References:

[1] M.B. Esch, J.H. Sung, J. Yang, C. Yu, J. Yu, J.C. March, M.L. Shuler, "On chip porous polymer membranes for integration of gastrointestinal tract epithelium with microfluidic 'body-on-a-chip' devices", Biomed Microdevices, Vol.14, pp.895-906, 2012.