Microfluidics for the Study of Breast Cancer

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Abstract:

We present a microfluidic system for determining the efficacy of chemotherapy drugs on breast cancer. The volume of the device is approximately 12 μL total, including a control chamber and an experiment chamber. A protocol for fabrication, preparation for cell introduction, culture and introduction of the experimental and control reagents is described. We were able to use the protocol to prepare MCF-7 human breast cancer cells in the device. The cells were able to adhere to the culture surface, proliferate and survive the culture protocols. However, the assessment of the efficacy of the chemotherapy drug tamoxifen was not successful due to the crystallization of the drug as the temperature in the device dropped during the observation periods.

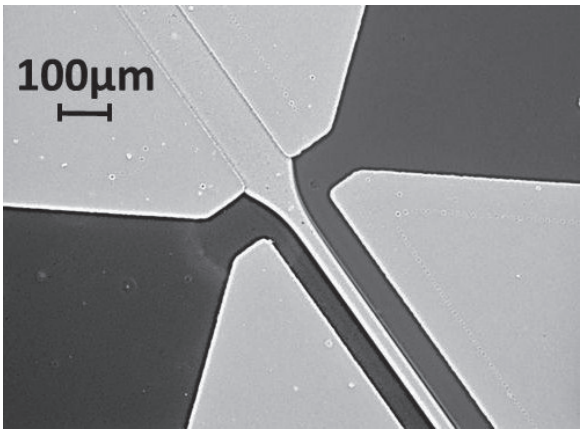

Figure 1: Separation of fluids in the two chambers by flow in the central channel.

Introduction:

Early diagnosis and treatment are a cancer patient's best chance of survival. To that end, microfluidic systems show great promise for developing point of care diagnostic technologies and quick evaluation of treatment options. Microfluidic systems allow precise manipulation of cells in a controlled environment, and can be cheaply and easily fabricated using standard soft lithography techniques. Microscale systems use smaller sample and media volumes, and provide fast results. Testing chemotherapy drugs on a biopsy within a microfluidic system can give patient-specific results so high efficacy treatment can begin as soon as possible.

The objective of this project was to determine the time for a known chemotherapy drug to have an observable effect on cancer cells in a microfluidic device. The cells used were from the MCF-7 cell line, immortalized human breast cancer epithelial cells. The cells were dosed with 20 μM tamoxifen, a chemotherapy drug proven to be effective against MCF-7 cells after a minimum of seven days with traditional methods [1].

Methods:

A microfluidic device was fabricated using standard soft lithography replica molding. A polydimethylsiloxane (PDMS) microfluidic structure was bonded to a glass microscope slide to form a device with two identical 5.95 μL chambers, experiment and control, separated by a central channel. This design allowed mixing between the two chambers initially to establish uniform cell growth in the experimental and control chambers. Diffusion between the chambers could be prevented simply by the flow of fluid through the central channel at a rate ten times higher than the flow into the chambers. The functionality of the design was confirmed by filling the chambers with food coloring and running water through the central channel. This effectively separated the chambers, as shown in Figure 1.

To set up the experiment, each input and output of the microfluidic device was connected to Tygon® Microbore tubing with 20 gauge blunt needles. A three-way stopcock was attached to the end of each tube to facilitate input changes. Three-milliliter syringes were used at the side inputs In1 and In2 to fill the chambers shown in Figure 2. The cell culture chambers were initially filled with gelatin to facilitate cell attachment. Phosphate-buffered saline (PBS) was used to wash out the excess gelatin

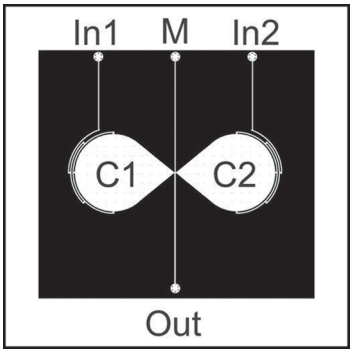

Figure 2: Diagram of the microfluidic device.

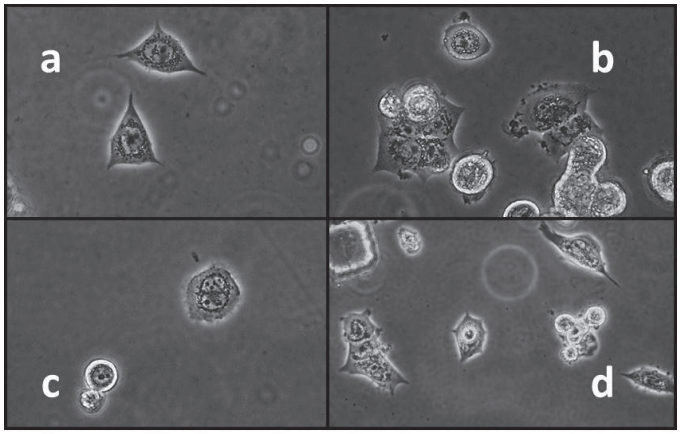

Figure 3: (a) Control group before adding the control solvent, and (b) after 20 hours with the control solvent. (c) Experimental group before adding tamoxifen, and (d) after 20 hours with tamoxifen.

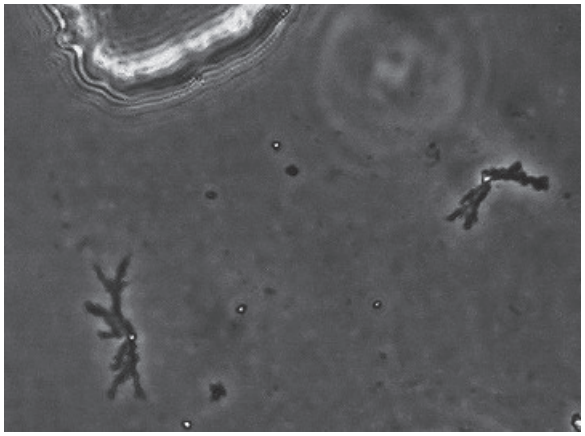

Figure 4: Tamoxifen crystals at 12 hours.

and wet all the surfaces. The PBS was then replaced with cell culture media, Dulbecco's modified eagle medium, plus 5% fetal bovine serum and 1% penicillin-streptomycin. MCF-7 cells were introduced into the chambers, and the microfluidic device was placed in an incubator until the cells adhered to the surface, forming a monolayer.

When healthy cell cultures had been established in each chamber, a syringe pump was used to run media through the middle channel and separate the experimental and control groups. Another syringe pump was then used to introduce 20 μM tamoxifen into the experimental chamber and the same amount of tamoxifen solvent, ethanol, into the control chamber. Cell viability of the two groups was then compared over the next 24-hour period. The flow rates into the chambers were 1 mL/h for 50 min to introduce the tamoxifen, then 2 μL/h complete media to maintain the cells. The corresponding flow rates for the central channel were 10 mL/h and 20 μL/h. These flow rates were shown to maintain the separation of fluids and provide sufficient media for cell growth without introducing harmful levels of shear stress to the cells.

Results and Conclusions:

After 14 hours with tamoxifen outside of the incubator, the MCF-7 cells still appeared to be healthy. The microfluidic device was placed in the incubator. Six hours later both the control and experimental groups showed new cell growth (Figure 3). Tamoxifen crystals were visible in the experimental chamber, indicating conditions were too cool outside the incubator (Figure 4).

The MCF-7 cells failed to exhibit inhibited growth or cell death within 24 hours of tamoxifen introduction. Removing the cells from the incubator for observation cooled the tamoxifen causing crystals to form that the cells couldn't absorb.

Future Work:

Future work includes improving the experimental procedure to prevent crystallization of tamoxifen and broadening the scope to simultaneously test different chemotherapy drugs at varying concentrations.

Acknowledgments:

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References:

[1] E.R.L. Sutherland, R.E. Hall, and I.W. Taylor, "Cell proliferation kinetics of MCF-7 human mammary carcinoma cells in culture and effects of tamoxifen on exponentially growing and plateau-phase cells", Cancer Research, 43, pp. 3998-4006, 1983.