A MICROFLUIDIC DEVICE FOR EXPOSING TUMOR BIOPSY TISSUE TO MULTIPLE DRUGS

Tim Chang[1*] ,Raymond J. Monnat, Jr and Albert Folch
[1]*Department of Bioengineering,University of Washington, Seattle, USA and*
[2]*Department of Pathology, University of Washington, Seattle, USA*

ABSTRACT

This paper reports a novel and practical microfluidic approach for culturing live tumor biopsies and multiplexing their exposure to chemotherapy drugs. Our microfluidic device offers 80 individually addressable streams which enables stimulation of multiple areas on a biopsy tissue and allows explant culture of a full core biopsy tissue while preserving the tumor microenvironment. Biopsy tissues from mouse livers were used to provide data including staining of distinct micro-domains on single biopsy tissues and intracellular delivery. Our results demonstrate the feasibility of a novel preclinical paradigm with great potential for predicting drug responses in cancer patients.

KEYWORDS: Microfluidics, Biopsy, Personalized Chemotherapy, Cancer, Tumor, Drug Testing

INTRODUCTION

Drug prediction in cancer patients is usually inaccurate because of the heterogeneity of the tumors and the difficulty of reconstructing the tumor model. In personalized chemotherapy, DNA sequencing is used to capture key genetic mutations and find the drugs that specifically target these key mutants. However, this genetic approach truly ignores the importance of the tumor microenvironment. Growing evidence suggests a pivotal role of tumor-stromal interactions in tumorigenesis and tumor progression and is responsible for drug responses in cancer patients [1]. Several research groups have used tumor slice cultures as a model for drug prediction [2] but only one group has attempted to culture biopsy tissue [3]. Tumor slices can only be produced from large tumors and after surgical extraction, whereas the extraction of a needle core biopsy is relatively less invasive and is routinely used for diagnostics. Using tumor slices or tumor biopsies capture the importance of maintaining tumor microenvironment for drug prediction. Nevertheless, there is not a systematic model to describe the method of using a piece of tissue from a tumor as a predictive model. In this paper, we report a device which preserves the tumor microenvironment, works as a platform for needle core biopsy culture, can test large amounts of drugs, and is user-friendly to operate. We believe that our approach can be combined with DNA sequencing as a model for drug testing and will have a better prediction about drug responses in cancer patients.

THEORY

Core biopsy tissue from a tumor is a suitable model for drug testing because the biopsy tissue preserves the tissue architecture and is compatible with the clinical approach. To achieve this model, we cultured the core biopsy following the "organotypic culture" technique used for brain slices, whereby the tissue is placed on top of a PET porous membrane, the cell culture medium is placed below the PET membrane, and the tissue is left in contact with air to maintain a good viability (Fig. 1a). The tissue is hydrophilic and is in contact with humidified air, so it is permanently moist, evaporation at the air-fluid interface, plays a crucial role in nutrient transport through the tissue to maintain viability. In order to achieve user friendliness, the device has a microfluidic input interface based on a 96-well plate and enables the multiplexed delivery of large numbers of reagents (Fig. 1b). The reagents can be pipetted into the wells of the 96-well plate and are delivered to the tissue (Fig. 1c) through a network of microchannels that are bonded underneath the 96-well plate.

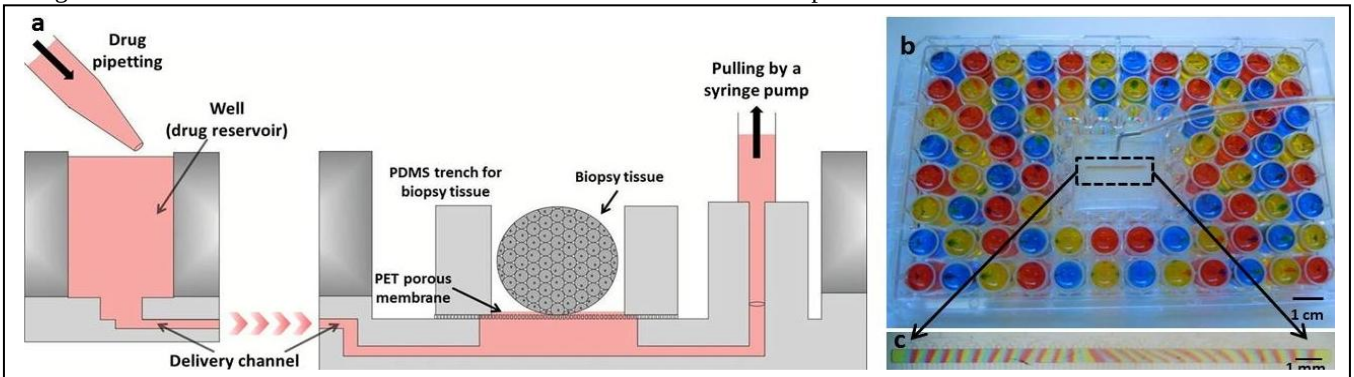

Figure 1. a) Cross-sectional view of the biopsy device. b) Biopsy device with coloring dye. 80 wells can be individually loaded with different reagents and laminar flows are created perpendicularly to the biopsy chamber. c) Multiple laminar flows. The distortion of the streams is because few channels in the center were not open.

EXPERIMENTAL

Device Fabrication and Operation The device includes a 96-well plate, a poly(dimethylsiloxane) (PDMS) interconnect layer, a microchannel network layer ,and the central area of the chip (where the tissue is placed) (Fig. 2a). The first layer is a bottomless 96-well plate with a modification of a square reservoir in the center of the plate. The modification is done by cutting the plate with a rotary tool and sacrificing an area equal to 4x4 = 16 wells, thus the device only accepts 80 inputs instead of 96. The second layer is a PDMS interface containing 3-mm holes and a central square. This is done by exclusion molding from a laser-cut rectangular mold to create a uniform rectangular PDMS block, followed by punching 3-mm holes and cutting the central square to match the modified 96 well plate. The third layer consists of two separate PDMS molds to assemble a complete channel network to distribute the fluids from the well inlets to the tissue. This layer is fabricated using photolithography to create a SU8 master of microchannels on a silicon wafer, laser-cut acrylic sheet to attach to the wafer to define the shape of the PDMS replica, and exclusion molding to create a uniform thickness of the PDMS replica (the thickness is the same as the acrylic sheet). Then, a 0.5-mm punch is used to punch through the PDMS replica to allow the fluids to flow into the microfluidic chip. The fourth layer is a microfluidic chip which has 80 individual channels in parallel. These channels sit underneath a PET porouse membrane, on top of which the biopsy tissue culture chamber sits. The microchannel network layer connects the wells with the central chip in order to deliver the reagents. The flow rates of all channels are equilibrated by adjusting the microchannel resistances (i.e. changing their widths according to their lengths). Bonding of PDMS to PDMS is done by oxygen plasma and PDMS to plastic is done by a surface modification using aminopropyltriethoxysilane (APTES) coating [4]. The central microfluidic chip is integrated within the well plate to accommodate standard microscopy platforms. The device is operated by gravity flow and the total flow rate is driven by a syringe pump through an outlet. Therefore, one tube controlled by a syringe pump is able to create 80 fluidic streams.

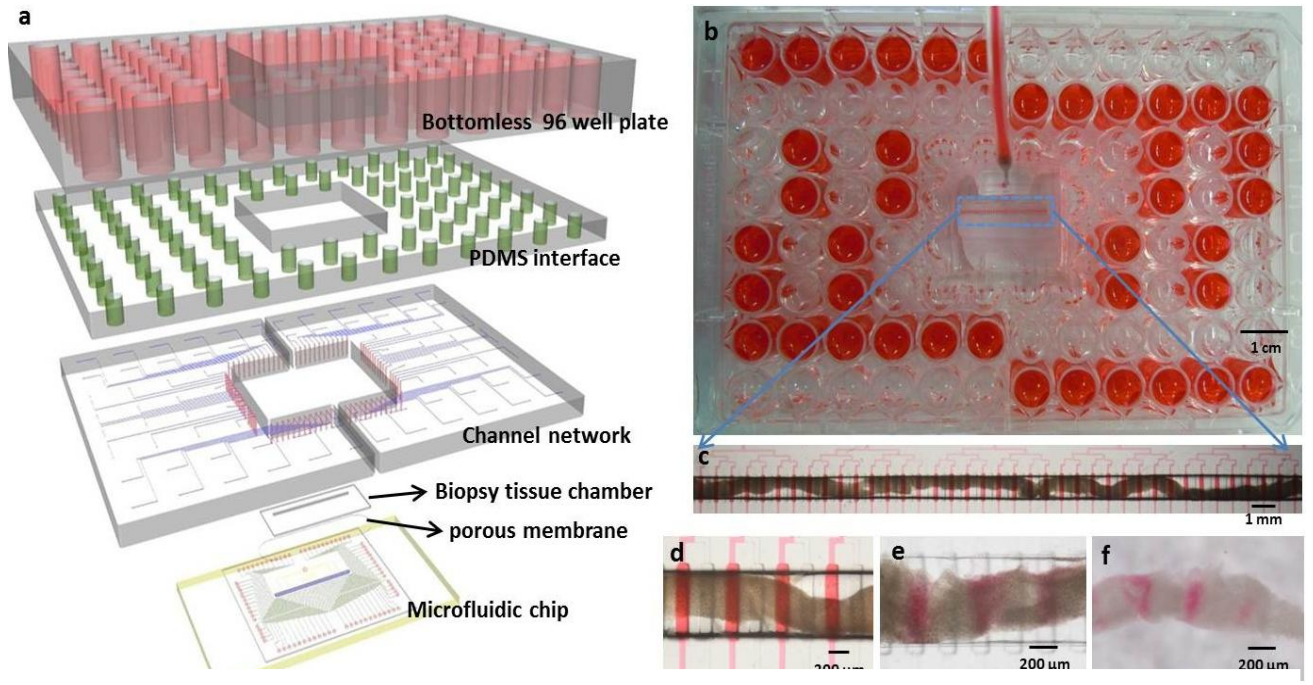

Figure 2. a) Layer-by-layer schematic view of the device. b,c) Two liver biopsy tissues are placed within a culture chamber and on the PET porous membrane while the red colored streams were delivered underneath the tissues. d~f) Red dye staining of liver biopsy tissues. After red colored dye was flowed for two hours (d) and flushed with HBSS (e), the dye was selectively transferred from the bottom streams to the liver tissue.

Mouse liver biopsy extraction Since the heterogeneity of tumor tissue can be a confounding factor in the analysis of results, we used healthy mouse liver as our tissue samples for our first proof-of-concept experiments. Mouse liver slice culture has been shown in many research groups as a model for toxicity testing [5] and is able to provide homogeneous tissue biopsies to allow us to verify our diffusion model in the device. To extract liver biopsies, the liver was first resected from a mouse, then was quickly transferred to ice cold Hanks' balanced salt solution (HBSS). A 20-gauge biopsy instrument (Angiotech, PA) was used to extract multiple liver biopsies with a diameter of 600 µm from one mouse liver, after which the liver biopsy tissues were transferred to the device (Fig. 2b,c) using a transfer pipette. The distinct micro-domains of red food-coloring stains on liver biopsy tissues are shown in Fig. 2d~f.

RESULTS AND DISCUSSION

Intracellular micro-domain delivery Green fluorescent chloromethyl derivatives of fluorescein diacetate (CMFDA, CellTracker Green) were used as a staining reagent to visualize diffusion transport to live cells. We first seeded GM639 human fibroblast cells in our device and waited for 3 hours to allow cells to attach to the PET membrane. We then pipetted CellTracker solution (1 μM) and HBSS in the alternative channels to allow both solutions to flow beneath the membrane and the cells for 40 minutes before the images were acquired (Fig. 3a). The micro-domain staining of the monolayer of attached cells was distinguishable and allowed us to verify the diffusion through the PET porous membrane from the bottom fluids to the cells. Additionally, we used a mouse liver biopsy tissue with the same experimental conditions as the dissociated cells to test the diffusion transport to the tissue. As seen in the results in Figure 3b, we were only able to see the micro-domain staining in a 2-dimensional view but there was no cross diffusion seen in this CellTracker experiment. The additional use of confocal microscopy will be applied to see the diffusion in a three-dimensional manner.

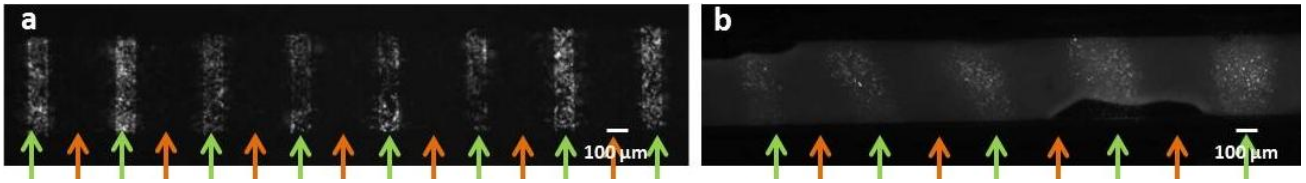

Figure 3. a) Monolayer of human fibroblast cells and b) a liver biopsy tissue were selectively stained with CellTracker.

CONCLUSION

We have successfully fabricated a novel microfluidic device that enables the culture and multiplexing of large numbers of reagents on one piece of biopsy tissue. Using a biopsy tissue allows for extracting a large amount of biological information with minimum invasiveness to the patients. Moreover, using an intact biopsy tissue preserves the tumor microenvironment, requires no additional procedures to the tissue (no additional tissue sectioning or dissociation) and matches the standard procedure of tumor diagnosis. This microfluidic device enables us to test large amount of drugs on a tumor biopsy to predict drug responses in cancer patients and is able to provide fast and accurate results. The device also has a user-friendly interface which is operated by pipetting. Our preliminary results demonstrate the feasibility of a new paradigm for predicting personalized chemotoxicity response profiles based on intact biopsy tissues.

ACKNOWLEDGEMENTS

This research was funded by the Coulter Foundation.

REFERENCES

[1] A. Orimo and R. A. Weinberg, Stromal fibroblasts in cancer - A novel tumor-promoting cell type, *Cell cycle*, 2006, **5**, 1597-1601.

[2] V. Vaira, G. Fedele, S. Pyne, E. Fasoli, G. Zadra, D. Bailey, E. Snyder, A. Faversani, G. Coggi, R. Flavin, S. Bosari and M. Loda, Preclinical model of organotypic culture for pharmacodynamic profiling of human tumors, *Proceedings of the National Academy of Sciences of the United States of America*, 2010, **107**, 8352-8356.

[3] S. M. Hattersley, C. E. Dyer, J. Greenman and S. J. Haswell, Development of a microfluidic device for the maintenance and interrogation of viable tissue biopsies, *Lab on a Chip*, 2008, **8**, 1842-1846.

[4] V. Sunkara, D. K. Park, H. Hwang, R. Chantiwas, S. A. Soper and Y. K. Cho, Simple room temperature bonding of thermoplastics and poly(dimethylsiloxane), *Lab on a Chip*, 2011, **11**, 962-965.

[5] I. A. M. de Graaf, P. Olinga, M. H. de Jager, M. T. Merema, R. de Kanter, E. G. van de Kerkhof and G. M. M. Groothuis, Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies, *Nature protocols*, 2010, **5**, 1540-1551.

CONTACT

Tim Chang, tel: +1-206-6169036; tim7252@u.washington.edu