CIRCULATING TUMOR CELL DETECTION FOR HUMAN BREAST CANCER DIAGNOSIS

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ABSTRACT

Detection and diagnosis of early stage tumors is normally accomplished by a combination of mammogram screening and biopsy. Mammography can detect only highly developed and calcified tumors, usually larger than 1 cm in diameter, even though detection of tumors at an early stage is the main factor in successful treatment of breast cancer. Detection of circulating tumor cells in the human body by a microfluidic biosensor can be the key to overcoming this limitation. Chang and Hammer [1] developed mathematical models of optimal flow velocity for antigen/antibody binding reactions. Demonstration of capture of MCF-7 cells using a microfluidic biosensor device was repeated by Feng et al. [2] This device was not for suitable routine clinical use since the cancer cell concentration was too high (10^5 cell/ml). The critical number of circulating tumor cells in human blood to determine cancer is 1~10 cell/ml [3]. New methods of preparation, delivery, and capture for a single or precise number of cancer cells without any cell loss or clogging was introduced to support experiments with very low cell counts. Capture of MCF-7 cells, human breast cancer, was successful in anti-EpCAM functionalized PMMA micro device with a predicted optimum flow velocity of 2 mm/s. The current objective of this project is to determine an optimized design of the microfluidic device. The flow rate in a micro channel to detect very rare cancer cells in whole blood is less than 10 minutes.

METHODS ACCOMPLISHMENTS

The design of the channel is the one of the important factors for maximizing the capture efficiency. The dimensions of channel were 100 µm wide and 20 µm high since capture rates will improve as the channel depth or width approaches the size of the cancer cells, which are typically 10-20 µm in diameter. The dead volume must be minimized to ensure capture of very rare cells from whole blood so that the target cells are not lost or trapped in other regions of the microfluidic network, including the interconnects. In addition, the channel has to be wide enough or deep enough to reduce the pressure drop and allow adequate throughput. The solution was to locate a capillary at the center of the entrance to a microfluidic channel. Assembly of the PMMA channel chip, PMMA cover chip, and capillary tubes was achieved by using passive alignment on the both PMMA chips to minimize the dead volume and to avoid unexpected cell loss on the flow path from cell preparation to cells in the micro channel (see Figure 1). Mold insert for the channel PMMA chip and cover PMMA chip is micro-milled (Kern MMP, Germany) and hot embossed using a Jenoptik HEX 02 system at the Center for Advanced Microstructures and Devices (CAMD).

The hot embossed PMMA chips were modified by exposure to UV, with 254 nm wave length and 15mW/cm² intensity for 8 min, and followed by an IPA rinse. The exposure time was selected based on a combination of the maximum water contact angle on the modified PMMA surface, the minimum physical surface damage, and the maximum FITC intensity of the anti-EpCAM functionalized PMMA surface for different conditions, materials, and doses. Sealing of the UV-modified PMMA channel was completed by thermoplastic fusion bonding with sub-micrometer deformation of the micro channel. Thermoplastic fusion bonding for UV-modified PMMA surfaces was optimized at 103°C for 35 min under minimal pressure - the temperature was lower than the pristine PMMA Tg (105°C) due to the UV modification. The cross-section of the fusion bonded channel is shown in Figure 2. Sealing tests of the thermally bonded channels was done by pumping dyed buffer.

NH2-modified and anti-EpCAM functionalized PMMA channel was attained by ethylenediamine, anti-EpCAM, and EDC coupling. Precise, small numbers of MCF-7 cells have to be collected and delivered to the micro channel from original sample solution to ensure accurate evaluation of the capture efficiency. A new protocol was introduced. A method using a hemacytometer, a capillary with polimide removed, and a 5 µl syringe was used to collect single or precise, small numbers of MCF-7 cells. A 105 µm diameter capillary was visible and movable in the 100 µm depth
hemacytometer after flaking off polyimide coating over a 600°C flame. From 1 to 10 MCF-7 cells were collected in the capillary tube (see Figure 3). The cell-containing capillary was inserted into a larger diameter capillary tube which is already inserted and sealed into the PMMA chip during the thermo plastic fusion bonding process (see Figure 4). The cells in the capillary tube were pumped into and through the functionalized microchannel. Delivering the collected MCF-7 cells from inside the capillary to the micro channel was done successfully without cell lose. MCF-7 cells were stained with DAPI and trypan blue to mark then as target cells and flowed at a 2mm/s average flow velocity in the channel. The channel was rinsed by PBS at greater than 10 mm/s flow velocity to confirm the strong binding of the anti-EpCAM and MCF-7 cells. Captured cells are shown in Figure 5 with both a DAPI filter and open filter. An untreated PMMA channel was used for the control experiments at the same conditions and no cells were captured. A series of experiments with ten MCF-7 cells and a range of flow velocities were completed to evaluate the capture efficiency. MCF-7 cells will then be added into whole human blood to determine the effect of other elements on the capture efficiency.

ACKNOWLEDGMENTS
This work is supported by a grant (NIH R21/R33-CA-099246-01) from the National Cancer Institute (NCI) of the National Institutes of Health (NIH), the National Science Foundation under Grant Number EPS-0546411, and the State of Louisiana Board of Regents Support Fund

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