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SEPARATION OF BREAST CANCER CELLS FROM BLOOD USING ANTIBODIES FIXED IN MICRO-CHANNEL

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ABSTRACT

This work demonstrates the application of Bio-Micro Electro Mechanical System (Bio-MEMS) technology to breast cancer detection and diagnosis. In early breast cancer detection and diagnosis, instead of using conventional mammogram screening followed by biopsy diagnosis, this detection micro-device is developed to identify and collect specifically tumor cell of low abundance from blood. By immobilizing the Anti-EpCAM antibodies on micro-channel walls with the use of the chemical surface modification of the PMMA, breast cancer cells, over-expressing EpCAM on their surfaces, are caught by the strong binding affinity between antibody and antigen. This detection micro-device can be assembled with other lab-on-chip for follow- up gene and protein marker analysis. The anti-EpCAM was successfully immobilized on the micro-channel and breast cancer cell stained with DAPI (blue) was detected in the micro-channel under florescence microscopy. Also, the optimal and critical velocities were determined in the models of antibody-antigen binding under blood pressure flow.

INTRODUCTION

Breast cancer is a leading cause of cancer death in women in the United States. Each year invasive breast cancer is diagnosed in 180,000 women, and more than 40,000 women die from this disease [1]. Despite considerable progress in the early detection of breast cancer by using screening techniques like X-ray mammography and ultrasound imaging, breast cancer mortality has remained unchanged. Mammogram screening produces inconclusive results, which require patients to undergo follow-up procedures, such as the surgical biopsy, in which suspicious tissue is removed and sent to pathology laboratory for analysis to determine if the tumor is benign or malignant. Fine Needle Aspiration (FNA) and Core Needle Biopsy (CNB), as well as surgical biopsy, are used increasingly as the initial diagnostic procedures. However, breast cancers metastasize very early in their course, and these procedures cannot reliably detect all cancerous tumor, so there is a need for improved early detection and diagnostic techniques for breast cancer.

Bio Micro-Electro-Mechanical Systems (Bio-MEMS) has been a topic of growing interest recently. The appealing advantage of this technology is that individual micro-components can be assembled into an instrument having specific functions. In this work, initial experiments to support the design of a device for detecting low abundant ($\sim 10^{-7}$) cancer cells in whole blood were carried out.

BACKGROUND

Tumor cells (ectoderm origin) differ from normal blood cells (mesoderm origin) in their gene expression, and each of these two cell populations has tissue-specific molecules on its surface [2]. Breast cancer cells over-express the epithelial cell adhesion molecule (EpCAM), which is 280,000 EpCAM molecules per cell. The EpCAM expression of breast cancer cells makes these cells suitable targets of the Anti-EpCAM antibody. At the early stage of tumorigenesis, tumor cells are shed into circulating blood, which makes it possible to detect cancer cells in the blood stream before the primary tumor is large enough to be detected by standard screening examinations [2].

In antibody-antigen binding, an increase of the flow velocity of cells relative to the micro-channel substrate leads to an increase in the encounter rate of antibody and antigen, while the encounter time for reaction is reduced [3]. Also, the mechanical strength of receptor-ligand adhesion is related to the chemical affinity of the receptor/ligand pair [4].

METHODS

A surface modification protocol for PMMA channels was performed and a detection method utilizing antibodies to recognize the tissue-specific molecules was proposed and applied. To catch and detect the rare breast cancer cells in blood (1 tumor cell among 10^7 normal blood cells), three fluorescence markers, each identified by a separate color were used to reliably identify the cancer cells. The cancer cells are defined by nucleic acid$^+$ (blue), CD45$^-$, and cell

membrane linker$^+$(green). White blood cells, which will interfere in detection of the cancer cells, are identified nucleic acid$^+$(blue), CD45$^+$(red), and cell membrane linker$^+$(green).

Three EpCAM/Anti-EpCAM binding models [3,4,5] were applied to determine the optimal velocity to guarantee the maximum binding and identify critical velocity, at which the existed bonds will break down due to high flow velocity through the micro-channel.

RESULTS

The rare breast cancer cells of interest are blue in the nuclei and green outlining the cell membrane but not blue and red shown by white blood cells. The properties that the rare breast cancer cells are simultaneously positive for two separate markers showing different colors, and negative for the Anti-CD45 (red) allow for the detection of one cancer cell out of one million blood cells. Figure 1 shows that breast cancer cell line (MCF-7) stained with DAPI was detected in micro-channel without antibody immobilization.

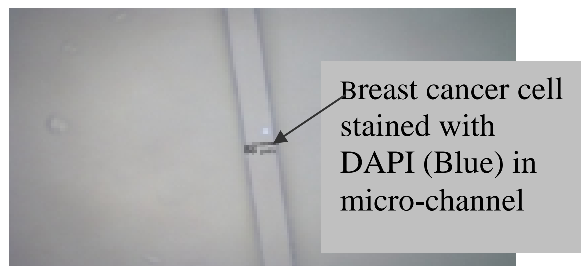

Figure 1 Breast Tumor Cell line (MCF-7) stained with DAPI in micro-channel (channel width is 58 μm).

In Figure 2, the green line along the channel displays that Anti-EpCAM and secondary antibody-FITC were immobilized on the micro-channel wall after PBS rinse.

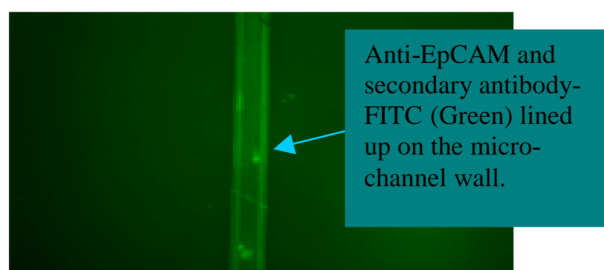

Figure 2 Mouse IgG Anti-EpCAM (Primary Antibody) and Donkey Anti-Mouse IgG-FITC (Secondary) immobilized on micro-channel wall.

Figure 3 shows that in the case of delta equaling 300, the effective binding reaction reaches maximum when Peclet number is 1000, thus he optimal velocity, at which antibody-antigen reaches maximum binding is determined.

Also, the critical velocity for breaking down the existed antibody-antigen binding was calculated by comparing the total force integrated on the contact surface area with the shear force on the cells. The total antibody-antigen binding force in the contact zone was determined by using two models. Both of them gave the same result.

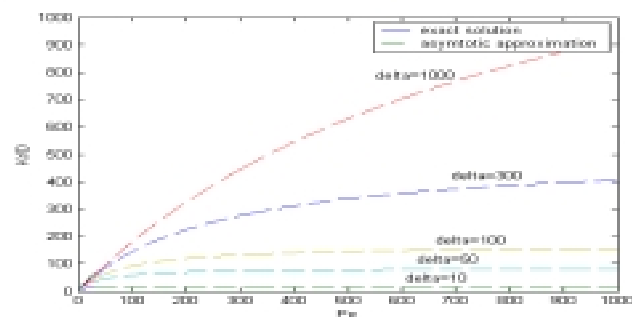

Figure 3 Dimensionless forward reaction rate constant K_f / D as a function of Peclet Number.

CONCLUSION

Breast tumor cell was collected by anti-EpCAM and detected by separate immunoflorescence, DAPI$^+$ (blue), CD45$^-$, and cell membrane linker$^+$(green). Optimal flow velocities for EpCAM/anti-EpCAM binding is 2mm/sec, and critical force to uproot adherent cell from substrate is 4.3×10^{-3} dyne.

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