A SELF-ASSEMBLED MONOLAYER CELLS ARRAY FOR RAPID TARGETED CELLS IDENTIFICATION

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ABSTRACT

We present a simple technique to arrange large quantity of living mammalian cells into a self-assembled two-dimensional (2D) monolayer array for rapid targeted cells identification, which is potentially useful for the early detection of circulating tumor cells (CTC). By controlling the concentration of cells suspension, 2 x 10^4 cells/μl gives the optimized density for 2D cells assembly. With the condition which enable cells align into a 2D monolayer array, the lowest ratio of the targeted cells to the white blood cells this simple method can provide is 1/10,000 by using a fluorescence microscope.

KEYWORDS: Cells Identification, Self-Assembled Monolayer

INTRODUCTION

Several methods such as flow cytometry [1], immunomagnetic enrichment [2], polymerase chain reaction (PCR) based array [3] have been developed for detecting circulating tumor cells [4] and widely used in medical. However, none of the described method is cheap or achievable in most cell biological laboratories. Furthermore, it takes a long time for the tumor cells to be separated one after another one from the whole blood cells in a large number. To solve these problems, this paper proposes a technique to align cells in a 2D array on substrate for effectively identifying the targeted cells rapidly and in parallel in a very short time.

THEORY

A cell culture insert, which has a Polyethylene terephthalate (PET) membrane with 0.4 μm diameter holes on top, is used as the cells aligning platform in our study. Figure 1 is the schemes of the designed chip. First, the water is added into the insert. Then the superabsorbent polymer (Poly(acrylic acid) partial salt, or PAA) powders (< $1mm^3$) are added into the water, and they absorb the water and swell. During the swelling process, under the PET membrane is filled with PAA. Next, the insert is flipped over, and the suspended cells inside a droplet (5μl) of buffer solution are brought into contact with the PET membrane substrate. The solution is then rapidly absorbed into the polymer and a flow field toward the inner and bottom direction is generated according to the patterns designed on the membrane. As the solution becomes thinner and thinner, stacked cells will be washed away from the first layer and a dense 2D cells array will be self-formed on the membrane in 3 minutes.

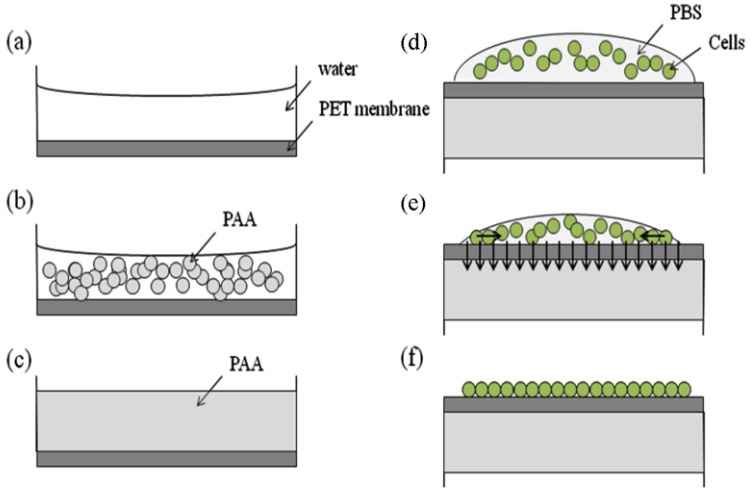

Figure 1: (a) The water is added into the insert. (b)The PAA powder is added into the water. (c) PAA powders absorb the water and swell in few minutes. (d)The insert is flipped over, and the cells suspension is dispensed onto the PET membrane. (e)The solution is then rapidly absorbed into the polymer and a flow field toward the inner and bottom direction is generated according to the patterns designed on the membrane. (f)As the solution becomes thinner and thinner, stacked cells will be washed away from the first layer and a dense 2D cells array will be self-formed on the membrane in 3 minutes

EXPERIMENTAL

To demonstrate the feasibility of this concept, HL-60 cells and BT-474 cells (both fixed by glutaraldehyde (GTA) and stained with fluorescent wheat germ agglutinin (WGA)) were employed. The different concentrations (1x10^4 cells/µl, 1.5 x10^4 cells/µl, 2 x10^4 cells/µl and 3 x10^4 cells/µl) of HL-60 cells suspension are tested to acquire the self-assembly monolayer cells array. After the optimized concentration of cells suspension is determined, the identification of the targeted cells from white blood cells is also tested. HL-60 cells stained with fluorescence dye with excitation spectrum at 488 nm represent the targeted cells, and HL-60 cells expressing weak green fluorescent protein (GFP) represent the white blood cells. Besides of using one kind of cell stained with different dyes, the two different kinds of cells were also demonstrated. BT-474 cells (as the targeted cells) and HL-60 cells (as the white blood cells) were stained with fluorescence dye with excitation spectrum at 555nm and 488 nm, respectively. The targeted cells are identified by the difference of their fluorescence spectrum with a fluorescence microscope.

RESULTS AND DISCUSSION

Figure 2 shows the cells self-assembled on the substrates from cells suspension of different concentrations. Under the concentration of 1 x 10^4 cells/µl, the cells concentration is too low to assemble a dense pack(Figure 2(a)). Therefore, the concentration is increased to 1.5 x 10^4 cells/µl to obtain the more densely packed cells (Figure 2(b)). At a concentration of 2 x 10^4 cells/µl, cells form a dense monolayer array with hexagonally close pack(Figure 2(c)). The monolayer arrangement of cells array is desired to prevent signal blockage from stacked cells of multiple layers, as the concentration of 3 x 10^4 cells/µl shown in Figure2 (d). In Figure 3, it is shown that cells concentration of 2 x 10^4 cells/µl gives the optimized density for 2D cells assembly.

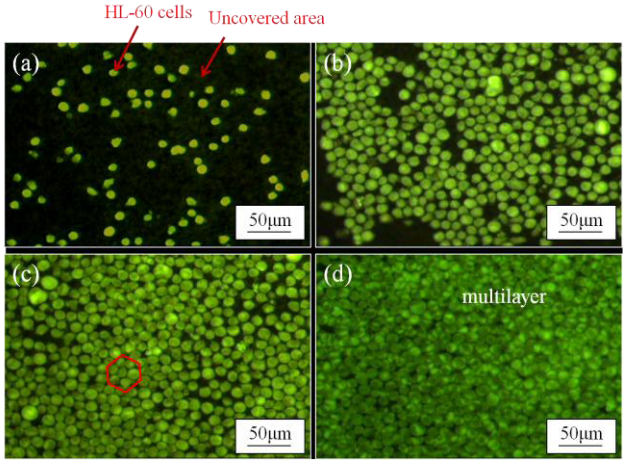

Figure 2: View of the cells with concentration of (a) 1 x 10^4 cells/µl (b) 1.5 x 10^4 cells/µl (c) 2 x 10^4 cells/µl (d) 3 x 10^4 cells/µl on the substrate after PBS in cell suspension was absorbed.

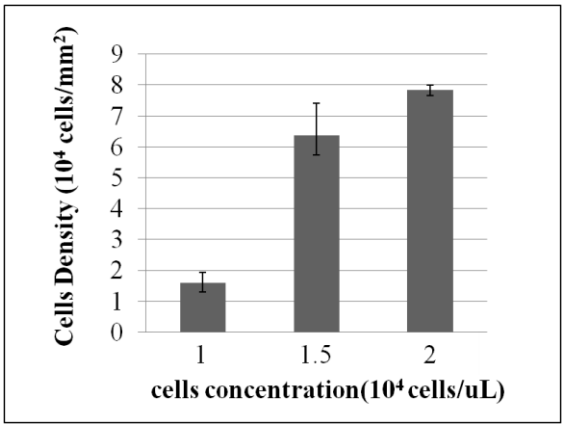

Figure 3: The cells density on the substrate with different cells concentration.

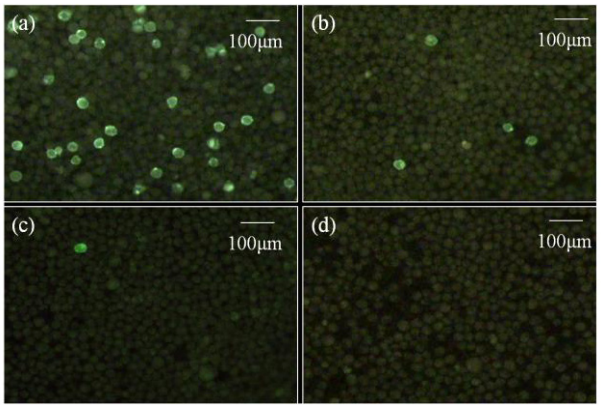

Figure 4: View of different ratio (a) 1:10 (b) 1:100 (c) 1:1,000 (d) 1:10,000 of targeted cells (brighter) to white blood cells (darker).

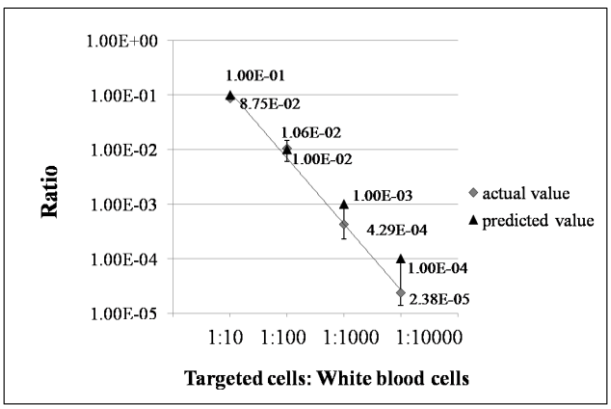

Figure 5: Comparison of the actual value and the predicted value with different ratio of targeted cells to white blood cells.

Once the optimized density was obtained, targeted cells (HL-60 cells stained with fluorescence dye with excitation spectrum at 488 nm) mixed inside white blood cells (HL-60 cells expressing weak green fluorescent protein (GFP)) with different ratios (1:10-1:10,000) is tested under the suspension concentration of 2 x 10^4 cells/μl by this scheme, as shown in Figure 4. The individual targeted cells can be easily identified from the white blood cells arranged in 2D dense array. In Figure 5, it is obvious that the numbers of HL-60 cells detected by this scheme is close to that of the prediction with 5.58% error. The lowest concentration this simple method can provide is 1/10,000.

In addition to HL-60 cells stained with two fluorescent dyes, HL-60 cells and BT-474 cells stained with different fluorescent dyes were also mixed and tested for the identification of the targeted cells. In Figure 6(a)-(c), we can see that the cells self-assembled a monolayer array and the individual cells can be easily observed. Figure 6(d)-(f) shows the different ratios (1:10-1:1000) of BT-474 cells to HL-60 cells. It is obvious that the amount of BT-474 cell (red) can be counted from the HL-60 cells (green) by different excitation spectrum. This simple method opens up a new opportunity for rapid and in parallel targeted cells detection.

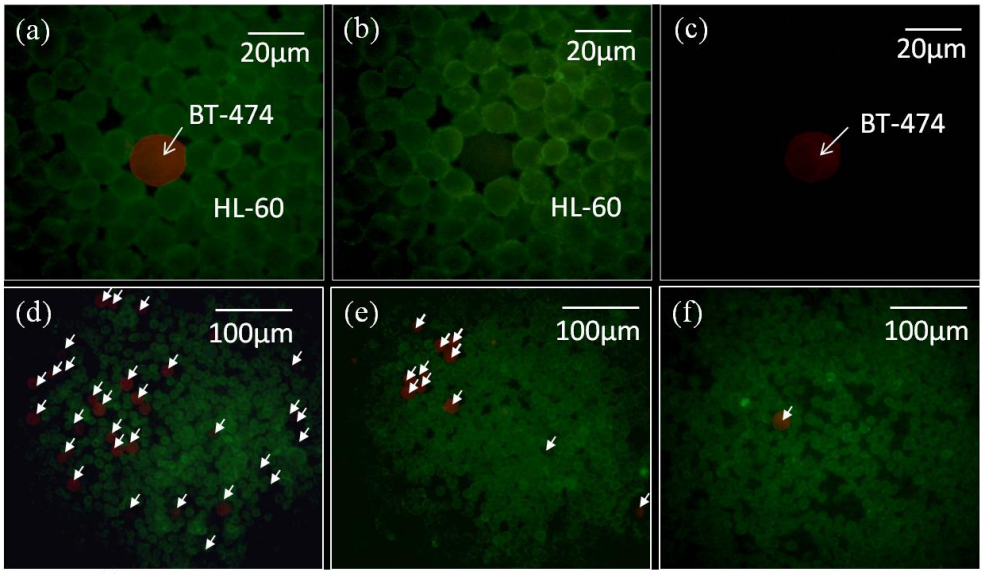

Figure 6: (a) The rare BT-474 (red) cell is identified from a large quantity of HL-60 cells (green). (b) The self-assembly monolayer cells with excitation at 488nm fluorescence. (c) The self-assembly monolayer cells with excitation at 555nm fluorescence. View of different ratio (d) 1:10 (e) 1:100 (f) 1:1,000 of BT-474 cells (red) to HL-60 cells (green) self-assembled on the substrate.

CONCLUSION

We have demonstrated a technique for the early detection of circulating tumor cells by making the cells in whole blood align on the substrate. The fabrication of the chip is very simple and the materials are cheap. It only takes less than 3 minutes for 5μl samples, and it could be compatible with whole blood sample approximately 100μl by increasing the area of platform in the future. This report provides the appropriate design for individual self-monitoring of early detection of tumor cells immediately.

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