SINGLE CELL SUSPENSION CULTURE USING POLYHEMA COATING FOR ANOIKIS ASSAY AND SPHERE FORMATION

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ABSTRACT

In this work, we report a single cell suspension chip which can perform anoikis assay and sphere formation at single cell resolution. The proposed chip uses a hydrodynamic capturing scheme based on difference in flow resistance to guide cell loadings to an exact position in the array of microchambers. PolyHEMA coating is integrated with microfabrication to achieve a single cell suspension culture in each microchamber. We performed two sets of experiments: anoikis assay of skov3 cells and sphere formation of SUM159 and MCF-7 cells; and the behavior of each single cell was successfully traced for more than ten days.

KEYWORDS: Suspension Culture, Anoikis Assay, Sphere Culture, Single Cell, polyHEMA coating

INTRODUCTION

Cell adhesion to the extracellular matrix (ECM) is essential in maintaining cellular homeostasis. Disruption of cell attachment leads to anoikis, which triggers the cell death by apoptosis [1]. However, in the metastasis process, tumor cells are required to detach from the ECM and be viable in circulation [2]. Anoikis serves as a natural barrier for metastasis and the ability to evade apoptosis in suspension marks the malignancy of cancer cells [3]. In conventional anoikis assays, thousands of cells were loaded in the suspension culture dish and cell viability was monitored after a few days [4]. However, cell aggregation, which inevitably happens, may skew survival rates in the conventional dish assays. Also, during the media exchange, some cells can be easily lost in pipetting operation; as a result the length of assay is limited. Here, we propose a single cell anoikis chip that can exactly load single cell in a chamber and provide continuous perfusion culture over time for viability test without interruption of cell positioning.

In the process of metastasis, detached cells should be able to form a new colony from metastatic cancer cells. Among many sub-populations in cancer cells, it is believed that cancer stem-like cells (CSC) are critical in tumorigenesis [5]. The ability to form a sphere in suspension environment (mammosphere assay) indicates stemness [6-7]. Compared to conventional mammosphere assays, which load a few thousand cells in a suspension dish, the proposed single-cell sphere-formation platform can easily trace each single cell and monitor its sphere formation process; thus, allowing the extensive study of different sub-population behaviors.

Microfluidic technologies allow single cell analyses for heterogeneous population cell groups, but most platforms have been limited to adherent culture. We demonstrated suspension culture by integrating hydrophobic surface inside the microchambers in our previous work [8]; however, the patterned surface requires expensive DRIE tools and deteriorates the quality of image. In this work, we report a chip with the surface modified using Poly(2-hydroxyethyl methacrylate) or polyHEMA. Cells can be loaded in each microchamber at single cell resolution in the same way reported previously [8], and cultured for more than ten days in continuous media perfusion. To the best of our knowledge, this is the first attempt to integrate polyHEMA in microfluidics and the first microfluidic platform for single cell anoikis assays.

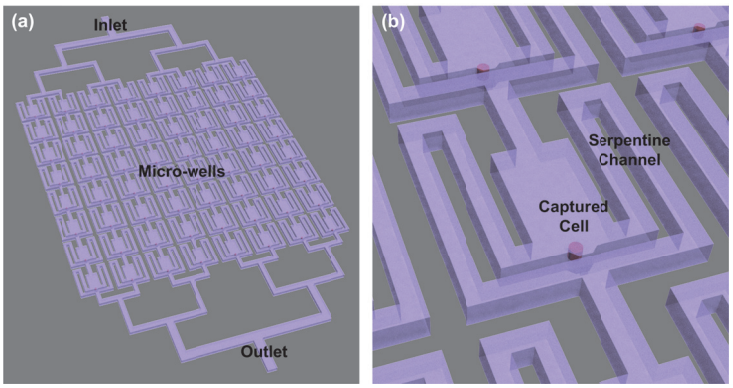

Figure 1. Schematic diagram of a single cell suspension culture chip: (a) the entire device and (b) an enlarged micro-chamber.

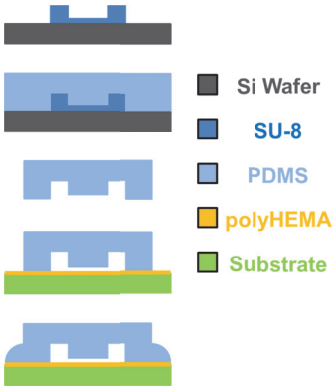

Figure 2. Fabrication process steps.

DESIGN AND FABRICATION

Figure 1 shows the schematic diagram of a single cell suspension culture chip. Flow channels are designed to give an optimal ratio of flow rates in two microfluidic paths for hydro-dynamic capture of single cells by gravity flow [9]. For suspension culture, the polyHEMA, which can inhibit cell adhesion, is coated on substrate by slow evaporation from 60mg/mL of polyHEMA in 95% ethanol. Both the PDMS layer and substrate are treated by oxygen plasma (300 Watt, 60 second), and then bonded together. Since polyHEMA absorbs water and swells, bonding strength may degrade. The uncured PDMS is used as a glue to fasten the device. The PDMS glue is then cured at a lower temperature (65 degrees) overnight to minimize any residue stress as illustrated in Figure 2.

EXPERIMENTAL

Skov3 cells were provided from Dr. Buckanovich's Lab (University of Michigan, MI, USA) and cultured in RPMI with 10% FBS and 1% penicillin/streptomycin. SUM159 and MCF-7 cells were obtained from Dr. Wicha's Lab (University of Michigan, MI, USA) and cultured in DMEM with 10% FBS and 1% penicillin/streptomycin. Same culture media are used in anoikis assays. For sphere formation, cells were grown in serum-free DMEM-F12 (1:1) supplemented with B27, 20 ng/ml bFGF, 20 ng/ml EGF, 2% and other supplements suggested by Dr. Wicha. Before cell loading, trypsin/EDTA is used to re-suspend the cells in solution, which is diluted to 10^6 cells/mL and injected to the inlet. Liquid height difference between the inlet and outlet can generate a gravity flow, and thus cells can be captured hydrodynamically. After 10 minutes, the cell solution in the inlet is replaced by fresh media for the assay.

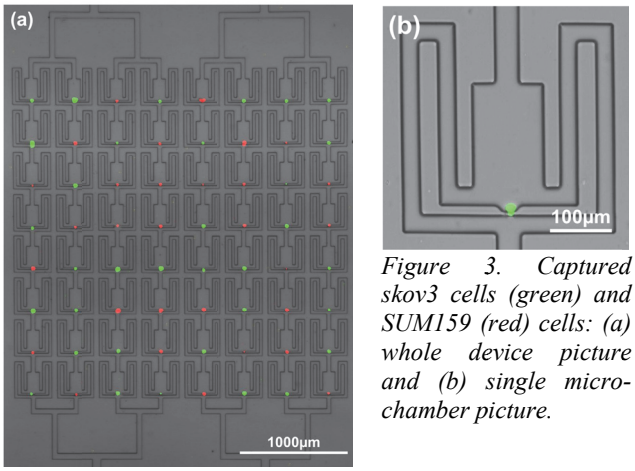

Figure 3. Captured skov3 cells (green) and SUM159 (red) cells: (a) whole device picture and (b) single micro-chamber picture.

RESULTS AND DISCUSSION

Figure 3 shows cells captured in the fabricated device with a single-cell capture rate over 90%. The capture mechanism outperforms the other previous trials for miniaturized anoikis assays [10]. Two types of cells are loaded to represent heterogeneous cell groups. As a proof of concept, skov3 (ovarian cancer) cells are used for anoikis assays. Hepatocyte growth factor (HGF) is believed to enhance the cell survival in suspension culture [11]. Figure 4 shows the comparison of suspension culture and adherent culture. The attached skov3 cells proliferated during the four days culture, while the suspended cells went apoptosis. The live/dead (green/red) staining is used to monitor cell viability. Figure 5 summarizes anoikis experiments, confirming the enhanced survival rate is observed when cells are exposed to 50ng/mL HGF.

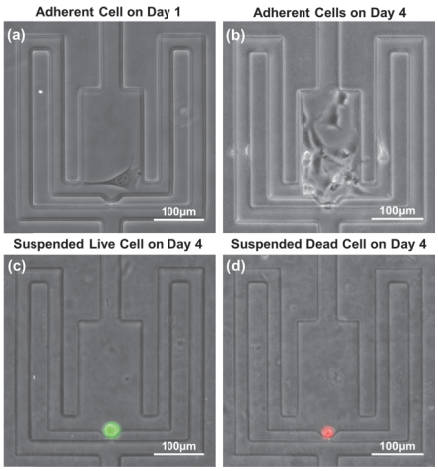

Figure 4. Skov3 cells in adherent and suspension culture: (a, b) adherent cells in a micro-well on day 1 and day 4, (c) suspended live cell on day 4 and (d) suspended dead cell on day 4.

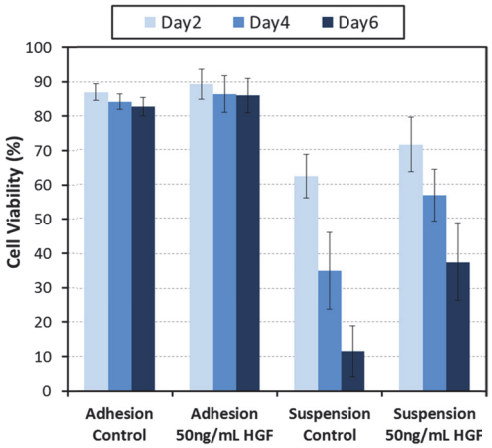

Figure 5. Single cell anoikis assay of skov3 cells for 6 days in polyHEMA treated microchambers for suspension culture.

Figure 6 shows the sphere formation of SUM159 (breast carcinoma) cells from a single cell in ten days. 72% of SUM159 cells in the devices formed spheres after ten days. The variation was 6.84% from four repeated experiments using the 64-mircowell devices. MCF-7 (breast adenocarcinoma) cells were also studied in the suspension culture platform. 3.14% of MCF-7 cells in the devices formed sphere after ten days, and the variation was 2.46% from eight repeated experiments using the 64-mircowell devices. The preliminary result matches well with that in the suspension dish culture. In addition to the quantitative analysis of sphere formation rate, qualitative observation can be also performed by contin-

uously monitoring the process of sphere formation. The behavior difference between different sub-population can be further studied by labeling the cells after loading and continuously tracing them.

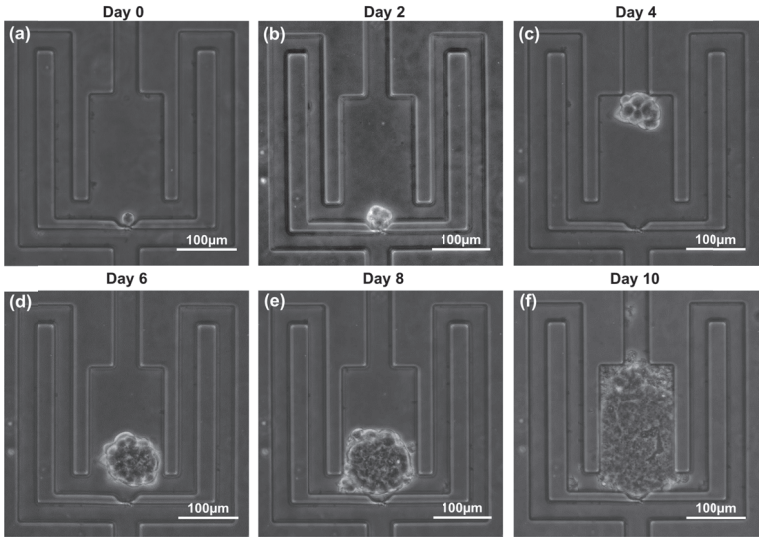

Figure 6. The development of a SUM159 sphere from a single cell in suspension culture inside a polyHEMA surface-coated micro-chamber: (a) day 0, (b) day 2, (c) day 4, (d) day 6, (e) day 8 and (f) day 10.

CONCLUSION

We successfully developed a microfluidic platform for single cell anoikis assay and sphere formation by incorporating polyHEMA coating inside the microwell array. We demonstrated anoikis assays for skov3 cells and sphere formation of SUM159 and MCF-7 cells using the fabricated chip. The behavior of heterogeneous cells could be successfully traced from the captured single cells for more than ten days. The proposed single-cell suspension-culture chip can facilitate the study of cancer metastasis in multiple stages.

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