CANCER CELL ASSAYS BY USE OF IMMUNOCAPTURE, SUBCELLULAR IMAGING, AND PROGRAMMED CELL RELEASE IN GEDI MICRODEVICES

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ABSTRACT

We report the use of Geometrically Enhanced Differential Immunocapture (GEDI) μdevices, which employ staggered obstacle arrays functionalized with a prostate-specific antibody, to capture and analyze prostate cancer cells on chip. GEDI μdevices have been demonstrated to have high capture efficiency and purity [2] as compared similar devices found in the literature [4]. GEDI μdevices have been used to isolate cells from cancer patient samples and are used herein to carry out in situ chemotherapeutic efficacy experiments with a model cell line, simulating a point-of-care diagnostics situation. In addition, we also demonstrate release of captured prostate cancer cells using desthiobiotin and standard antibody conjugation chemistry.

KEYWORDS: Immunocapture, Cell capture, Point-of-care diagnostic, Circulating tumor cells

INTRODUCTION

We report the use of Geometrically Enhanced Differential Immunocapture (GEDI) μdevices to capture prostate cancer cells, assay them for chemotherapeutic response in situ, and release immobilized cells from functionalized surfaces for further analysis. We have captured prostate cancer cells with GEDI μdevices using staggered obstacle arrays functionalized with a prostate-specific biotinylated antibody. We use this device to image the effects of a chemotherapeutic drug (Paclitaxel) on cellular processes. GEDI μdevices have been used previously to isolate cells from cancer patient samples[2] and are used here to carry out in situ drug efficacy experiments with a model cell line, simulating a point-of-care diagnostics situation. In addition, we also demonstrate release of prostate cancer cells adhered to a surface functionalized with desthiobiotinylated antibody.

Figure 1: GEDI μdevices were cleaned and functionalized using standard conjugation chemistry to produce biotinylated and desthiobiotinylated antibody surfaces. Using this chemistry, we have demonstrated capture of cancer cells over a range of shear stresses (right) as well as in GEDI μdevices[2].

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EXPERIMENTAL

GEDI μdevices were chemically functionalized with a monoclonal antibody (J591 mAb) targeted to Prostate Specific Membrane Antigen (PSMA) (Figure 1), a transmembrane protein expressed by prostate cells. Model prostate cancer cells were captured on GEDI μdevices and assayed to determine response to Paclitaxel, a chemotherapeutic drug. In addition to on-chip chemotherapeutic efficacy experiments, prostate cancer cells were immobilized on glass coverslips with desthiobiotinylated-J591 mAb. Immobilized cells were exposed to 100 μg/ml of exogenous biotin in PBS to induce release.

RESULTS AND DISCUSSION

GEDI μdevices have been previously characterized as having high capture efficiency and purity as compared to the literature [2]. Using these devices, we have successfully imaged immobilized prostate cancer cells at subcellular resolution. The ability to investigate changes in cellular structure in the presence of outside chemotherapeutic stimulus in situ is previously undemonstrated in cancer cell capture microdevices. Comparison of confocal images of cells immobilized on coverslips and GEDI microdevices showed no qualitative differences in nuclear, tubulin, or AR structure (Figure 2). To validate that cell capture does not inhibit normal biochemical processes, we demonstrated that captured prostate cancer cells were still sensitive to the presence of DHT (a testosterone metabolite) (Figure 3). We have also shown that DHT induces nuclear AR localization, both on coverslides and in situ (Figure 3). In contrast, cells treated with PTX and then incubated with DHT showed a mitigated AR response (Figure 3). These experiments show that in situ biological assays can be carried out in GEDI μdevices with the same level of precision as traditional systems. Using a simple substitution to standard conjugation chemistry, programmed cell release was achieved without the use of sacrificial layers [3], excessive fluid forces, or exposure to enzymatic digestion. Following exposure to exogenous biotin, desthiobiotin conjugated surfaces released 94% of immobilized cells. Cell viability staining revealed no changes in viability to cells from the release process. This is the first demonstration of the use of desthiobiotin to release immobilized cells from device surfaces. The ability to temporally release captured cells will allow researchers to investigate many different routes of biochemical and genetic analyses to further understand prostate cancer progression.

CONCLUSION

We demonstrate the use of GEDI μdevices for the capture, release, and analysis of prostate cancer cells and model cell lines. GEDI μdevices captured cells from cancer patient samples and in situ chemotherapeutic efficacy experiments were conducted to investigate changes in cellular structure, which has not perviously been demonstrated in cancer cell capture μdevices. We also demonstrated programmed cell release of viable prostate cancer cells adhered to a surface functionalized with a desthiobiotinylated antibody. The ability to temporally release captured cells could allow for further investigation of prostate cancer development and treatment.
Figure 4: Cancer cells were incubated on functionalized glass coverslips using either biotin or desthiobiotin linkers. Samples were incubated with exogenous biotin (100μg/mL) for 20 min. Surfaces were imaged and released cells were counted and their viability quantified.