Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Capture of circulating tumor cells from whole blood of prostate cancer patients using geometrically-enhanced differential immunocapture (GEDI) and a prostate specific antibody

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Computational modeling

Basic system design employed isolated prediction of sphere impact on obstacles, as made possible by the relatively large spacing of obstacles in the presented device. Particle displacement was predicted and the distribution of cells was calculated using a custom MATLAB code as a function of transport through a 50-row array of obstacles. This analysis has several key assumptions, including (a) target cells are rigid and spherical, (b) the fluid is Newtonian, and (c) net margination of cells owing to rigidity can be ignored. While effects of nonspherical cells are critical in biorheology in general, our model currently ignores margination in this system owing to the variable location of the obstacles which interferes with the coherent but subtle action of margination of rigid cells in blood. Non-spherical cells leads to a smoothing of predicted data, but again the well-known coherent effects of non-spherical geometry in biorheological flows are suppressed by staggered obstacle arrays. Blood is a colloidal suspension with a shear-thinning serum component; it is well known to exhibit Casson fluid-type behavior rather than Newtonian behavior. However, the shear stress in the region near an obstacle is not more than twice the shear stress in regions far from obstacles, and the shear-thinning of serum is minor, so a full analysis of the fluid constitutive relation leads to only minor changes in the observed results. The diffusion of cells used in the model was set at 1e-12 m^2/s, based on typically observed shear-induced diffusion at the flow rates, hematocrits, and cell sizes considered, and Stokes-Einstein-type diffusion was ignored. A shooting algorithm was employed which modeled the displacement of a randomly distributed set of cells (n=1000). Randomness of the initial condition is seen in the noise in the presented final data in Figure 1B; this noise is retained, in part, so the reader is aware that a statistical technique has been used.

GEDI μdevice fabrication

All device fabrication was carried out at the Cornell NanoScale Facility. Standard photolithography techniques were used to define array geometries on silicon wafers. The wafers were etched with an oxygen plasma reactive ion etcher (Uniaxis SLR770) to a depth of 100 μm, and cleaned using sulfuric acid and hydrogen peroxide prior to antibody surface functionalization. J591 monoclonal antibody [produced and purified at Weill Cornell Medical College, NY, NY] was immobilized on the device surfaces using the same protocol described in the main text for 2D glass experiments.

Polydimethylsiloxane (PDMS) sheets (5:1 base:curing agent), approximately 4 mm thick, were polymerized for 18 hours at 60°C and trimmed to form covers for the GEDI μdevices. A PDMS sheet was clamped to the top of the device with a custom jig to create closed channels populated with post arrays. Inlet and outlet holes were created with a biopsy punch, and 23 gauge metal tubes inserted into the PDMS served to connect inlet and outlets to external tubing.

Cell culture / labeling / handling

LNCaP (androgen-sensitive) and PC3 (androgen-insensitive) human prostate adenocarcinoma cell-lines were purchased from American Type Culture Collection (ATCC) [Manassas, VA] and cultured in standard cell culture conditions (37°C, 5% CO_2) in cell culture flasks. Complete culture medium consisted of RPMI-1640 [Lonza, Walkersville, MD] supplemented with 10% fetal bovine serum [Gemini Bio-Products, W. Sacramento, CA] and 1% penicillin/streptomycin [Mediatech Inc., Herndon, VA]. Upon reaching confluence, cells were passaged with trypsin at a subcultivation ratio of 1:5.

Whole blood samples (non cancer patient control, and castrate-resistant prostate cancer patient) were collected via venipuncture under Institutional Review Board (IRB) approved protocols and processed within 48 hours. Peripheral blood mononuclear cells (PBMCs) were isolated from control blood samples using standard ficoll gradient separation protocols. PBMCs were stored in complete culture medium in standard culture conditions, as described above, until use (< 48 hours).

Fluorescent labeling of LNCaPs for spiking experiments was performed using a standard cell labeling kit [Invitrogen, Eugene, OR]. Briefly, the cells were suspended in serum-free culture medium and incubated with 5μL/mL Vybrant DiO cell labeling solution for 20 minutes at 37°C. Cells were centrifuged at 1500 rpm for 5 minutes, washed with PBS twice, and resuspended in appropriate volumes of either PBS or control whole blood for experiments.

To minimize cell settling prior to GEDI μdevice processing, the infusion syringe containing the cell suspension/blood was agitated frequently by hand to homogenize the solution. In addition, for all

samples analyzed, the entire volume of the sample in the syringe was run through the device, ensuring all spiked and hematological cells were processed.

Prostate-specific membrane antigen (PSMA), expressed by PCTCs and not hematological cells, was used to identify captured prostate circulating tumor cells from castrate-resistant prostate cancer patient blood samples using FITC-conjugated J591. Following blood processing and flushing of the device with PBS to remove unbound cells as described in the main text, the device was unclamped and the PDMS cover was removed. A 50 µg/ml FITC-conjugated J591 in PBS solution was incubated on the device for 10 minutes, and then rinsed with PBS to remove excess J591. Devices were then imaged under a fluorescent microscope to enumerate PCTCs.

J591 mAb PSMA specificity

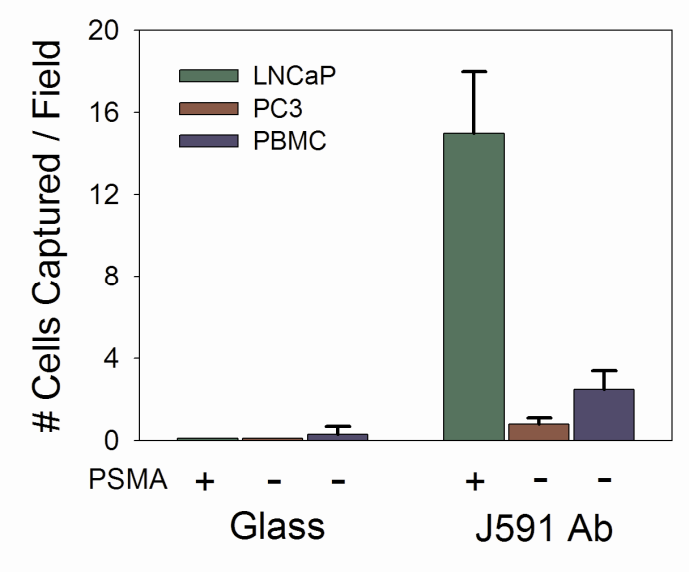

Figure S1: # of captured cells on plain glass compared to glass functionalized with J591 mAb confirm high specificity for PSMA expressing cells and low non-specific binding of PSMA negative cell types.

Cell spiking experiments

Labeled cells used for spiking experiments were counted on a hemacytometer to determine the number of spiked cells. Counting errors were minimized by counting 4 different samples from the well-mixed concentrated cell suspension. The cell counts from each sample were averaged together to produce a mean cell count and standard deviation. For all experiments, standard deviation of the cell counts was fewer than 5 cells. The concentrated cell suspension was appropriately diluted, processed through the

device and labeled cells were enumerated directly. The capture efficiency percentages were calculated using equation (1), taking into account the errors associated from the hemacytometer count.

$$CaptureEfficency = \frac{Captured}{Spiked} \pm \frac{Captured}{Spiked}\sqrt{\left(\frac{SD_{Captured}}{Captured}\right)^2 + \left(\frac{SD_{Spiked}}{Spiked}\right)^2} \quad (1)$$