Published in final edited form as:

Anal Chem. 2017 November 07; 89(21): 11545–11551. doi:10.1021/acs.analchem.7b02878.

Continuous Sorting of Cells Based on Differential P Selectin Glycoprotein Ligand Expression Using Molecular Adhesion

Bushra Tasadduq^{‡, #}, Brynn McFarland[⊥], Muhymin Islam[§], Alexander Alexeev[§], A. Fatih Sarioglu[‡], Todd Sulchek^{*, §, †}

[‡]The School of Electrical and Computer Engineering, Georgia Institute of Technology Atlanta, Georgia 30332, United States

[#]NED University of Engineering & Technology, Karachi Pakistan

[⊥]Department of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[§]Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[†]Wallace H Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

Abstract

Cell surface molecular adhesions govern many important physiological processes and are used to identify cells for analysis and purifications. But most effective cell adhesion separation technologies use labels or long-term attachments in their application. While label-free separation microsystems typically separate cells by size, stiffness, and shape, they often do not provide sufficient specificity to cell type that can be obtained from molecular expression. We demonstrate a label-free microfluidic approach capable of high throughput separation of cells based upon surface molecule adhesion. Cells are flowed through a microchannel designed with angled ridges at the top of the channel and coated with adhesive ligands specific to target cell receptors. The ridges slightly compress passing cells such that adhesive contact can be made with sufficient surface area without unduly affecting cell trajectories because of cell stiffness. Thus, sorting is sensitive to cell adhesion but not to stiffness or cell size. The enforced interactions between the cells and the ridges ensure that a high flow rate can be used without lift forces quenching adhesion. As a proof of principle of the method, we separate both Jurkat and HL60 cell lines based on their differential expression of PSGL-1 ligand by using a ridged channel coated with P selectin.

*Corresponding Author todd.sulchek@me.gatech.edu.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Bushra Tasadduq wrote the manuscript and performed the experiments.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.analchem.7b02878](https://doi.org/10.1021/acs.analchem.7b02878). HL60 cell size distribution and FITC BSA used to determine the degree of detachment of immobilized P selectin after flow experiment (PDF)

The authors declare no competing financial interest.

We demonstrate 26-fold and 3.8-fold enrichment of PSGL-1 positive and 4.4-fold and 3.2-fold enrichment of PSGL-1 negative Jurkat and HL60 cells, respectively. Increasing the number of outlets to five allows for greater resolution in PSGL-1 selection resulting in fractionation of a single cell type into subpopulations of cells with high, moderate, and low PSGL-1 expression. The cells can flow at a rate of up to 0.2 m/s, which corresponds to 0.045 million cells per minute at the designed geometry, which is over 2 orders of magnitude higher than previous adhesive-based sorting approaches. Because of the short interaction time of the cells with the adhesive surfaces, the sorting method does not further activate the cells due to molecular binding. Such an approach may find use in label-free selection of cells for a highly expressed molecular phenotype.

Graphical Abstract

Cell molecular interactions regulate important physiological processes, such as cell homing, immune modulation, and cancer metastasis. Identifying and isolating cells that express desired molecular surface markers is thus critical to a variety of applications in the biological sciences, cell therapy, and medical diagnostics.¹⁻⁴

Label-free separation techniques that manipulate physical biomarkers, such as size, stiffness, and shape, to sort cells have been successfully demonstrated during the past decade, but often lack the specificity⁵⁻¹¹ that can be achieved by using cell surface biomarkers. Cell surface biomarkers are typically determined through adhesion-based cell isolation platforms to specifically purify by immunophenotype.¹² Labeling methods include using antibodies that are fluorescently tagged to enable fluorescence activated cell sorting (FACS)¹³ or tagged with magnetic nanoparticles to enable MACS.^{14,15} Although FACS¹³ and MACS offer high purity with high enrichment possible, the techniques do not yet offer the capability for fractionation into multiple outputs of finer sensitivity to the molecule of interest. In other words, it provides a binary picture of the analog expression. Other drawbacks of these methods include the need to detach these labels from the cells for further downstream uses and the risk of tag-induced activation of the sorted cells.¹⁶ Recently, several label-free

adhesion-based cell sorting methods were also demonstrated. Microfluidic approaches have used shear flow to select cells that are adherent to nonspecific substrates to result in high enrichment of mesenchymal stem cells but require culturing of cells on a substrate and hence are not continuous.¹⁷ Other methods require harsh release reagents to retrieve the sorted cells. For example, a CD4 cell counting device¹⁸ uses antibodies to capture CD4 cells and requires successive rinses with several buffers to enumerate the captured cells. There are also challenges associated with release of affinity based captured cells without perturbing the cells' morphology, viability, molecular content, activation state, and phenotype. Most methods of cell release by shear^{19,20} require a number of attachment points between the cells and the surface which can damage fragile cells.²¹ Alternatively, label-free cells can be captured on a solid substrate using affinity based columns but the process requires a low flow rate to maintain rolling interactions with the adhesive surface.²² Microfluidic approaches to label-free sorting often require cell rolling at low flow rates to avoid hydrodynamic lift forces.²³

We propose a novel microfluidic platform capable of high throughput separation of cells by differences in molecular adhesion. The device operates by flowing cells through a ridged microchannel such that the channel surfaces are decorated with an adhesion molecule providing specificity to a cell surface receptor. As cells transiently interact with ligands at the ridge and wall interfaces, the net forces are altered to redirect the flowing cells toward one side of the channel. Thus, cells with high expression of the target molecule are concentrated toward one side of the channel for collection. The unique aspect of this sorting design is the ridge gap spacing that uses cell compressions to increase the surface area for interaction between the ligand on cell surface and coated receptor molecule, but without sufficient strain to unduly influence the cell trajectory by biomechanical properties such as stiffness¹¹ and viscoelasticity.²⁴ Thus, ridge compression is used to optimize adhesive interactions in a manner that cell stiffness does not influence the cell separation mechanism. As a result, receptor-specific cell separation occurs while maintaining a high flow rate and throughput. By designing chips with multiple outlets, the sorting is capable of fractionation of cells based upon the amount of receptor expressed.

To demonstrate cell separation, we use the lectin molecules, which previously have been used for sorting cells through affinity columns.²⁵ Since cell binding to lectins depends on factors like metabolic state, and stage of cell division and differentiation, the method is useful for applications like isolating stem cells based on differentiation or homing potential.^{26–29} We chose the specific lectin P selectin which binds to the P selectin glycoprotein ligand 1 (PSGL-1) to demonstrate sorting due to the potential applications in understanding the role of PSGL in T cell immune response^{30,31} and developing effective therapeutics.^{2,3,23,32} Therefore, in this study PSGL-1/P selectin, ligand–receptor binding is used to sort target cells.

EXPERIMENTAL METHODS

Microfluidic Device Fabrication.

Microfluidic sorting devices were designed in SolidWorks. The microfluidic devices were fabricated by replica molding Polydimethylsiloxane (PDMS) (Sylgard 184 Dow Corning

Corp) on a permanent mold. The mold is made from SU-8 2007 using a two mask photolithography process. The mold dimensions were characterized with profilometry (Dektak 150 profiler) and verified with confocal microscopy imaging (Olympus LEXT). Uncured PDMS was mixed in a 10:1 ratio of elastomer to curing agent, then poured onto the SU-8 molds to a thickness of 1 cm and cured in an oven at 60 °C for 6 h. The cured PDMS layer was peeled off the mold, cut into chips, and inlet and outlet holes were formed with a 1 mm biopsy punch. The PDMS device was treated with oxygen plasma (Harrick plasma cleaner) for 2 min then bonded to a glass microscope slide. We designed the ridged microchannels to have gap sizes of 9.3 μm for Jurkat cells and 10.3 μm , which imposes a cellular strain of ~15% on each cell type. The channel width is 560 μm and length is 3.8 mm with 25 skew ridges equally spaced along the channel length. The ridges are 20 μm wide and distance between two consecutive ridges is 70 μm . The ridges are at the top of the microchannel and are inclined 30 degrees. We use three and five outlet devices to study the resolution of separation.

Sample Preparation and Experimental Setup.

Jurkat cells (CRL-1990) and HL60 (CCL 240) were purchased from ATCC. Cells were cultured and maintained in RPMI-1640 medium (Millipore Sigma) with the addition of 10% FBS (Millipore Sigma) and 1% penicillin–streptomycin. Cells were incubated at 37 °C supplied with 5% carbon dioxide. Recombinant human P selectin was purchased from R&D Systems (Minneapolis, MN) and resuspended in PBS at a concentration of 3 $\mu\text{g}/\text{mL}$. The assembled device was degassed in a vacuum chamber for 10 min, filled with P selectin solution by pipetting, and then incubated at room temperature. After 3 h incubation, the device was washed with 1% bovine serum albumin (BSA). Cells suspended in medium at 0.5×10^6 or 1×10^6 cells/mL were flowed into the device at 0.045 and 0.1 mL/min using a syringe pump. A high speed camera (Phantom V7.3 Vision Research) and inverted microscope setup was used as described previously.³³ Following collection at outlets, cells are incubated at 37 °C with blocking solution for 15 min and then incubated for 30 min with primary monoclonal antibodies and then incubated with fluorescently labeled secondary antibodies for 30 min at final concentrations of 30 and 50 $\mu\text{g}/\text{mL}$ respectively. Between primary and secondary antibodies incubation and after secondary antibody incubation and flow analysis, cells were washed with PBS. To detect cell-surface PSGL-1, we used mouse antihuman PSGL-1 clone KPL-1 (Millipore Sigma), followed by secondary antibody PE-conjugated goat antimouse IgG (Invitrogen). Solutions composed of primary and secondary antibodies were preincubated for at least 1 h prior to incubation with cells. Cells were analyzed with flow cytometer (BD Acuri). Fluorescent imaging was used to check the degree of detachment of P selectin from cells after flow experiment. For this purpose, we have used 1% FITC BSA (Millipore Sigma) as a blocking agent. To measure the activation of cells, antibodies, anti CD69-FITC, and CD11b-APC (Biolegends Inc.) were used according to manufacturer's manual. We conducted a set of three experiments for the study of cell activation. In the first experiment we incubated Jurkat cells with P selectin for 24 h at 37 °C with 5% carbon dioxide. In the second experiment, we incubated Jurkat cells for 2 h with P selectin coated PDMS surface, in which the PDMS surface was first activated by incubating it with P selectin for 3 h at room temperature, at 37 °C with 5% carbon dioxide. In the third experiment, Jurkat cells were collected after sorting through the proposed

device. Expressions of CD69 and CD11b were compared for all the three experiments using flow cytometry.

Cell Stiffness Measurement with Atomic Force Microscopy.

We utilized atomic force microscopy to measure the stiffness of cells. All cells were measured in suspended states after slight attachment to the surface. To measure cells in a suspended state, a monolayer of poly-L-lysine (Millipore Sigma) was grafted onto the glass slide substrate. This operation provided anchorage of the cell to the glass substrate while maintaining roundedness of morphology for cells and improved the cell stability during the AFM measurements. We carried out our AFM experiment immediately after the washing step and poly-L-lysine cell attachment treatment and all measurements were finished within 2 h. We did not observe a change in measured stiffness during the course of these measurements. Measurements were conducted using a MFP-3D AFM (Asylum Research) attached to an inverted optical microscope (Nikon Eclipse Ti). A silicon nitride cantilever with a spring constant measured to be 37.1 pN/nm and a spherical tip was positioned above the center of individual cells before indentation. Prior work showed that the Young's modulus is a function of loading force and loading rate.³⁴ The force-indentation curve was obtained for each measurement at a 40% strain and then analyzed with a Hertzian model for a spherical tip (Wavemetrics, IgorPro software routines) from which the Young's modulus values were calculated.

Fluid Flow Simulations.

Finite element simulations of fluid flow were performed using COMSOL Multiphysics software (COMSOL Inc., Burlington, MA). Simulations were performed for channel width of 560 μm and ridge inclination angles of 30°. PDMS was selected as the material of interfacing structure. The flow profiles in the channel were obtained by solving the Navier–Stokes equations for incompressible fluid using fluid–structure interaction physics. At the outlet, the pressure was set to zero with no viscous stress on the boundary. Because of low Reynolds number of the fluid, it was assumed that the suspended particles would follow the fluid streamlines.

RESULTS AND DISCUSSION

The sorting device shown in Figure 1A uses the flow of cells through a microchannel decorated with diagonal ridges and coated with P selectin specific to PSGL1. For negative control, flow experiments were conducted without P selectin incubation of the device. First, we examined the behavior of cells flowing in the device with and without P selectin. Figure 1B shows the trajectories of Jurkat cells flowing through the device with P selectin (black) and without (blue) coating. The trajectories indicate that the adhesion force induced by ridges and P-selectin coated bottom glass surface on cells as they flow results in a net lateral displacement that distributes the cells at different y positions hence at different outlets based on the binding between P selectin on device surface and PSGL-1 on cell surface. The cells flowing in the device without P selectin follow the fluid streamlines¹¹ as no adhesion is observed in this case. The unique aspect of this sorting design is the use of optimized gap size H, defined as the distance between the ridge and bottom of the channel, to lightly

squeeze the cells while flowing under the ridged part of the channel while offering a high surface area for specific interaction between the cells and ligand molecules coated on the ridges.

To understand the sorting of cells by expression of PSGL-1, we examined the behavior of two model leukocytes flowing in the device, HL60, and Jurkat cell lines. The gap size in each case was optimized so that biomechanical compression forces resulting from stiffness and viscoelasticity^{11,24} are minimized to not dominate the cell separation process. In these flow experiments, we used 9.3 μm gap size for adhesive sorting Jurkat cells, which is larger than prior gap sizes reported for stiffness separation of Jurkat cells of 8 μm .¹¹ Thus, the 9.3 μm gap size is sufficient to lightly squeeze Jurkat cells as they are 11 μm in diameter but large enough that stiffness does not dominate the sorting. In the case of HL60 cells, a 10.3 μm gap size was used as these cells are 12 μm in diameter. Specificity to PSGL-1 expression was obtained through device functionalization by ligand found on surface of HL60 and Jurkat cells and shows binding to P selectin coated surfaces.^{22,35}

To characterize the sorting of different cell types by the device we examined the sorted cells after incubating them with primary and secondary antibodies using the protocol described in methods with flow cytometry. For both HL60 and Jurkat cells, we were able to separate cells based on expression of PSGL-1 ligand by using a ridged channel coated with P selectin. HL60 cells were flowed at flow rates of 0.045 and 0.1 mL/min in the device with P selectin incubation and at 0.045 mL/min without P selectin incubation. The flow cytometry data for outlets for the three different cases are shown in Figure 2A. We see the adhesion dependence of outputs faces an upper limit of flow rate in which sorting of PSGL-1 positive cells at high flow rates is diminished, as shown in flow cytometer data in Figure 2A. Figure 2B shows the fluorescent mean values of collected sample at different outlets for 0.045 mL/min flow rate with P selectin incubation. A two way ANOVA and Tukey tests on the collected data were performed to show a significant difference in fluorescent mean values of three outlets. The flow rate of 0.045 mL/min was substantially higher than that used in other microfluidic label-free adhesion based sorting. We demonstrate 3.8 and 3.2-fold enrichment of PSGL-1 positive and negative HL60 cells respectively as shown in Figure 2C. We have also used fluorescent microscopy to validate outlet characterization of our device, as shown in Figure 2D. The PSGL-1++ outlet in Figure 2D shows more cells with secondary antibody attached to them, hence showing more PSGL-1 expression.

Figure 3 analyzes the trajectory of HL60 cells with and without P selectin functionalization and at increased flow rates. In Figure 3D, the data from trajectories are extracted to determine the lateral displacement of cell flows between two ridges, defined as y/ridge . The trajectories for uncoated devices show displacement only in the negative y direction whereas the P-selectin coated device shows displacement with a range of values, either positive, negative or in the middle of the device. Analysis of trajectories of cells through the P selectin coated device in Figure 3D and 3E shows that 40% of the cells are displaced in the negative y direction with 7.3 μm and 60% in the positive y direction with 5.3 μm y/ridge and at much higher velocities as compared to 0.045 mL/min flow rate. This lack of enrichment at high flow rates (Figure 2A right) indicates hydrodynamic forces dominate adhesive forces, in part due to larger hydrodynamic forces and in part to insufficient time for

cells to form adhesions with the coated surface. Therefore, low enrichment of cells based on adhesion is observed at a flow rate of 0.1 mL/min.

The working mechanism of the device is explained in Figure 4A. As cells flow under the ridge, they bind to P-selectin and when leaving the ridges cells with more ligand expressed on their surface resist the secondary drag force and stay adhered to bottom of the channel and experience the flow in negative y direction (red arrow). On the other hand cells with less ligand detach and pull away from the surface and enter the streamline in positive y direction (green arrow). Cells flowing through the device without P selectin coated ridges move with the fluid flow streamlines. The trajectories of Jurkat cells are compared with COMSOL streamlines at height equal to half of the gap size in *xy*-plane (Figure 4B–D). Cells closely follow the simulated streamline in the case of a device with no P selectin. Since cells in the stream are located near the bottom channel wall with weak elastic force and no adhesion force they are transported by the circulating flow, created by ridges, in the negative transverse direction as shown in Figure 4B. In the case of less PSGL-1 on the cell surface (Figure 4C), as a cell leaves the ridge it is pulled off and detached from the surface and follows the streamlines that moves up in y direction. On the other hand a cell with more PSGL-1 (Figure 4D) is attached to the surface once it enters the ridge and rolls nearly straight on the bottom glass surface as it leaves the ridge.

In previous studies,¹¹ we tested gap sizes ranging from 4 to 12 μm for sorting Jurkat cells by stiffness and found that 8 μm gap size was the best option that emphasized biophysical differences with minimal occlusion. Gap sizes that were greater than 8 μm were found to not provide sufficient constriction for sensitivity to stiffness differences. On the basis of these conclusions, we tested gap sizes of 9 and 14 μm to evaluate sensitivity to adhesion differences without sensitivity to stiffness differences. As can be seen in Figure 5A and B, increasing the gap size decreases the enrichment factor because of a decrease in cell strain, which leads to decrease in surface area for interaction between cell surface and coated device but also a decreased elastic force acting on the cell. After cell separation, we also examined stiffness of the cells collected at the three outlets, as shown in Figure 5C. A lack of significant difference in stiffness indicates that stiffness was not a determining factor in the cell sorting. The measured cell size distribution, representing the average plus and minus the standard deviation of HL60 cells, was between 10.0 and 15.4 μm (Figure S1). From this distribution and the ridge gap dimension, approximately 8% of cells were exposed to <3% cell strain and ~92.7% of cells were exposed to cell strain between 3%–33%. We previously found¹¹ that a threshold of >40% cell strain is required for cell stiffness to dominate the sorting mechanism in a ridged microchannel. While large cell heterogeneity in size or stiffness may indeed impact adhesion separation, for the conditions of our study, cell stiffness did not vary at the sorting outlets. We anticipate that if cell heterogeneity significantly exceeds that studied here, i.e. if cell size variation exceeds 25%, then decreased accuracy of adhesion dependence is expected. Fluorescent imaging of the device (Figure S2) after the flow experiment verified that P selectin was not observed to be removed after flow experiments and remains intact throughout the flow experiment.

We have demonstrated 26-fold and 4.4-fold enrichment of PSGL-1 positive and negative Jurkat cells respectively shown in Figure 6C. We have showed fractionation of a single cell

type based on the expression of a ligand at high flow rates and with significant population enrichment, as shown in Figure 6D. One potential application for fractionation of T cells is sorting based on the density of specific chimeric antigen receptors (CAR). Potential targets for CAR-based therapies are cell surface antigens expressed at higher densities on cancer cells but may lead to severe adverse effects owing to the recognition of minimal Ag expression outside the target tumor. The microfluidic sorting device can therefore be used to determine threshold Ag densities for CAR based therapies in order to avoid off target tumor toxicity.³⁶

A major concern of adhesion-based isolation methods is the modification and activation of the target cells after isolation by long-term binding (greater than 1 h). Beads used for binding and pull-down isolation can activate cells.³⁷ Studies have also indicated that engagement of selectin ligands on leucocytes directly transduces signals.³⁸ For example, interactions of PSGL-1 with immobilized P-selectin rapidly induce tyrosine phosphorylation of multiple proteins.³⁹ However, the data presented show that increase in tyrosine phosphorylation is observed at least 2 min after P selectin and PSGL-1 binding. Our device provides another application where cell sorting is possible without cell activation due to very short binding contact between receptor and ligand. We have conducted three sets of experiments in order to compare activation of cells due to short-term binding of PSGL-1 and P selectin in the microfluidics sorting approach. To test activation of the sorted cells (data presented is for Jurkat cells), CD69 staining and CD11b were used to detect the activation of cells⁴⁰ as classical markers. The results in Figure 7 show a slight up regulation of activation markers after cells are incubated with P selectin for 24 h but a significant change when removed from P selectin coated surface. There is no change in expression of activation markers in case of cells collected after sorting. Also, no change in shape was observed. Taken together, these results indicate sorting produces no cell activation due to very short binding time ($<10 \mu s$) between P selectin and PSGL-1 while cells are flowing through the shear in the device and the association and dissociation are sufficiently gentle to not activate cells. In this work, we have separated HL60 cell line at a final concentration of 10^6 cells/mL at a flow rate of $45 \mu L/min$ which results in a throughput of approximately 45 000 cells per minute. The high throughput is over 2 orders of magnitude higher than previous microfluidic approaches using asymmetric adhesive patches that require cell rolling to maintain contact.²² The improvement results from our ability to flow cells at a faster velocity due to the forced contact at the narrow-gap ridges.

CONCLUSION

We have demonstrated a novel microfluidic device capable of high throughput separation of cells by differences in specific cell adhesion. We show that the adhesion is mediated by specific adhesive events from cell surface PSGL-1 receptors to surface immobilized selectins. The device displays sufficient sensitivity to adhesion so that differential receptor expression can be distinguished and enriched. We have demonstrated a 26-fold and 3.8-fold enrichment of PSGL-1 positive and 4.4-fold and 3.2-fold enrichment of PSGL-1 negative Jurkat and HL60 cells, respectively. We have also demonstrated the enrichment of PSGL-1 positive Jurkat cells to 3-fold using a five outlet fractionation device. This is the first study that shows fractionation of a single cell line based on the ligand it expresses at high flow

rates with significant population enrichment. Heterogeneity in cell stiffness or size show minimum effect on sorting at optimized gap size. Because of the skew ridge design, we observe no clogging and the ability to clear cells with less interrupted fluid flow. Further, applications using the device to sort desired cell phenotypes with high throughput are possible to allow downstream purification and analysis.

The ligand-based sorting also has potential applications in understanding the role of PSGL-1 in T cell immune response and developing effective therapeutics. It has been reported that PSGL-1 on T cells dampens TCR signals, limits survival of effector T cells, and promotes immune inhibitory receptor expression, thereby supporting establishment of exhaustion in viral and tumor models. PSGL-1-deficiency enhances T cell antitumor immunity to melanoma, promotes viral control and T cell survival is reported to be increased in PSGL-1 deficit T cells after chronic virus infection.^{2,3} The proposed device also offers direct measurement of transient interactions between important physiological ligands and cells. The device can also be used as a tool to monitor stem cell differentiation.⁴ It also has potential applications in measuring degree of changes in adhesion signature to cancer cells,^{41,42} hence better therapeutics. Unlike MACS and panning, the separation can fractionate by the amount of surface antigen on a cell and also eliminate the limitation posed by the choice of antibodies is limited within a pool of commercially available antibodies that do not activate the cells.¹⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This research was supported by NSF project number CBET-0932510, NIH 1R21EB020977-01, the Centre for Pediatric Nanomedicine Seed Grant, and the Fulbright Scholarship Program. We would like to acknowledge Betsy Campbell for her valuable suggestions regarding activation experiments.

REFERENCES

- (1). Watanabe K; Terakura S; Martens AC; van Meerten T; Uchiyama S; Imai M; Sakemura R; Goto T; Hanajiri R; Imahashi N; et al. *J. Immunol* 2015, 194, 911–920. [PubMed: 25520398]
- (2). Tinoco R; Carrette F; Barraza ML; Otero DC; Magaña J; Bosenberg MW; Swain SL; Bradley LM. *Immunity* 2016, 44, 1190–1203. [PubMed: 27192578]
- (3). Tinoco R; Otero DC; Takahashi AA; Bradley LM. *Trends Immunol* 2017, 38, 323. [PubMed: 28262471]
- (4). Karp JM; Leng Teo GS. *Cell stem cell* 2009, 4, 206–216. [PubMed: 19265660]
- (5). Ji HM; Samper V; Chen Y; Heng CK; Lim TM; Yobas L. *Biomed. Microdevices* 2008, 10, 251–257. [PubMed: 17914675]
- (6). Crowley TA; Pizziconi V. *Lab Chip* 2005, 5, 922–929. [PubMed: 16100575]
- (7). Choi S; Ku T; Song S; Choi C; Park J-K. *Lab Chip* 2011, 11, 413–418. [PubMed: 21072435]
- (8). Choi S; Park J-K. *Lab Chip* 2009, 9, 1962–1965. [PubMed: 19532973]
- (9). Choi S; Park J-K. *Lab Chip* 2007, 7, 890–897. [PubMed: 17594009]
- (10). Inglis DW. *Appl. Phys. Lett* 2009, 94, 013510.
- (11). Wang G; Mao W; Byler R; Patel K; Henegar C; Alexeev A; Sulchek T. *PLoS One* 2013, 8, No. e75901.

- (12). Zola H; Swart B; Banham A; Barry S; Beare A; Bensussan A; Boumsell L; Buckley CD; Bühring H-J; Clark G; et al. *J. Immunol. Methods* 2007, 319, 1–5. [PubMed: 17174972]
- (13). McPherson R; Pincus MR; Henry JB. *Henry's Clinical Diagnosis and Management by Laboratory Methods*; Saunders Elsevier: Philadelphia, PA, 2007.
- (14). Tannenberg RK; Lauridsen LH; Kanwar JR; Dodd PR; Veedu RN. *Curr. Alzheimer Res* 2013, 10, 442–448. [PubMed: 23270374]
- (15). Miltenyi S; Müller W; Weichel W; Radbruch A. *Cytometry* 1990, 11, 231–238. [PubMed: 1690625]
- (16). Lee W, Tseng P, Di Carlo D, Eds. *Microtechnology for Cell Manipulation and Sorting*; Springer, 2016.
- (17). Singh A; Suri S; Lee T; Chilton JM; Cooke MT; Chen W; Fu J; Stice SL; Lu H; McDevitt TC; et al. *Nat. Methods* 2013, 10, 438–444. [PubMed: 23563795]
- (18). Cheng X; Irimia D; Dixon M; Sekine K; Demirci U; Zamir L; Tompkins RG; Rodriguez W; Toner M. *Lab Chip* 2007, 7, 170–178. [PubMed: 17268618]
- (19). Yu X; He R; Li S; Cai B; Zhao L; Liao L; Liu W; Zeng Q; Wang H; Guo SS; et al. *Small* 2013, 9, 3895–3901. [PubMed: 23650272]
- (20). Cheung LSL; Zheng X; Stopa A; Baygents JC; Guzman R; Schroeder JA; Heimark RL; Zohar Y. *Lab Chip* 2009, 9, 1721–1731. [PubMed: 19495456]
- (21). Parkinson DR; Dracopoli N; Gumbs Petty B; Compton C; Cristofanilli M; Deisseroth A; Hayes DF; Kapke G; Kumar P; Lee JS; et al. *J. Transl. Med* 2012, 10, 138. [PubMed: 22747748]
- (22). Bose S; Singh R; Hanewich-Hollatz M; Shen C; Lee C-H; Dorfman DM; Karp JM; Karnik R. *Sci. Rep* 2013, 3, 2329. [PubMed: 23900203]
- (23). Choi S; Karp JM; Karnik R. *Lab Chip* 2012, 12, 1427–1430. [PubMed: 22327803]
- (24). Wang G; Crawford K; Turbyfield C; Lam W; Alexeev A; Sulchek T. *Lab Chip* 2015, 15, 532–540. [PubMed: 25411722]
- (25). Janshoff A; Steinem C. *ChemBioChem* 2001, 2, 798–808. [PubMed: 11948865]
- (26). Yuan Y; Oberholzer M; Lenhoff A. *Colloids Surf., A* 2000, 165, 125–141.
- (27). Shah MA; Schwartz GK. *Drug Resist. Updates* 2000, 3, 335–356.
- (28). Simon A; Nagel D; Kitchen B; Bode U. *International Journal of Pediatric Hematology/Oncology* 1997, 4, 401–414.
- (29). Ruardy T; Schakenraad J; Van der Mei H; Busscher H. *Surf. Sci. Rep* 1997, 29, 3–30.
- (30). Barthel SR; Schatton T. *Immunity* 2016, 44, 1083–1085. [PubMed: 27192573]
- (31). Nishimura Y; Shimojima M; Tano Y; Miyamura T; Wakita T; Shimizu H. *Nat. Med* 2009, 15, 794–797. [PubMed: 19543284]
- (32). Greenberg AW; Hammer DA. *Biotechnol. Bioeng* 2001, 73, 111–124. [PubMed: 11255159]
- (33). Tasadduq B; Wang G; El Banani M; Mao W; Lam W; Alexeev A; Sulchek T. *Flow Meas. Instrum* 2015, 45, 218–224.
- (34). Li Q; Lee G; Ong C; Lim C. *Biochem. Biophys. Res. Commun* 2008, 374, 609–613. [PubMed: 18656442]
- (35). Herman CT; Potts GK; Michael MC; Tolan NV; Bailey RC. *Integrative biology: quantitative biosciences from nano to macro* 2011, 3, 779–791. [PubMed: 21614364]
- (36). Fisher J; Abramowski P; Wisidagamage Don ND; Flutter B; Capsomidis A; Cheung GW-K; Gustafsson K; Anderson J. *Mol. Ther* 2017, 25, 1234–1247. [PubMed: 28341563]
- (37). Yu ZTF; Aw Yong KM; Fu J. *Small* 2014, 10, 1687–1703. [PubMed: 24515899]
- (38). McEver RP. *Cardiovasc. Res* 2015, 107, 331. [PubMed: 25994174]
- (39). Hidari KI-PJ; Weyrich AS; Zimmerman GA; McEver RP. *J. Biol. Chem* 1997, 272, 28750–28756. [PubMed: 9353345]
- (40). Kishimoto TK; Jutila MA; Berg EL; Butcher EC. *Science* 1989, 245, 1238–1242. [PubMed: 2551036]
- (41). Reticker-Flynn NE; Malta DFB; Winslow MM; Lamar JM; Xu MJ; Underhill GH; Hynes RO; Jacks TE; Bhatia SN. *Nat. Commun* 2012, 3, 1122. [PubMed: 23047680]

- (42). Kim G; Davidson B; Henning R; Wang J; Yu M; Annunziata C; Hetland T; Kohn EC. Cancer 2012, 118, 1543–1553. [PubMed: 22009736]

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Figure 1. (A) Adhesion based sorting device. The top figure shows the device and its schematic showing it consists of ridges coated with cell adhesion molecules. The bottom enlarged figure under the ridge shows the device working mechanism. (B) Shows the trajectories of Jurkat cells flowing through the device with (black) and without (blue) P selectin coating.

Figure 2.

Enrichment and fractionation of HL60 cells through adhesion. (A) Shows the flow cytometer data for HL60 cells collected at different outlets showing a peak shift in their mean fluorescent values only in the case when device is coated with P selectin and flow rate is 0.045 mL/min. The first and third top figures show the data with no P selectin coated device (flow rate 0.045 mL/min) and with coated P selectin device but flow rate of 0.1 mL/min. There is not any significant shift in the fluorescent peak values for the these two cases. (B) Shows the mean fluorescent values at different outlets. (C) Shows the enrichment factor, and (D) shows the images of cells at outlets for P selectin coated device and 0.045 mL/min flow rate.

Figure 3.

(A, B, C) Trajectories of HL60 cells with and without P selectin incubated device at different flow rates and their (D) $\Delta y / \text{ridge}$ and (E) velocity analysis based on data extracted from these trajectories.

Figure 4.

(A) As cells flow under the ridge, they bind to P selectin. When they leave the ridges cells with more ligand expressed on their surface resist the secondary drag force and stay adhered to bottom of the channel and experience the flow in negative y direction (red arrow). On the other hand cells with less ligand detach from the surface and enter the streamline in positive y direction (green arrow). Panel B shows the cell trajectory in without P selectin coated device, and panels C and D show the trajectories of cells with less and more PSGL-1, respectively, with P selectin coated device, and all are compared with streamline at the middle height of the gap size with z velocity set to zero.

Figure 5.

(A and B) Enrichment factors for (PSGL1+/PSGL1+) and (PSGL1-) Jurkat cells for gap size optimization of the adhesion based sorting device. As the gap size is changed from 9 to 14 μm, the device efficiency decreases as indicated by the enrichment factors. (C) Young's modulus of HL60 cells collected at different outlets measured using AFM and (D) their size using ImageJ. The data suggests that the device can sort by adhesion, with minimal effect of mechanics.

Figure 6. Adhesion-based fractionation of cells. (A) Shows the flow cytometer data for Jurkat cells collected at different outlets showing a peak shift in their mean fluorescent values. (B) Mean fluorescent intensity at each outlet. (C) Enrichment factor for three outlet device and (D) for five outlet device.

Figure 7.

CD69 and CD11b markers are used to study if the cells are activated after sorting. Results are compared with cells incubated with P selectin for 24 h and with cells incubated with P selectin coated surface for 2 h. There is a significant up regulation of activation markers after cells removed from P selectin coated surface. There is no change in activation markers in case of cells collected after sorting.