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Open-Tubular Capillary Cell Affinity Chromatography: Single and Tandem Blood Cell Separation

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In this paper, an open-tubular capillary cell affinity chromatography (OT-CAC) method to enrich and separate target cells is described. Open tubular capillaries coated with anti-CD4, anti-CD14, or anti-CD19 antibodies were used as affinity chromatography columns to separate target blood cells. Cells were eluted using either shear force or bubbles. Bubbles were used to elute the captured cells without diluting the captured cells appreciably, while maintaining viability (the viability of the recovered cells was 85.83 ± 7.34%; the viability of the cells was 90.41 ± 3.49% before separation). Several aspects of the OT-CAC method were studied, such as the affinity of one antibody between two different cell lines, the effect of shear force, and the recovery of captured cells. Single- and multicell type separations were demonstrated by isolating CD4+ cells with antiCD4 coated capillary and isolating CD4+ and CD19+ cells with two capillaries in tandem from blood samples. In the one cell type isolation test, an average of 87.7% of the recovered cells from antiCD4 capillary were lymphocytes and an average of 97.7% of those lymphocytes were CD4+ cells. In the original blood sample, only 14.2% of the leukocytes were CD4+ cells. Two capillary columns were also run in tandem, separating two blood cell types from a single sample with high purity. The use of different elution shear forces was demonstrated to selectively elute one cell type. This method is an inexpensive, rapid, and effective method to separate target cells from blood samples.

The separation of cells is important in the fields of chemistry, biology, and medicine. The ability to detect and isolate cells by phenotype is also crucial in the fields of bioengineering and pharmaceutical research, especially in the implementation of proteomics and genomics to human diseases. Cell separation by affinity methods have been studied since the 1970s.[1] Affinity cell separation methods include cell affinity chromatography (CAC), immunomagnetic cell separation (or magnetic cell sorting (MACS)), and microfluidic methods including flow cells and microarrays.[2] CAC exploits differences in cell surface macromolecules by passing mixtures of cells through a column. This column typically contains beads to which affinity ligands are added,[3,4] porous supports[5−10] or membranes.[11]

In early work, Hertz et al. separated T lymphocytes from peripheral blood and designed a simple model for cell binding.[3] Hammer et al. designed an adhesion model of CAC and showed the adhesion efficiencies vary as a function of flow rate and temperature.[12] Ujam et al. improved the affinity separation of monocytes with a 77% yield of the captured cells at a purity of 90% and ≥65% viability using expanded-bed adsorption.[5] Affinity cryogel monoliths were later used to separate *Saccharomyces cerevisiae* and *Escherichia coli* cells. At the optimal conditions, the *E. coli* cells in the flowthrough fraction were nearly in 100% purity, whereas the fraction eluted by compression of the adsorbent contained viable *S. cerevisiae* cells with 95% purity.[10] Hollow fibers coated with anti-CD34 were also demonstrated to enrich CD34+ cells.[13,14]

Recently, microchannels (and arrays) coated with antibodies were designed to capture cells.[15,16] Antibodies were coated on the surface of the microchannel or the microarray to capture the antigen expressing cells, and a stop-flow technique was used to remove the interference cells. While arrays can separate multiple phenotypes from a single sample, they cannot effectively elute the cells without remixing the separated cell types.[16−20] Another

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cell separation approach, capillary electrophoresis (CE), has been demonstrated for separating bacteria and viruses.[21−23] While CE has been demonstrated as an effective technique in these studies, cells with similar properties, such as lymphocyte subsets, may be difficult to separate.

The interactions between cells and surfaces are critical for immune response and have been studied in the parallel-plate flow chamber and microfluidic chambers.[15,24] In another area of cell separation, antibody-coated microspheres have also been attached to glass to serve as a cell capture surface.[25] Affinity arrays have also been developed for immunophenotyping of leukemia based on antibody−antigen capture.[26−31] In a similar approach, Zhang et al. used an antibody array to detect different blood cells but the device required overnight incubation for analysis.[18] The Semibio Corporation also uses antibody-coated arrays to isolate CD4+ T cells for analysis.[32] A recent work from Du et al. describes the separation of normal and cancerous breast cells in a poly(dimethylsiloxane) microchannel using two antibodies. The separation efficiency for cancer cells was 30% while the separation efficiency of normal cells was 5%.[33] In a more recent study, Cheng et al. have developed a similar flow channel for CD4+ T cell counting, showing that CD14+ monocytes (which also express CD4) can be removed selectively by wash pressure.[34]

Cells separated by affinity methods may be recovered by adding a soluble competitive agent in the buffer.[35] Alternately, flow-induced elution of target cells has been reported using mechanical shear flow with a recovery from 60% to 80%.[5−7] The high shear rates required to remove cells result in sample dilution and reduced viability of the cells. Compared with flow-induced detachment, elastic deformation of the separation medium can increase the cell detachment ratio but the viability of cells is still low, ranging from 40%−70%.[9] Bubble-induced detachment of affinity-adsorbed cells achieve a higher recovery, up to 90% of attached cells can be recovered with a five-bubble string.[36]

Open tubular capillary separations are particularly attractive for bubble-induced cell detachment. The irregular flow patterns of packed CAC columns are problematic for bubble detachment and cell collection. In this paper, an open-tubular capillary cell affinity chromatography (OT-CAC) method is reported, using the open tubular capillary and affinity cell separation to obtain higher capture efficiency. The method was used to capture target blood cells using shear- or bubble-induced detachment of captured cells. The ability to separate and recover cells while maintaining viability and avoiding sample dilution is also demonstrated. Cells were tested for viability and apoptosis following mechanical elution from the capillary. The effect of shear force on cell purity has been demonstrated.

One main advantage of the OT-CAC approach is that multiple capillaries can be placed either in parallel or series, allowing for either higher throughput or for multiple immunophenotyping. In this article, the separation of two different cell types (CD19+ and CD4+ cells from blood) is demonstrated with separate collection of each purified fraction. Also, removal of one cell interference cell type (CD14+/CD4+ monocytes) from the target analyte cell (CD4+ lymphocytes) has been demonstrated in a tandem capillary.

EXPERIMENTAL SECTION

Cells and Cell Culture. Human T lymphocyte (HuT 78) and B lymphocyte (RPMI 8226) transformed cell lines were both purchased from American Type Culture Collection. The HuT 78 T cell antigen expression is as follows: CD3+, CD4+, and CD71+; RPMI 8226 B cells are CD71+ and CD19−. Cells were maintained in RPMI 1640 medium (VWR Scientific) supplemented with 10% fetal bovine serum (Sigma-Aldrich) in an incubator set to 37 °C and 5% CO_2. Cells were subcultured by removing cell suspension and replacing the removed volume with fresh medium 1−2 times each week. Cell density approached 10^6 cells/mL before subculture and 10^5 cells/mL after subculture. Cell density varied between 10^5−10^6 cells/mL for analysis. Whole blood standards (Multi-Check Control) were purchased from Becton-Dickinson.

Blood samples were injected after lysing erythrocytes with distilled H_2O for 30 s; osmolarity was then restored using concentrated saline and samples were injected into the capillary by a syringe pump. Whole blood was injected without the lysis procedure. Capillaries with inner diameters of 200 μm were obtained from Polymicro Technologies. The polyimide coating was burned off to facilitate microscope imaging.

Chemical and Reagents. Sterile phosphate buffered saline (pH = 7.4) was purchased from Invitrogen. Fetal bovine serum, bovine serum albumin, and biotinylated bovine serum albumin were purchased from Sigma-Aldrich. Neutravidin was obtained from Pierce. The following biotinylated antibodies were purchased from Becton Dickinson: mouse anti-human CD4, mouse anti-human CD19, and mouse anti-human CD71. Mouse anti-human CD14 conjugated to biotin was purchased from Ebioscience. The

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following fluorophore conjugated antibodies (purchased from Becton Dickinson) were used for flow cytometry and fluorescence microscopy: mouse anti-human CD4-Alexa Fluor 488, mouse anti-human CD19-PE, and mouse anti-human CD14-PE. To visualize the effect of mechanical elution on the antibody coating, rhodamine 110-biotin (Biotium) and *R*-phycoerythrin-streptavidin (VWR Scientific) were used.

Preparation of the Capillary. The protocol described by Flavell's group[37] was followed for preparing the inner surface of the capillary with a uniform layer of biotin-binding protein. Flavell's protocol was modified in the present work: neutravidin was chosen for reduced nonspecific binding with respect to streptavidin.[16] Antibodies were linked to the glass surface as follows: (i) biotin-conjugated albumin was coated onto the inner wall of the capillary; (ii) neutravidin was coated over the first layer, forming a uniform coating of the protein on the surface; (iii) monoclonal mouse anti-human IgG antibodies conjugated to biotin were used to create surfaces for cell capture. Capillaries were then stored in a refrigerator at 4 °C. The entire process required less than 1 h for a batch of capillary columns. For a capillary of 10 cm length, 200 μm inner diameter, the reagent loading volume is 3 μL.

Separation System. The open-tubular capillary cell separation system is a straightforward design. Capillary and syringe were connected by a piece of Teflon tube; sample or buffer solution was loaded by the syringe. The loading speed was controlled by a syringe pump. Phosphate buffered saline containing 3% bovine serum albumin (BSA) was used as the carrier fluid and to minimize nonspecific binding. For stop-flow separations, cell samples were injected and allowed to incubate for 20 min before unbound cells were washed away. For continuous flow separations, cells were passed through the capillary at the optimum speed by the syringe pump. The effluent was collected with 1.5 mL microcentrifuge tubes (VWR Scientific). Microscope images of the columns were taken before and after the washing steps to determine cell retention and removal efficiencies.

Cell Detection. Cells were counted on-column using either transmission or fluorescence microscopy. An inverted microscope (IX71, Olympus, Center Valley, PA) was used to obtain all of the images of the columns. A 0.10 NA, 4× objective was used for white light imaging and a 0.25 NA, 10× objective was used for fluorescence imaging. Cell viability was determined using a 5 μg/mL solution of propodium iodide and acquiring fluorescence images at the appropriate wavelength. Images were recorded using a 12-bit, cooled charge-coupled device (CCD) camera (Orca-285, Hamamatsu, Hamamatsu, Japan) using the manufacturer's software. 16-bit tagged image file format (TIFF) images were processed using ImageJ (version 1.33, National Institutes of Health).

The antigen expression of the captured cells and cells in the effluent was verified using a BD FACSCalibur flow cytometer (Becton-Dickinson) and dye-conjugated antibodies. Cell samples were diluted 1:5 in PBS prior to analysis. HuT 78 T cells and RPMI 8226 B cells were tested for relative CD71 antigen expression. Lymphocytes from blood samples were identified by forward and side scatter; CD4+ and CD19+ lymphocytes were identified from this subset using anti-CD4 and anti-CD19 antibodies, respectively. Likewise, monocytes were identified by forward and side scatter as well as anti-CD14 antibody fluorescence.

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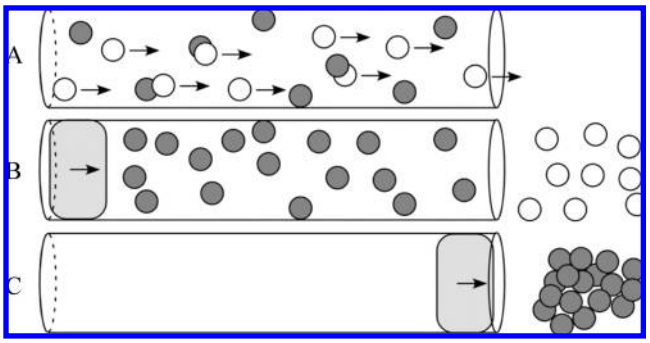

Figure 1. Cell recovery procedure: (A) cell loading, nontarget cells do not form bonds and are washed from the column; (B) bubble-induced recovery after removing nonspecifically bound cells; (C) cell detachment by bubbled elution. Concentrated cells are collected for additional analysis.

Captured Cell Recovery. Either shear flow or bubbles were used to elute cells retained in the column. Figure 1 shows the procedure of cell recovery via bubble injection. Nonspecific bound cells were washed out first (Figure 1a) at a low flow rate. Bubble sequences were initiated by introducing air by a syringe to dislodge cells from the capillary (Figure 1b). Recovered cells were collected into 1.5 mL tubes containing phosphate buffered saline (Figure 1c), and cell numbers were counted using a hemacytometer.

Safety Precautions. Both HuT 78 T cell and RPMI 8226 B cell lines used in this study were transformed cell lines derived from humans and are rated at a biosafety level of 1. Universal precautions for blood-borne pathogens were followed for all cell lines and blood samples. All personnel were trained for blood-borne pathogen safety procedures and used appropriate personal protective equipment (gloves, lab coats, etc.). All materials were disinfected with 10% bleach when necessary. Disposable materials were deposited in biohazard containers, which were autoclaved prior to disposal. A laminar flow biosafety cabinet appropriate for the required biosafety level was used when handling cell cultures to maintain culture sterility and minimize exposure risk.

RESULTS

Capillary Coating. In order to verify that the antibody coating was responsible for cell retention, as opposed to nonspecific adhesion to glass or a BSA layer, untreated glass capillaries and capillaries coated with BSA were compared to capillaries with the complete antibody coating. Lysed blood was loaded into the capillary at 0.04 mL/h for 30 min. Capillaries were washed using a flow rate of 2 mL/h (0.08 mL total volume), and the remaining cells were collected for flow cytometry analysis.

Capillaries coated in BSA showed minimal nonspecific binding (<1%). After sample loading, too few cells were adhered to the capillary to measure by flow cytometry, indicating that nonspecific binding of any cell leukocyte type to the BSA layer was insignificant. With the uncoated, glass-surface capillaries, few cells were detected and there was no selective retention of any particular cell type on the column surface. All major leukocyte types tested were present in the glass-only capillary column, with no depletion or enrichment of other cell types. Clearly, the coating of antibodies on the column surface is responsible for selective cell retention as outlined below.

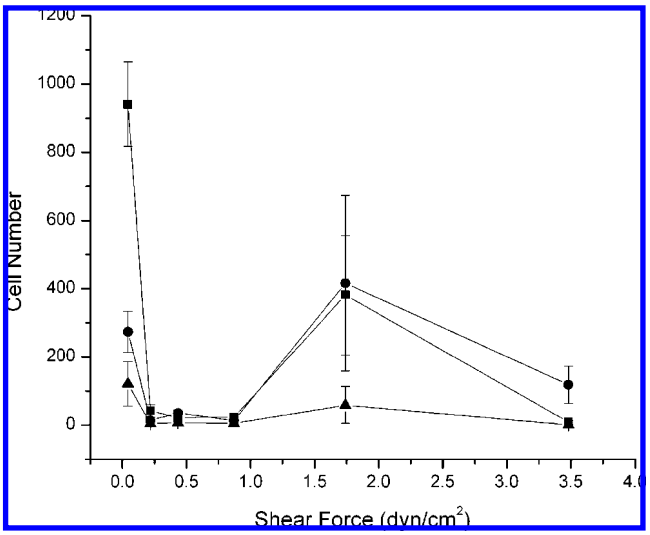

Figure 2. Blood cells eluting from the capillary column as a function of shear force. Unbound cells are washed away at low shear force, while the retained cells are dislodged at shear forces about 1.5 dyn/cm^2. ■ = granulocytes, ● = lymphocytes, ▲ = monocytes (all cell types determined by forward and side scatter).

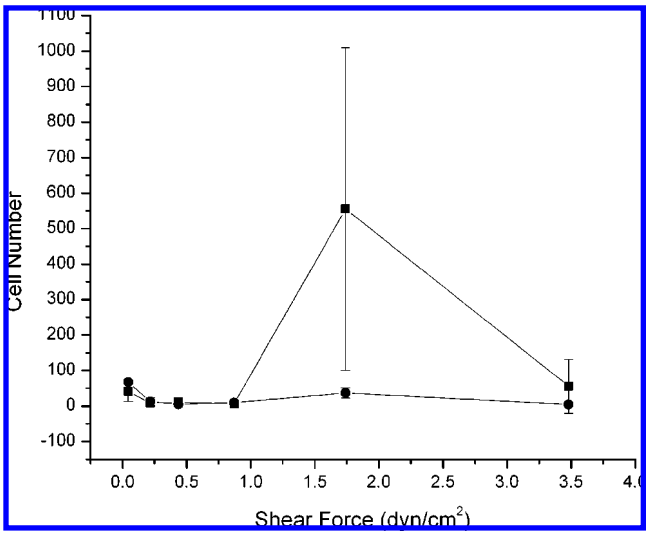

Figure 3. Lymphocyte and monocyte cells eluting from the capillary column. In a single-column arrangment, most CD4+ cells are CD14−, indicating they are helper T lymphocytes. ■ = CD4+, CD14− lymphocytes; ● = CD4+, CD14+ monocytes (all cell types determined by fluorescence measurements).

When cells were eluted, either by shear flow or bubbles, the cell-surface linkage could be broken at a number of points. For example, the antibody−antigen bond, either of the two biotin−neutravidin bonds, or the BSA−glass interaction could be disrupted. In order to determine the dissociation point for eluted, specifically bound cells, recovered cells were stained with either biotin-rhodamine 110 or streptavidin-phycoerythrin (PE). If cells were positively stained with biotin-rhodamine 110, then the cells detached along with the neutravidin. Cells positively stained with streptavidin-PE would show that the biotinylated antibody remains attached to the cell after elution.

Microscope images of cells eluted by bubbles showed no biotin-rhodamine 110 or streptavidin-PE staining, indicating that the likely breakage site is the antigen−antibody bond or the BSA−glass interface. Once capillaries were used, there was significant nonspecific binding, indicating that the antibody coating had been compromised. We are currently evaluating the attachment scheme to improve capillary longevity. This is not a critical weakness, however, as the cost of producing capillaries on a mass scale, even manually in the laboratory, is low. Each capillary column costs approximately $0.50 U.S. dollars to fabricate.

Shear Force Effects. The expected bond strength between an adhered cell and the surface depends on the antigen/antibody density, the interaction time, and interaction area. This result is consistent with Lauffenburger's model,[3]

$$B^* = \tau_c A_c B$$

where B is the density of bonds formed per unit contact area, B^* is the estimated number of bonds formed during a collision, τ_c is the collision duration, and A_c is the contact area.

Lysed blood was continuously loaded into an anti-CD4 capillary for 30 min. Cells in the capillary were then subjected to increasing shear forces, and the cell phenotype was identified by flow cytometry. As shown in Figure 2, nonspecifically bound cells, essentially those resting on the capillary surface, are comprised largely of granulocytes and are removed with minimal shear force. Cells remaining on the capillary wall remain essentially in place until the shear force approached 1.7 dyn/cm^2. At this point, the majority of cells are washed out of the column. Figure 2 shows that a large fraction of granulocytes remained in the capillary after the lower shear force elutions. However, when one observes the CD4+ lymphocyte and CD14+ monocyte fractions (identified by antibody fluorescence and laser scatter on the flow cytometer), the majority of those cell types are comprised of CD4+ lymphocytes (Figure 3). The high granulocyte fraction at the 1.7 dyn/cm^2 shear force may be due to apparent granulocyte affinity for the anti-CD4 antibodies used. In our staining tests with both anti-CD4-biotin and anti-CD4-FITC, we have observed that granulocytes will bind to the antibodies in free solution (i.e., no surface immobilization) with affinities similar to CD4+ lymphocytes (see Supporting Information). It is possible that different antibody clones, or aptamer-based capture, will reduce the nonspecific affinity of granulocytes. We intend to investigate the nature of this nonspecific binding in the future.

Figure 4 shows that the CD4+ lymphocyte fraction eluting from the column varies in purity (both relative to the total cell number and to the cells identified as lymphocytes by laser scatter). At 1.7 dyn/cm^2, 58 ± 3% of the total cells are identified at CD4+. When compared to the lymphocytes only, the purity of CD4+ cells rises to 92 ± 1%. There is therefore an optimal shear force for elution where a majority of the CD4+ cells are removed from the column with high purity in the lymphocyte fraction. Lymphocytes are relatively straightforward to discern from monocytes and granulocytes using forward and side scatter of the laser beam in the cytometer. In this case no antibody or other stain is required. In future work, we will incorporate a laser beam to count cells eluting from the column and use two low-cost detectors and a counting board to identify lymphocytes using forward and side scatter alone. This will result in label-free detection with purity that is comparable with many flow cytometers without the need for sample preparation or fluorescent antibodies.

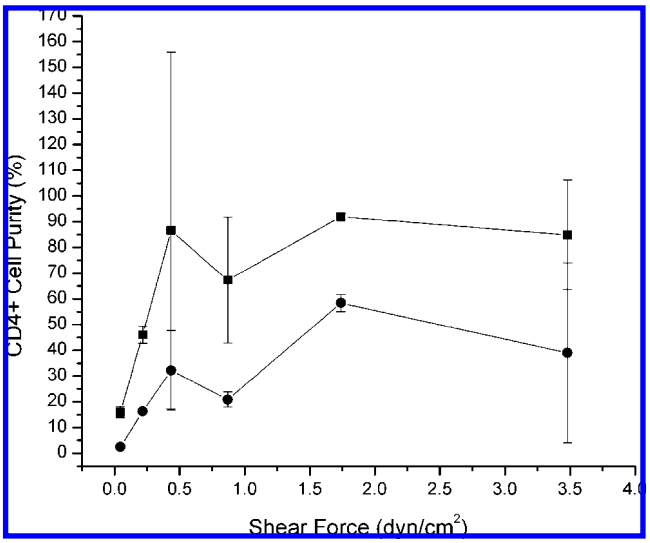

Figure 4. CD4+ lymphocyte purity as affected by shear force of buffer elution: ■ = CD4+ lymphocyte purity of all cells in the lymphocyte scatter gate; ● = CD4+ lymphocyte purity of all cells (no lymphocyte gate).

Table 1. Enrichment Difference between B and T Lymphocytes Using Anti-CD71 Columns

position[a] (cm)	enrichment factor		T:B cell enrichment ratio
	B cells	T cells	
1	5.42	26.01	4.80
1.5	4.10	20.89	5.08
2	3.39	17.16	5.06
4	2.02	9.48	4.69
6	0.76	5.75	7.51
average	3.14	15.86	5.43

[a] Position along capillary.

Affinity Comparison between T and B Cells. The capture of T and B cells was compared at different positions of an anti-CD71 coated capillary by continuous separation. The enrichment ratio was calculated as the concentration of the enriched cells in the capillary divided by the initial concentration. The HuT 78 T cells were enriched 5.4 ± 1.2 times as much as the RPMI 8226 B cells (Table 1), indicating that the T cells have a higher affinity than B cells using an anti-CD71 capillary. This retention ratio is consistent with flow cytometry data of CD71 expression of these two cell lines (data not shown). The variation in capture ratio between T and B cells along the length of the capillary is due, in part, to the counting statistics, with the exception of the final postion (6 cm). The B cell enrichment ratio at this point was too low to measure with substantial error, which corresponded to the larger enrichment ratio (7.51 T/B cells).

Cell Enrichment and Recovery. In continuous flow separation, target cells were enriched in the capillary as nontarget cells were passed to waste. The enriched cells were recovered by injecting a sequence of air bubbles to avoid dilution and maintain viability. By continuously flowing cells through the antibody coated capillary, a volume of sample larger than the column volume can be loaded over time, allowing a statistically sufficient number of low-abundance cells to be captured. After cell recovery, the concentration of the elution cell solution is measured; an average enrichment ratio of 1.93 ± 0.73 was obtained with T cells in the anti-CD4 coated capillary.

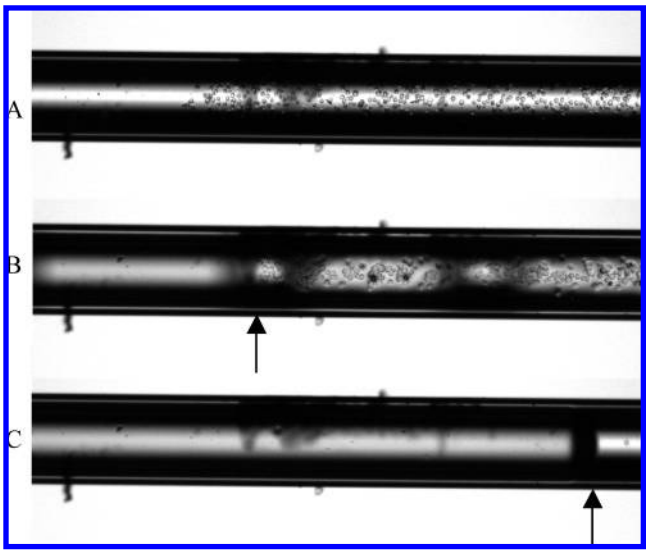

Figure 5. Bubble-induced cell recovery. Images of the capillary column (A) before bubble introduction, (B) after several bubbles, and (C) after 0.1 mL of air bubbles injected. (Arrows indicate bubble interfaces).

The main benefit of bubble-induced detachment is that the cells are removed without dilution. Figure 5 shows images of the column before, during, and after the introduction of bubbles to elute the target cells. From previous work by Hubble,[35] more bubbles of small volume are more effective than fewer bubbles of large volume, presumably because it is the air−liquid interface that is critical for cell detachment.

The cell elution efficiency can be calculated as

$$r_{\text{elution}} = \left(1 - \frac{N_{\text{after}}}{N_{\text{before}}}\right) \times 100\%$$

where N_{after} is the number of cells retained in the capillary after bubble-induced elution. N_{before} is the number of cells loaded onto the capillary prior to elution. The advantage of using the elution efficiency is that it is rapid and accurate, since it is easy to measure cells directly in the capillary under the microscope. In this work, eight to nine different positions along the column were compared in each test. An amount of 99.94 ± 0.06% of captured cells in this study were recovered with 0.1−0.5 mL air bubble sequence. The recovered cells had a viability of 85.8% (the original cell sample viability was 90.4%)

Cells were also separated in a sterile manner for subsequent reculture. An anti-CD4 column was operated in a laminar flow hood under sterile conditions to capture HuT 78 lymphocytes. Cells eluted using a bubble sequence showed a drop in viability (to 37%) over 1 day but recovered after several days in culture. The sorted cells were grown and tested for 13 days and showed 91% viability on day 6 and 98% viability on the last day of culture. The recultured cells were discarded after 31 days of continuous culture.

Blood Cell Separation with One Antibody (anti-CD4). Lysed blood was resuspended with 3% BSA in PBS and then

Table 2. CD4+ Blood Cell Separation

		CD4+ T cells in total (%)	CD4+ cells in lymphocyte (%)	lymphocyte (%)	monocyte (%)	granulocyte (%)	ratio of CD4+ Vs monocyte
before		14.24	46.20	30.84	4.80	63.96	2.97
recovery	av	85.70	97.65	87.75	2.37	5.13	53.03
	std	2.72	1.00	2.22	1.84	2.61	30.66
waste	av	2.43	13.30	17.45	3.16	76.69	0.75
	std	1.57	6.35	2.44	0.93	2.97	0.30
flush out	av	8.92	26.43	29.43	9.07	59.52	0.95
	std	7.62	17.44	13.60	2.39	14.20	0.86

continuously injected into an anti-CD4 coated capillary at the speed of 0.04 mL/h. Whole blood was tested, but the high density of erythrocytes prevented adequate leukocyte−surface interactions. The waste solution, flush solution (the cells suspended in the carrier solution at the end of the loading period), and captured cells (recovered by bubbles) were analyzed for phenotype by flow cytometry. Table 2 summarizes the results of the four tests.

After continuous flow, most of the CD4+ T cells were captured by the capillary and most of the granulocytes and monocytes were not retained. An amount of 87.7% of the recovered cells were lymphocytes and an average of 97.7% of those lymphocytes were CD4+ T cells. The enrichment factor for CD4+ T cells was 6× after a 1 h loading time. It is important to stress that the loading time affected total cell number, not the fraction of target cells retained. There were some CD4+ monocytes in all of the three fractions of cells in these experiments. With comparison of the recovered fraction, waste, and flushed (suspended) cells with the original blood sample, the number of monocytes in the recovered fraction was the lowest (it is about 50% of that in original blood). An amount of 3.2 ± 0.9% of the waste was comprised of monocytes, which is lower than the ratio of monocytes in the original blood. The percentage of monocytes in the flushed out solution (suspension) is 9 ± 2%, which is 189% of that in original blood. This method could therefore be used for negative depletion, where one cell type is removed and the flushed out cells are then collected for analysis.

Blood Cell Separation with Tandem Capillaries. One of the most interesting strengths of the OT-CAC approach is the ability to place several capillaries in either a series or parallel arrangement. In the parallel approach, one could use a battery of identical columns for increased throughput. Each capillary in the parallel arrangement could also have separate antibodies for multiple immunophenotyping. The advantage to this approach is that each column would be exposed to the same sample. In the series arrangement, the preceding column can be used to deplete a cell type prior to the second column separation. For example, one could deplete CD14+, CD4+ monocytes in an anti-CD14 column prior to separating CD4+ lymphocytes. For this article, an anti-CD19 column was connected to an anti-CD4 column in series to simultaneously capture two different cell types. Lysed blood was loaded into the tandem capillaries for 30 min as described in the single column separation. Prior to bubble-induced elution, the capillaries were separated so that each column output is collected separately. The recovered fractions were verified using flow cytometry.

Figure 6 shows the cytometry results of the tandem capillary experiment. The phenotype purity of the recovered fractions was

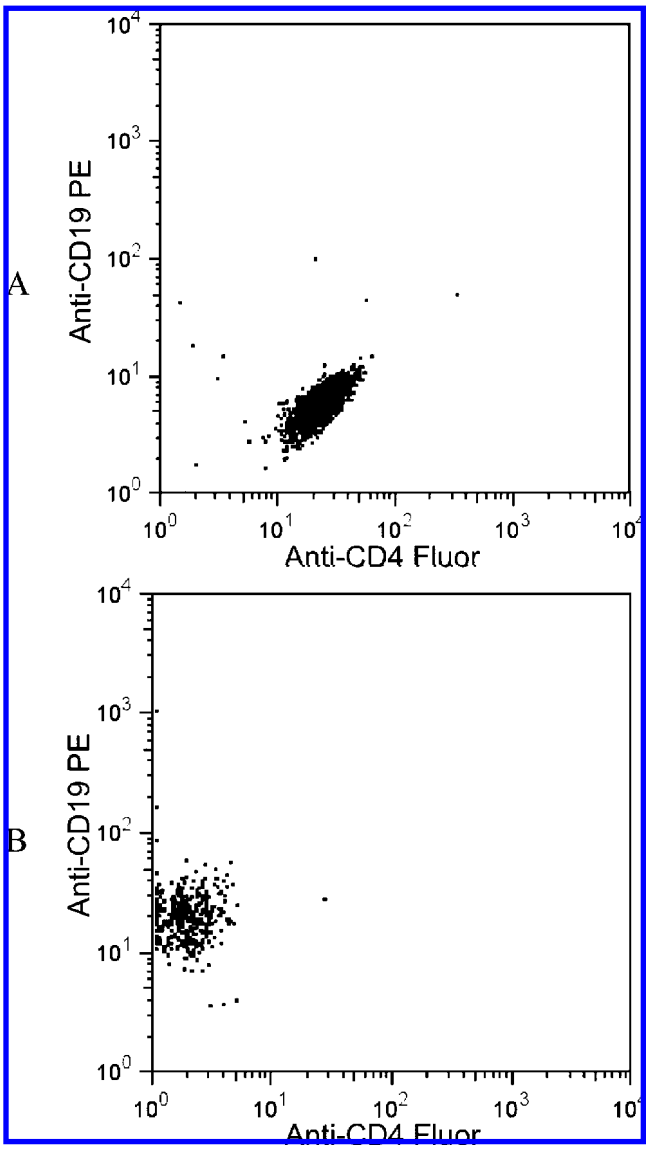

Figure 6. Flow cytometer analysis of blood separated by two columns in series: (A) fluorescence of CD4+ leukocytes separated in the anti-CD4 column, CD4+ purity is 87 ± 7% (CD19+ cells are 0.2 ± 0.2%); (B) fluorescence of CD19+ lymphocytes in the anti-CD19 column, CD19+ purity is 82 ± 6% (CD4+ cells are 0.1 ± 0.2%).

consistent with the single column experiments. In both the anti-CD19 and anti-CD4 capillary columns, the nonspecific binding was due primarily to granulocytes. Both the anti-CD19 and anti-CD4 columns exhibited minimal capture of the counterpart target cells. That is, the anti-CD19 capillary only retained an average of 0.10%

of the CD4+ cells, and the anti-CD4 capillary only retained an average of 0.23% of the CD19+ B lymphocytes.

Series-arrangement tandem columns were also evaluated to deplete cells from the sample prior to the second column. Two approaches were used. First, an anti-CD14 capillary and a downstream anti-CD4 capillary were used to separate CD14+ cells and CD4+ cells from lysed blood. Second, a neutravidin capillary was coupled to a downstream anti-CD4 capillary, and the lysed blood samples were prestained with anti-CD14 biotin.

In the first approach, the anti-CD14 capillary did not efficiently retain CD14+ monocytes. Even in the single-capillary arrangement, anti-CD14 capillaries tested did not yield a large number of monocytes. The hypothesis for this result is that either the surface antigen density was too low or the CD14 affinity for the antibody was insufficient to cause a binding event at any of the shear forces tested (0.04−3.5 dyn/cm^2). In the second approach, where monocytes were prestained with anti-CD14-biotin antibody, the stained, lysed blood sample was passed through both columns and the bound cells from each column were separated. The prestaining approach resulted in monocytes retained in the anti-CD14 column, but the separation purity of the anti-CD4 column remained roughly the same as in the single-capillary experiments (see Supporting Information). As mentioned previously, granulocyte affinity for the anti-CD4 antibodies used in these experiments remains the major source of impurity in these separations. Nevertheless, the separation of multiple blood types has been demonstrated, and in future work multiple (>2) capillaries will be tested in series and parallel arrangements.

DISCUSSION AND CONCLUSION

In this work an open-tubular affinity capillary approach was used for target cell enrichment, detection, elution, and recovery. Bubble-induced cell detachment was used to ensure a high elution ratio with high viability. The OT-CAC method can selectively enrich target cells in complex samples. In the OT-CAC method, the sample concentration does not affect the cell enrichment and detection. For a sample with very low-target cell concentration, the sample loading volume can be increased at the expense of analysis time. For the upper limit of cell concentrations, cell capacity can be increased by increasing the length of the capillary.

Compared with the other blood separation methods, the OT-CAC method exhibits high purity. For example, Ujam et al. improved monocyte isolation to reach a 77% yield with a purity of 90% and ≥65% viability.[5] These are the results of the positive isolation method; the negative method results are lower. With the use of their evaluation standard, the OT-CAC method had an 85.70 ± 2.72% yield with a purity of 97.65 ± 1.0% and 85.83 ± 7.34% viability with CD4+ T cells.

With open-tubular capillary cell affinity chromatography, one can easily elute almost all the captured cells with bubbles, without decreasing their viability significantly. Compared with the traditional flow-induced detachment and mechanical compression methods, bubble induced detachment is more efficient. In the compression method, 40−80% of bound cells can be detached and only 10−40% of cells can be detached by flow induced detachment methods.[9] Bubble induced elution can get a higher detachment ratio, about 99.9% with high precision and viability.

For applications requiring further study of the target cells, such as culturing or testing the metabolism of the cells, viability is paramount. Traditional elution methods with buffer exchange may affect the cell membrane, which decreases the viability of the recovered cells. The mechanical compression recovery of cells in a cryogel monolith achieved 85 ± 4% viability, but in this condition, the recovery was only 75 ± 3%. With the use of a proper bubble eluting speed, the average viability of the recovered cells is 85.8 ± 7.3% with a recovery of 99.94 ± 0.06%. In these tests, the cell viability before separation was 90.41 ± 3.49%. It is important to note that the bubble speed can affect viability. For example, increasing the bubble speed to 1 mL/s air injection, the viability of the recovered cells decreased to 50%.

The multiparameter measurement capability of OT-CAC has also been demonstrated. It is possible to attach multiple columns together in several arrangements to separate multiple cell types. In the future, the individual column performance will be optimized to improve the tandem separation approach, allowing for multi-immunophenotyping.

OT-CAC is an effective cell separation method with the abilities of cell capture, enrichment, and recovery. The excellent recovery, purity, and viability of the method make it amenable to many cellular analyses. The ability to use multiple capillaries in series allows for multiparameter separation and sorting.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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