Automating recovery of spiked tumor cells from blood

AVIVA BIOSCIENCES

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RedSift

ABSTRACT # 369

The isolation and study of circulating tumor cells (CTC) enables personalized medicine solutions, monitoring of therapeutics (theranostics), and monitoring of minimal residual disease (MRD); culturing these rare cells is a frequent goal in cancer research, however the common methods for their isolation (gradient centrifugation, lysis buffer) are damaging to these cells, and many emerging technologies leave the cells bound in a consumable device, bound to a biomarker or in a state that is difficult to use in cell culture. Further, anuclear cells in blood greatly outnumber nucleated cells and present significant challenges to the study of the nucleated cells, for example, erythrocytes can absorb excitation or emission wavelengths in fluorescence studies, and their shape promotes rouleaux formation which can sequester the cells of interest; platelets also have a tendency to be activated by physical handling and cause aggregation that can sequester and damage the cells of interest, and these all contain the greatest content of RNA and other markers that increase background noise in many assays. We demonstrate a new technology that automates the depletion of erythrocytes, thrombocytes, and plasma markers from, initially, small volumes of blood, using a micromachined membrane filter. We spiked small counts (10 to 1000 estimated from dilution) of tumor cell lines BT474 (breast cancer), DLD1 (colorectal cancer), and K562 (myelogenous leukemia) and other cell types into aliquots of blood. Using calcein-AM-loading to distinguish the spiked cells from blood cells, the recovery rates were determined in a flow cytometer, indicating an average of 80% ±4% (SE, N=27) recovery of the rare cells after depletion of anuclear cells and plasma. The technology is amenable to use of beads for initial negative depletion of common nucleated cells by magnetic beads and has a recovery capacity of a few to as much as 3 million cells per specimen. Tumor cell lines alone that were run through this automation process then seeded for cell culture demonstrated growth rates indistinguishable from those seeded in similar counts in a regular cell passage. In further studies we demonstrate depletion of leukocytes with magnetic beads and of anuclear cells by filtration to handle larger blood volumes. The filtration system may be used for automated recovery of nucleated cells or cell clusters from blood and other fluid samples without use of harsh chemicals or centrifugation, removing a common barrier for emerging approaches.

BACKGROUND

The cells of clinical relevance are usually sick cells or remodeled and dedifferentiated cells such as stem cells, tumor cells, that express higher levels of anion exchanger than mature leukocytes. For these cell types, lysis has been problematic, resulting in selective loss of those cells of highest interest (eg, Resnitzky and Reichman, 1978. Blood, 51(4):645). Further, cytosolic alkalization from dissolved ammonia in lysis buffers can activate carbonic anhydrase and many other cellular processes, ultimately changing homeostasis. Another common method for rare cell enrichment is to employ high osmolality solutions to produce density gradient which, under centrifugation, is able to separate cells based on their density. This tedious procedure results in loss of many cells found in blood including granulocytes and other nucleated cells of interest, in addition to presenting an osmotic challenge to sick or displaced cells. Finally, centrifugation itself is known to result in cell loss, and it is integral to gradient centrifugation method and most lysis methods.

We present a novel system using micromachined filters to automate cell preparation and provide samples enriched for total nucleated cells. The RedSift Cell Processor system can handle any blood volume as small as 10µL and as much as 3 million nucleated cells per pass. With four filtration lanes per filter array, the cell processor can recover 25-30 million nucleated cells per hour. The cells are separated by a gentle technique that does not lyse cells, nor does it liberate cytosolic contents and RNA into the sample; and configurable input and output volumes can remove centrifugation steps in cell preprocessing.

METHOD

Various carcinoma cell lines (ATCC), including K562 (myelogenous leukemia), DLD-1 (colorectal tumor), BT474 (breast carcinoma), and A549 (lung tumor), were cultured in DMEM with F12 supplement and 10% BSA (Thermo Fisher Scientific). Aliquots of cells were counted on a microscope using a hemocytometer, and counts were confirmed on a CBC instrument (Sysmex) to determine cell density per aliquot.

Peripheral blood was collected from healthy donors and aliquots of each sample were used up to 72 hours after collection. Blood counts were done on a CBC instrument. Some aliquots were enriched for TNC using RedSift method to determine efficiency of recovery of TNC's. Enrichment is performed on RedSift Cell Processor, an automated microfluidics platform that recovers total nucleated cells in 15 minutes.

In some experiments, carcinoma cells were stained using Calcein-AM-loaded fluorescent biomarker (Becton Dickinson, or eBioscience). These unfixed marked carcinoma cells were then assayed immediately after staining to determine cell counts using a CBC instrument. Aliquots of the marked carcinoma cells were spiked into 50µL whole blood in in quantities of 10 to 1000 cells then filtered on RedSift system. Additional aliquots of same quantities were spiked into the same volume of whole blood and left unfiltered. Finally, aliquots of 1000 cells were spiked into 50µL of PBS as controls. All test aliquots were assayed by flow cytometry (Beckman Coulter, FC500) to determine counts of marked spiked carcinoma cells.

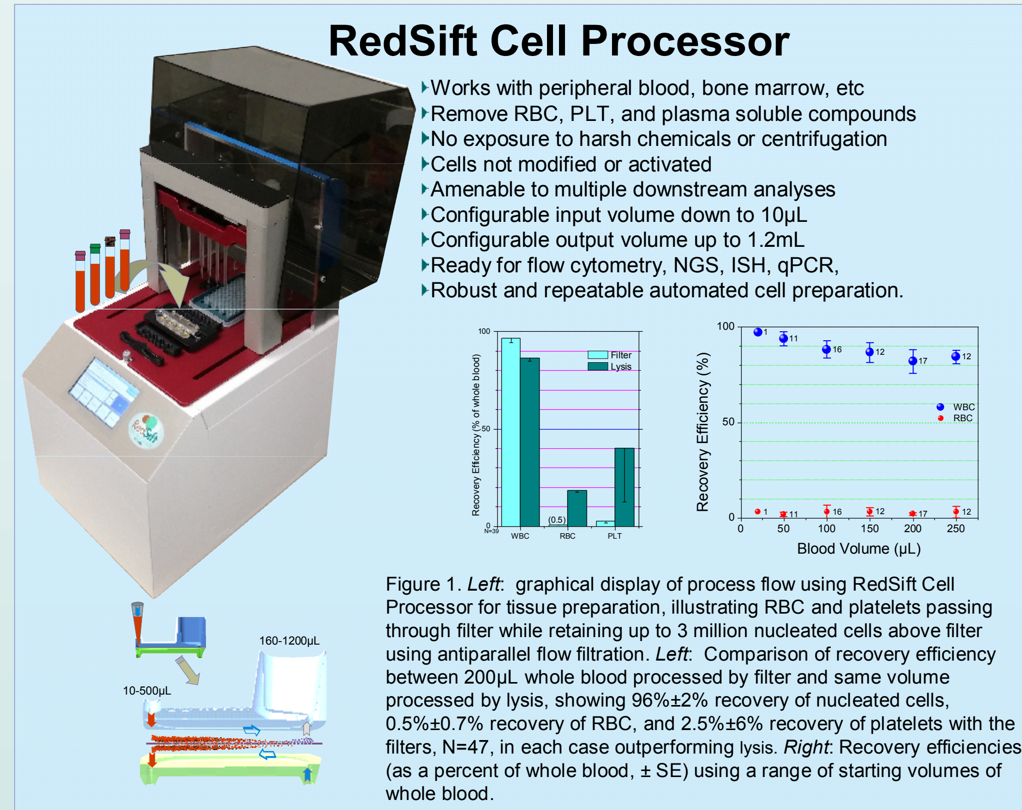

Figure 1. *Left*: graphical display of process flow using RedSift Cell Processor for tissue preparation, illustrating RBC and platelets passing through filter while retaining up to 3 million nucleated cells above filter using antiparallel flow filtration. *Left*: Comparison of recovery efficiency between 200µL whole blood processed by filter and same volume processed by lysis, showing 96%±2% recovery of nucleated cells, 0.5%±0.7% recovery of RBC, and 2.5%±6% recovery of platelets with the filters, N=47, in each case outperforming lysis. *Right*: Recovery efficiencies (as a percent of whole blood, ± SE) using a range of starting volumes of whole blood.

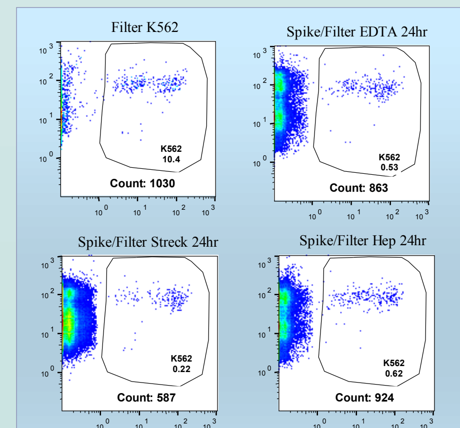

Figure 2. K562 leukemia cell line spiked into **buffer** (*top left*), or whole peripheral blood buffered in **EDTA** (*top right*), **Streck** (*bottom left*) or **Heparin** (*bottom right*) then filtered prior to measuring calcein fluorescence in the spiked tumor cells. Filtration did not reduce the tumor cell counts.

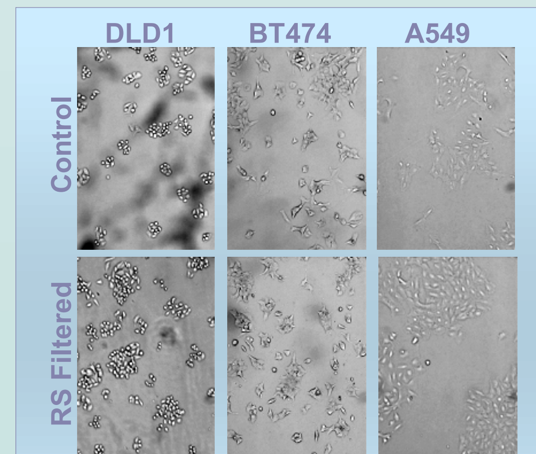

Figure 3. Filtered cells are amenable to cell culture. Various cancer cells from equal aliquots that were not spiked or filtered (Control) or were spiked into whole peripheral blood then recovered using RedSift method (RS Filtered) were imaged after 4 days in culture.

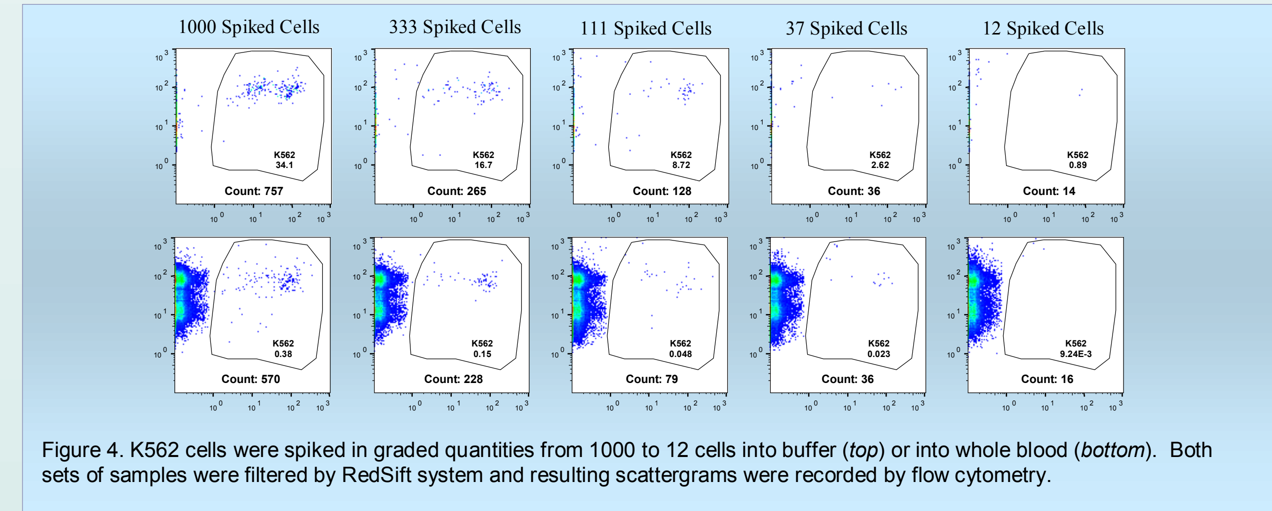

Figure 4. K562 cells were spiked in graded quantities from 1000 to 12 cells into buffer (*top*) or into whole blood (*bottom*). Both sets of samples were filtered by RedSift system and resulting scattergrams were recorded by flow cytometry.

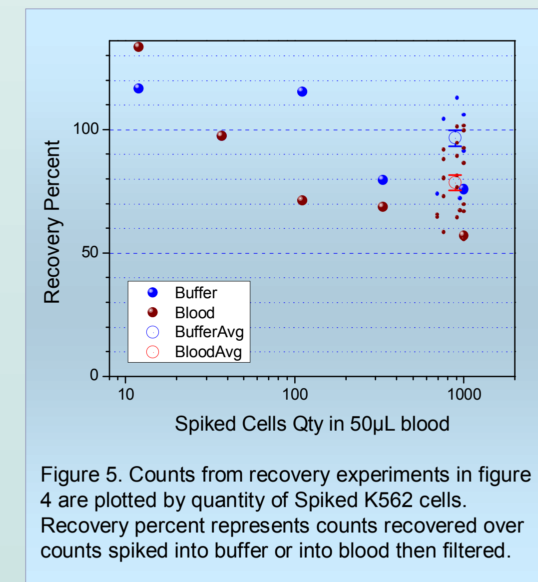

Figure 5. Counts from recovery experiments in figure 4 are plotted by quantity of Spiked K562 cells. Recovery percent represents counts recovered over counts spiked into buffer or into blood then filtered.

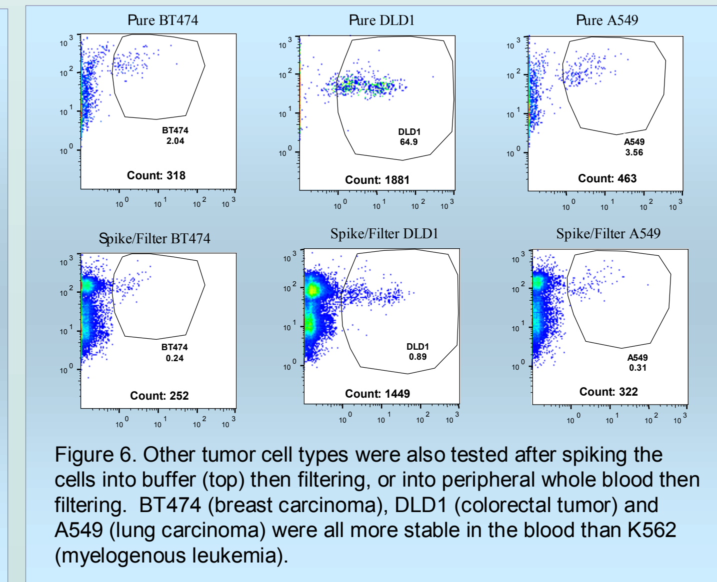

Figure 6. Other tumor cell types were also tested after spiking the cells into buffer (top) then filtering, or into peripheral whole blood then filtering. BT474 (breast carcinoma), DLD1 (colorectal tumor) and A549 (lung carcinoma) were all more stable in the blood than K562 (myelogenous leukemia).

CONCLUSIONS

RedSift filters are able to recover 80-97% of all nucleated cells from small blood samples, while reducing red blood cell (RBC) counts by more than 3 orders, and removing unbound platelets (PLT).

Small counts of cancer cells introduced into the blood sample were recovered in high efficiency, undamaged, and allow subsequent culturing of the recovered cells. This may prove useful for recovery of rare cells including CTCs, stem cells or fetal cells from blood.

RedSift system removes user-dependent variance and facilitates standardization across multiple sites for recovery of rare cells.

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