REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
19-09-2008	Final Report	1-Sep-2005 - 31-Aug-2007

4. TITLE AND SUBTITLE
Final Progress Report for "Impact of Radiation on Blood Cell Deformability"

5a. CONTRACT NUMBER
W911NF-05-1-0392

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
5D10U7

6. AUTHORS
Prof. James C. Sturm, Prof. Robert H. Austin

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
Princeton University
Office Of Research & Project Administration
The Trustees of Princeton University
Princeton, NJ 08544 -0036

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
49034-LS-DRP.1

12. DISTRIBUTION AVAILIBILITY STATEMENT **Approved for Public Release; Federal Purpose Rights**
~~Distribution authorized to U.S. Government Agencies Only; Contains Proprietary Information~~

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
This project had two exploratory goals. The first is the investigation the effect of radiation on the deformability of blood cells, as detected by novel microfluidic chips. The long-term goal would be to have a rapid low-cost method to screen people for exposure to radiation. For this project we have developed chips to measure deformability of both red and white blood cells in human blood, and observed an effect from radiation. We found that at very high doses, there was a clear difference in the behavior of irradiated and non-irradiated cells. The physical reason behind the behavior was more complicated that originally postulated. Further work needs to be done to understand such behavior, and to examine clear trends at lower radiation

15. SUBJECT TERMS
Blood, radiation, deformability, microfluidics, fractionation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			James Sturm
U	U	U	SAR		19b. TELEPHONE NUMBER 609-258-5610

Report Title

Final Progress Report for "Impact of Radiation on Blood Cell Deformability"

ABSTRACT

This project had two exploratory goals. The first is the investigation the effect of radiation on the deformability of blood cells, as detected by novel microfluidic chips. The long-term goal would be to have a rapid low-cost method to screen people for exposure to radiation. For this project we have developed chips to measure deformability of both red and white blood cells in human blood, and observed an effect from radiation. We found that at very high doses, there was a clear difference in the behavior of irradiated and non-irradiated cells. The physical reason behind the behavior was more complicated that originally postulated. Further work needs to be done to understand such behavior, and to examine clear trends at lower radiation doses.

Second, the project had a goal of using novel microfluidic fractionation technologies to rapidly find lymphoblasts in blood. These are early marker of disease and/or vaccine response. If they can be detected, it would allow the early detection of immune system response and allow fast testing of vaccine efficacy. This would shorten vaccine development cycles in times of emergency and improve mass population vaccinations. Towards this end, we have recently for the first time successfully detected large white blood cells in a regular human blood, and indeed observed the changes using rapid microfluidic techniques in white blood cell size populations due to infection from staphylococcal enterotoxin B (SEB), a potential bioterror agent.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

D.W. Inglis, J.A. Davis, R.H. Austin, J.C. Sturm, "Critical particle size for fractionation by deterministic lateral displacement," Lab on a Chip., 6, pp. 655 - 658 (2006)

D.W. Inglis, R.H. Riehn, J.C. Sturm, and R.H. Austin, "Microfluidic high-gradient magnetic cell separation," J. Appl. Phys. 99, Art. No. 08K101 (2006).

David W. Inglis, John A. Davis, Thomas J. Zieziulewicz, David A. Lawrence, Robert H. Austin, James C. Sturm, "Determining blood cell size using microfluidic hydrodynamics," Journal of Immunological Methods, 329, pgs. 151-156 (2007).

John A. Davis, D.W. Inglis, R.H. Austin, and J. C. Sturm. “White blood cell deformation in a microfluidic environment and effective cortical tensions” in preparation.

Number of Papers published in peer-reviewed journals: 3.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts: 0.00

Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED
Suberr Liu Chi	1.00
John Alan Davis	0.50
David Inglis	0.50
Chih-kuan Tung	0.50
FTE Equivalent:	**2.50**
Total Number:	**4**

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Robert Riehn	0.10
Kapeeleshwar Krishana	1.00
FTE Equivalent:	**1.10**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
David Inglis
John Alan Davis
Total Number: 2

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

1 a. Health Research, Inc.

1 b. One University Place

Rensselaer NY 12144-3447

Sub Contractor Numbers (c):

Patent Clause Number (d-1):

Patent Date (d-2):

Work Description (e): Circulating white blood cells (WBC), especially lymphocytes, are known to be cell types with high s

Sub Contract Award Date (f-1): 2/1/2005 12:00:00AM

Sub Contract Est Completion Date(f-2): 8/31/2007 12:00:00AM

Inventions (DD882)

Final Report
For Army Research Office
Award Title: Impact of Radiation on Blood Cell Deformability
Award number: W911NF0510392

Authors: James C Sturm, Robert H Austin

Blood, radiation, deformability, microfluidics, fractionation

Distribution: unlimited

Abstract and Goals

This project had two exploratory goals. The first is the investigation the effect of radiation on the deformability of blood cells, as detected by novel microfluidic chips. The long-term goal would be to have a rapid low-cost method to screen people for exposure to radiation. For this project we have developed chips to measure deformability of both red and white blood cells in human blood, and observed an effect from radiation. We found that at very high doses, there was a clear difference in the behavior of irradiated and non-irradiated cells. The physical reason behind the behavior was more complicated that originally postulated. Further work needs to be done to understand such behavior, and to examine clear trends at lower radiation doses.

As a first step towards establishing this basic understanding, we performed an initial study on the deformability behavior of white blood cells in a microfluidic environment. The fundamental properties relating to deformability appear similar to those seem previously at much slower time scales in classical experiments.

Second, the project had a goal of using novel microfluidic fractionation technologies to rapidly find lymphoblasts in blood. These are early marker of disease and/or vaccine response. If they can be detected, it would allow the early detection of immune system response and alllow fast testing of vaccine efficacy. This would shorten vaccine development cycles in times of emergency and improve mass population vaccinations. Towards this end, we have recently for the first time successfully detected large white blood cells in a regular human blood, and indeed observed the changes using rapid microfluidic techniques in white blood cell size populations due to infection from staphylococcal enterotoxin B (SEB), a potential bioterror agent.

Papers, Peer reviewed journals: 3

D.W. Inglis, J.A. Davis, R.H. Austin, J.C. Sturm, "Critical particle size for fractionation by deterministic lateral displacement," Lab on a Chip., 6, pp. 655 - 658 (2006)

D.W. Inglis, R.H. Riehn, J.C. Sturm, and R.H. Austin, "Microfluidic high-gradient magnetic cell separation," J. Appl. Phys. 99, Art. No. 08K101 (2006).

David W. Inglis, John A. Davis, Thomas J. Zieziulewicz, David A. Lawrence, Robert H. Austin, James C. Sturm, "Determining blood cell size using microfluidic hydrodynamics," Journal of Immunological Methods, 329, pgs. 151-156 (2007).

John A. Davis, D.W. Inglis, R.H. Austin, and J. C. Sturm. "White blood cell deformation in a microfluidic environment and effective cortical tensions" in preparation.

Princeton PhD Theses Resulting from this work (2)
They can be found at http://www.princeton.edu/~sturmlab/phd_theses.htm . Go to the appropriate link at the end of the list.

David Inglis, "Microfluidic Devices for Cell Separation," (2007).

John A. Davis, "Microfluidic Separation of Blood Components through Deterministic Lateral Displacement," (2008).

Patents filed:

"Microfluidic Device for Continuous Flow Fractionation of Particles in Fluid with a Very Large Range of Sizes," D. Inglis, J.Davis, R. Austin, and J. Sturm,
US Provisional Patent number 60/809,933, filed June 2005

Technical Report

Outline:
I. Radiation Effects and Deformability
II. Rapid Detection of Immune System Response Using Microfluidic Chips.
III. Study of Deformability of White Blood Cells in a Microfluidic Environment

I. Radiation Effects and Deformability

The first part of our work is aimed at detecting radiation dose through the resulting change in deformability of blood cells as sensed by microfluidic chips – where the cells are squeezed into tapered channels. We carried out a series of experiments involving the response of red blood cells and white blood cells from whole blood to high doses of ionizing radiation. The idea is to use microfabrication to make "squeezing arrays" to test cell deformability after large radiation doses, we hoped to quickly determine if a person has sustained a high rad dose in the event of a nuclear event, and we focused on gamma radiation because gamma rays are the deepest penetrating and the ones most likely to damage the bone stem cell population. We used a Cs137 source (3 Gy/min) at Albany (Wadsworth Center.)

The sensitivity of the human body in terms of mortality to deeply penetrating ionizing radiation is not due to primary materials damage, such as cross-linking of the membrane surface of the red blood cell membrane but rather to biological network response to

genomic damage to the hematopoietic stem cells in the bone marrow which are the progenitors of the entire immune system. These stem cells are in a sense "dangerous" in that they are immortal, constantly dividing and non-differentiated: in a sense they are cancer cells that the body accepts because they are vital. However, damage to the genome of these stem cells could result in rapid generation of cancers throughout the body, so the hematopoietic stem cells have exquisitely sensitive ways to detect genomic damage and enter the apoptotic pathway of cell suicide at ionizing radiation dosages long before obvious material damages can be detected.

When we began this work, we were hopeful that at lethal doses of ionizing radiation that cause changes in red blood cell membrane deformability could be determined by measurements of red blood cell deformability in our narrow channel arrays. We did in fact detect changes in the red blood cell deformability, but at doses far exceeding the mean lethal dose of the human body, which is approximately 600 Gray. Channel blockage began to be apparent at doses of approximately 5000 Gray. We realized at this point that physical changes of membrane cross-linking events due to ionizing radiation were not sensitive to the actual biological network events that lead to apoptosis of the hematopoietic stem cells. We decided then to see if we could detect changes in the white blood cell physical properties since the white blood cells lie at the key point where the immune system begins to power down after high radiation doses.

Runs where done with finger prick blood, with white blood cell stains and a new chip with a 20 micron deep etched and tapered channels designed to look not at RBC deformation but rather WBC deformation. Figure 1 (attached) shows this chip: the array consists of 10 rows of a particular funnel, starting with 10 micron openings that taper to 5 microns, 5 to 3.5, 3.5 to 2.5, 25 to 2.0, 2.0 to 1.5 microns. 10 microns wide bypass channels allow cells flow to continue even when funnels are blocked. Pressure from a precision syringe applied pressure to drive the fluid for a fixed time of 0.5 hr.

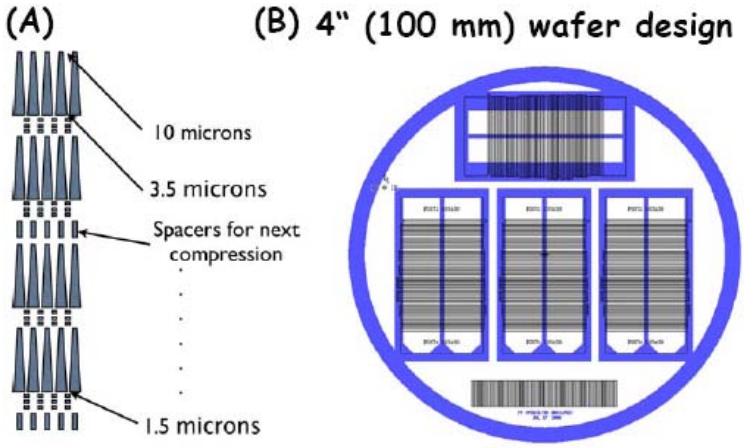


Fig 1. Schematic diagram of (a) microfluidic channels (white) and barriers (shaded) and (b) overall chip layout for deformability testing. Flow direction is top to bottom in (a).

We also finally listened to the edict of Enrico Fermi and did the experiment between two extremes: if you see nothing at the extreme, unlikely to be anything interesting in between. The last run used an exposure of 12000 rads (120 Gy, which is a 40 minute exposure to the source). This is about x20 a lethal dose, but remember that this is a deep lethal dose number, not superficial burn dose.

Fig. 2 (next page) shows the result from that run. RBC deformability, which is in fact tested at the 1.5 micron gap, seems weakly affected even at these huge doses using freshly drawn finger prick blood from one of the investigators. There is a dramatic WBC response, but the opposite sign from what we expected.

In a control blood sample without radiation 1.5 hours old the WBCs get stuck way up in the 5 micron gaps. Therefore few cells are trapped down in the 2.5 or 3.5 micron gaps (Fig. 2(a). It is not clear whether this is from their becoming physically stuck due to inability to further deform, or from adhesion to the device walls. With time (hours) sitting in a tube this stickiness increases. Standard anti-adhesion mechanisms and coatings were not sufficient to ameliorate this effect.

The highly irradiated blood (and it takes an hour just to do the radiation) appears to penetrate much further down into the array at equal incubation times. Fig. 2 shows side by side comparisons of equal incubation time blood that was not (0 gy) and was (120 Gy) irradiated. More cells are clearly visible and trapped at high radiation dose in the 2.5 and 3.5 gaps, although the mechanism is complex. At present, it does not seem that a lower deformability is causing more cells to get stuck further down in the array (at small gap spacings). Higher fluid velocities through the microchannels at high radiation dose, due to less trapping at larger cell sizes, and the resulting higher cell flux, leads to more trapping, not necessarily a change in deformability. Further work is needed to clarify the mechanism. As a first step, we then studied the isolated effect of deformability in a microflidic environment, reported in final technical section of this report.

Related to this work related to radiation is a mutagenic effects project we launched on directed evolution; part of this work is funded from the AFOSR via Dr. Walter Kozumbo for biosolarH2 production. In the course of this directed evolution work we were interested to find out that actually the response of cells to radiation damage is intimately involved with evolution, a cell senses genomic damage at a very deep level and turns out mutations quickly to try and work its way out of possible stops in transcription due to radiation damage. This so-called "SOS" response is very important in all sorts of aspects of cancer, aging and evolution. This is an interesting area for future research, since measuring markers of SOS signals may be a more direct way of sensing the effect of radiation on cells, than final byproducts such as a change in cell stiffness (or stickiness).

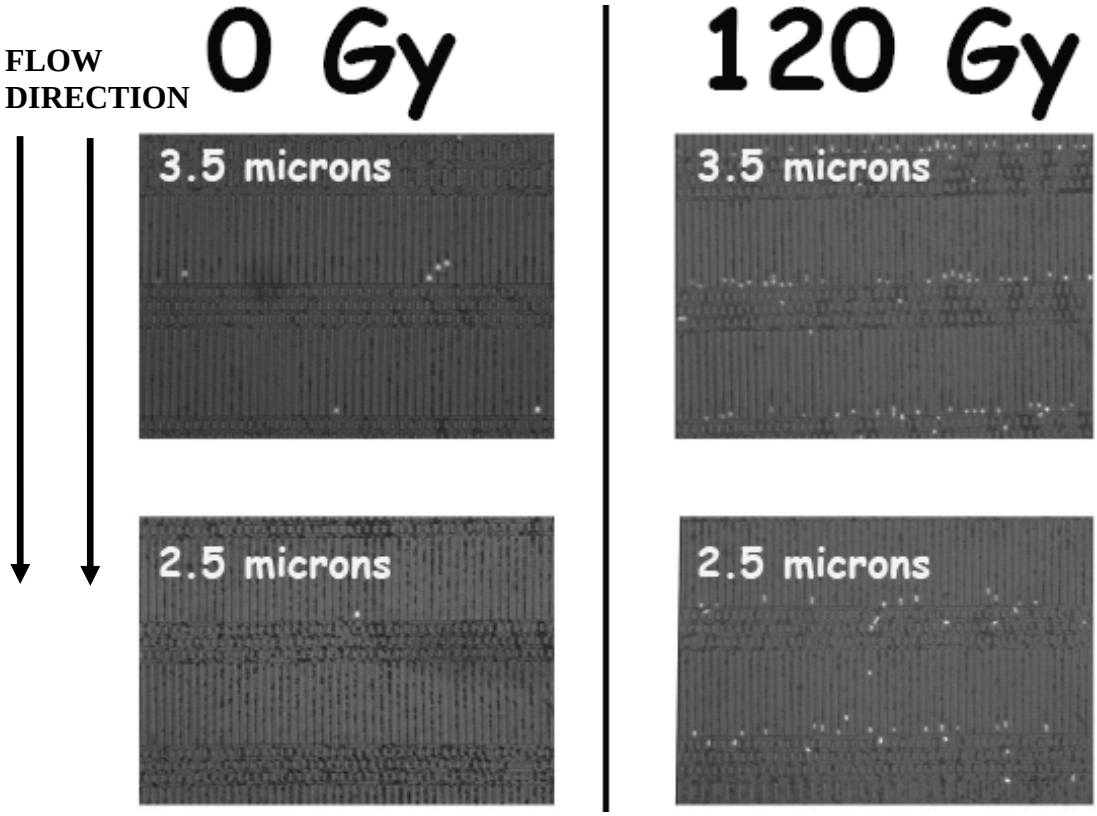


Fig 2. Trapping of white blood cells for 0 and 120 Gy does for different gap sizes. More cells are clearly visible and trapped at high radiation dose, although the mechanism is complex. Higher fluid velocities through the microchannels at high radiation dose, and the resulting higher cell flux, leads to more trapping, not necessarily a change in deformability. Further work is needed to clarify the mechanism.

II. Rapid Detection of Immune System Response Using Microfluidic Chips.

There is a need for microfluidic devices that analyze more and or new cell parameters with compact and minimal means. Prime targets are early stages of disease detection (e.g. for screening of troops before critical missions), and quick feedback on immunological response to vaccines. Because such chips can give a readout within minutes of blood being drawn, this will decrease the development times of vaccines, and allow faster determination to determine if vaccination of an individual was effective.

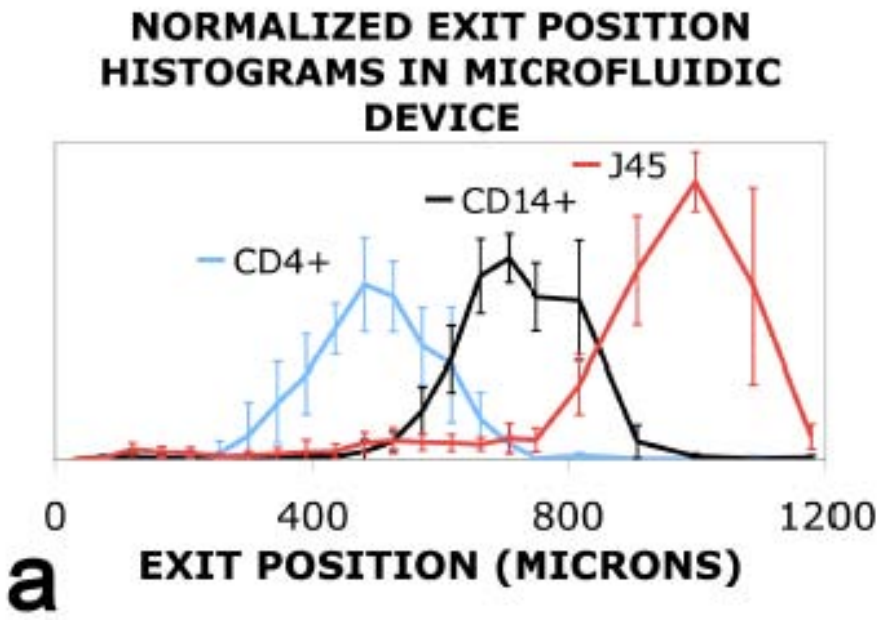
Here we show a new microfluidic device can be used to count cells rapidly based on size. This is used to differentiate healthy lymphocytes from cancerous lymphocytes by size alone and we use the device to detect increased numbers of activated lymphocytes in blood as a result of exposure to staphylococcal enterotoxin B (SEB), a potential bioterror agent. Together the results demonstrate a microfluidic device that performs some of the

measurement and separation tasks of a flow cytometer but at a fraction of the cost and complexity.

The device is a microfluidic device based on our previous work sorting sub-micron polystyrene beads with record precision quickly [Huang, L. R., Cox, E. C., Austin, R. H., and Sturm, J. C. “Continuous particle separation through deterministic lateral displacement,” Science 304, 987-990 (2004)] but applied here to blood cells.

Initial results are in Fig 3-4. Fig 3(a) attached shows the displacement of cells at the end of the device. Larger cells are displaced more to the right. Note the CD4+ cells (a lymphocyte subset) are smaller than the CD15+ cells (monocytes), as expected, and that the J45 cells (CD3+) (J45 lymphocytes from the American Type Culture Collection,a model of a cancerous lymphocyte which is much larger than normal lymphocyte). Fig 3(b) shows forward scatter histograms from the established flow cytometry method, which requires very sophisticated expensive instrumentation. The microfluidic device in principle could be very cheap, and thus detect the enhanced-size lymphocytes quickly.

The next step is to measure the results of whole human blood exposed to exposure to staphylococcal enterotoxin B (SEB), a potential bioterror agent. Clinical symptoms depend on the route of exposure but ingestion of SEB typically causes food poisoning within 1 to 6 hours. Whole venous blood (100microL) diluted with minimal essential medium (100 uL) was incubated with 1 ug SEB at 37 C in 7% CO_2, 5% O_2 for 18 hr. When exposed to a stimulating antigen a portion of competent lymphocytes dedifferentiate into more immature forms and proliferate mitotically. The more immature form is called a transformed or activated lymphocyte or a lymphoblast and is larger in diameter by a few microns than normal lymphocytes. It is these larger cells we seek to detect as a marker of immune system response.



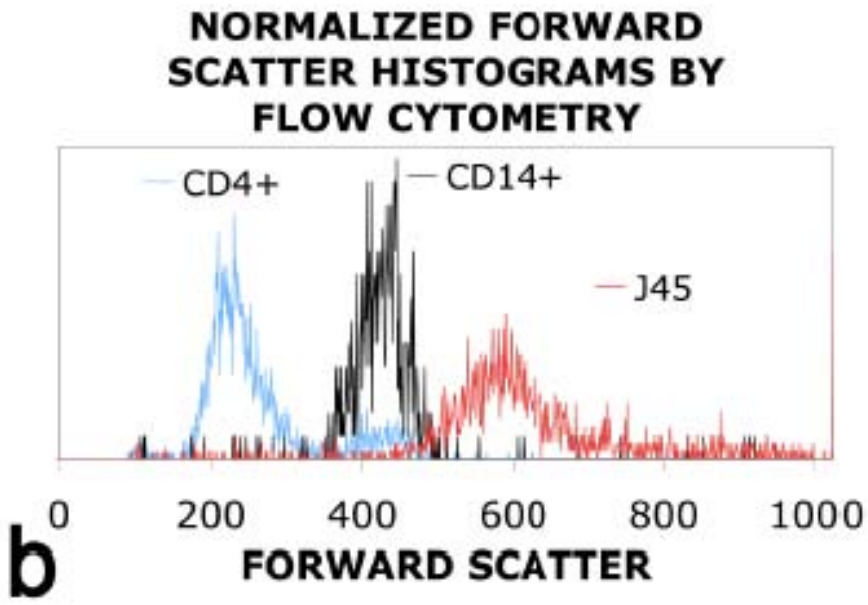


Fig. 3. Comparison of size measurements for three cell types from the experimental device (a) and conventional flow cytometry (b). CD4+ (blue) and CD14+ (black) cells are from whole blood, CD4+ labeled J45 T-lymphocytes (red) from cell culture. (a) The error bars express the standard deviation observed between six independent tests. (b) The forward scatter value for only those cells whose PE fluorescence value was over 1200 (of 2024) are included in the plot.

Figure 4 (attached) shows size histograms comparing the SEB and control sample analyzed in the microfluidic device (a) and by flow cytometry (b). Both samples were labeled with PE-CD3. In both the experimental device and the industry-standard flow cytometry measurement, a pronounced tail representing the larger cells, indicating a clear immune system response, was observed. Using the experimental device 0.4% of CD3+ cells in the control sample were larger than 10.3 microns in diameter, (exited the device at greater than 700 microns displacement). In the SEB sample 7.0% of CD3+ cells were larger than 10.3 microns. This confirms the microfluidic device can be used to detect immune system response. More information can be found in David W. Inglis, John A. Davis, Thomas J. Zieziulewicz, David A. Lawrence, Robert H. Austin, James C. Sturm, "Determining blood cell size using microfluidic hydrodynamics," Journal of Immunological Methods, 329, pgs. 151-156 (2007).

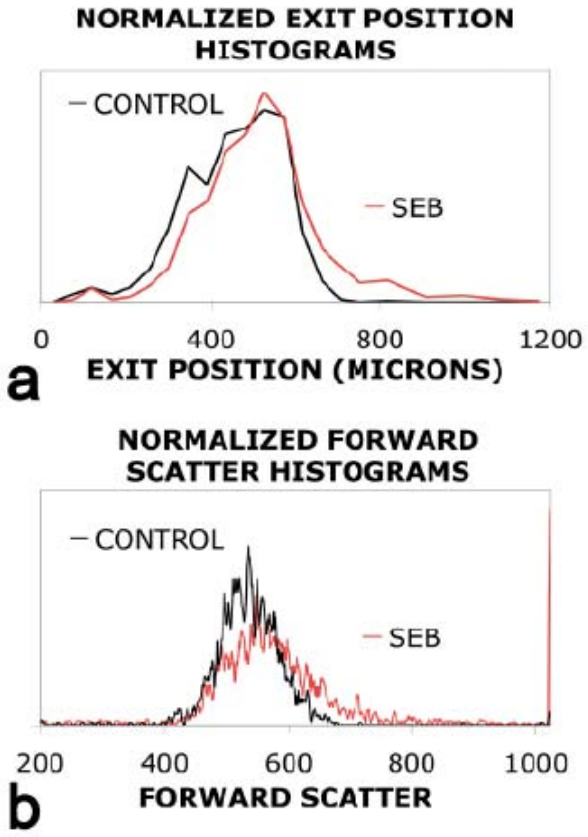


Fig. 4. Comparison of size measurements for blood incubated with the activating toxin SEB using the microfluidic device (a) and conventional flow cytometry (b). Both plots show that the SEB sample (red) has a higher proportion of the moderately larger cells, indicating a clear immune system response, was observed. Using the experimental device 0.4% of CD3+ cells in the control

III. Study of Deformability of White Blood Cells in a Microfluidic Environment

The first section (radiation effects) of this report described that we experimentally found it difficult to separate "stickiness" from "deformability" effects on white blood cells in the microfluidic chip used in that experiment due to its design. Therefore we designed a special chip to measure deformability of white blood cells in a microfluidic environment

The work was done using a deterministic lateral displacement array similar to that described in the previous section, which laterally displaced white blood cells as they moved through an array of posts, with larger cells displacing farther. If one performs such an experiment with rigid plastic beads suspended in water instead of blood, one finds the displacement of the beads does not depend on the speed of the fluid (speeds in the range of 0.1 to 10 mm/s). However, when blood was run through the chip, it was found that white the average displacement of white blood cells decreased as the fluid flow increased (Fig 5). . Because the lateral displacement of particles and cells was designed to increase with their size, this means that the white blood cells were going through the post array acting as if their size was decreasing as the velocity was increasing.

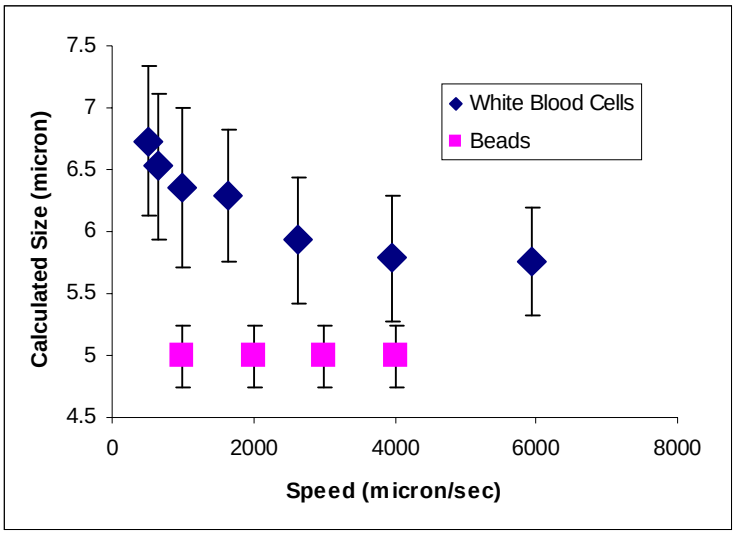


Figure 5: Change in effective size of white blood cells compared to polystyrene beads, as function of velocity.

This was attributed to the cells becoming deformed as they interacted with (literally ran into) the posts in the array. The deformation of white blood cells is typically measured/modeled by poking them with a sharp tip, and measuring deformation vs force. The cells are typically modeled as consisting of an incompressible fluid surrounded by a membrane (the cell wall) which exerts a tension to keep the cell spherical. The strength

of this “cortical tension” is a measure of the cell stiffness. It can be mathematically related to the deformation amount at a given deformation force.

In the experiments of Fig. 5, the change in apparent cell size was attributed to the deformation. The force acting on the cell in a post collision was calculated by modeling the hydrodynamic forces acting on a cell using finite element software. Using the deformation and the force, the cortical tension of the white blood cells in the microfluidic environment could be found (Fig. 6).
At low flowspeeds (which is the rate at which the cells would be deformed by running into the posts) (1000 microns/sec = 1 mm/s) our extracted cortical tensions are in the

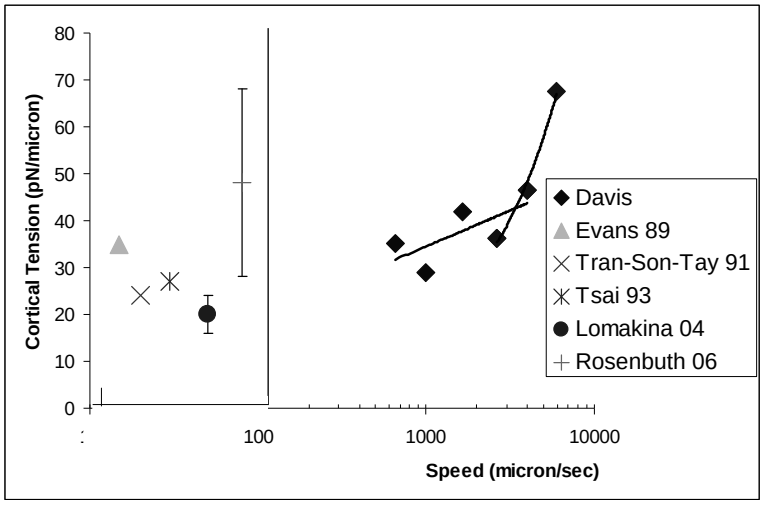


Figure 6: The calculated values of cortical tension for our measured data which is compared to the existing values from literature.

range of 30-40 pN/micron. This is on the same order as found by classical cell deformation experiments (which occur in the range of 1-100 microns sec). Thus the cell “deformability” is similar in the microfluidic environment compared to the environment at lower speeds in previous work. It is interesting to note that the extracted cortical tension increases at higher speeds (above 5 mm/s). The nature of this dynamic process is not understood, but is probably due to the characteristic time constants of the deformation relaxation in the cells. Further modelling is in progress.

For more details and references see Chapter 6 of the Ph.D. thesis of John A. Davis, "Microfluidic Separation of Blood Components through Deterministic Lateral Displacement," (2008), at http://www.princeton.edu/~sturmlab/phd_theses.htm . A publication of this work is in preparation.