Chapter

# Assessing the Vascular Deformability of Erythrocytes and Leukocytes: From Micropipettes to Microfluidics

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## Abstract

Among the most crucial rheological characteristics of blood cells within the vasculature is their ability to undergo the shape change (i.e., deform). The significance of cellular deformability is readily apparent based solely on the disparate mean size of human erythrocytes (~8 μm) and leukocytes (10–25 μm) compared to the minimum luminal size of capillaries (4–5 μm) and splenic interendothelial clefts (0.5–1.0 μm) they must transit. Changes in the deformability of either cell will result in their premature mechanical clearance as well as an enhanced possibility of intravascular lysis. In this chapter, we will demonstrate how microfluidic devices can be used to examine the vascular deformability of erythrocytes and agranular leukocytes. Moreover, we will compare microfluidic assays with previous studies utilizing micropipettes, ektacytometry and micropore cell transit times. As will be discussed, microfluidics-based devices offer a low-cost, high throughput alternative to these previous, and now rather ancient, technologies.

**Keywords:** deformability, hemorheology, red blood cells, white blood cells, micropipette assay, ektacytometry, cell transit analysis, microfluidic analysis, transfusion medicine

## 1. Introduction

The circulating cellular elements of blood consist of erythrocytes (red blood cells; RBC), leukocytes (white blood cells; WBC) and platelets. The hemorheology of these blood cells is unique in that these cells exist in a fluid phase subjected to variable, and often extreme, rheological shear stress, viscosity changes and biomechanical obstacles (e.g., capillaries and splenic filtration). Hemodynamically, shear stress is induced by the highly variable flow rate of blood within the ~100,000 kilometers of the human vasculature bed which encompasses both large arteries and veins to the capillary beds (**Figure 1A**) [1]. With an average resting cardiac output of approximately 5 L/min, blood flow in the largest artery (i.e., aorta) is approximately 50 cm/s while flow rates drop to only about 0.03 cm/s in the smallest capillaries and return to about 15–40 cm/s in the largest veins (e.g., superior and inferior vena cava) [1, 2]. In high flow conditions, RBC reside in the fast flowing central axial column of the vessel while WBC (and platelets) are located more peripherally

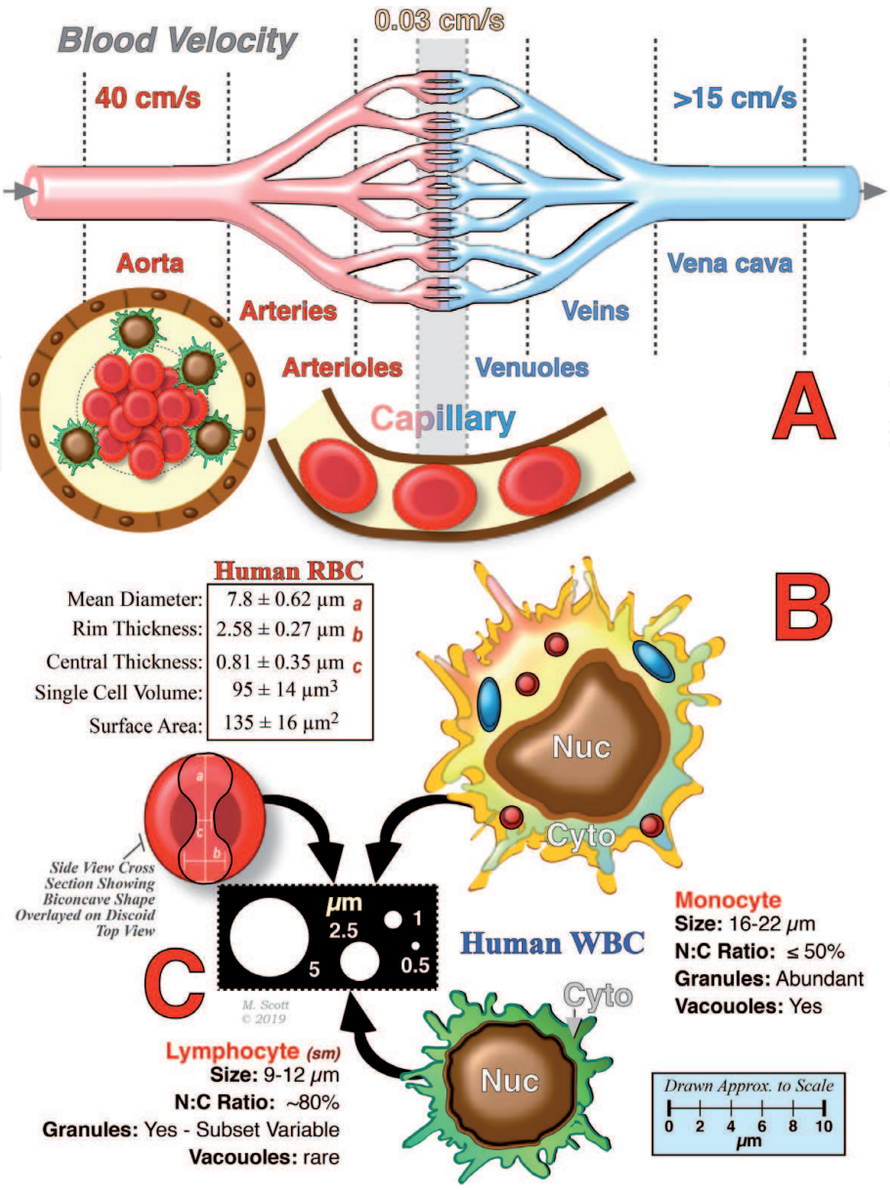


**Figure 1.**
*The physiology and morphology of the vascular bed and blood cells imparts unique rheological stress on circulating blood cells. Panel A: the vascular bed is composed of blood vessels of various sizes which create significant disparity in blood (fluid and cellular) velocity consequent to vessel diameter. The fluid flow induces rheological sheer stress while the vessel size can create biomechanical deformation of cellular elements. Panel B: shown are the general physical parameters of human RBC and WBC. Note that the biconcave RBC is a anuclear while within the WBC, the nucleus:cytoplasm (N:C) ratio of monocytes and lymphocytes are quite divergent. RBC cytoplasmic viscosity is primarily defined by hemoglobin while in WBC, in addition to the nucleus, the presence of granules and vacuoles also impact intracellular viscosity and the aggregate cellular deformability. Panel C: blood cell deformability is crucial during vascular flow due to the size disparity between red blood cells and various leukocytes (e.g., monocytes and lymphocytes) and the capillary (4–58 µm) and splenic interendothelial clefts (0.5 µm). Panels B,C are drawn approximately to scale.*

and prone to mechanical interaction with the endothelial cells lining the blood vessels. WBC also have adhesion molecules on their membrane and, if appropriate signals (e.g., inflammation) are present, they actively roll on the endothelial cells prior to attachment and extravasation (**Figure 1A,B**). Moreover, the viscosity of blood is also variable and is a function of, primarily, red blood cell (RBC) number and flow rate. At high RBC counts and high flow rates, blood is highly viscous while at low RBC counts and low flow rates (capillaries), blood viscosity is greatly reduced. Moreover, as shown in **Figure 1C**, the rheological stress is further exacerbated by the biomechanical stresses induced by the extreme disparity in the size of RBC (~8 µm) and WBC (10–25 µm) to the minimum diameter of the vascular

capillary beds (4–5 μm) and splenic interendothelial clefts (0.5–1.0 μm) [3, 4]. Hence, consequent to both the shear forces, viscosity and biomechanical stresses placed on blood cells, a key biologic/physiologic requirement of both RBC and WBC within the vascular space is rheological deformability. Biomechanically, the intracellular viscosity and membrane rigidity of the RBC and WBC are the key factors in imparting their vascular rheological deformability.

For the anuclear RBC, intracellular viscosity is primarily determined by hemoglobin content (both absolute content and hemoglobin structure (**Figure 1B**)). RBC membrane deformability/flexibility is primarily imparted by the cytoskeletal structure of the cells and, to a lesser extent, the composition of the bilayer itself (lipid species, protein content, integral versus peripheral membrane proteins, and carbohydrates). For normal RBC the intra- and inter-individual variability of both intracellular viscosity is relatively invariant; however, genetic mutations affecting hemoglobin structure (e.g., HbS, α and β thalassemia, HbE mutations) will dramatically affect both hemoglobin content and the viscosity of the hemoglobin itself. Similarly, the cytoskeletal structure of normal red blood cells is both well characterized and consistent within humans. But, as with hemoglobin variants, mutations in any component of the cytoskeleton can dramatically affect the discoid shape of the RBC and result in size changes and/or altered rigidity or stability of the cytoskeleton and cell itself. Indeed, numerous studies have documented that changes in either the hemoglobin content or structure (the major determinant of viscosity) or mutations to cytoskeletal components (the major determinant of membrane rigidity) can exert significant effects on RBC deformability, biologic function and in vivo circulation. In evidence of this, both biological conditions and pharmacologic agents that affect hemoglobin content and/or viscosity or the RBC cytoskeleton alter cellular deformability and have profound in vivo and in vitro effects on RBC function and survival [5–16]. Indeed, RBC deformability can be a diagnostic indicator of RBC abnormalities and the quality of stored RBC prior to transfusion [17–28].

Intracellular viscosity and membrane structure are similarly key to the rheological deformability of WBC. However, in contrast to RBC, WBC intracellular viscosity is more complex and affected by multiple components including the: nuclear to cytoplasm (N:C) ratio; intracellular granule composition; presence of cytoplasmic vacuoles; as well as the activation state of the immune cell (**Figure 1B**) [28–30]. Similarly, membrane rigidity is also more complex due to: abundance of membrane proteins and protein rafts; changes in protein structure and polymerization consequent to immune activation; and the variability of the membrane and cytoskeletal protein composition of immune cell populations (e.g., monocytes, lymphocytes, granulocytes) and subsets (e.g., T cells versus B cells; CD4+ versus CD8+ T cells; NK cells) [30–35]. Perhaps surprisingly, despite the biologic importance of its rheological deformability within the vasculature, WBC deformability is both poorly defined and much less understood. Indeed, previous studies on WBC have most commonly defined "deformability" as cellular shape change or spreading under extrinsic suction (e.g., micropipette aspiration), compression pressure (e.g., centrifugation and cell poker/probe), or upon activation induced motility [30–32, 34–36]. However, vascular deformability is vastly different from cellular shape change or spreading which are most commonly induced by immune cell activation and, importantly, the actual loss of vascular rheologically-mediated (i.e., fluid motion and spatial confinement) deformability. The paucity of data relating to vascular deformability of WBC has, in large part, been due to the absence of suitable tools for measuring deformability across the broad range of cell types encompassed within leukocyte population. However, the complexity of the leukocyte population and resultant changes in rheological deformability upon activation (e.g., granule

release) potentially arising in peripheral blood WBC may be of clinical importance as a biomarker of acute or chronic immune activation.

## 2. Measuring the vascular (rheological) deformability of blood cells

Because of the crucial role that cellular deformability plays in vascular circulation of RBC, methods to quantitate this biomechanical-aspect of normal and abnormal RBC has been of interest to hematologists since the 1960s [3, 5–7, 9, 10, 37–40]. Historically, multiple technological tools have been employed to study RBC (but rarely WBC) deformability including: micropipette aspiration; ektacytometry; cell transit times; and, most recently, microfluidic analysis.

### 2.1 Micropipette aspiration

Perhaps the earliest experimental approach to measure RBC deformability was the micropipette aspiration (**Figure 2**). Initial studies examined the ability of normal and stored RBC to traverse the length of a micropipette of known diameter [38]. This early "microfluidic" single cell analytical approach, while very low throughput and time consuming, did demonstrate that damaged or stored RBC were less deformable than fresh normal RBC. Subsequent variations of these micropipette studies further examined the localized elasticity of the membrane in both intact cells and RBC ghosts using ever smaller micropipettes to deform a small segment of the membrane to characterize static deformability via membrane extensional rigidity and bending rigidity. To further characterize dynamic deformability of the cells, the time constants for rapid elastic recovery from extensional and bending deformations were also quantitated [41–47]. However, micropipette, single-cell aspiration, measurements did not adequately reflect the biomechanical heterogeneity of even a relatively homogenous cell population (e.g., normal RBC), much less, the highly divergent population of cells encompassed within the WBC population. Hence newer methods were devised in an attempt to study large number of RBC under flow-like conditions. In contrast to RBC, micropipette studies are still commonly used to examine leukocytes; though these approaches tend not to be focused on rheological deformability [22, 35, 48–53].

### 2.2 Ektacytometry

Perhaps the most glaring flaw of the various micropipette aspiration approaches were their limitation to single cell analyses. To overcome this limitation, ektacytometry

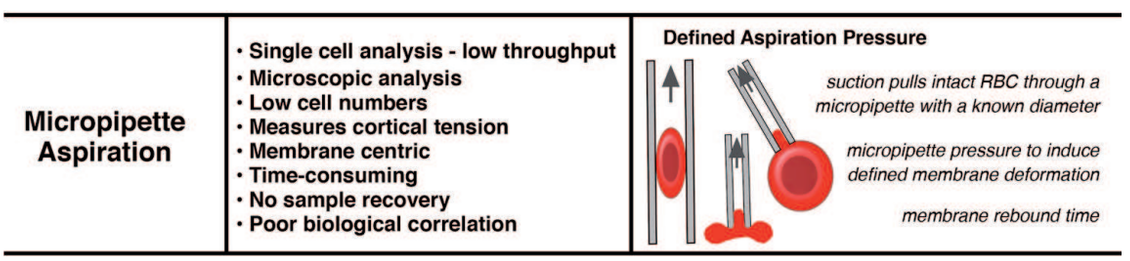


**Figure 2.**
*Overview of micropipette aspiration analysis of blood cells. Micropipette-based analyses were first used to explore the crucial role of cellular deformability in the circulation of red blood cells. As noted, these single cell analyses were low throughput and time consuming. Multiple variation of this technique have been developed ranging from whole cell aspiration to localized membrane deformation. Studies could be done on intact cells or membrane ghosts.*

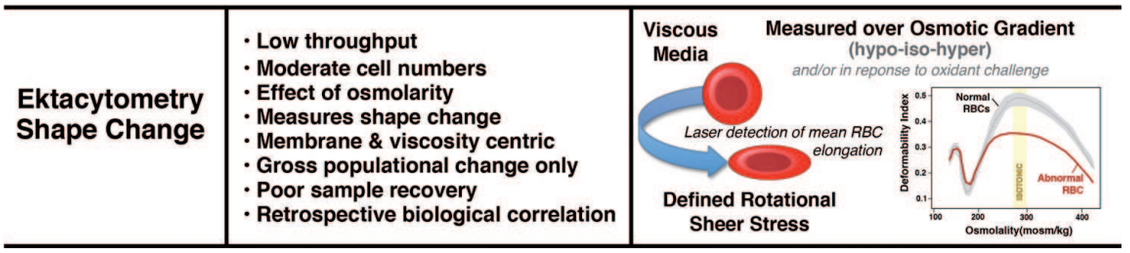


**Figure 3.**
*Overview of ektacytometric analysis of blood cells. To overcome the single cell limitations inherent to micropipette aspiration, the ektacytometer can analyze the shear-induced deformability of a much larger population of RBC; though at the expense of information of single cell data acquisition. Ektacytometry measures deformability by subjecting RBC suspended in a viscous solution to rotational shear stress such that the normal cells form ellipsoids. The scatter intensity pattern from laser diffraction produces isointensity curves and deformability indices. Additionally, the most common approach of ektacytometry examines RBC deformability over a broad osmotic gradient (hypotonic → isotonic → hypertonic). Deformability is measured via laser diffraction as the shear stress forces the RBC to assume an elongated shape.*

was developed. Ektacytometry measures deformability by suspending RBC in a viscous solution and applying rotational shear stress such that the normal discoid cells form ellipsoids which is measured by laser diffraction (**Figure 3**) [13, 14, 54–57]. The extent of ellipsoid formation is dependent on the deformability of the sample population. Abnormal RBC can be detected by shifts relative to the scatter intensity pattern of normal cells. Abnormal (i.e., non-deformable) cells can result in any combinations of left or right shifts in response to hypo- or hypertonicity, and/or a decrease in the maximum deformation observed under isotonic conditions. Relative to micropipette studies, ektacytometry provided a relative rapid assay to examine RBC. Numerous ektacytometry studies have elucidated the profound influence that mean corpuscular hemoglobin concentration (hence intracellular viscosity), abnormal hemoglobins, cytoskeletal aberrations, drugs and oxidant challenge exert on the cellular deformability [13, 14, 18, 54–64]. Importantly, ektacytometry only measures the "average deformability" of a cell population and cannot accurately and efficiently quantify the abundance of rigid cells in a bimodal population where both normal and abnormal cells are present [57, 65]. In the context of blood banking, ektacytometry has been used for assessing RBC following blood bank storage [66–68]. Of note, ektacytometry has been used exclusively in the context of erythrocytes; with no known studies examining the shear-induced deformability of lymphocytes, neutrophils, monocytes or other leukocytes. Thus, despite some promising data regarding its clinical use in transfusion medicine, ektacytometry has not become commonly used in transfusion medicine due to both the cost of instrumentation and the relatively low throughput of the existing testing protocols. Moreover, ektacytometry does have some significant drawbacks as it cannot, without experimental manipulations (e.g., density separation), provide any information on subsets of cells within the larger population—the results obtained are simply the "average" of the population. This limitation is, perhaps, the critical failure of ektacytometry because, in many pathologic states, abnormal RBC represent a minor (<10%) fraction of the overall RBC mass hence subtle changes will not be clearly obvious. Moreover, it is difficult to recover RBC subsequent to ektacytometric analysis for further biologic testing due to the viscous media utilized and, using traditional ektacytometry, the fact that the RBC are irreversibly (in most cases) altered by the osmotic gradient employed during the assay.

### 2.3 Cell transit analysis

In contrast to micropipette analysis and ektacytometry, cell transit analysis provides information at both the single cell and populational level (**Figure 4**).

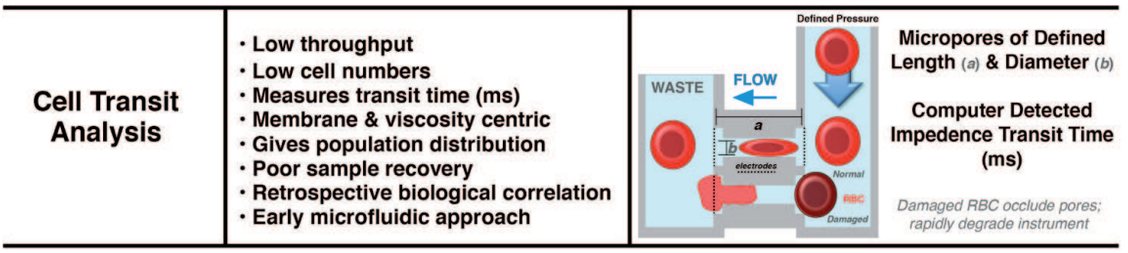


**Figure 4.**
*Overview of cell transit analysis of blood cells. In contrast to micropipette analysis and ektacytometry, cell transit analysis provides information at both the single cell and populational level. Cellular deformability is indirectly measured via transit time (ms) of RBC through pores of defined diameter and length. Transit time is measured by the change in electrical resistance as an RBC passes through a micropore. Cell transit analysis is, in essence, an early micro (macro) fluidics approach.*

To accomplish this, cell transit analysis combines features of both the traditional micropore filtration assay and the micropipette aspiration methodology, in that deformability of each RBC constitutes a single data point and can be used to then generate a populational distribution curve. In a cell transit analyzer, a single RBC passes through a micropore of fixed diameter and length with the transit time (in milliseconds; ms) of the cell calculated using the electrical resistance generated by the RBC within the channel as detected via a conductometer. However, the sensitivity of this method varies with cell size. Smaller cells, even if less deformable, pass through the pores with less resistance. In contrast, abnormally large or rigid cells, which are clinically important, are also be problematic as they block the micropore and are excluded from analysis [17, 69, 70]. Despite these limitations, cell transit analysis is very useful in that it provides subset/heterogeneity analysis via binning of the cells based on the transit time thus providing a continuous measure of the deformability profile of a sample and/or the severity of the deformability defect. The comparative utility of ektacytometry and cell transit analysis of RBC can be seen in normal and model ß thalassemic RBC in which purified alpha-hemoglobin chains are entrapped within normal RBC (**Figure 5**) [17, 19, 61–63]. While the ektacytometry and cell transit analysis have proven very useful as research tools, they have not been used to any great extent clinically. This is in large part due to the expense and complexity of the devices as well as their slow throughput making them impractical for clinical laboratories. Moreover, these in vitro studies often lack biological validation to the very low throughput of the assay (e.g., micropipette aspiration studies), overly small cell numbers, difficulty/impossibility of cell recovery post assessment, or more importantly, an inability to either identify or collect specific sample subsets (e.g., low versus high deformability) following analysis (e.g., Ektacytometry and Cell Transit Analysis studies).

## 2.4 Microfluidics

As noted in the preceding discussion, multiple micro/macro fluidic approaches have been used to model hemorheology of circulating blood cells; albeit almost exclusively RBC. Despite their valuable contributions to our understanding of blood cell deformability, these methods are inherently low throughput and dependent on relatively expensive instrumentation. But perhaps one of the biggest issues challenging these previous methodologies is the inability to recover substantial, or any, subpopulations (e.g., highly versus poorly deformable cells) from the analyzed sample. This weakness precludes additional in vitro or in vivo studies to tease out biological variations leading to the differential deformability profiles. Microfluidics approaches (**Figure 6**) potentially offers a cost-effective, high throughput,

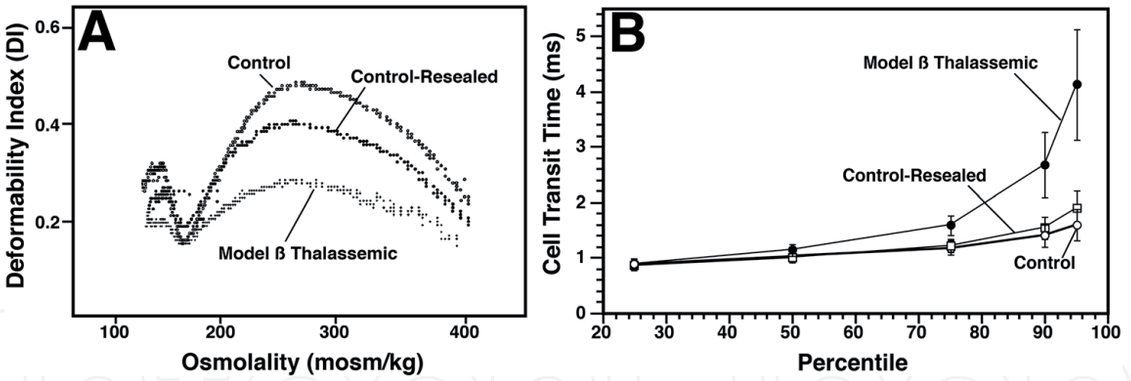


**Figure 5.**
*Comparative data of normal versus model ß thalassemic RBC as assessed by ektacytometry and cell transit analysis. Panel A: as shown, ektacytometry provides the mean diffraction profile of a population of cells over a broad osmotic gradient; however, it does not provide any information as to the deformability distribution of the cells within the total population. Panel B: cell transit analysis gives information regarding both the deformability profile of the entire population and the individual cells within the tested population. Data derived from Refs. [17, 63].*

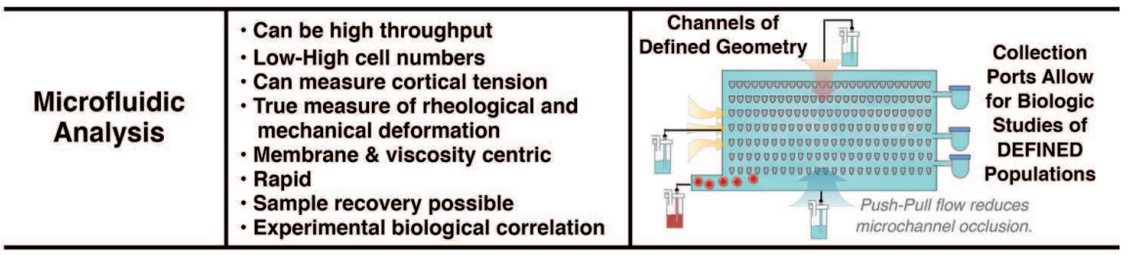


**Figure 6.**
*Overview of microfluidic analysis of blood cells. Recent advances in microscale fabrication technologies have allowed for the development of an exceedingly broad array of microfluidic devices that may have utility in assessing the deformability of blood cells. These approaches range from single to multi-channel devices with channels of single or variable lengths and diameters. In addition, some designs incorporate collection ports so that cell exhibiting differential deformability profiles can be collected for further in vitro or in vivo study. Device shown is adapted from Guo et al. and Kang et al. [26, 28].*

alternative to assessing blood cell deformability relative to these previous, and now rather ancient (as reflected by the key research papers relating to these approaches) technologies [22–25, 27, 28, 71–78]. Deformability measurement using microfluidics uses minute amounts of a whole blood or purified RBC/WBC in suspension flowing through a funnel-shaped micro-constriction(s) in a disposable plate. As demonstrated in our previous publications, and discussed in the following section, microfluidics devices are capable of providing reproducible intra- and inter-individual data, detecting oxidatively damaged RBC, identifying changes in RBC deformability consequent to storage, and identifying leukocytes [20–28].

## 3. Utility of microfluidics in transfusion medicine

As evidenced by the number of publications and patents being generated annually, the promise of microfluidic devices in medicine is seemingly unbounded. One area of particular interest to our laboratories has been in the field of transfusion medicine [20–28]. Annually over 100,000,000 units of blood are collected worldwide for transfusion purposes. Despite the volume collected, our tools for assessing the quality of the stored blood products remains primarily centered on 1950–80s technology. Upon collection of whole blood in Canada the blood is processed to produce 3 major components: RBC, platelets and plasma. The RBC component for

use in blood transfusion therapy are stored at 4°C for up to 42 days. The maximum storage window for RBC is based on studies dating from the 1950s on that defined a ≥ 75% recovery rate at 24 hours post-transfusion as the clinical "quality control" standard for stored donor RBC [79, 80]. Despite decades of research into RBC biology and advances in other aspects of transfusion medicine, the 24 hour survival rule remains the current gold standard for determining acceptable donor RBC quality in transfusion medicine. Currently there are no other established biomarkers by which blood services can discriminate "good" versus "bad" units. Note however, that ultimately the survival of the donor RBC is consequent to their vascular deformability (which is in turn governed by a multitude of biologic/metabolic factors). Hence, cost effectively assessing the deformability of stored RBC could serve as an excellent biomarker for the quality of stored donor RBC. Intriguingly, RBC deformability may also be a potent pre-screening tool that could be used to exclude potential donors from RBC donations. RBC which demonstrate poor initial deformability upon collection do not store well and may lead to adverse events in patients who receive these units. Poor deformability of potential donor RBC may arise from a broad range of issues including: undiagnosed RBC abnormalities (e.g., cytoskeletal, hemoglobin or metabolic aberrations); vascular inflammation; or dietary or drug-mediated alterations of the RBC.

To assess the deformability of blood cells, our laboratories have utilized a variety of microfluidic devices ranging from a simple, low throughput, funnel chain (prone to clogging) to a much more advanced and robust high throughput ratchet device. The ratchet microfluidic approach has proved better at assessing vascular deformability as blood cells are pushed laterally and vertically through tapered microchannels of decreasing size thus modeling the process of cellular deformation in microvasculature (**Figure 7**). Vertical movement is done via an oscillatory vertical pressure deferential that allows both a net vertical filtration flow and a downward declogging flow to minimize microchannel obstruction by blood cells as they reach their deformability limit. Importantly, this design also incorporates collection outlets allowing for recovery, and further testing, of cell populations with differential deformability profiles. Our research to date has demonstrated that this microfluidic microfiltration device is capable of isolating circulating tumor cells

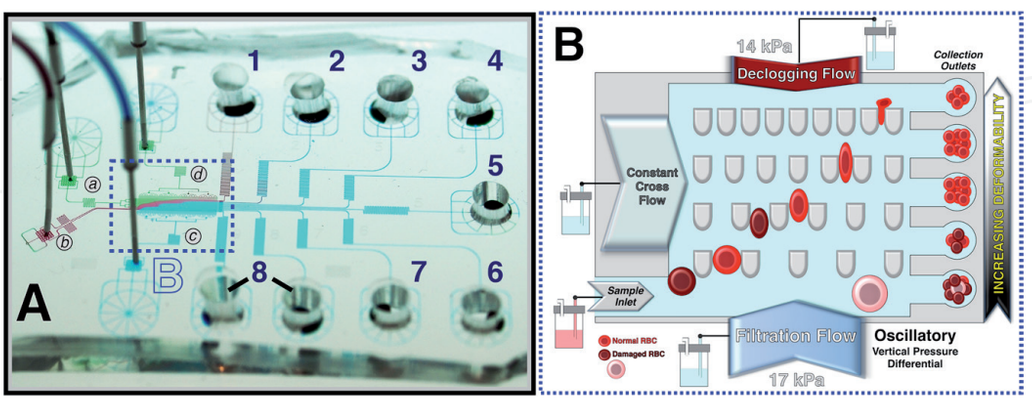


**Figure 7.**
*General schematic of a ratchet microfluidic device. Panel A: shown is a photograph of the ratchet microfluidic device infused with different color dyes to highlight the design features: cross flow inlet (a), sample inlet (b), upward (c) and downward (d) oscillatory flow inlets, sorting region (dashed blue box) and outlets 1–8. In this design, outlets 8–1 corresponds to blocking pore sizes of ≥6.5, 5.5, 4.5, 4.0, 3.5, 3.0, 2.5 and 2.0 μm, respectively. Panel B: schematic of the sorting region showing the decreasing size of the tapered microchannels as well as the deformability of normal and oxidized RBC through these microchannels. Poorly deformable cells (e.g., oxidized RBC) are collected in outlets 8 and 7 while highly deformable cells are collected in outlets 3 thru 1. The downward oscillatory pressure minimizes channel obstruction by poorly deformable cells which are pushed horizontally into the collection outlets by the cross flow pressure. This device is suitable for use for both human RBC and WBC.*

from leukocytes, malaria-infected and oxidized RBC from normal cells, granulocytes and lymphocytes from whole blood, and detecting early immune cell activation consequent to degranulation [26–28, 81].

Key to the use of microfluidic devices in RBC blood banking is documenting the ability of the device(s) to discriminate between "normal" and abnormal cellular deformability and document that the loss of deformability is associated with diminished in vivo circulation. Loss of cellular deformability can arise from a host of causes, most of which, due to the iron and oxygen rich environment of the RBC, leads to cellular oxidation [17–19, 23, 57, 61, 63, 82]. As shown in **Figure 8**, human or murine RBC oxidized by exposure to 50 μM phenazine methosulfate (PMS) were readily discriminated from normal RBC as measured by the cortical tension required to push the RBC through a funnel shaped micropore However, as noted by the differences between the human and murine RBC, the microchannel size (2–2.5 μm in this experiment) relative to the mean diameter of the RBC itself (~8 versus 6.7 μm for human and mouse RBC, respectively) will also play a role. Most importantly however, the loss of murine deformability in the oxidized RBC sample, as noted in the microfluidic device, correlated closely with the loss of in vivo survival. These findings suggest that microfluidic devices could prove useful for both diagnostic purposes (e.g., hemoglobinopathies such as sickle cell disease and thalassemia) as well as in evaluating the quality of stored human RBC prior to transfusion into a patient.

Indeed, microfluidics analysis of stored human RBC suggests that deformability is affected by storage time. As demonstrated by Matthews et al., using a microfluidic device, there is a significant loss of RBC deformability as early as 2 weeks into storage [25]. This finding confirms single-cell deformability studies that similarly

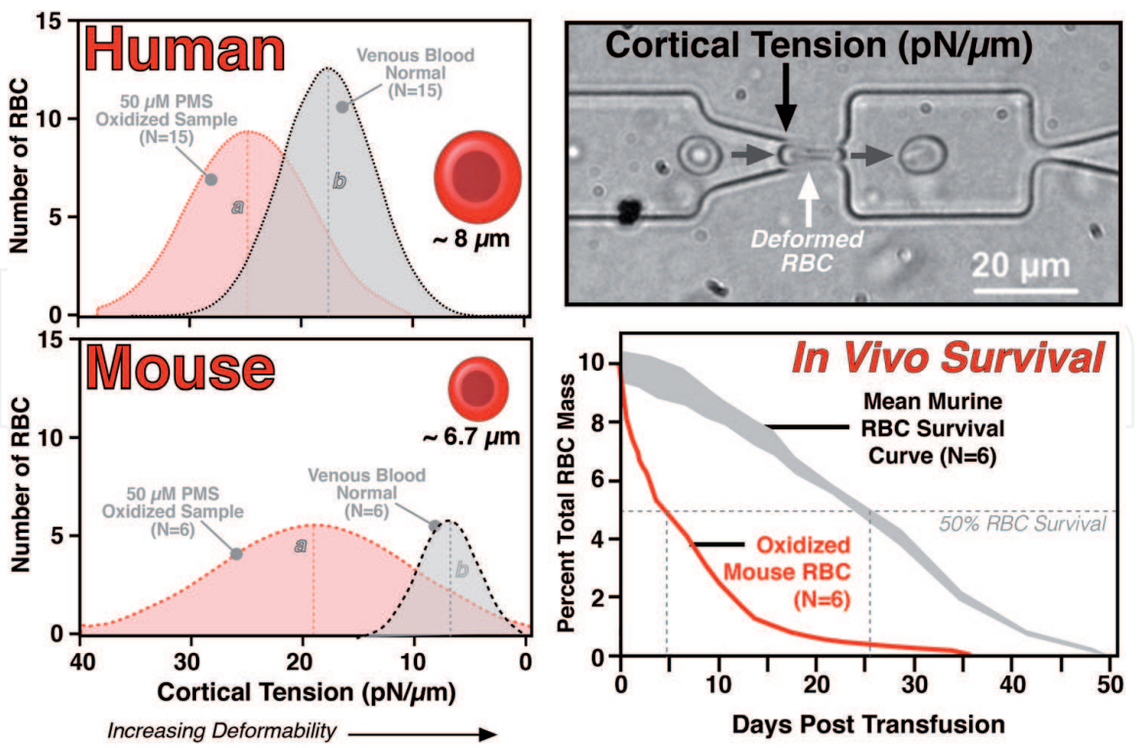


**Figure 8.**
*Analysis of human and murine RBC deformability using a conical microfluidic array. The width of the funnel shaped micropore constriction used to measure RBC deformability was approximately 2–2.5 μm in size at its minimum. (a) and (b) equals peak count for oxidized and normal RBC respectively. Human blood was obtained via a finger prick while mouse blood was obtained by saphenous bleed. Also noted is the 50% in vivo survival point for oxidized (~5 days) and normal (~26 days) murine RBC. Data derived from Kwan et al. [23].*

indicated that RBC deformability remained fairly constant in the first 2–3 weeks of storage and then rapidly decreased [83, 84]. However, in contrast to these single cell studies, our high throughput device can rapidly assess the proportion of individual RBCs that are too rigid to transit the microconstrictions and may, upon transfusion into an individual, be cleared by the spleen. Indeed, by day 42 of storage, 30% of all donor RBCs were too rigid to transit the device. Interestingly, a small subset of donors had RBC that demonstrated poor storage in that >50% of their RBC were too rigid to passage the microconstriction. These research findings suggest that the RBC quality of individual donors are, not unexpectedly, variable. The source of inter-individual variability causing the poor storage could be either inherent to the donor RBC itself (e.g., metabolic, structural or hemoglobin abnormalities) or transient (e.g., inflammation, food or drug induced).

The prescreening questionnaire completed by both new and repeat blood donors is focused, in part, on identifying factors that could adversely affect the quality of the blood product(s) produced from a donation. While most biologically-mediated RBC defects are likely to have been previously detected during normal medical surveillance of the prospective donor, transient inflammatory-mediated effects, such as those arising from viral, bacterial, drug or autoimmune events, are most likely to impact blood component quality. To address these potential risks, at the time of blood donation, all donors are asked if they feel ill or have had a recent fever. While the primary purpose of these self-reporting questions is to avoid transfusion of blood-borne infective agents or plasma that may contain potent immunomodulatory chemokines and cytokines, systemic inflammatory events may also result in bystander injury to the RBC that may compromise RBC storage and safety. The described microfluidics ratchet device may also provide a means of assessing both the WBC population and activation state of an individual [26, 28]. As shown in **Figure 9**, the ratchet microfluidic device described in **Figure 7**, is capable of differentially sorting monocytes from lymphocytes. The same device can also differentiate between resting (granule containing) from activated (degranulated) CD8+ T lymphocytes. Further refinement of the microchannel geometry will be capable of improving cell separation making it possible to readily prescreen individuals for evidence of immune activation thus improving blood component safety consequent to empirical donor evaluation versus self-reporting. Finally, microfluidic devices

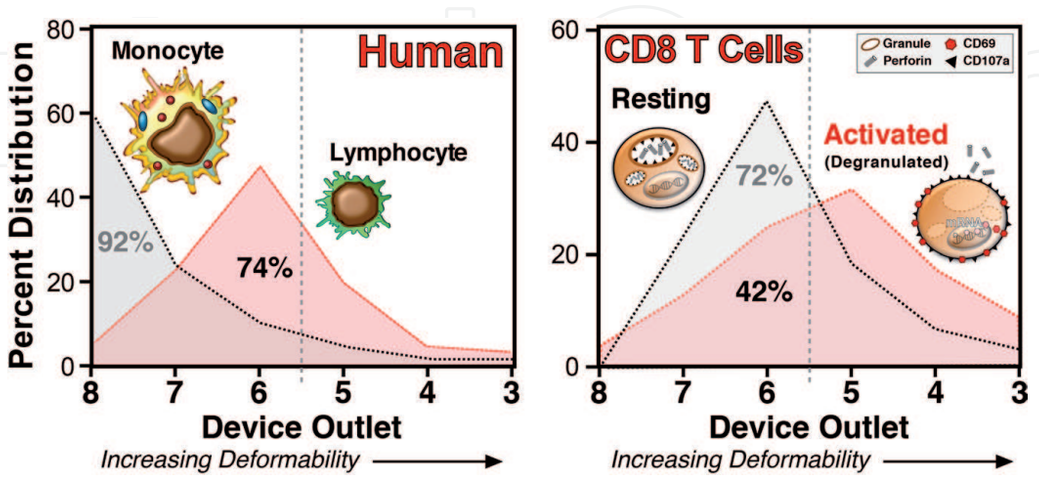


**Figure 9.**
*Analysis of human monocyte and lymphocyte populations showing differential sorting on the ratchet microfluidic device. Note that the prototype device can also detect degranulation of lymphocytes (shown are CD8+ T cells) which occurs upon inflammatory activation. The vertical dashed line separates cells based on less deformable (collection outlets 8–6) and more deformable (collection outlets 5–3). Data derived from Kang et al. [28].*

could also be used during the blood collection process, as well as in the field, to screen individuals who have reported recent travel to malarial endemic areas, for actual malaria infection [27, 85–88]. Currently, individuals traveling to malarial endemic regions are deferred from blood donation; an action that often results in their permanent loss from the blood donor pool.

## 4. Conclusions

Microfluidics devices have the potential to dramatically, and cost-effectively, change the practice of transfusion medicine. As illustrated, purpose-specific development of ratchet microfluidics devices will make it possible, via a finger prick (e.g., as shown in **Figure 8**), to prescreen donors at the time of pre-donation testing (i.e., simultaneously with determining the donor's hematocrit prior to unit donation) to select donors whose RBC show normal deformability profiles prior to storage. Donors with RBC deformability profiles outside of the normal range would be deferred from RBC donation, though potentially, still donating plasma for fractionation into plasma protein components. Moreover, the same microfluidic approach could improve the detection of patients with recent/current systemic immune activation that could result in the presence of undesirable cytokines/chemokines within the donated blood or that might have adversely affected normal RBC deformability. Hence, the cost-effective microfluidic-based prescreening process would potentially diminish the risk to patient safety that accompanies ineffectual RBC transfusion and/or the presence of inflammatory mediators in blood products. Not inconsequentially, prescreening for good donors would reduce the expense to the blood operator associated with the production and distribution of a potentially ineffectual, or unsafe, blood unit. Beyond prescreening donors, patient safety would also be enhanced by doing point-of-care deformability analysis of stored RBC prior to transfusion. Such analysis would enhance patient safety by reducing the aggregate transfusion needs of a patient by preventing the transfusion of RBC which would have poor in vivo survivability. Such an approach would be of particular value in the chronically transfused patient (e.g., sickle cell, thalassemic and myelodysplastic) populations.

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## Conflict of interest

The University of British Columbia and HM have pending patent applications relating to the described microfluidic devices.

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