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Sublethal mechanical shear stress increases the elastic shear modulus of red blood cells but does not change capillary transit velocity.

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Abstract

Blood exposure to supraphysiological shear stress within mechanical circulatory support is suspected of reducing red blood cell (RBC) deformability and being primal in the pathogenesis of several secondary complications. No prior works have explored RBC dynamics with the resolution required to determine shear elastic modulus, and/or cell capillary velocity, following exposure to mechanical stresses.

Healthy RBCs were exposed to 0,5,50, and 100Pa in a Couette shearing system. For comparison, blood was also exposed to heat treatment – a method that predictably increases RBC rigidity. Shear modulus assessment required aspiration of single RBCs through narrow micropipettes at known suction force. Cell transit velocities were measured within micro-channels in regions of fully-developed flow.

Supraphysiological shear stress increased the elastic shear modulus by 39% and 69% following exposure to 50 and 100Pa, respectively. Cell transit velocity, however, did not change following shear, with concurrent decreases in cell volume likely nullifying increased shear modulus-friction interactions. Differences observed were consistent with our internal control (heat treatment), supporting that cell mechanics are significantly impaired following supraphysiological-sublethal shear exposure.

Given mechanical circulatory support operates at shear stresses consistent with the present study, it is plausible that these devices induce fundamental impairment to the material properties of RBCs.

Keywords: haemorheology; mechanobiology; micropipette aspiration; RBC deformability; rheology; subhaemolytic;

Background

Mechanical circulatory support is commonly used to assist (or replace) the function of native organs during acute surgical interventions and/or chronic organ failure. Over the past half-century, these devices have been continually improved; however, significant complications continue to plague recipients, with “blood trauma” being implicated (1-3). Blood interactions with foreign

surfaces, excessive turbulence, and mechanical stresses can cause dangerous complications of altered blood rheology and organ perfusion (for review, see Nemeth et al., 2018).

The capability of red blood cells (RBCs) to deform is vital for successful blood circulation and fluidity. The human RBC has a resting diameter of $\sim 8\ \mu\text{m}$, and must be able to reversibly stretch, fold, and deform through the smallest capillaries of the body ($< 3\ \mu\text{m}$ lumen) to facilitate gas exchange and tissue perfusion (5). The unique capacity of RBCs to endure large deformations and subsequent recovery is predominantly determined by a few key attributes: unlike most cells, RBCs are anuclear and have no organelles; RBCs have a biconcave morphology with a large surface-area-to-volume ratio ($\sim 40\%$ larger than a sphere of the same volume); the RBC membrane is comprised of a highly elastic lipid bilayer connected to a stable latticed protein cytoskeleton; and, RBCs contain a cytosol (principally comprised of haemoglobin) that is more viscous than the extracellular plasma, facilitating energy/force dissipation across the cell membrane. While an RBC is well equipped to repeatedly traverse circulation during its 120-d lifespan, alterations to the cell membrane, cytoskeleton, and cytosol may greatly impact cellular deformability and thus its ability to transit the microvasculature (6).

Particle deformability is an important determinant of wall friction forces, and thus transit velocity through a tube. The different biophysical properties of RBCs have been demonstrated to significantly impact flow through microchannels of various sizes, where cell geometry (e.g., volume and surface area) predominantly govern entry into small pores/capillaries ($\sim 3\ \mu\text{m}$), and cell viscosity factors (i.e., intracellular *and* membrane viscosity) dictate entry into larger pores/vessels ($\sim 7\ \mu\text{m}$) (7). The rigidity of the RBC membrane in response to shear stress (i.e., the shear elastic modulus) largely influences RBC-wall friction forces, and transit times while cells traverse smaller vessels (8). In a recent microfluidic investigation of cancer cells, this notion was reaffirmed as evidenced by stiffer cells traversing more slowly through artificial channels due to stronger friction forces at the wall (9). Driessen et al. (10), and Lipowsky et al. (11), employed *in vivo* rat models to identify that infusion of partially-hardened RBCs (with increased shear moduli) decreased blood flow velocity through capillaries, diminished the number of available perfusion pathways in tissue beds by plugging vessels, and even led to stasis of flow. Further, hardened RBCs were observed to be shunted through large thoroughfare channels, thus skimming past smaller vessels, supporting the notion that blood flows through pathways of least resistance, and

especially when cellular deformability is impaired. It follows that ensuing malperfusion of the microcirculation would lead to increased likelihood of localised ischaemic events.

Within circulation, the physiological shear stresses that RBCs are exposed to typically range from 0–10 Pa (12). Mechanical stresses >10 Pa therefore represent a supraphysiological condition; initially, cells tolerate these stresses without substantial effects, although biophysical properties of cells become impaired when exposed to 40–80 Pa (depending on exposure duration (13)) and eventually haemolyse at shears >150 Pa (again, depending on exposure duration (12, 14, 15)). The range of shears that induce biophysical impairment to RBC are progressively termed “sublethal”, while stresses that induce overt cell destruction are haemolytic and/or “lethal”. Recent advances in characterising mechanically-induced blood damage identified that shear stress magnitudes that are supraphysiological, yet non-lethal, can negatively impact RBC structure and function as evidenced through: i. aberrations to cell morphology (16); ii. decreased cellular deformability (17); iii. altered cell electronegativity and cell surface chemistry and thus thresholds for cell aggregation/disaggregation (18); and iv. cell fragmentation (19). The extent to which these alterations influence *in vivo* haemodynamics remain to be elucidated, although it is hypothesised that altered RBC mechanobiology may propagate malperfusion and/or flow stagnation that lead to poor tissue oxygenation (11, 20). Despite recent advances in understanding how RBCs are functionally affected by sublethal shear stresses, it remains unknown whether the cell membrane mechanical properties *per se* are affected. The current study thus examined whether a vital material property – the shear elastic modulus – is altered following RBC exposure to sublethal shear stress, to elucidate the mechanisms underlying altered RBC behaviour.

Methods

Blood sampling and preparation

In the present study, healthy men (i.e., free from cardiovascular and metabolic comorbidities, and haematological/immune disorders) were recruited to donate blood samples via venepuncture from a prominent vein in the antecubital region using a 21-g needle and syringe; the syringe method was employed with an aspiration rate of $\sim 0.5 \text{ mL} \cdot \text{s}^{-1}$. Once collected, blood was immediately transferred into tubes containing $1.8 \text{ mg} \cdot \text{mL}^{-1}$ K₂-EDTA anticoagulant which were gently mixed for $\sim 10 \text{ s}$. Using a haematology analyser (DxH 600, Beckman Coulter, Brea, CA), RBC

parameters were subsequently inspected with complete blood cell analysis and confirmed to be within normal ranges. All experimental procedures were completed within 4 hours of initial blood collection. The experimental protocols of the present study were reviewed and approved by the Griffith University Human Research Ethics Committee (reference number: 2016/712), which conforms with the Declaration of Helsinki.

Experimental design

To investigate the influence of sublethal shear stress exposure on RBC shear modulus and subsequent capillary transit velocity, the current study design comprised three main steps: 1. treatment phase – exposing blood samples to discrete magnitudes of shear stress; 2. shear modulus determination via cell aspiration through a minimally-tapered glass micropipette with an inner diameter of 1.2 μm ; and 3. for the cells that had been examined in Step 2, capillary transit velocity and volume measurements were performed immediately after Step 2 with a different minimally-tapered glass micropipette, this time having an inner diameter of 3 μm .

RBC preparation and treatment phase

Following blood collection, to facilitate accurate shear/viscosity control, the non-Newtonian properties of blood were overcome by diluting the sample ~ 200 times (i.e., $\sim 0.2\%$ haematocrit) with a standard-viscosity suspending medium (360 kDa polyvinylpyrrolidone in 0.1 M phosphate buffered saline; viscosity = 29.9 mPa $\cdot$ s, pH = 7.4, osmolality = 291 mOsmol $\cdot$ kg $^{-1}$). Subsequently, samples were separated into seven groups: 1 $\times$ untreated control (Con); 3 $\times$ shear conditioned at 5, 50, and 100 Pa (*N.B.*, 1 Pa = 10 dyn $\cdot$ cm $^{-2}$); and 2 $\times$ heat treatment at 48°C for 5- and 10-min. Shear conditioning was performed in a commercial annular Couette shearing system (LORRCA MaxSis, Mechatronics, Hoorn, Netherlands) operating at $37 \pm 0.2^\circ\text{C}$. Following blood exposure to the experimental condition, 10 μL of the diluted blood sample was immediately mixed into 500 μL of PBS containing 2% w/v bovine serum albumin – the presence of additional plasma proteins in solution minimised cell adhesion to the glass slide and micropipette tip (21, 22). Samples were then promptly placed atop of a glass coverslip and transferred to the microscope rig for analyses.

Micropipette aspiration: rig design

The micropipette aspiration rig used in the present study was retrofitted to an inverted microscope (IX73, Olympus, Melville, NY). As depicted in Fig 1, the micropipette aspiration rig was constructed of three primary components: micromanipulated glass micropipettes, semi-automated water column pressure control, and microscopic cell imaging using a 60 × objective and a CMOS camera (QImaging Corporation, Surrey, Canada). The minimally-tapered glass micropipettes were initially fabricated from borosilicate glass capillary tubes using a micropipette puller (P-1000, Sutter Instrument Company, Novato, CA). To cut the pipettes to an inner diameter (I.D.) suitable for the measurements of the present study, a custom-made microforge was constructed with nichrome wire and a borosilicate glass microbead. Given the micropipette and microbead consisted of the same material properties, a variation of the method described by Engström and Meiselman (23) was deployed to cut the tips. Briefly, the microbead was heated beyond its softening point before the micropipette tip was advanced into the microbead to the desired cutting diameter. Once advanced to the required depth, the micropipette was rapidly withdrawn from the microbead and the electric current through the nichrome wire was stopped, allowing the micropipette, microbead, and wire filament to cool and harden. Once all components were hardened, the micropipette was rapidly advanced into the solid microbead with a single action, where the mechanical shock caused the micropipette to bend and fracture at the point where it was previously advanced into the softened bead; a visible impurity/weakness in the shape of the micropipette could be observed at this location.

For the current study, the micropipette rig was constructed with dual micropipettes of different diameters, with the same two micropipettes being used for the entirety of the study (1.2 μm I.D. for shear modulus assessments, and 3 μm I.D. for capillary transit velocity). To preserve the micropipettes between days of testing, each was primed and stored similar to the method reported by Engström and Meiselman (23), except rather than storing in buffer solution, an enzymatic cleaner was used. The micropipettes were thoroughly rinsed prior to priming with a 30-g needle. During each measurement, precise suction pressure was controlled with the use of water columns constructed with 30 mL fluid reservoirs connected to computer controlled microfluidic syringe pumps. Prior to each measurement the height of the water columns was initially 'zeroed' for

suction pressure (note 1 mm of water equates to ~9.98 Pa) by ensuring they caused no movement of microparticles or cells near the micropipette tip. To minimise evaporation from the reservoirs throughout the duration of the experiment, the water columns were covered with pierced parafilm.

FIG 1 ABOUT HERE

Micropipette aspiration: shear elastic modulus

For the determination of the shear elastic modulus, the smaller 1.2 μm glass pipette was manipulated to be positioned at the surface of a single cell. Using visual feedback of cell/microparticle movement as a guide, the pressure induced by the water column was again confirmed to be zeroed and level with the height of the stage. Using the syringe pump fluidics control, 200 μL was removed and subsequently replaced in the water column to level the meniscus of the fluid and align the direction of pumping to remove motor backlash (if unchecked, this would induce erroneous results in the initial stages of the measurement). Suction pressure was then increased in 5 Pa increments to 50 Pa (or until “cell folding” occurred); this pressure range facilitated measurement of a cell’s shear elastic modulus without inducing hysteresis and shear-hardening. At each pressure increment (ΔP) it was ensured that the aspirated portion of the cell (L_p) had stabilised and reached equilibrium prior to capturing the micrograph image for analysis. Each micrograph frame was analysed for L_p , which was subsequently normalised for the radius of the pipette tip (r_p). Given ΔP and r_p were known parameters, $L_p \cdot r_p^{-1}$ was linearised against the suction force (pN); suction force was determined by calculating the ΔP exerted across the area of the micropipette tip ($\pi \cdot r_p^2$; Fig 2. The experimental determination of the slope gives the shear modulus (μ) for a single cell in units $\text{pN} \cdot \mu\text{m}^{-1}$ (Fig 3).

FIG 2 ABOUT HERE

FIG 3 ABOUT HERE

Micropipette aspiration: volume and capillary transit velocity

After measurement of a cell's shear modulus, the larger glass micropipette (i.e., 3 μm I.D. tip) was used with the micropipette aspiration rig to estimate cell volume and assess capillary transit velocity. Following the initial zeroing of the pressure water column, the larger micropipette was also manipulated to the cell membrane, and 30 Pa of suction pressure was applied to the system. Following entry of the entire cell into the micropipette, micrograph images were captured at 1 Hz. In each captured frame, the length of the cell was measured, and cell volume was estimated by calculating cylindrical volume (assuming excess volume due to curvature at the ends of the cell would account for the fold in the biconcave region). Cell transit velocity (v) was determined by $d(x)/d(t)$ of RBC centroids (Fig 4).

FIG 4 ABOUT HERE

Statistical analysis

For each individual cell in each experimental condition, shear modulus (μ ; the gradient of $L_p r_p^{-1}$ vs F), cell volume, and capillary transit velocity (v) were determined. Following confirmation of normality with Shapiro-Wilk test and investigation of Q-Q normality plots, Pearson's correlation and regression analysis were performed. Data (shear modulus, volume, and velocity) from each experimental condition were grouped and compared using a one-way ANOVA with matched repeated measures applying the Geisser-Greenhouse correction where required, and the Bonferroni post-hoc analysis. Significance was determined at an alpha of 0.05. Unless otherwise stated, results are presented as mean $\pm$ standard error.

Results

Haematology

Relevant clinical haematology values are presented in Table 1. All parameters were confirmed to be within normal references ranges for healthy individuals.

Table 1. Relevant haematology results obtained from complete blood count test.

		Normal Range (24)
RBC count ($\times 10^{12} \cdot \text{L}^{-1}$)	4.93 ± 0.12	4.5 – 5.6
Haemoglobin ($\text{g} \cdot \text{L}^{-1}$)	154 ± 3	137 – 172
Haematocrit ($\text{L} \cdot \text{L}^{-1}$)	0.45 ± 0.01	0.40 – 0.50
MCV (fL)	90.3 ± 0.7	83 – 98
MCH (pg)	31.3 ± 0.3	28 – 33
MCHC ($\text{g} \cdot \text{L}^{-1}$)	347 ± 3	320 – 360

RBC: red blood cell. MCV: mean corpuscular volume. MCH: mean corpuscular haemoglobin. MCHC: mean corpuscular haemoglobin concentration.

Shear elastic modulus ($\text{pN} \cdot \mu\text{m}^{-1}$)

The calculated shear modulus values for individual and grouped cell responses to each experimental condition is presented in Fig 5. The rigidity of the RBC membrane significantly increased with accumulated shear exposure as well as heat treatment conditions ($F=65.07$, $p<0.001$). All shear conditions (except 5 Pa) increased shear modulus when compared with untreated Con. The heterogeneity of shear modulus values – that is, the spread – increased following experimental treatment incorporating either of shear or heat.

FIG 5 ABOUT HERE

Cell volume

The volumes of the discrete cells analysed in the present study are illustrated in Fig 6, clustered for experimental group (Fig 6A); correlations with shear modulus were also examined (Fig 6B). Typical values for apparently healthy donors were observed in the untreated (Con) cells, with the average measured volume comparing favourably with the MCV values determined using a commercial cell analyser (Table 1). Significant decreases in cell volume were detected following exposure to 50 and 100 Pa shear stress, and also after heat treatment (HT) – a known mediator of impaired cell mechanics. For each individual cell, cell volume was negatively correlated with changes in measured shear modulus ($p<0.001$; Fig 6B). Qualitative microscopy observation of the cells (Fig 7) supported the measured change in cell volume, noting increased prevalence of

echinocytes, stomatocytes, and crenated cells following exposure to 100 Pa shear stress, and also after 5 and 10 min of HT.

FIG 6 ABOUT HERE

FIG 7 ABOUT HERE

Capillary transit velocity

Capillary transit velocity for individual cells is reported for each experimental condition in Fig 8A; correlations with shear modulus were also examined (Fig 8B). Relative to Con (mean transit velocity = $22.5 \mu\text{m}\cdot\text{s}^{-1}$), no significant differences were detected between groups for capillary transit velocity, nor for correlation with shear modulus values. That is, shear modulus did not dictate capillary velocity in the developed flow region of the defined geometry employed in the present study. Qualitative observations indicated that cells with larger shear modulus values required a longer duration to enter the $3 \mu\text{m}$ micropipette; e.g., a few extreme cases were observed in the 10HT condition, where complete cell entry into the microchannel required greater than 60 s

FIG 8 ABOUT HERE

Discussion

The salient findings of the present study indicate that when RBCs are exposed to dose-increases of shear stress in a step-wise manner: i) cell volume is decreased; ii) membrane shear modulus and cell rigidity are increased; and iii) cell capillary transit velocity remains unaltered. It was also noted that considerable heterogeneity was observed within each cell suspension – i.e., individual RBCs responded differently to shear stimuli. The collective findings of the present study indicate sublethal mechanical trauma can alter the physical and morphological properties of RBCs that influences cell alignment and entry into small capillaries. The potential for impaired cell entry into small apertures typical of the microcirculation, and thus also the subsequent challenge to oxygen distribution across the microvascular beds is likely to propagate profound complications. It is

possible that these results may explain, in part, the heightened risk of ischaemic complications observed following surgical interventions that expose blood to high-shear environments, such as mechanical circulatory support.

Cell volume significantly decreased following exposure to discrete shear stresses (50 and 100 Pa) that were supraphysiological, yet below the haemolytic threshold (i.e., sublethal domain of shear stress); this was not observed when cells were exposed to physiological shears (5 Pa). Changes in cell volume induce several effects that alter the mechanical properties of RBCs. At a morphological level, direct imaging of cells demonstrates that exposure to increased shear stress is associated with an increased frequency of echinocytes, stomatocytes, and crenation (3, 16, 21); this was confirmed in the present study (Fig 7). These observations of aberrant morphology are associated with increased surface-area-to-volume-ratio, which is consistent with our observation of decreased cell volume following high shear exposure (Fig 6). Intracellular viscosity of RBCs may also increase following high shear exposure, given cytosolic viscosity is largely dependent upon the concentration of haemoglobin (25); efflux of fluid in the absence of membrane rupture results in a relatively increased haemoglobin concentration. While the underlying mechanism of a shear-mediated cytosol loss remains to be determined, it is plausible that fluid-shifts may occur due to passive development of 'leaky' membrane pores, or active mechanotransductive pathways, with evidence supporting Piezo and Gardos channel involvement (6, 26). Both surface area-to-volume ratio, and cytosolic viscosity, are primary determinants of RBC deformability, and thus the present data suggest these physical properties contribute to cell rigidification following exposure to sublethal shears. The implications of altered cell properties on blood flow are complex, given that while increased cytosolic viscosity bares negative implications for cell deformability and thus fluid properties, Chien (6) reported that decreased cell volume may be beneficial for cell transit through narrow capillaries due to decreased wall friction forces.

Exposure of blood to supraphysiological shear stresses, below haemolytic levels at which cell rupture is induced, alter the physical and electrochemical properties of RBCs by remodelling the membrane, denaturing cytoskeletal proteins, and thinning the glycocalyx (18, 27-29). A number of recent reports have identified that exposure of RBCs to sublethal mechanical trauma also decreases RBC deformability as measured using laser diffractometry (13, 16, 17, 30). While laser diffractometry provides high-throughput assessment of the ensemble-average response for RBC suspensions, it lacks single cell resolution which facilitates interrogation of cell subpopulations

and cannot provide absolute measurements of cell rigidity (e.g., shear modulus) that are essential for discrete mathematical modelling.

The present study employed classical micropipette aspiration techniques to yield high-resolution data, for the first time in this context, to quantitatively measure the change in membrane shear modulus of RBCs exposed to sublethal shear stress. Exposure to physiological shear stress (i.e., 5 Pa) did not alter the shear elastic modulus of RBCs; however, when RBCs were exposed to sublethal shear stress conditions (i.e., 50 and 100 Pa), the shear elastic modulus significantly increased by >50% (Fig 5B). Indeed, a step-wise increase in cell rigidity was observed when RBCs were exposed to 50 and 100 Pa, when compared with unsheared samples, towards levels approaching heat treatment at 48°C for 5 min – a “classic” model of experimental cell rigidification (31). To our knowledge, this is the first definitive measurement of the cell mechanical properties of RBCs following exposure to sublethal shears and demonstrates that in addition to cell dehydration induced by mechanical stimulation, cell membrane rigidity is independently increased. Decreases in cellular deformability following exposure to sublethal shears are thus caused by both intracellular and extracellular alterations, which may be caused by cross-linking of cytoskeletal proteins (27), formation of pores in the RBC membrane (32), and spectrin fragmentation (28, 33).

The current findings also demonstrated that when RBCs are exposed to treatments that increase membrane rigidity, a considerable heterogeneity may be observed in the magnitude of change in the shear elastic modulus (Fig 5A). When RBCs were exposed to sublethal shear stress, for example, it was clear that while the bulk of cells (and thus the average response) presented with augmented shear elastic modulus, some cells appeared to remain unaffected. A similar response was even observed for a handful of cells that were heat treated at 48°C for 10 min – an extreme treatment used to induce rigidification. The present data thus highlights that blood cells do not respond uniformly to “damaging” interventions, including sublethal mechanical stresses. This heterogenic response for the shear elastic modulus of single RBCs following exposure to sublethal mechanical stress has not been previously reported, and precise mechanisms dictating (non)response to shear damage remain to be elucidated. Recent work (34) presents a plausible explanation, however, for such disparity in the present individual cell responses: subpopulations that arise due to physiological “aging” respond differently to mechanical shear, where the most

dense RBCs (older cells) accumulate shear rigidification at more than twice the rate of the less dense counterparts (younger cells).

Particle deformability has consistently been identified to be an important determinant of wall friction forces, and thus transit velocity. In a recent microfluidic investigation of cancer cells, this notion was reaffirmed with stiffer cells flowing slower due to stronger friction forces at the wall (9), with the membrane viscous interface likely being more important than the elasticity of the particle itself (35). In contrast to prior studies (8, 10, 20) which observed that diamide rigidified RBCs had reduced flow velocity and even resulted in irreversible clogging of small apertures, no changes in cell capillary transit velocity were observed in the present study. It is plausible that the changes in cell volume following shear-rigidification resulted in reduced wall contact, compensating for increased friction forces, and thus yielding no net change in transit speed. Moreover, given tube geometry is a primary determinant of flow, the interaction between cell membrane mechanics, cell volume, and capillary geometry needs to be considered, with our present findings being representative of similar (i.e., $\sim 3 \mu\text{m}$) rigid tubes. It was directly observed, however, that despite transit velocities being unchanged by shear modulus, the most rigid cells in the present study required longer duration to orientate and *enter* the micropipette at a given suction force. It is plausible that this novel observation may have more profound implications for biological function than cell transit velocity itself: if cells do not orientate with flow or are margined in bulk flow due to altered cell mechanics, malperfusion and/or flow stagnation may propagate in smaller vessels that lead to poor oxygen delivery within the vascular bed, and potential localised ischaemic events (11, 20).

Conclusions

The findings of the present study are meaningful given the increased reliance on mechanical circulatory support in various forms of medical intervention. The shear stresses that exist within mechanical circulatory support, while low enough to avoid overt cell rupture, have been recently thought to negatively impact blood cell and plasma protein function (36). The present study provides direct evidence, for the first time, that sublethal shear stresses also increase the shear elastic modulus of RBCs. This finding has profound implications for microvascular function; if severely rigid cells remain in circulation and are not promptly sequestered or phagocytosed, sluggish/slow flow is likely to ensue and lead to localised ischaemic events. These findings thus

also lend support for further evaluation of current medical pumps that exert supraphysiological stresses on blood, and the potential clinical complications that may be independent of the primary pathology itself. This knowledge will tangibly lead to improved blood compatibility and thus design of blood-contacting medical devices.

Declarations

Ethics approval and consent to participate: The present study protocol was reviewed and approved by the Griffith University Human Research Ethics Committee (reference number: 2016/712), which conforms with the Declaration of Helsinki.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: Experimental concept and design: AM. Development of infrastructure: AM. Conduction of the experiment: AM. Data analysis and interpretation: AM, MS, GT. Contributed to reagents/materials/analysis tools: AM, MS, GT. All authors wrote, reviewed, and approved the final manuscript.

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Declaration of Interests

The authors declare that they have no competing interests.

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Figure Legends

Fig 1. Schematic of the dual micropipette aspiration rig that was developed for assessment of mechanics and transit velocity of blood cells.

Fig 2. Micropipette aspiration principle for determining the shear modulus (μ) of single red blood cells. Step 1: the micropipette was manipulated to the surface of the cell membrane. Step 2: aspiration pressure (ΔP) was applied to the inside of the pipette. Step 3: the length of aspirated portion of the cell at the centre of the pipette (L_p) was normalised for r_p and plotted as a function of suction force ($F = \Delta P \cdot \pi \cdot r_p^2$).

Fig 3. Aspiration of a red blood cell into a micropipette with a 1.2 μm inner diameter. The reciprocal of the gradient of the line of identity yields the shear modulus (μ), and the y-intercept represents the bending modulus of the membrane.

Fig 4. Identification of RBC $d(x)/d(t)$ for capillary transit velocity (v) measurement.

Fig 5. Individual (A) and grouped (B) shear modulus (μ) of red blood cells determined by micropipette aspiration technique following exposure to varied magnitudes of shear stress or duration of heat treatment (HT; positive control). * significantly different to Con, $p < 0.05$.

Fig 6. Cell volume of individual RBCs derived from the micropipette aspiration technique. Shear exposure at 5, 50, and 100 Pa, and heat treatment (HT) for 5 and 10 min, altered the mean and range of cell volume (A). Decreased cell volume was significantly correlated with increased shear modulus (B). * significantly different to Con, $p < 0.05$.

Fig 7. Micrograph of RBCs undergoing micropipette aspiration for unsheared Con RBCs (A) and RBCs following exposure to the 100 Pa shear condition (B).

Fig 8. Capillary transit velocity (A) and correlation of cell velocity with shear modulus (B) did not significantly change between untreated (Con), shear, and heat treatment (HT) conditions.

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