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Red blood cell (RBC) suspensions in confined microflows: Pressure-flow relationship

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Abstract

Microfluidic-based assays have become increasingly popular to explore microcirculation *in vitro*. In these experiments, blood is resuspended to a desired haematocrit level in a buffer solution, where frequent choices for preparing RBC suspensions comprise notably Dextran and physiological buffer. Yet, the rationale for selecting one buffer versus another is often ill-defined and lacks detailed quantification, including ensuing changes in RBC flow characteristics. Here, we revisit RBC suspensions in microflows and attempt to quantify systematically some of the differences emanating between buffers. We measure bulk flow rate (Q) of RBC suspensions, using PBS- and Dextran-40, as a function of the applied pressure drop (ΔP) for two hematocrits (~0% and 23%). Two distinct microfluidic designs of varying dimensions are employed: a straight channel larger than and a network array similar to the size of individual RBCs. Using the resulting pressure-flow curves, we extract the equivalent hydrodynamic resistances and estimate the relative viscosities. These efforts are a first step in rigorously quantifying the influence of the ‘background’ buffer on RBC flows within microfluidic devices and thereby underline the importance of purposefully selecting buffer suspensions for microfluidic *in vitro* assays.

Keywords

In vitro models; Microfluidics; Red blood cells (RBCs); Hydrodynamic resistance; Suspensions; Pressure-flow measurements

1 Introduction

To quantify the properties of blood in the microcirculation, red blood cell (RBC) flows have been widely investigated *in vitro* as a proxy for the innate microvasculature [1–3]. In this context, microfabrication techniques have facilitated the proliferation of *in vitro* studies on blood flows where the use of microfluidic models has helped address questions pertaining to

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Conflict of interest

No conflict of interest.

the role of microvascular morphology [4,5], blood viscosity [6,7] and haematocrit [8], as well as RBC deformation [9–11]. In these experiments, blood is commonly resuspended to a desired haematocrit (Hct) level in a buffer solution (ranging from non-physiological values of 10% and lower [8,12] to near-physiological values of 35%–50% [4,13]) and higher, thereby avoiding the problematic use of plasma and allowing RBCs to sustain physiological-like behaviour. Frequent choices for preparing RBC suspensions *in vitro* comprise notably Dextran and physiological buffer [7,8,14], where studies have focused for example on velocimetry measurements (e.g. with Dex-40 [5,15–18], or physiological buffer [19–22]) and RBC deformation assays [4,10,13,23].

On the one hand, Dextran-based solutions represent a mixture of glucose polymers that serve as a nontoxic plasma substitute [24], where Dextran 40 (Dex-40) with its low molecular weight is commonly used [5,17,23,25,26]. As recapitulated in Table 1, the advantages of Dex-40 include preventing aggregation [27] and thus improving microcirculation [28]. Furthermore, the sedimentation of RBCs within tubing occurs at a slow rate [10,11,29], thereby easing the maintenance of experiments, especially when the RBC suspension is stagnant or infused at relatively low flow rates [30]. In contrast to plasma ($\mu \sim 1.1$ cP [31]), the viscosity of Dex-40 is significantly higher with values near ~ 4 cP [32] such that Dextran-based suspensions remain non-physiological when compared to *in vivo* conditions.

As an alternative (see Table 1), salt-based physiological buffer is an effective choice where phosphate-buffered saline (PBS) is commonly used with a viscosity ($\mu = 0.889$ cP [33]) much closer to that of plasma. In turn, sedimentation of RBCs suspended in PBS occurs within shorter times compared with Dex-40 [34] where ensuing flow behaviour is acknowledged to be different but has not been thoroughly characterized [29]. One consequence of such property is the necessity to agitate or stir the suspension during experiments [35,36] or frequently mix it to avoid sedimentation [37]. To address the sedimentation problem, vertically-based setups have also been developed but remain typically uncommon and often cumbersome to implement [38,39]. Despite such drawbacks, PBS represents a more sensible choice to mimic physiological properties of RBCs *in vivo*. Nevertheless, some groups prefer to suspend RBCs in Dextran due to the easier maintenance of the experiment compared to PBS, as mentioned above (Table 1). Alternatively, given its high viscosity, Dextran may be used to control the Reynolds numbers of the system [16,23] as well as explore the influence of viscosity on flow properties by adjusting the buffer viscosity [3,7]. Beyond such examples, however, the rationale for selecting one buffer versus another is most often not firmly established and the ramifications therein (e.g. flow modifications) remain frequently ill-defined and lack detailed quantification.

Motivated by ongoing questions on the role of buffer for *in vitro* blood flow dynamics, we revisit here RBC suspensions in microflows and attempt to quantify systematically flow differences originating between physiological buffer and Dextran. To this end, we investigate the bulk flow of RBCs in PBS- and Dex-40-based suspensions using two distinct microfluidic devices: (i) a straight channel with a square cross section of $50 \mu\text{m} \times 50 \mu\text{m}$, and (ii) a network array with a characteristic cross section ($10 \mu\text{m} \times 10 \mu\text{m}$) similar to the size of individual RBCs ($\sim 7 \mu\text{m}$). Together, these devices capture varying degrees of RBC confinement at the microscale where we measure bulk flow rate (Q) as a function of the

applied pressure drop (ΔP) for two haematocrit levels ($\sim 0\%$ and 23%). Using the resulting pressure-flow curves, we extract the corresponding hydrodynamic resistance and estimate the ensuing relative viscosity for the various *in vitro* setups (i.e. combinations of buffer, Hct and device). These results underscore how influential the ‘background’ buffer may be in altering ensuing RBC flows across microdevices. To the best of our knowledge and with the ongoing lack of discussions explicitly addressing such issue, our efforts represent a first quantitative step in differentiating how a buffer suspension modulates the relative viscosity and may help select more purposefully a suitable buffer depending on the specific end point of the microfluidic *in vitro* assay.

2 Methods

2.1 Device fabrication

Two master wafers were fabricated for Polydimethylsiloxane-based (PDMS) molding using either SU-8 photolithography [40] for the straight channel or deep reactive ion etching (DRIE) of a silicon on insulator wafer [41] for the network channel. Devices were punched and sealed onto a glass-slide using O_2 plasma. Further details on device fabrication are discussed in the Supplementary Material (SM) and in previous work [42].

Briefly, the microfluidic straight channel holds a square cross section of dimensions $50\ \mu\text{m} \times 50\ \mu\text{m}$ ($w \times h$) with a length of $1\ \text{cm}$ (Fig. 1a). The microfluidic network array is constructed of a repeating lattice with regularly positioned circular posts that are spaced with a separation distance of $10\ \mu\text{m}$ and arranged in a staggered array (Fig. 1b); the height of the circular posts is constant and fixed at $10\ \mu\text{m}$ such that the local cross-sectional area through which RBCs flow is effectively a square. The entire array is centred within the microfluidic flow device (Fig. 1b), where RBC suspensions (see Blood Preparation below) are perfused through the domain inlet via a rectangular channel of width $w = 120\ \mu\text{m}$ that smoothly expands into the network. Past the network, the flow returns to the outlet in a symmetrical fashion where RBCs are drained (Fig. 1b).

2.2 Blood preparation

Whole blood was taken from healthy human volunteers. Plasma was removed by centrifugation ($800 \times g$ for 5 minutes, $22\ ^\circ\text{C}$) and discarded. Pelleted RBCs were re-suspended in $50\ \text{mL}$ of phosphate buffered saline (PBS, Sigma, USA) and passed through a leukoreduction filter (RN, Haemonetics, USA). The leukoreduced RBC suspension was washed in PBS ($800 \times g$ for 5 minutes, $25\ ^\circ\text{C}$) and adjusted to 0.01% and 23% haematocrit (Hct) by resuspending the RBCs in either PBS ($283\ \text{mOsm/kg}$) or 10% Dex-40 ($5\ \text{g}/50\ \text{mL}$, $295\ \text{mOsm/kg}$) where suspensions are considered isotonic [43,44]. All procedures were approved for *in vitro* experiments by the Carmel Hospital Institutional Review Board (IRB), Haifa Israel.

2.3 Experimental setup

The experimental setup consists of an inverted microscope (Nikon Instruments) mounted with $\times 40$ or $\times 60$ objective lenses, and a high-speed CMOS camera (Photron). For each experiment, the pressure drop (ΔP) between the inlet and outlet of the microfluidic device is

maintained and controlled by an automated computer-controlled pressure-driven system (Fluigent MFCS-EZ, France), with values ranging between 0.1–10 kPa. The range of pressure drops measured is selected according to the specific microfluidic geometry (i.e. straight channel or network array) and the corresponding RBC suspension (i.e. PBS or Dex-40, ~0% or 23% Hct). In turn, the ensuing Reynolds numbers are calculated to range between 0.01–0.56 in the straight channel and 0.001–0.093 in the microfluidic network array, and are in line with physiological values anticipated in arterioles, venules and capillaries, respectively [45].

RBC suspensions are perfused into each model via a 22-gauge polyethylene tubing system (Instech, USA). To avoid sedimentation of RBCs, the experimental setup is repeatedly agitated between individual experiments. Flow rates are digitally measured using a dedicated commercial flow sensor (Small Flow Unit, Fluigent SA, France), where measurements are sampled at 10 Hz and standard deviations (SD) of the temporal fluctuations off the mean value may be up to 5% (see examples in SM Fig. S1). For each RBC suspension, a minimum of $n = 6$ independent experiments are conducted in each geometry. For each case, data are fitted with a linear regression (see Results). Average and standard deviations are extracted for the measurement ensembles. For each distinct geometry, we conduct a Student t -test for normally-distributed data at different Hct levels of the same suspension; statistical significance is taken for p values $p < 0.01$.

3 Results

We have conducted bulk flow rate (Q) measurements as a function of applied pressure drop (P) for two RBC suspensions (Dex-40 and PBS) flowing under confinement in two distinct microfluidic models. The straight channel holds characteristic dimensions (i.e. 50 μm) that are sizeably larger than individual RBCs (see Fig. 1c and SM Videos 1 and 2); meanwhile, the network array is captured by length scales (i.e. 10 μm) at pair with the size of individual RBCs that qualitatively give rise to extensive flow confinement (see Fig. 1d and SM Videos 3 and 4).

Fig. 2a presents raw data measurements of the pressure-flow rate relationship across the straight channel (SC) for RBC suspensions in Dex-40 (black) and PBS (red), shown at two haematocrit levels (Hct ~0% and 23%). For each case, data are fitted with a linear regression, underlining a strong linear relationship independent of buffer solution or the addition of RBCs to the suspension ($r^2 > 0.97$ across all cases). Following the classic hydrodynamic relationship ($P = Q \cdot R$), slopes of the linear fit correspond to the equivalent hydrodynamic resistance (R) for a given RBC suspension flowing in the SC model. Results are summarized in the histograms of Fig. 2b for the corresponding mean (and standard deviation SD) of the hydrodynamic resistance (R) with SD ranging between 0.51% and 7.44% of the mean depending on the case. We recall that the ratio of viscosities between Dex-40 and PBS is ~4.44; our measurements for ~0% Hct corroborate this observation with a ratio of the (mean) hydrodynamic resistance of ~4.61. Interestingly, however, this ratio is reduced to ~2.95 for measurements in 23% Hct suspensions, underlining the presumably heightened influence of the RBCs in determining ensuing flow dynamics (discussed further below).

Raw data measurements for the corresponding pressure-flow rate relationship across the microfluidic network array (Net) are presented in Fig. 3a for RBC suspensions in Dex-40 (black) and PBS (red), at ~0% and 23% Hct levels. Following an analogous fitting scheme for the Net model (Fig. 3a, $r^2 > 0.98$ across all cases), the histograms of Fig. 3b summarize the mean (and SD) of the equivalent hydrodynamic resistance (R), with SD ranging between 0.70% and 7.2% of the mean depending on the case. Note that the magnitudes of R (i.e. y -axis) are significantly larger than those observed in the SC model (on average ~3.8 times); an indication of the extensive hydrodynamic resistance imposed by the intricate microfluidic network array relative to the straight channel. A similar trend to that seen in Fig. 2b arises, where the ratio of the hydrodynamic resistances at ~0% Hct is ~3.98 (Fig. 3b), highlighting again the largely Newtonian properties of such suspensions at near 0% Hct and their anticipated independence to the model in which they are perfused through. For the 23% Hct suspensions, on the other hand, our measurements yield a ratio of ~2.4. While qualitatively similar to that observed in the SC model, this lower value points however to the more likely prominent role of RBCs under strenuous constriction in the Net model.

In a final step, we make use of the estimations for the hydrodynamic resistances (R) to explore the notion of a ‘relative apparent viscosity’ of the *in vitro* RBC suspensions. Here, we adapt the classic definition of the relative viscosity ($\mu_{rel} = \mu_{blood}/\mu_{plasma}$) to our data sets [1,3]. Following the linear relationship for volumetric flow rate versus resistance, we may extract the resistance of the entire microfluidic device for each distinct suspension. Since resistance depends both on fluid viscosity and geometrical properties, the ratio of the resistances of varying suspensions within an identical microfluidic device underlines the importance in the change of the fluid viscosity due to the presence of RBCs therein. In particular, for a straight circular channel governed by Poiseuille flow it may be assumed to a first approximation that $R_1/R_2 = (8\mu_1/\pi r^4)/(8\mu_2/\pi r^4) = \mu_1/\mu_2$; a strategy originally employed in seminal works investigating blood flows across network arrays [46]. Hence, we may compute the ratio $R_{Hct=23\%}/R_{Hct\sim 0\%}$ for the SC and Net models, respectively, both for Dex-40 and PBS suspensions. Results are summarized in the histograms of Fig. 4 where clear trends arise. For Dex-40 suspensions (Fig. 4, left two columns), differences in the ratio $R_{Hct=23\%}/R_{Hct\sim 0\%}$ between the SC and Net model are minor and both ratios are only just slightly larger than unity. In contrast, differences between the SC and Net models are stressed for PBS suspensions (Fig. 4, right two columns). There, our estimations of the relative apparent viscosity is highest in the network array and in general agreement with the range of relative apparent viscosities anticipated for RBC suspensions in glass capillary tubes [3].

4 Discussion

While both Dex-40 and PBS buffers are commonly used for *in vitro* preparations of RBC suspensions in microfluidic assays, the justification for selecting either one is oftentimes overlooked or poorly discussed. With the present work we have set out to quantify in experiments the role of buffer solution on ensuing RBC bulk flows, using two distinct microdevices. On the one hand, the straight channel holds features (i.e. 50 μm) that are larger than the size of RBCs but where anticipated flows still fall well within regimes of the Fahraeus-Lindqvist effect [1] (e.g. tubes with diameters in the range of ~7–500 μm). Despite

some degree of geometrical confinement, the morphology of RBCs along the channel does not change sensibly during their transit time as qualitatively observed in the SM Videos 1 and 2. In contrast, by specifically employing a staggered microfluidic network array, RBCs must constantly reorient along the constrictions where the shapes of RBCs are qualitatively seen to change (see SM Videos 3 and 4); a feature that would not be emphasized in a straight channel of similar cross section [47]. To quantify the role of the buffer solution on RBC flows within these devices, we have compared the hydrodynamic resistance of a suspension with an Hct level of 23% relative to that at nearly 0%. Within a fixed geometry, the relative resistance yields a first estimate of the relative viscosity of the RBC suspension and thus the influence of RBCs on the viscosity of the whole suspension.

One of the key differences between the examined buffers lies in their intrinsic viscosities. As anticipated for ~0% Hct, the measured slopes of the pressure-flow relationships reflect the buffer viscosities independently of the examined device configurations (Figs. 2a and 3a); a known feature of Newtonian fluids. This latter property is quantitatively captured by the ratio of the hydrodynamic resistances for Dex-40 and PBS, i.e. ~4.61 and ~3.98 in the SC and Net model, respectively, compared with $\mu_{\text{Dex-40}}/\mu_{\text{PBS}} \sim 4.44$. For 23% Hct suspensions, RBCs affect differently outcomes of the pressure-flow relationships (i.e. slopes). On the one hand, when Dex-40 is used the presence of RBCs is largely overshadowed by the ‘background’ viscosity of the buffer ($R_{\text{Hct}=23\%}/R_{\text{Hct}\sim 0} \sim 1.05\text{--}1.13$), both for the SC and Net models (Fig. 4, left two columns); namely, the relative viscosity increases by only 13% within the networks and a mere 5% within the straight channel. We recall that a Hct level of 23% is considered relatively high for microfluidics-based blood flow assays [15,17,23]. In turn, for lower Hct levels commonly used in the literature [16–23,48], the impact of RBCs to the overall viscosity is anticipated to be further reduced.

In contrast when PBS is used as the ‘background’ buffer, the influence of RBCs is suddenly brought to light following our estimations of the relative apparent viscosity ($R_{\text{Hct}=23\%}/R_{\text{Hct}\sim 0} \sim 1.64\text{--}1.88$; Fig. 4, right two columns). Specifically, the presence of RBCs now increases the relative viscosity by 88% within the microfluidic networks and 64% within the straight channel. These results underline how much the addition of RBCs increases the suspension viscosity. We note that for both suspensions the relative viscosity is indeed higher in the Net models, where significant confinement and deformation of RBCs in such micro-environments are known to arise and have been recently characterized [42].

Overall, our quantitative efforts emphasize that for *in vitro* microflow assays selecting the proper background buffer solution can influence ensuing flow outcomes of RBC suspensions and must thus be accounted. On the one hand, our results imply that for application-driven assays and lab-on-chip technologies such as cell separation [49–51], both Dex-40 and PBS-based suspensions may both be suitable and thereby somewhat freely selected. In contrast, if the scope of the study is focused on mimicking more closely *in vivo* conditions, selecting an appropriate buffer is significant including for example in the context of velocimetry measurements [20,23]. In particular, for microfluidic-based assays conducted in small channels (well below <300 μm that fall within the Fahraeus-Lindqvist regime) or alternatively within network arrays where characteristic length scales are similar to or approach the size of individual RBCs, our results underscore how PBS is anticipated to be a

more appropriate buffer in an effort to preserve a ‘background’ viscosity (see Table 1) that mimics more closely physiologically- and rheologically-realistic conditions for flows exhibiting strong RBC confinement. As a final remark, we note that within the scope of this technical note our measurements are limited to an Hct level of 23% such that measurements at higher Hct levels would be required to investigate whether the trends observed here in the differences in relative viscosity between buffers (i.e. Dex-40 vs. PBS) are further emphasized under microflow confinement.

5 Conclusions

In this technical note, we have reported on the influence of buffer suspensions (i.e. Dex-40 vs. PBS) on the flow of RBCs where measurements are conducted within two distinct microfluidic setups highlighting different levels of micro-confinement. To date, the question of selecting an appropriate ‘background’ buffer and its impact on microfluidic-based assays of RBC suspensions has often been overlooked or simply not discussed. By systematically investigating pressure-flow rate relationships to extract the equivalent hydrodynamic resistances and estimate relative viscosities, our efforts represent to the best of our knowledge a first step towards establishing end-user guidelines in purposefully selecting buffer suspensions for *in vitro* microfluidic blood flow assays.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic layout of the microfluidic devices illustrating the geometries of the straight channel (SC) and the network array (Net); flow is from left to right. (a) The straight channel holds a square cross section of $50\mu\text{m} \times 50\mu\text{m}$ ($w \times h$) and is 1 cm in length. (b) The microfluidic network is composed of a repeating lattice of circular posts arranged in a staggered array across the domain (dimensions shown). Note that the dashed red rectangle is discussed in the inset (d). (c) Instantaneous snapshot of an RBC suspension diluted in Dex-40 (Hct= 23%) flowing in a microfluidic SC model ($P= 0.2\text{ kPa}$); see SM Video 1 for the original movie and SM Video 2 for the corresponding flow of an RBC suspension diluted in PBS (Hct= 23%). (d) Instantaneous snapshot of an RBC suspension diluted in Dex-40 (Hct= 23%) flowing across the Net ($P= 2\text{ kPa}$); see SM Video 3 for the original movie and SM Video 4 for the corresponding flow of an RBC suspension diluted in PBS (Hct= 23%). The Net holds a fixed height of $10\mu\text{m}$ with a distance between neighbouring

posts of 10 μm (see arrows). The repeating lattice is hexagon-shaped with 6 circular posts and an additional one in the centre.

Fig. 2.

Pressure drop (ΔP) as a function of flow rate (Q) measured across the straight channel (SC) for RBC suspensions in Dex-40 (black) and PBS (red), shown at two haematocrit levels (Hct $\sim 0\%$ and 23%). Data are fitted with linear trends where slopes correspond to the equivalent hydrodynamic resistance for a specific RBC suspension flowing in the SC (for all cases $r^2 > 0.97$). (b) Histograms of the corresponding mean (and standard deviation) hydrodynamic resistances (R) extracted from the linear fits shown in (a), for each RBC suspension. The equivalent hydrodynamic resistance for RBC suspensions in Dex-40 is significantly higher than that in PBS, in particular for 23% Hct compared to $\sim 0\%$ Hct. * describes $p < 0.001$ following a Student t -test for normally distributed data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3.

Pressure drop (ΔP) as function of flow rate (Q) measured across the microfluidic network array (Net) for RBC suspensions in Dex-40 (black) and PBS (red) suspensions, shown at two haematocrit levels (Hct $\sim 0\%$ and 23%). Data are fitted with linear trends where slopes correspond to the equivalent hydrodynamic resistance for a specific RBC suspension flowing across the Net (for all cases $r^2 > 0.98$). (b) Histograms of the corresponding mean (and standard deviation) hydrodynamic resistances (R) as extracted from the linear curves shown in (a), for each RBC suspension. The equivalent hydrodynamic resistance for RBC suspensions in Dex-40 is significantly higher than that in PBS, in particular for 23% Hct compared to $\sim 0\%$ Hct. Note that the magnitudes of R (on y-axis) are significantly larger than those shown for the SC models (Fig. 2). * describes $p < 0.001$ following a Student t -test for normally distributed data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4.

Ratio of mean hydrodynamic resistances (R) (and SD) for 23% Hct RBC suspension compared to ~0% Hct, for Dex-40 (black) and PBS (red). For a given geometry (i.e. SC or Net), this ratio indicates a measure of the relative viscosity of the RBC suspension. Results show that it is significantly lower for Dex-40 than for PBS. Moreover, this ratio is much larger in the microfluidic network array (Net) compared with the straight channel (SC), emphasizing the impact of RBC suspensions flowing within a domain where confinement (10 μm) is on a scale similar to individual RBC sizes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Comparative summary between properties of Dextran 40 and PBS solutions commonly used in (microfluidic) *in vitro* experiments for RBC suspensions.

Dextran 40	PBS
Non-toxic glucose polymer-based plasma substitute established in 1950 [28].	Water based salt solution.
Antithrombogenic.Prevents aggregation.	
$\mu \sim 4$ cP	$\mu = 0.888$ cP
$\rho = 1.04$ [g/mL]	$\rho = 0.995$ [g/mL]
295 mOsm/kg	283 mOsm/kg
Sedimentation of RBCs is relatively slow.	Sedimentation of RBCs is relatively quick.
Suspension can be operated for longer times.	Demands occasional mixing or stirring of suspension.