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Technical note

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 rational 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.

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 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 (μ~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

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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]. Antithrombogenic.Prevents aggregation.	Water based salt solution.
$\mu \sim 4$ 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.

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 rational 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 µm x 50 µm, and (ii) a network array with a characteristic cross section (10 µm x 10 µm) similar to the size of individual RBCs (~7 µm). 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 (~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 µm x 50 µm (w x h) with a length of 1 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 µm and arranged in a staggered array (Fig. 1b); the height of the circular posts is constant and fixed at 10 µm 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 $w = 120$ µm 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).

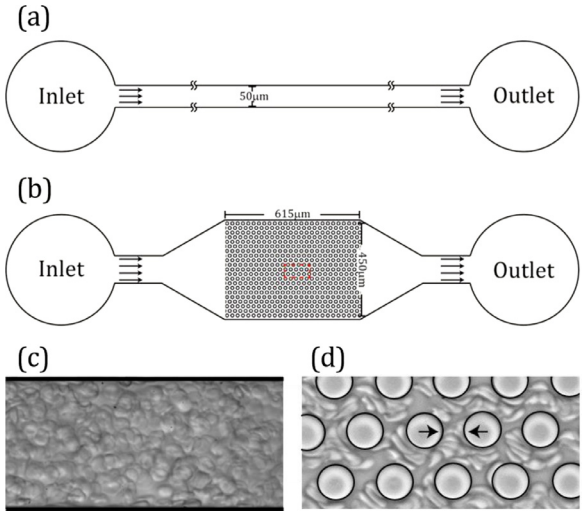

Fig. 1. Schematic layout of the microfluidic devices illustrating the geometries of the straight channel (SC) and the network array (Net); flow is from left to right. (a) The straight channel holds a square cross section of 50 µm x 50 µm (w x h) and is 1 cm in length. (b) The microfluidic network is 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 (ΔP= 0.2 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 (ΔP= 2 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 µm 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.

2.2. Blood preparation

Whole blood was taken from healthy human volunteers. Plasma was removed by centrifugation (800 × g for 5 minutes, 22 °C) and discarded. Pelleted RBCs were re-suspended in 50 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 × g for 5 minutes, 25 °C) and adjusted to 0.01% and 23% haematocrit (Hct) by resuspending the RBCs in either PBS (283 mOsm/kg) or 10% Dex-40 (5 g:50 mL, 295 mOsm/kg) where suspensions are considered isotonic [43,44]. All

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