Haemorheology of dense suspension of red blood cells under oscillatory shear flow

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We present a numerical analysis of the rheology of a suspension of red blood cells (RBCs) for different volume fractions in a wall-bounded, effectively inertialless, oscillatory shear flow. The RBCs are modeled as biconcave capsules, whose membrane is an isotropic and hyperelastic material following the Skalak constitutive law, and the suspension examined for a wide range of applied frequencies. The frequency-dependent viscoelasticity in the bulk suspension is quantified by the complex viscosity, defined by the amplitude of the particle shear stress and the phase difference between the stress and shear. Our numerical results show that deformations of RBCs weakly depend on the shear frequency, and the normal stress differences, membrane tension and amplitude of the shear stress are reduced by the oscillations. The frequency-dependent complex viscosity is nevertheless consistent with the classical behavior of non-Newtonian fluids, where the real part of the complex viscosity \( \eta' \) decreases as the frequency increases, and the imaginary part \( \eta'' \) exhibit a maximum value at an intermediate frequency. Such local maximum frequency is the same in both dense and dilute conditions. The effect of the viscosity ratios between the cytoplasm and plasma, volume fractions of RBCs, and oscillatory amplitudes represented by a capillary number on the complex viscosity are also assessed.

Key words: red blood cell, suspension rheology, oscillatory shear flow, computational biomechanics.

1. Introduction

Human blood is a dense suspension consisting of 55% plasma (which is typically assumed Newtonian), and 45% blood cells, with over 98% of the cells being red blood cells (RBCs), which are non-spherical deformable particles. Hence, the bulk rheology of human blood, modelling hydrodynamic interactions among RBCs from the cellular

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to the macroscale level, possesses a non-Newtonian character, the well-known shear-thinning behaviour (Chien et al. 1967; Chien 1970; Cokelet & Meiselman 1968; Goldsmith & Skalak 1975). RBC deformability highly complicates the problem, being key to determining the haemorheology. Indeed, the shear-thinning character of the blood disappears when RBCs are hardened (Chien et al. 1967; Chien 1970), with the blood suspension exhibiting an almost Newtonian behaviour and higher viscosity compared to a suspension of normal RBCs. Researchers have attempted to relate the rheological nature of the blood to the dynamics of RBCs, starting from experimental observations of the individual cell behaviours under controlled flows, usually simple steady shear flows, e.g., Schmid-Schönbein & Wells (1969); Fischer et al. (1978); Abkarian et al. (2007); Lanotte et al. (2016). More recently, numerical simulations have been also adopted to investigate the motion of RBCs at different steady shear rates; in particular, it was found that RBCs experience transitions from rolling/tumbling motions to kayaking (or oscillating/swinging) and swinging, following tank-treading motions as the shear rate increases for a wide range of ratios between the internal and external fluid viscosity (≈ 0.1–10) (Cordasco et al. 2014; Sinha & Graham 2015; Lanotte et al. 2016; Takeishi et al. 2019b). In our previous numerical analysis, we have related the aforementioned single-cell behaviour to the bulk suspension rheology, and successfully demonstrated the shear-thinning character of blood flow examining the cellular-scale dynamics under steady shear (Takeishi et al. 2019b).

In real human blood, RBCs are constantly under mechanical stimulation from the plasma flow due to the heart beat (~ 1 Hz) and from the vessel walls in various organs. It is also known that RBCs travel in the body as an oxygen carrier, and easily alter their shapes to pass through 3-to-4 µm capillaries (Skalak & Branemark 1969), where the upper limit of shear stress in the human circulatory system has been estimated to be 15 Pa (Koutsiaris et al. 2013). However, when they travel through artificial blood pumps, the cells may experience much higher shear stresses, up to 1000 Pa (= 10^4 dynes/cm^2) (Deutsch et al. 2006). From a physiological viewpoint, the relationship between the deformation, as a mechanical response to oscillatory loading, and the oxygen transport, as a biological function, is therefore of great interest, and several studies have shed some light on this (Parthasarathi & Lipowsky 1999; Wei et al. 2016; Zhou et al. 2019). The rheological description of blood under oscillatory flow is thus of fundamental importance not only for a physiological understanding, but also in the design of novel artificial blood pumps that minimise mechanical stimuli that may cause the rupture of RBCs, the so-called hemolysis. The first question in this study is, therefore, whether the cell deformation can be reduced or enhanced by varying the oscillatory frequency. Although the recovery of RBCs under oscillatory flow has been investigated in the past with experimental observations (Nakajima et al. 1990; Watanabe et al. 2006), and model analysis (Noguchi 2010; Li et al. 2014; Cordasco & Bagchi 2016; Zhu & Asaro 2019), much is still unknown, especially in relation to the bulk suspension rheology of RBCs under oscillatory shear flow. Hence, our second question is how the bulk suspension rheology of RBCs differs in an oscillatory shear flow with respect to the steady case.

The linear mechanical response of soft materials to weak oscillatory shear strain γ(t) is generally frequency dependent and viscoelastic, so that the oscillatory stress τ can be expressed with the help of a complex shear modulus \( G^* \): \( \tau(t) = G^* \gamma(t) \) (Squires & Mason 2010; Mewis & Wagner 2012). The complex modulus \( G^* \) can be decomposed into its two components, \( G^* = G' + iG'' \), where the real part \( G' \) is the storage modulus representing the elastic component of the stress, and the imaginary part \( G'' \) is the loss modulus representing the viscous dissipative part (Squires & Mason 2010; Mewis & Wagner 2012). Traditional (macroscopic) rheometers enable direct mechanical measurements of the
frequency dependent $G^*$. To cite few relevant examples, Mason & Weitz (1995) extracted the rheological properties from the thermal motion of colloidal probes embedded within the material, whereas Wagner (1993) and Lionberger & Russel (1994) calculated $G'$ at high frequency in hard-sphere dispersions from theory, and compared the results to the experiments by Shikata & Pearson (1994). Although numerical simulations allow us to quantify the dynamical viscoelasticity by calculating the complex moduli, the analysis of deformable particle suspensions is still a challenge because the hydrodynamic coupling among the particles, their deformation and the solvent motion must all be taken into account (Wagner 1993; Lionberger & Russel 1994).

Some numerical analyses of (semi-)dilute or jammed particle suspensions under oscillatory shear flow have been reported recently, e.g., rigid spherical particles at finite inertia (Villone et al. 2019, 2021), soft particle glasses (Khabaz et al. 2018), bubbles (Lin et al. 2019), and spherical capsule in dilute condition (Matsunaga & Imai 2020). The recent theoretical work by Armstrong et al. (2018) compared viscoelastic moduli obtained with several viscoelastic(-thixotropic) models and laboratory measurements under oscillatory shear flow. Matsunaga & Imai (2020) systematically investigated the effect of viscosity ratio on the viscoelastic character of capsule suspension for a wide rage of oscillatory shear rate frequencies. By employing continuum modeling, in particular the Oldroyd 8-constant framework, Saengow et al. (2019) assessed the non-Newtonian character of human blood under uni-directional large-amplitude oscillatory shear (LAOS) flow, which is generated by superposing LAOS onto a steady shear flow. Clarifying the cellular-scale dynamics under oscillatory flow allows us to build precise continuum models of suspensions (Oldroyd 1958; Anand et al. 2013; Alves et al. 2021), and may lead us to novel biomedical applications (Mutlu et al. 2018), such as phenotype cell screening, and circulating tumor cell isolation in a chip (Martel & Toner 2014). However, this can be achieved only after fully grasping the effect of the particle deformations induced by the oscillations of the bulk suspension.

Therefore, the objective of this study is to clarify the relationship between the behaviour of individual RBCs under small (SAOS) and large (LAOS) amplitude oscillatory shear flows and the viscoelastic character of dense suspension of RBCs. The contribution of the individual deformed RBC to the bulk suspension rheology is quantified by the stresslet tensor (Batchelor 1970). Here, the RBC is modeled as a biconcave capsule, whose membrane follows the Skalak constitutive law (Skalak et al. 1973). The internal and external fluid of the RBCs are modeled as Newtonian fluids and solved by the lattice-Boltzmann method, while the membrane mechanics with a finite-element method. The fluid and structure are fully coupled by an immersed boundary method (Peskin 2002). We resort to GPU computing to speed-up the above-mentioned numerical procedure. Note that the same tools have been successfully applied to the analysis of the bulk suspension rheology of RBCs under steady shear flow in Takeishi et al. (2019b).

The remainder of this paper is organised as follows. Section 2 gives the problem statement and numerical methods. Section 3 presents the numerical results for dense suspensions of RBCs, and the effect of parameters such as viscosity ratio $\lambda$, capillary number, and volume fraction of RBCs on the viscoelastic behaviour, characterised by the storage and loss moduli. Section 4 presents a discussion of the main findings, followed by a summary of the main conclusions.
2. Problem statement

2.1. Flow and cell models

We consider a cellular flow consisting of plasma and RBCs with radius $a_0$ in a rectangular box of size $16a_0 \times 10a_0 \times 16a_0$ along the span-wise $x$, wall-normal $y$, and stream-wise $z$ directions, with a resolution of 16 fluid lattices per radius of RBC. The size of the domain and the numerical resolution have been justified in our previous works (Takeishi et al. 2014, 2019b). Periodic boundary conditions are imposed on the two homogeneous directions ($x$ and $z$ directions). The fluid is modeled as an incompressible, Newtonian viscous fluid. Each RBC is modeled as a biconcave capsule, i.e., a Newtonian fluid enclosed by a thin elastic membrane, with a major diameter of $8 \mu m$ ($= 2a_0$), and maximum thickness of $2 \mu m$ ($= a_0/2$). The initial shape of the RBC is set to be the classical biconcave shape. A sketch of the computational domain, with the coordinate system used, is shown in figure 1 with an instantaneous visualisation of one of the dense cases, volume fraction $\phi = 0.41$.

The oscillatory shear flow is generated by moving the top and bottom walls located at $y = \pm H/2$, where $H$ ($= 10a_0$) is the domain height. The snapshot depicts a dense suspension of RBCs ($\phi = 0.41$) with viscosity ratio $\lambda = 5$ and capillary number $Ca_0 = 0.05$. Here, $\gamma_0$ is the shear-rate amplitude and $f$ its frequency. The oscillatory strain $\gamma(t)$ is therefore defined as:

$$\gamma(t) = \gamma_0 \exp(i2\pi ft).$$

(2.1)

Here, $\gamma_0$ is the shear-rate amplitude and $f$ its frequency. The oscillatory strain $\gamma(t)$ is therefore defined as

$$\gamma(t) = \int \dot{\gamma}(t) dt = \frac{\dot{\gamma}_0}{i2\pi f} \exp(i2\pi ft) = -i\gamma_0 \exp(i2\pi ft),$$

(2.2)

where $\gamma_0 (= \dot{\gamma}_0/(2\pi f))$ is the strain amplitude. For the following analysis, we define the non-dimensional input frequency as $f_m = f/\gamma_0$.

The membrane is modeled as an isotropic and hyperelastic material. The surface deformation gradient tensor $F_s$ is given by

$$dx_m = F_s \cdot dX_m,$$

(2.3)

where $X_m$ and $x_m$ are the membrane material points of the reference and deformed
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states, respectively. The local deformation of the membrane can be measured by the Green–Lagrange strain tensor

\[ \mathbf{E} = \frac{1}{2}(\mathbf{C} - \mathbf{I}) , \] (2.4)

where \( \mathbf{I} \) is the tangential projection operator. The two invariants of the in-plane strain tensor \( \mathbf{E} \) can be given by

\[ I_1 = \lambda_1^2 + \lambda_2^2 - 2 , \quad I_2 = \lambda_1^2 \lambda_2^2 - 1 = J_s^2 - 1 , \] (2.5)

where \( \lambda_1 \) and \( \lambda_2 \) are the principal extension ratios. The Jacobian \( J_s = \lambda_1 \lambda_2 \) indicates the ratio of the deformed to the reference surface areas. The elastic stresses in an infinitely thin membrane are replaced by elastic tensions. The Cauchy tension \( \mathbf{T} \) can be related to an elastic strain energy per unit area, \( w_s(I_1, I_2) \):

\[ \mathbf{T} = \frac{1}{J_s} \mathbf{F}_s \cdot \frac{\partial w_s(I_1, I_2)}{\partial \mathbf{E}} \cdot \mathbf{F}_s^T , \] (2.6)

where the strain energy function \( w_s \) satisfies the Skalak (SK) constitutive law (Skalak et al. 1973)

\[ w_s = \frac{G_s}{4} \left( I_1^2 + 2I_1 - 2I_2 + C I_2^2 \right) . \] (2.7)

In the expression above, \( G_s \) is the surface shear elastic modulus, and \( C \) a coefficient representing the area incompressibility. Substituting equation (2.7) into equation (2.6), we can obtain the principal tensions in the membrane \( T_1 \) and \( T_2 \) (with \( T_1 \geq T_2 \)):

\[ T_1 = \frac{G_s \lambda_1}{\lambda_2} \left[ \lambda_2^2 - 1 + C \left( \lambda_1^2 \lambda_2^2 - 1 \right) \right] , \quad \text{(likewise for } T_2) . \] (2.8)

In this study, we set \( G_s = 4 \mu \text{N/m} \) and \( C = 10^2 \). Bending resistance is also considered (Li et al. 2005), with a bending modulus \( k_b = 5.0 \times 10^{-19} \text{J} \) (Puig-de-Morales-Marinkovic et al. 2007); these values have been shown to successfully reproduce the deformation of RBCs in shear flow and the thickness of the cell-depleted peripheral layer (Takeishi et al. 2014).

The internal (cytoplasm) and external (plasma) fluids are treated as Newtonian viscous fluids, which obey the incompressible Navier–Stokes equations. It is known that the usual distribution of hemoglobin concentration in individual RBCs ranges from 27 to 37 g/dL corresponding to the internal fluid viscosity being \( \mu_1 = 5–15 \text{cP} \) (Mohandas & Gallagher 2008), while the normal plasma viscosity is \( \mu_0 = 1.1–1.3 \text{cP} \) at \( 37^\circ \text{C} \) (Harkness & Whittington 1970). Hence, physiologically relevant values of the viscosity ratio lie in the range \( \lambda = \mu_1/\mu_0 = 4.2–12.5 \) if the plasma viscosity is set to be \( \mu_0 = 1.2 \text{cP} \). The viscosity ratio \( \lambda = 5 \) is adopted for most of the results presented in this study, except when specifically investigating the role of \( \lambda \) on the suspension rheology. In this case, the range \( \lambda = 0.1–10 \) is investigated.

In the inertialess limit, the problem is characterised by the capillary number,

\[ Ca_0 = \frac{\mu_0 U_c}{G_s} = \frac{\mu_0 \gamma_0 a_0}{G_s} , \] (2.9)

in addition to the non-dimensional input frequency \( f_m = f / \gamma_0 \) and the viscosity ratio \( \lambda \) introduced above. The capillary number quantifies the relative importance of elastic and viscous forces, with highly deformable cells characterised by large values of \( Ca_0 \). Note that, to limit the computational cost and yet obtain results not affected by inertial effects, we set the Reynolds number \( Re = \rho \gamma_0 a_0^2 / \mu_0 = 0.2 \), where \( \rho \) is the plasma density. This value well reproduces the capsule dynamics in unbounded shear flows obtained with
In this study, a relatively small $Ca_0 (= 0.05)$ is used to investigate the small amplitude oscillatory shear (SAOS) flow regime; this value is much smaller than typical wall-shear rates of 333 s$^{-1}$ (Koutsiaris et al. 2013) corresponding to $Ca_0 = 0.4$. To provide some insight on the effect of large amplitude oscillations on the results (LAOS), we also consider suspensions at $Ca_0 = 0.8$, corresponding to a typical arteriole wall shear rate of 670 s$^{-1}$ (Koutsiaris et al. 2007).

2.2. Numerical method

The finite-element method (FEM) is used to solve the weak form of the equations governing the inertialess membrane dynamics and obtain the load $q$ acting on the membrane:

$$\int_S \hat{u} \cdot q dS = \int_S \hat{\varepsilon} : T dS,$$

where $\hat{u}$ and $\hat{\varepsilon} = (\nabla_s \hat{u} + \nabla_s \hat{u}^T)/2$ are the virtual displacement and virtual strain, respectively. The in-plane elastic tension $T$ is obtained from the Skalak constitutive law, see equation (2.7).

The Navier–Stokes equations for internal and external fluids are discretised by the LBM based on the D3Q19 model (Chen & Doolen 1998; Dupin et al. 2007). In the LBM, the macroscopic flow is obtained by collision and streaming of hypothetical particles described by the lattice-Boltzmann-Gross-Krook (LBGK) equation (Bhatnagar et al. 1954), which is given as

$$f_i (x_f + c_i \Delta t, t + \Delta t) - f_i (x_f, t) = -\frac{1}{\tau} [f_i (x_f, t) - f_i^{eq} (x_f, t)] + F_i \Delta t,$$

where $f_i$ is the particle distribution function for particles with velocity $c_i (i = 0–18)$ at the fluid node $x_f$, $\Delta t$ is the time step size, $f_i^{eq}$ is the equilibrium distribution function, $\tau$ is the non-dimensional relaxation time, and $F_i$ is the external force applied from the membrane material points, obtained with the immersed boundary method (IBM) (Peskin 2002). The membrane node $x_m$ is updated by Lagrangian tracking with the no-slip condition, i.e.

$$\frac{dx_m}{dt} = v (x_m).$$

The explicit forth-order Runge–Kutta method is used for the time-integration.

Note that, in our coupling of LBM and IBM, the hydrodynamic interaction between individual RBCs is solved without modeling a non-hydrodynamic inter-membrane repulsive force in the case of vanishing inertia. The volume-of-fluid (VOF) method (Yokoi 2007) and front-tracking method (Unverdi & Tryggvason 1992) are employed to update the viscosity in the fluid mesh. A volume constraint is implemented to counteract the accumulation of small errors, leading to changes in the volume of the individual cells (Freud 2007): in our simulation, the volume error is always maintained lower than $10^{-3}\%$. All numerical procedures are fully implemented on graphics processing unit (GPU) to accelerate the numerical simulation. For further details of the methods we refer to our previous work (Takeishi et al. 2019b). The mesh size of the LBM for the fluid solution is set to be 250 nm, and that of the finite elements describing the membrane is also approximately 250 nm (an unstructured mesh with 5,120 elements is used for each cell). This resolution has been shown to successfully represent single- and multi-cellular dynamics (Takeishi et al. 2014); we have verified that the results of
multi-cellular dynamics are not changing with twice the resolution for both the fluid and membrane (see also Takeishi et al. 2014).

2.3. Analysis of capsule suspensions

For the following analysis, the behaviour of RBCs subjected to oscillatory shear flow is quantified by the length of the semi-major axis \( a_{\text{max}} \) and orientation angle \( \theta \) between the major axis of the deformed RBC and the shear direction. The length of the semi-major axis \( a_{\text{max}} \) of the deformed RBC is obtained from the eigenvalues of the inertia tensor of an equivalent ellipsoid approximating the deformed RBC (Ramanujan & Pozrikidis 1998). Note that, the orientation angle \( \theta \) of a single deformable spherical capsule converges to \( \pi/4 \) in shear flow as \( Ca \to 0 \) (Barthes-Biesel 1980; Barthes-Biesel & Sgaier 1985).

The suspension rheology of RBCs, or the contribution of the suspended RBCs to the bulk viscosity, is quantified by the particle stress tensor \( \Sigma^{(p)} \) (Batchelor 1970). Specifically, for a deformable capsule and any viscosity ratio, Pozrikidis (1992) analytically derived an expression for the corresponding stresslet, so that the particle contribution to the total stress can be written as:

\[
\Sigma^{(p)} = \frac{1}{V} \sum_{i=1}^{N} S_i, \quad \text{(2.13)}
\]

\[
= \frac{1}{V} \sum_{i=1}^{N} \int_{A_i} \left[ \frac{1}{2} (r \hat{q} + \hat{q} r) - \mu_0 (1 - \lambda) (vn + nv) \right] dA_i, \quad \text{(2.14)}
\]

where \( V \) is the volume of the domain, \( S_i \) the stresslet of the \( i \)-th RBC (or capsule), \( r \) is the membrane position relative to the centre of the RBC, \( \hat{q} \) the load acting on the membrane including the contribution from the bending rigidity, \( \mu_0 \) the outer fluid (plasma) viscosity, \( v \) the interfacial velocity of membrane, and \( A_i \) the membrane surface area of the \( i \)-th RBC. Here, the suspension shear viscosity \( \mu_{\text{all}} (= \mu_0 + \delta \mu) \) is represented by the viscosity \( \mu_0 \) of the carrier fluid (plasma) and a perturbation \( \delta \mu \). This leads to the introduction of the relative viscosity \( \mu_{\text{re}} \) and of the specific viscosity \( \mu_{\text{sp}} \) defined as:

\[
\mu_{\text{re}} = \frac{\mu_{\text{all}}}{\mu_0} = 1 + \mu_{\text{sp}}, \quad \text{(2.15)}
\]

\[
\mu_{\text{sp}} = \frac{\delta \mu}{\mu_0} = \frac{\Sigma^{(p)}}{\mu_0 \gamma_0}, \quad \text{(2.16)}
\]

where the subscript 1 represents the streamwise direction (i.e., the \( z \)-direction in this study) and the subscript 2 the wall-normal direction (i.e., the \( y \)-direction in this study). Using the diagonal components of the particle stress tensor \( \Sigma_{ii}^{(p)} \), the first and second normal stress differences, typically used to quantify the suspension viscoelastic behaviour, can be defined as:

\[
\frac{N_1}{\mu_0 \gamma_0} = \frac{\Sigma_{11}^{(p)} - \Sigma_{22}^{(p)}}{\mu_0 \gamma_0}, \quad \text{(2.17)}
\]

\[
\frac{N_2}{\mu_0 \gamma_0} = \frac{\Sigma_{22}^{(p)} - \Sigma_{33}^{(p)}}{\mu_0 \gamma_0}. \quad \text{(2.18)}
\]

In the case of small oscillations, the particle stress \( \Sigma_{12}^{(p)}(t) \) is also an oscillatory function, defined by the amplitude \( |\Sigma_{12}^{(p)}|^{\text{amp}} \) and the phase difference \( \delta (-\pi/2 \leq \delta \leq 0) \) between
The non-dimensional time \( \dot{\gamma}(t) \) and the input shear rate \( \dot{\gamma}(t) \):

\[
\Sigma_{12}^{(p)}(t) = |\Sigma_{12}^{(p)}|^{\text{amp}} \exp \{i(2\pi ft + \delta)\},
\]

where \( |\Sigma_{12}^{(p)}|^{\text{amp}} \) is the amplitude of oscillatory particle stress \( \Sigma_{12}^{(p)} \). Following classical rheological analyses, the frequency-dependent rheology of particle suspensions is expressed in terms of a complex modulus \( G^* \) and a complex viscosity \( \eta^* \). Using the fluid strain \( \gamma(t) \), \( G^* \) is given by:

\[
G^* = \frac{\Sigma_{12}^{(p)}(t)}{\gamma(t)} = \frac{i|\Sigma_{12}^{(p)}|^{\text{amp}}}{\gamma_0} \exp (i\delta) = \frac{2\pi|\Sigma_{12}^{(p)}|^{\text{amp}}}{\gamma_0} (\sin \delta + i \cos \delta) = G' + iG'',
\]

where \( G' \) is the storage modulus and \( G'' \) is the loss modulus defined as:

\[
\begin{align*}
G' &= -\frac{2\pi|\Sigma_{12}^{(p)}|^{\text{amp}}}{\gamma_0} \sin \delta, \\
G'' &= \frac{2\pi|\Sigma_{12}^{(p)}|^{\text{amp}}}{\gamma_0} \cos \delta.
\end{align*}
\]

Using the applied shear \( \dot{\gamma}(t) \), instead, one can introduce a complex viscosity \( \eta^* \), given by:

\[
\eta^* = \frac{\Sigma_{12}^{(p)}(t)}{\dot{\gamma}(t)} = \frac{|\Sigma_{12}^{(p)}|^{\text{amp}}}{\dot{\gamma}_0} \exp (i\delta) = \frac{|\Sigma_{12}^{(p)}|^{\text{amp}}}{\dot{\gamma}_0} (\cos \delta + i \sin \delta) = \eta' - i\eta'',
\]

where \( \eta' \) and \( \eta'' \) are defined as:

\[
\begin{align*}
\eta' &= \frac{|\Sigma_{12}^{(p)}|^{\text{amp}}}{\dot{\gamma}_0} \cos \delta = \frac{G''}{2\pi\dot{\gamma}_0}, \\
\eta'' &= -\frac{|\Sigma_{12}^{(p)}|^{\text{amp}}}{\dot{\gamma}_0} \sin \delta = \frac{G'}{2\pi\dot{\gamma}_0}.
\end{align*}
\]

In the following analysis, \( \eta' \) and \( \eta'' \) are normalised by \( \mu_0 \), and hence, the moduli are rewritten as:

\[
\begin{align*}
\frac{\eta'}{\mu_0} &= |\mu_{\text{amp}}| \cos \delta, \\
\frac{\eta''}{\mu_0} &= -|\mu_{\text{amp}}| \sin \delta,
\end{align*}
\]

where \( |\mu_{\text{amp}}| = |\Sigma_{12}^{(p)}|^{\text{amp}}/(\mu_0\dot{\gamma}_0) \) is the magnitude of the specific viscosity.

The oscillations are examined after the fully-developed regime has been reached. The dominant frequency for input \( \dot{\gamma}(t) \) and output amplitude \( \mu_{\text{amp}}(t) \) are obtained with a discrete Fourier transform (DFT), using the Fastest Fourier Transform in the West (FFTW) library. Note that, the frequency peak of the output signal always corresponds to the frequency of the applied shear for all cases considered here. The details of the DFT analysis adopted in this study are reported in the appendix §A.1. To reduce the influence of the initial conditions, the DFT analysis and the spatial-temporal average start once the amplitude of \( \mu_{\text{amp}} \) became stable; as an example, for \( f_{\text{in}} = 0.1 \), the analyses start from the non-dimensional time \( \dot{\gamma}_0 t = 800 \), and continues for 12 periods (see figure 2c). For all the cases, one output wave is resolved by at least 100 discrete points, and over 10 wave periods are considered in the DFT analysis. The effect of the number of periods on the calculated values are discussed in the appendix §A.2. When presenting the results, we
will initially focus on the analysis of suspensions in physiological conditions, with volume fraction $\phi = 0.41$, $\lambda = 5$, and $Ca_0 = 0.05$, and later consider variations of the viscosity ratio, volume fraction and oscillation amplitudes.

3. Results

3.1. Dense suspension of RBCs under oscillatory shear flow

First, we investigate the oscillatory behaviour of dense suspension of RBCs under SAOS flow at $Ca_0 = 0.05$. A snapshot of the numerical results for the lowest input shear frequency investigated in this study $f_{in} = 0.01$ are shown in figure 2(a), while the time history of the specific viscosity $\mu_{sp}(t)$ and shear-rate $\dot{\gamma}(t)$ are displayed in figure 2(b), where the value of $\mu_{sp}$ obtained under steady shear flow is also displayed with dashed line for comparison. As expected, the magnitude of $\mu_{sp}$ at the lowest shear frequency $f_{in}$ is similar to that obtained under steady shear flow, and the output wave, $\mu_{sp}$, responds to the imposed shear, $\dot{\gamma}$, without significant delay. When the shear frequency $f_{in}$ increases, the resultant amplitude $|\mu_{sp}|^{amp}$ is reduced, as shown in figures 2(c) and 2(d). Furthermore, there is a clear phase delay between the $\mu_{sp}$ and $\dot{\gamma}$ oscillations, with the phase difference increasing as $f_{in}$ increases.

The frequency-dependent deformation and orientation angle $\theta$ of individual RBCs are investigated to understand their link to the flow rheology. The deformation is characterised by the maximal radius $a_{max}$, with the ratio $a_{max}/a_0$ chosen as a deformation index. The spatial-temporal averages of these two observables are shown in figures 3(a) and 3(b), respectively. The value of $\langle a_{max} \rangle/a_0$ is slightly non-monotonic, first growing till $f_{in} \approx 1$ and then decreasing. Especially for the middle range of $f_{in}$ ($0.05 \leq f_{in} \leq 1$), $\langle a_{max} \rangle/a_0$ is greater than in steady shear flow. However, the frequency-induced mean deformation differs by only 0.8% from that in steady shear flow even for the maximum $\langle a_{max} \rangle/a_0$ at $f_{in} = 0.5$ (figure 3a). The amplitude of the fluctuations around the mean keeps instead reducing as $f_{in}$ grows. The spatial-temporal averages of the orientation angle $\theta/\pi$ fluctuate around zero for all $f_{in}$, and thus, the values are lower than that obtained under steady shear flow, with no significant frequency dependence, as shown in figure 3(b).

The first and second normal stress differences $\langle N_i \rangle/\mu_0 \dot{\gamma}_0$ (with $i = 1$ and 2), the resultant amplitude $|\mu_{sp}|^{amp}$ and phase difference $\delta$ are shown in figures 3(c), 3(d) and 3(e) for the different frequencies under consideration. The magnitude of $|\langle N_i \rangle|/\mu_0 \dot{\gamma}_0$ decreases monotonically from that in the steady shear flow as $f_{in}$ increases, reaching nearly zero for the highest value of $f_{in}$. Note that $\langle N_1 \rangle/\mu_0 \dot{\gamma}_0$ and $\langle N_2 \rangle/\mu_0 \dot{\gamma}_0$ have positive and negative values at low frequencies and for zero shear. The error bars for $\langle N_i \rangle/\mu_0 \dot{\gamma}_0$ are displayed only on one side of the mean value for major clarity. $|\mu_{sp}|^{amp}$ also decreases from the value in steady shear flow as $f_{in}$ increases above the lowest value (figure 3d). $|\mu_{sp}|^{amp}$ only weakly changes in the intermediate range of $f_{in}$ ($0.05 \leq f_{in} \leq 0.5$), before decreasing again at higher $f_{in}$. The phase delay $\delta$ is also found to be a function of the shear frequency, as documented in figure 3(e), where we observe that $\delta$ decreases from almost zero at the lowest $f_{in}$ (= 0.01) until $f_{in} = 1$, and then increases for $f_{in} > 1$.

The frequency-dependent $|\mu_{sp}|^{amp}$ and $\delta$ define the complex viscosity, with real and imaginary part $\eta'$ and $\eta''$, which are reported in figure 3(f). The variations of $\eta'$ with $f_{in}$ well follow $|\mu_{sp}|^{amp}$, whereas $\eta''$ first increases, reaches a maximum at $f_{in} \approx 1$, and then decreases again for larger frequencies. While $\eta''$ almost coincides with $\eta'$ at large $f_{in}$, the increase of $\eta''$ at low $f_{in}$ can be well approximated by a power law: $\eta'' \propto f_{in}^{0.47}$. This result indicate that dense suspensions of RBCs cannot be modeled as Maxwell fluids, where
Figure 2. (a) Snapshot of a dense suspension of RBCs ($\phi = 0.41$) zooming on the central part of the rectangular computational box for the lowest shear frequency $f_{in} = 0.01$ under consideration. (b) Time history of the specific viscosity $\mu_{sp}$ and input shear rate $\dot{\gamma}/\dot{\gamma}_0$ under $f_{in} = 0.01$, where $\dot{\gamma}$ is normalised by the amplitude $\dot{\gamma}_0$. The result of $\mu_{sp}$ obtained under steady shear flow is also displayed as red dashed line. (c and d) Time history of $\mu_{sp}$ and $\dot{\gamma}$ for shear frequencies $f_{in} = (c) 0.1$ and (d) 1. The results are obtained with $\phi = 0.41$, $\lambda = 5$, and $Ca_0 = 0.05$.

the complex shear moduli $\eta'' (= G'/(2\pi f)) \propto f^2$ and $\eta' (= G''/(2\pi f)) \propto f$ for low $f$; on the other hand, the present results are similar to those obtained with the Oldroyd-B and Giesekus fluids in the absence of rigid particles (Villone et al. 2021, Fig. 2b).

3.2. Effect of the viscosity ratio

Next, we investigate the effect of the viscosity ratio $\lambda = (0.1, 1, 5$ and $10)$ on the system viscoelasticity for relatively low and high frequencies $f_{in} = (0.05$ and $0.5)$. The behavior of $\langle a_{max}/a_0 \rangle$ and $\langle \theta \rangle/\pi$ as a function of $\lambda$ is reported in figures 4(a) and 4(b). $\langle a_{max}/a_0 \rangle$ slightly increases with $\lambda$, similarly to what found in steady shear flows. The mean deformation of individual RBCs progressively increases with $f_{in}$, with only a slight increase at low $f_{in} (= 0.05)$, and a more significant one for $f_{in} = 0.5$, when comparing with the case of steady shear flow (figure 4a). On the other hand, $\langle \theta \rangle/\pi$ decreases under oscillatory shear flow, with no significant differences in the mean values for the different $\lambda$ considered (figure 4b). When the shear frequency is small ($f_{in} = 0.05$), the RBCs are able to attain a similar orientation (i.e., small standard deviations of $\langle \theta \rangle/\pi$), while when the shear frequency is large ($f_{in} = 0.5$), the ordered orientation is disrupted, as shown by the
Figure 3. (a) Spatial-temporal average of the deformation index $\langle a_{\text{max}} \rangle/a_0$, (b) orientation angle $\langle \theta \rangle/\pi$ between the major axis of the deformed RBC and the shear direction, (c) the first and second normal stress differences $\langle N_i \rangle/\mu_0 \dot{\gamma}_0$ ($i = 1$ and $2$), (d) amplitude of the specific viscosity $\mu_{sp}$, (e) phase difference $\delta$, and (f) complex viscosity $\eta'/\mu_0$ and $\eta''/\mu_0$ as a function of the input frequency $f_{in}$. The results obtained under steady shear flow are also displayed in (a)–(d) as dashed (or dash-dot) lines. The error bars in (a)–(c) represent standard deviations. The error bars in panel (c) are displayed only on one side of the mean value for major clarity. The results are obtained with $\phi = 0.41$, $\lambda = 5$ and $Ca_0 = 0.05$. 
Figure 4. (a) Spatial-temporal average of the deformation index $\langle a_{max}\rangle/a_0$, (b) orientation angle $\langle \theta \rangle/\pi$, (c) the first and second normal stress differences $\langle N_i \rangle/\mu_0\dot{\gamma}_0$ ($i = 1$ and 2), (d) amplitude $|\mu_{sp}|_{amp}$, (e) phase difference $\delta/\pi$, and (f) complex viscosity $\eta'/\mu_0$ and $\eta''/\mu_0$ as a function of viscosity ratio $\lambda$ ($= 0.1, 1, 5$ and $10$). The results obtained under steady shear flow at each $\lambda$ are also displayed in (a)–(d) as black dash-dot lines. The results are obtained with $\phi = 0.41$ and $Ca_0 = 0.05$ for relatively low and high shear frequencies, $f_{in} = 0.05$ and $f_{in} = 0.5$.

large value of standard deviations in figure 4b. This is consistent with the observations from the data for $\lambda = 5$ discussed above, cf. figure 3(b).

As also described before in figures 3(c) and 3(d), $|\langle N_i \rangle|/\mu_0\dot{\gamma}_0$ and $|\mu_{sp}|_{amp}$ decrease under oscillatory shear flow, and this behaviour is consistently observed also for the
different values of $\lambda$ under consideration (figures 4c and 4d). $\langle N_1 \rangle$ only slightly increases with $\lambda$ at low $f_{in}$, while it exhibits an opposite tendency at high $f_{in}$; furthermore, there are no significant variations in $\langle N_2 \rangle$ between high and low $f_{in}$. In particular, $|\mu_{sp}|^{amp}$ increases with $\lambda$, similarly to what observed for steady shear flow. For low viscosity ratios, $|\mu_{sp}|^{amp}$ progressively reduces with $f_{in}$, while for large viscosity ratios, it does not vary significantly with the frequency of the imposed shear $f_{in}$. The phase difference $\langle \delta \rangle/\pi$ instead does not vary significantly with the viscosity ratio for small values of $f_{in}$, while it changes significantly (i.e., the magnitude of $\delta$ decreases as $\lambda$ increases) for the large value of $f_{in}$ (see figure 4e). In other words, large values of the viscosity ratios help to reduce the time-lag between the input and output signal at high shear frequency observed at steady shear.

The complex viscosity is displayed in figure 4(f) as a function of $\lambda$ for the two values of $f_{in}$ under investigation. For all the values of the viscosity ratio, $\eta'$ well follows $|\mu_{sp}|^{amp}$, while $\eta''$ is only weakly dependent on $\lambda$. At the large $f_{in}$ (= 0.5), $\eta'$ overcomes $\eta''$ between $\lambda = 1$ and 5, while such crossover is not found at low $f_{in}$ (= 0.05). $\eta'$ is higher at the low $f_{in}$ than at the large one, while $\eta''$ follows an opposite trend, i.e., the value is higher at the large $f_{in}$ than at the low one.

Overall, the rheological behaviour of the RBC suspension in oscillating shear flows appears to be only weakly dependent of the viscosity ratio, except for the lower phase lag observed at high $\lambda$.

### 3.3. Effect of the volume fraction

We performed simulations in a dilute condition ($\phi = 0.11$) to investigate the behavior at low volume fractions, characterised by less cell-cell interactions. The results for different oscillation frequencies at $\phi = 0.11$ are compared to those at $\phi = 0.41$ in figure 5, following the same order as in figure 3.

The deformation $\langle a_{\max} \rangle/a_0$ in dilute conditions remains almost the same as in dense conditions, but with smaller fluctuations than at larger volume fractions, as indicated by the error bar (figure 5a). Although frequency-induced deformations are found in the intermediate range of $f_{in}$ ($0.0333 < f_{in} < 1$), all the variations with respect to the case of steady shear flow are always less than 1%. The mean orientation $\langle \theta \rangle/\pi$ and its fluctuations also remain similar to those in the dense suspension, as shown in figure 5(b), with $\langle \theta \rangle/\pi$ fluctuating around zero for all $f_{in}$, resulting in a lower average $\langle \theta \rangle/\pi$ than under steady shear flow.

The spatial-temporal average of the first and second normal stress differences $\langle N_i \rangle/\mu_0 \gamma_0$ ($i = 1$ and 2), the specific viscosity $|\mu_{sp}|^{amp}$ and of the phase shift $\delta$ are shown as a function of $f_{in}$ in figures 5(c), 5(d) and 5(e). As for the flow of dense suspensions, $\langle N_i \rangle/\mu_0 \gamma_0$ and $|\mu_{sp}|^{amp}$ decrease from the steady-shear flow value as $f_{in}$ increases. The values of $\langle N_i \rangle$ decrease by one order of magnitude from $\phi = 0.41$ to $\phi = 0.11$. $|\mu_{sp}|^{amp}$ drops very quickly from the steady state value, by about 50% already at $f_{in} = 0.05$; further increasing $f_{in}$, the specific viscosity remains almost constant before decreasing for higher frequencies, $f_{in} > 0.25$. The trend is similar to that observed in dense conditions, although the specific viscosity is clearly lower at low volume fraction. For both volume fractions under investigation, a first plateau with lower $|\mu_{sp}|^{amp}$ amplitude occurs at $f_{in} \approx 0.05$ before the final decay for high frequencies. On the other hand, the behavior of $\delta/\pi$ in the dilute condition is different from that in the dense suspension: in this case, $\delta/\pi$ monotonically decreases as $f_{in}$ increases, see panel (e) of figure 5. Because of this, at relatively high forcing frequencies, $f_{in} \gg 1$, the magnitude of $\delta/\pi$ becomes greater in the dilute condition than in the dense case (cf. figure 5e).

Finally, figure 5(f) reports the complex viscosity. The real part $\eta'$ again well follows
Figure 5. (a) Spatial-temporal average of the deformation index \( \langle a_{max} \rangle / a_0 \), (b) orientation angle \( \langle \theta \rangle / \pi \), (c) the first and second normal stress differences \( \langle N_i \rangle / \mu_0 \gamma_0 \) \((i = 1 \text{ and } 2)\), (d) amplitude \( |\mu_{sp}|^{amp} \), (e) phase difference \( \delta / \pi \), and (f) complex viscosity \( \eta' / \mu_0 \) and \( \eta'' / \mu_0 \) as a function of \( f_{in} \) for dilute \((\phi = 0.11)\) and dense \((\phi = 0.41)\) suspensions. The results obtained under steady shear flow are displayed in panels (a)–(d) with dashed lines. The results are obtained with \( Ca_0 = 0.05 \), and \( \lambda = 5 \).

The trend discussed for \( |\mu_{sp}|^{amp} \), whereas \( \eta'' \) attains its maximum value around \( f_{in} = 0.5 \) and then crosses over \( \eta' \) for larger frequencies. At low frequencies, we estimate \( \eta'' \) to increase with \( f_{in} \) following a power law with exponent 0.53, i.e., \( \eta'' \propto f_{in}^{0.53} \).

Overall, we note that the volume fraction clearly affects the mean specific viscosity in
SAOS flows; however the differences due to volume fraction reduce at higher oscillations frequencies. The other observables appear instead to be almost independent of the volume fraction. High frequency or dilute conditions decrease the rate of hydrodynamic interaction between RBCs, resulting in a reduced contribution of the RBCs to the suspension bulk properties, as observed for the normal stress differences.

3.4. Effect of the oscillatory amplitude

Lastly, we consider large-amplitude oscillatory flows (LAOS), characterised by a capillary number $Ca_0 = 0.8$, and quantify the effects of the oscillatory amplitude on the complex viscosity for different values of the fraction $\phi$. Snapshots of the suspension of RBCs subject to low and large amplitude oscillations, $Ca_0 = 0.05$ and 0.8, at $f_{in} = 0.5$ and for different volume fractions $\phi = 0.11$–0.41 are shown in figure 6(a). Also, the spatial-temporal average of the deformation $\langle a_{\max} \rangle/a_0$ and of the orientation angle $\langle \theta \rangle/\pi$ are shown in figures 6(b)–6(e) for both low $Ca_0 (= 0.05)$ and high $Ca_0 (= 0.8)$.

Starting from figure 6(b), $\langle a_{\max} \rangle/a_0$ for low $Ca_0 (= 0.05)$ as functions of $\phi$ and $f_{in}$, we note that the the deformation remains almost the same, independently of $f_{in} (= 0.05$ and 0.5) and $\phi$, close to the values obtained for steady shear flow (see the black dashed line in the figure). On the other hand, for high-amplitude oscillations, $Ca_0=0.8$, we observe a difference between the low and high $f_{in}$; in particular, $\langle a_{\max} \rangle/a_0$ is larger at $f_{in} = 0.05$ than at $f_{in} = 0.5$, also with larger variations around the mean (see error bars). We therefore observe a decrease of the deformation in the case of large amplitude oscillations, which become more and more apparent when increasing the RBC volume fraction. For the lowest value considered, $\phi = 0.11$, we do not observe significant variations of the average deformation, suggesting that the decrease of deformation with frequency is due to hydrodynamic interactions among the different cells.

In the results presented so far, $\langle \theta \rangle/\pi$ is found to fluctuate around zero, resulting in a lower value of $\langle \theta \rangle/\pi$ than that for steady shear flow. This is observed also when $f_{in}$, $\phi$, and $Ca_0$ change as shown in figures 6(d) and 6(e). The fluctuations of $\langle \theta \rangle/\pi$ are larger for high-frequency oscillatory shear, which is similar to the results for different viscosity ratios $\lambda$ presented in figure 4(b).

The spatial-temporal average of the specific viscosity $|\mu_{sp}|^{amp}$ and of the phase delay $\delta/\pi$ are compared for low $Ca_0 (= 0.05)$ and high $Ca_0 (= 0.8)$ in panels (a)–(d) of figure 7. As expected, the specific viscosity increases with the RBC volume fraction: $|\mu_{sp}|^{amp}$ is maximum for steady shear flow and decreases under oscillatory shear flow. For small amplitude oscillations, $Ca_0=0.05$, $|\mu_{sp}|^{amp}$ is almost independent of the frequency of the imposed shear $f_{in}$, at least for the range considered in this study, deemed relevant for physiological flows. For high-amplitude oscillations, $Ca_0=0.8$, the specific viscosity appears to decrease monotonically with $f_{in}$. For both low and high $Ca_0$, the frequency-dependent decrease of $|\mu_{sp}|^{amp}$ becomes more evident as $\phi$ increases. We can also note in the figure that the specific viscosity is lower for the largest value of the capillary number investigated at steady shear rate. As high $Ca_0$ corresponds to large deformability in the limit $f_{in} \rightarrow 0$, this result confirms the shear-thinning behaviour with deformability in the case of suspensions of deformable objects (Rosti et al. 2018; Rosti & Brandt 2018; Chiara et al. 2020).

As discussed when examining the results in figure 4(d), the magnitude of $\delta/\pi$ increases at high $f_{in}$ for every volume fraction $\phi$, independently of $Ca_0$ as shown in figures 7(c) and 7(d). Furthermore, the magnitude of $\delta/\pi$ also depends on $\phi$: at low $f_{in} (= 0.05)$, $|\delta/\pi|$ slightly increases as $\phi$ increases both for low and high $Ca_0$ (figures 7c and d). On the other hand, at high $f_{in} (= 0.5)$, $|\delta/\pi|$ decrease as $\phi$ increases, especially for large
Figure 6. (a) Snapshots from our numerical results of suspensions with different $\phi=0.11$ and 0.41 at imposed shear frequency $f_{in} = 0.5$. The top raw shows the data at the lowest $Ca_0 = 0.05$ and the bottom raw those at the highest $Ca_0 = 0.8$. (b and c) Spatial-temporal average of the deformation index $(a_{max})/\alpha_0$, and (d and e) orientation angle $(\theta)/\pi$ as function of $\phi$ for two values of $f_{in}$ (0.05 and 0.5). The left column (panels b, and d) displays the results for $Ca_0 = 0.05$, and the right column (c, and e) those for $Ca_0 = 0.8$. The results are obtained for viscosity ratio $\lambda = 5$. 
amplitude oscillations. Overall, the phase difference $|\delta/\pi|$ is largest for the lowest $\phi (= 0.11)$, high $f_{in} (= 0.5)$, and high $Ca_0 (= 0.8)$.

Finally, the complex viscosity is reported in figures 7(c) and figures 7(f) versus the volume fraction $\phi$ for the two values of $Ca_0$ under consideration. Consistently with the
results described so far, $\eta'$ follows the trend displayed by $|\mu_{sp}|^{anmp}$ for both high and low $Ca_0$; in particular, $\eta'$ is higher for low $Ca_0$ than for high $Ca_0$, confirming the shear thinning with deformability of elastic objects. Furthermore, $\eta'$ at low $f_{in}$ ($=0.05$) is always higher than at high $f_{in}$ ($=0.5$) for each $\phi$. This is also true for $\eta''$, which increases with $\phi$. Overall, conditions of small $Ca_0$ and high $f_{in}$ are those to lead to the largest $\eta''$.

3.5. Membrane tension under oscillatory shear flow

Finally, we also investigate the maximum in-plane principal tension $T_1 (\geq T_2)$ and the isotropic tension $T_{iso}(= T_1 + T_2)/2$ in the deformed RBC, and display the results in figure 8. We indicate the spatial-temporal average of those tensions as $\langle T_1 \rangle$ and $\langle T_{iso} \rangle$, which are computed using equation (2.8). It is known that these tensions monotonically increase with $Ca$, as observed in narrow rectangular microchannels (Takeishi et al. 2019a). Similarly to the normal stress differences $\langle N_i \rangle$, the values of $\langle T_i \rangle$ also decrease with $f_{in}$, independently of $\phi$ (figures 8a and 8b), clearly indicating the decrease of the RBC deformation and a reduction in their hydrodynamic interactions. Indeed, the magnitude of $|\langle T_i \rangle|$ decreases for all $f_{in}$, compared to those in a steady shear flow, with the maximum principal tension $(T_1)$ being much lower especially in the dilute condition ($\phi = 0.11$). A monotonically frequency-dependent decrease of the membrane tensions is also found for all $\phi$ both in SAOS ($Ca_0 = 0.05$) and LAOS ($Ca_0 = 0.8$) (figure 8c and 8d, respectively).

Finally, the state of membrane tension is further investigated for different viscosity ratios $\lambda$, the results shown in figure 8(e). Compared with the previous results of single RBC in Omori et al. (2012); Takeishi et al. (2019a), the similarities or discrepancies in the values of $\langle T_i \rangle$ for different $\lambda$ arise here from the different input frequencies $f_{in}$. The observations above about the reduction in membrane tension with the oscillations are valid also for the different values of $\lambda$ examined.

Overall, the input frequency results to be the main parameter affecting the tension of the RBC membrane. Although we could not observe any particular frequency-induced variation of the deformation of the RBCs in terms of the index $\langle a_{max} \rangle/a_0$, the membrane tension is strongly affected by the input frequency. Therefore, the full viscoelastic behaviour of RBC suspensions cannot be simply estimated by the geometrical properties of individual deformed RBCs, but it should include the state of the membrane tension, which appears to be strongly related to the normal stress difference behaviour (see figures 8a and 8b).

4. Conclusions

We have performed numerical simulations of dense suspension of RBCs under oscillatory shear for a wide rage of shear frequencies $f_{in}$, and quantified the viscoelastic character of the bulk suspension by its complex viscosity; this is defined in terms of the amplitude of the particle stress tensor and the phase difference between the output particle stress and the sinusoidal applied shear (see eqn. 2.24). The flow is assumed to be inertialess, with the fluids inside and outside the RBCs modeled as Newtonian. With the notation adopted, small capillary number $Ca_0$ corresponds to SAOS flow, with the amplitude of the oscillations increasing with $Ca_0$ for the same membrane elasticity. The role of the viscosity ratio $\lambda$, the volume fraction of the RBCs $\phi$, and the $Ca_0$ on the complex viscosity have been evaluated and discussed.

The first important question we focused on is whether the cell deformation might be enhanced by varying the oscillatory frequency. Although frequency-induced deformation can be found both in dense and dilute conditions, especially for the intermediate range of
Figure 8. (a and b) Spatial-temporal average of the first and isotropic tensions ($T_1$ and $T_{iso}$, respectively) as a function of $f_{in}$ for (a) dense ($\phi = 0.41$) and (b) dilute ($\phi = 0.11$) conditions. The results are obtained for SAOS ($Ca_0 = 0.05$). In (c) and (d), the membrane tensions are shown as function of $\phi$ and $f_{in}$ ($= 0.05$ and $0.5$) for (c) SAOS ($Ca_0 = 0.05$) and (d) LAOS ($Ca_0 = 0.8$). The results shown in (a)–(d) are obtained for $\lambda = 5$. (e) The membrane tensions as function of the viscosity ratio $\lambda$ and $f_{in}$ ($= 0.05$ and $0.5$) for dense condition ($\phi = 0.41$). The result in (e) is obtained for SAOS ($Ca_0 = 0.05$).
frequencies, $0.05 \leq f_{in} \leq 1$ corresponding to $2.1 \, \text{Hz} \leq f \leq 42 \, \text{Hz}$ for $Ca_0 = 0.05$ ($\approx 42 \, \text{s}^{-1}$ assuming the reference radius of $a_0 = 4 \, \mu\text{m}$ and the surface shear elastic modulus of $G_s = 4 \, \mu\text{N/m}$), the differences with the deformations in steady shear flow are always less than 1%. Thus, enhancement of cell deformation under oscillatory shear is unlikely to occur for RBCs. Previous numerical analysis of a single spherical capsule, whose membrane follows the neo-Hookean constitutive law (strain softening character), demonstrated that frequency-dependent deformations become evident for high $Ca$ and large values of $\lambda$ (Matsunaga et al. 2015), specifically $Ca = 2.0$ and $\lambda = 10$, with a 26% deformation increase observed at $f_{in} = 0.01$. Our results, however, show that even in LAOS regime, characterised by $Ca_0 = 0.8$, the cell deformation by oscillatory shear flow is very limited for biconcave capsules, a possible reason being its ability to re-orient according to the imposed shear-flow. Focusing on biological systems, the oxygen-dependent regulation of RBC properties should be taken into considerations (Parthasarathi & Lipowsky 1999). For instance, it is known that the elongation of RBCs in response to shear stresses increases as oxygen tension is decreased (Wei et al. 2016). Thus, it will be interesting to study whether frequency-dependent deformation of RBCs under pulsatile flows results in passive regulation for oxygen transport. Differently from what observed for the cell deformation, the tension of the membrane decreases significantly with the input frequency, also resulting in a decrease of the normal stress differences. This result suggests that the viscoelastic character of RBC suspensions can be fully understood only by accounting for the state of the membrane tension.

Our next question is how the bulk suspension rheology is altered in oscillatory flows when compared to the case of steady shear flow, where most experimental measures are taken. As $f_{in}$ increases, $\eta'$ gradually decreases, while $\eta''$ attains its maximum value at frequency $f_{in} = 0.5$ corresponding to $f = 21 \, \text{Hz}$ (figure 5f). A similar trend was reported for a single spherical capsule with neo-Hookean constitutive law in Matsunaga & Imai (2020), where $\eta''$ was shown to reach its maximum at $f_{in} = 0.2$. Interestingly, the local maximum $f_{in}^{max} (= 0.5)$ remains the same in dilute condition ($\phi = 0.11$) for the RBC suspensions considered here (figure 5f); therefore, the discrepancy in the frequency of the maximum $\eta''$ with the previous numerical study by Matsunaga & Imai (2020) might be due to the different membrane constitutive law or the capsule shape rather than an effect of the volume fraction. Furthermore, we note that the behavior of the complex viscosity is consistent with that of a non-Newtonian liquid subjected to SAOS flow, such as polymer suspensions modelled by the Oldroyd-B, and Giesekus laws (Villone et al. 2021).

These observations suggest that the viscoelastic character of the suspension of RBCs is less sensitive to the particle volume fraction (at least for $\phi \leq 0.41$) and particle deformation, but depends on the oscillatory frequency. Effects of particle deformability and inertia on the viscoelastic character in suspensions of RBCs will be reported in a future study. A physiological interpretation for the local maximum of the $\eta''$ viscosity at a frequency of $\sim 21 \, \text{Hz}$ is still missing. Since this is 20 times higher than the physiological heart beat ($\sim 1 \, \text{Hz}$), such high frequency hydrodynamic interactions may only occur in local vascular areas, e.g., aneurisms, or in an artificial blood pumps. Thus, in the future, it will be interesting to also study whether such high-frequency oscillations can induce or delay blood clots.

Our numerical results and quantitative model analysis of the viscoelastic property of dense suspensions of RBCs will be helpful to build more rigorous non-Newtonian constitutive laws that consider multi-scale dynamics, and to gain insights not only into the passive cellular flow in physiological systems (Chien et al. 1967; Chien 1970; Cokelet & Meiselman 1968; Goldsmith & Skalak 1975), but also into the design of novel artificial
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Declaration of interests
The authors report no conflict of interest.

Appendix A. Numerical details

A.1. Discrete Fourier transform

The peak frequency and amplitude of the output signal, the specific viscosity $\mu_{sp}$, and of the imposed shear rate $\dot{\gamma}$ are calculated by the discrete Fourier transform (DFT), defined as:

$$X(f_k) = \sum_{j=0}^{n-1} x(t_j) \exp(-i2\pi jk/n),$$  \hspace{1cm} (A1)

where $X(f_k)$ is the Fourier coefficient of the variable $x$ for the $k$-th mode, and $n$ is the number of data points. Considering only the signal dominant component of frequency $f_p$, the one of the imposed shear as shown below, we can write the input and output signals as $\dot{\gamma}(t) \approx \cos(2\pi f_p t + \Theta_{\dot{\gamma}})$ and $\mu_{sp}(t) \approx |\mu_{sp}| \cos(2\pi f_p t + \Theta_{\mu_{sp}})$, with the phase difference $\delta (-\pi/2 \leq \delta \leq 0)$ expressed as $\delta = \Theta_{\mu_{sp}} - \Theta_{\dot{\gamma}}$. The phases $\Theta$ ($= \Theta_{\dot{\gamma}}$ or $\Theta_{\mu_{sp}}$) can be defined as the angle between the real and imaginary parts of the Fourier coefficient $X(f_p)$ at the peak frequency $f_p$ of $\dot{\gamma}(t)$ or $\mu_{sp}(t)$:

$$\Theta = \text{atan}2(\text{Im}(X(f_p)), \text{Re}(X(f_p))).$$  \hspace{1cm} (A2)

Indeed, the calculated peak frequencies of $\dot{\gamma}$ and $\mu_{sp}$ coincide with the input strain frequency $f_{in}(= f/\dot{\gamma}_0)$ as shown in figure 9.

A.2. Effect of wave periods on the solutions by DFT analysis

The accuracy of the analysis is important especially for small values of $\delta$ because the complex modulus is proportional to $\sin \delta \approx \delta$ for $\delta \ll 1$. We have therefore checked the
Figure 9. The peak frequency $f_p$ of the input shear rate $\dot{\gamma}(t)$ versus the peak frequency of the output specific viscosity $\mu_{sp}(t)$ calculated by the DFT analysis as a function of shear rate frequency $f_{in} = \dot{f}/\gamma_0$. The results pertain the simulations with $\phi = 0.41$, $\lambda = 5$, and $Ca_0 = 0.05$.

| Number of wave periods | 5     | 10    | 15    | 20    | 25    | 30 (ref) |
|------------------------|-------|-------|-------|-------|-------|----------|
| $\delta/\pi$           | -0.07956 | -0.08127 | -0.08117 | -0.08080 | -0.08045 | -0.08014 |
| $\varepsilon(\delta/\pi)$ | 0.00730 | 0.01400 | 0.01283 | 0.00818 | 0.00382 | -        |
| $|\mu_{sp}|_{amp}$      | 2.76387 | 2.43931 | 2.46789 | 2.49496 | 2.51260 | 2.52682 |
| $\varepsilon(|\mu_{sp}|_{amp})$ | 0.09381 | 0.03463 | 0.02332 | 0.01261 | 0.00382 | -        |
| $\eta'$                | 2.67798 | 2.36025 | 2.38808 | 2.41501 | 2.43278 | 2.44715 |
| $\varepsilon(\eta')$   | 0.09433 | 0.03551 | 0.02414 | 0.01313 | 0.00563 | -        |
| $\eta''$               | 0.68364 | 0.61603 | 0.62254 | 0.62654 | 0.62830 | 0.62950 |
| $\varepsilon(\eta'')$  | 0.08600 | 0.02141 | 0.01107 | 0.00470 | 0.00191 | -        |

Table 1. Effect of the number of wave periods used in the DFT analysis on the phase difference $\delta$, amplitude of the specific viscosity $|\mu_{sp}|_{amp}$, and moduli of the complex viscosity ($\eta'$ and $\eta''$). The error parameter $\varepsilon(\chi)$ is defined as $\varepsilon(\chi) = |\chi/\chi_{ref} - 1|$, where the subscript $ref$ indicates the reference values. The simulations are performed at $\phi = 0.41$, $Ca = 0.05$, $\lambda = 5$, and $f_{in} = 0.05$.

The effect of the number of wave periods used for the input and output signals in the DFT analysis: $\delta/\pi$, $|\mu_{sp}|_{amp}$, $\eta'$, $\eta''$. Specifically, we have computed these values for a number of periods ranging from 5 to 30, where each output wave is resolved by a sufficiently large number of discrete points ($\geq 100$). The values and errors obtained with respect to the data from the longest signal (30 periods) are summarised in table 1. All errors decrease as the number of wave periods increases. For a reasonable analysis, we deem necessary to use at least 10 periods, which gives a difference lower than 4% with respect to using the full 30 wave periods.

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