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Complex Dynamics of a Bilamellar Vesicle as a Simple Model for Leukocytes

Badr Kaoui, Timm Krüger, and Jens Harting

1Department of Applied Physics, Eindhoven University of Technology, P. O. Box 513, 5600 MB Eindhoven, The Netherlands
2Centre for Computational Science, University College London, 20 Gordon Street, WC1H 0AJ London, United Kingdom
3Institute for Computational Physics, University of Stuttgart, Allmandring 3, 70569 Stuttgart, Germany

The influence of the internal structure of a biological cell (e.g., a leukocyte) on its dynamics and rheology is not yet fully understood. By using 2D numerical simulations of a bilamellar vesicle (BLV) consisting of two vesicles as a cell model, we find that increasing the size of the inner vesicle (mimicking the nucleus) triggers a tank-treading-to-tumbling transition. A new dynamical state is observed, the undulating motion: the BLV inclination with respect to the imposed flow oscillates while the outer vesicle develops rotating lobes. The BLV exhibits a non-Newtonian behavior with a time-dependent apparent viscosity during its unsteady motion. Depending on its inclination and on its inner vesicle dynamical state, the BLV behaves like a solid or a liquid.

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I. INTRODUCTION

Unilamellar vesicles (ULVs) consisting of a single closed phospholipid membrane were extensively used as biomimetic model for erythrocytes (red blood cells) in the past. They succeeded to reproduce many known features, like the steady shapes in Poiseuille flow or the dynamical states under shear flow. However, for leukocytes (white blood cells), despite their relevant role in the immune system, the dynamics and rheology are still poorly understood since their complex internal structure dominated by the nucleus alters the mechanical properties in a non-straightforward manner. We use a bilamellar vesicle (BLV) as a model for biological cells, in particular, a leukocyte. While a ULV consists of a single vesicle, a BLV consists of two vesicles: an outer larger one (the cell) enclosing an inner smaller one (mimicking the nucleus). We study numerically the dynamics of a BLV under shear flow and investigate how the dynamical and rheological properties of a leukocyte are affected by varying the size and the deformability of the nucleus as well as the amount of fluid enclosed between the nucleus and the cell. We show that leukocytes cannot be described simply by fluid-filled particles enclosing a homogeneous fluid without an internal structure as it has been used, for example, in Refs. [8, 9]. This is because leukocytes adapt their mechanical properties and act as a solid or a liquid depending on how they are deformed by the imposed fluid.

II. SIMULATION METHOD

We consider two concentric vesicles in 2D in a shear flow generated between two parallel plates. We designate by $R_{\text{out}}$ and $R_{\text{in}} < R_{\text{out}}$ the effective radii of the outer and the inner vesicle ($R = P/(2\pi)$, where $P$ is the vesicle perimeter). All fluids are considered to be incompressible, Newtonian and of the same viscosity $\eta$. Their flow is solved by the lattice-Boltzmann method and the fluid-vesicle two-way coupling is achieved employing the immersed boundary method (see [10] for details). Both vesicle membranes are locally inextensible and experience resistance to bending with the same rigidity $\kappa$. They exert a reaction force per unit length (in 2D)

$$f = \kappa \left( \frac{\partial^2 c}{\partial s^2} + \frac{c^3}{2} \right) n + \frac{\partial^* \sigma}{\partial s}$$

on the surrounding fluid where $n$ and $t$ are the unit normal and tangent vectors, $c$ is the local curvature, $s$ is the curvilinear coordinate and $\sigma^*$ is the local effective surface tension. Both membranes interact purely hydrodynamically. The distance between the plates is chosen as such that the effect of wall confinement is negligible.

First, we investigate how the dynamics of the BLV is affected by varying two parameters: i) $R_{\text{in}}$, to study the effect of its internal structure and ii) the deformability number $C_a = \eta R_{\text{out}}^3/\kappa$, which we define in the style of a capillary number used for droplets, but based on the bending rigidity instead of the surface tension. Here, $\gamma$ is the shear rate. Second, we investigate how the dynamics of the inner vesicle is affected by the flow induced by the outer one by varying the swelling degree $\Delta_{\text{out}} = 4\pi A_{\text{out}}/P_{\text{out}}^2$ ($A_{\text{out}}$ is the outer vesicle area) while keeping all other parameters fixed. All simulations are performed in the Stokes regime: $\mathcal{O}(\text{Re}) = 10^{-2}$, where $\text{Re} = \rho R_{\text{out}}^2/\eta$ is the Reynolds number and $\rho$ is the fluid density. Both vesicles are deflated and have a swelling degree $\Delta_{\text{in}} = \Delta_{\text{out}} = 0.9$. While this is a typical situation for vesicles, for leukocytes it corresponds to large deformation encountered in capillaries or in micropipette experiments [8]. We vary $R_{\text{in}}$ while keeping $R_{\text{out}}$ fixed.
The obtained dynamics is shown in Fig. 1 as a function of $0 < R_{in}/R_{out} < 1$. For smaller inner vesicles (Fig. 1(a)−(c)), both vesicles perform a steady tank-treading motion (they assume a steady inclination angle with respect to the flow while their membranes undergo a tank-treading like motion). The BLV aligns more and more with the flow when $R_{in}$ is increased. Due to the symmetry, both vesicle centers are stationary. Beyond a threshold of $R_{in}$, the motion of the BLV transits from steady tank-treading to unsteady tumbling motion (rotation as solid elongated particle). Fig. 1(d) shows snapshots of a tumbling BLV with $R_{in}/R_{out} = 0.75$. The inner vesicle assumes a relative angle with respect to the main axis of the outer one. During the tumbling motion, the mean value of $\theta_{out}$ (the angle defined by the main long axis of the BLV and the flow direction) is zero. When plotting $\theta_{out}$ as function of $R_{in}/R_{out}$ (Fig. 2), we see that $\theta_{out}$ decreases with increasing $R_{in}/R_{out}$ until it vanishes at a critical value where tank-treading (TT) is replaced by tumbling (TB). The TT-TB transition is known for viscous ULVs where it is induced by increasing the viscosity contrast $\Lambda$ (ratio between the internal and external fluid viscosities) beyond a given threshold $\Lambda_{cr}$. Here, however, all fluids have the same viscosity and the transition is induced solely by the presence of the encapsulated vesicle and by enlarging its size. Veerapaneni et al. [12] predicted a similar transition for non-viscous ($\Lambda = 1$) ULVs enclosing a solid particle. They claim that the inclusion increases the apparent internal viscosity leading to the transition as observed for inclusion-free ULVs with $\Lambda_{cr} > 1$. To investigate the effect of the apparent internal viscosity, we follow [14, 15] and compute $\eta^* = \eta + \langle\sigma_{xy}\rangle/\langle S_{xy}\rangle$, where $\langle\sigma_{xy}\rangle = -\int_{\partial\Omega_{in}} d\mathbf{s} (f_x r_y)/A_{in}$ is the average excess shear stress caused by the presence of the inner vesicle. Therefore, the integration has to be performed on the surface of $\Omega_{as}$. The average shear rate within the outer vesicle domain $\Omega_{out}$ (consisting of the region between the two vesicles and the region within the inner vesicle) can be written as a surface integral by making use of Gauss’s theorem: $\langle S_{xy}\rangle = \frac{1}{A_{out}} \int_{\partial\Omega_{out}} d\mathbf{s} (n_x u_y + n_y u_x)$. Here, $\mathbf{r}$ and $\mathbf{u}$ are the position and the velocity of a membrane element, respectively. The apparent viscosity contrast of the BLV, $\Lambda^* = \eta^*/\eta$, vs. $R_{in}/R_{out}$ is depicted in Fig. 2. Increasing $R_{in}$ leads to a monotonic increase of $\Lambda^*$. The TT-TB transition for the BLV takes place at a critical value of $\Lambda_{cr}^* = 6.9$. This value is close to the critical viscosity contrast $\Lambda_{cr} = 6.6$ required for a viscous ULV to undergo the same transition (for the same swelling degree of 0.9). A systematic comparison of the inclination angle $(\theta, \theta_{out})$ vs. the viscosity contrast $(\Lambda, \Lambda^*)$ of a viscous ULV and a BLV (see Fig. 3) shows that both exhibit similar qualitative behavior: the angle decreases with increasing viscosity contrast until it vanishes at the transition point $R_{in}/R_{out} = 0.64$. The ratio $R_{in}/R_{out}$ is given below each image. For small inner vesicles (a)−(c), the BLV performs steady tank-treading. The angle between the main axis of the BLV and the flow direction $\theta_{out}$ decreases with $R_{in}$ (see Fig. 2). Beyond a threshold of $R_{in}$, the BLV starts tumbling (d). This is similar to the tank-treading-to-tumbling transition observed for viscous vesicles [5, 11], but is triggered here (in the absence of a viscosity contrast) by the presence of the inner vesicle.

III. OBSERVATIONS AND DISCUSSION

The ratio $R_{in}/R_{out}$ is given below each image. For small inner vesicles (a)−(c), the BLV performs steady tank-treading. The angle between the main axis of the BLV and the flow direction $\theta_{out}$ decreases with $R_{in}$ (see Fig. 2). Beyond a threshold of $R_{in}$, the BLV starts tumbling (d). This is similar to the tank-treading-to-tumbling transition observed for viscous vesicles [5, 11], but is triggered here (in the absence of a viscosity contrast) by the presence of the inner vesicle.
tion point. However, for all viscosity contrasts, the angle of the BLV is found to be larger than that of the viscous ULV ($\theta_{\text{out}} > \theta$), especially at larger $\Lambda^*$ corresponding to larger inner vesicles. This demonstrates that a BLV does not behave exactly as a viscous ULV for which the internal fluid is a homogeneous medium. An internal heterogeneous medium, as it is the case for a leukocyte, with viscosity contrasts between the intranucleus fluid, the cytoplasm and the plasma, would affect the critical value of the dynamical transition. For example, a tank-treading BLV with a given ratio $R_{in}/R_{out}$ is expected to transit to tumbling only by making the inner vesicle fluid more viscous.

Moreover, when tumbling, the apparent internal viscosity $\eta^*$ of the BLV is a time-dependent quantity and varies in a coherent manner with $\theta_{\text{out}}$, see Fig. 4. It diverges to the limit of a solid medium when $\theta_{\text{out}} = \pm \pi/4$ (direction of the elongation/compression of the shear flow) since the average shear rate $\langle S_{xy} \rangle$ vanishes at that point. This is a signature of the non-Newtonian rheological behavior. The BLV internal medium changes its apparent viscosity as a response to the orientation with respect to the flow. For biological systems, e.g., a leukocyte flowing in a vessel (Poiseuille flow), this behavior suggests that the apparent viscosity depends on the stresses experienced by the cell, which vary with the instantaneous lateral position within the vessel.

At higher Ca, the membrane deformability becomes important and thus the BLV deforms substantially. For a smaller inner vesicle, the BLV again tank-treads. However, for larger $R_{in}$, in contrast to the limit of small deformation, we surprisingly observe that the BLV does not tumble anymore. It rather performs a new type of unsteady motion (Fig. 5). The inner vesicle undergoes a swinging motion; the main axis oscillates about a positive mean angle ($\theta_{\text{in}}$ in Fig. 5(a)) while its shape does not deform. Such motion is known for red blood cells and capsules [13]. The outer vesicle exhibits a non-regular motion: although its main long axis performs oscillations about a positive mean angle as well ($\theta_{\text{out}}$ in Fig. 5(a)), its shape undergoes larger undulations, i.e., its membrane buckles. It develops two oscillating lobes for intermediate sized inner vesicles or four rotating lobes for larger inner vesicles (Fig. 5(b)). Both unsteady motions cannot be qualified as vacillating-breathing (VB) [16], swinging (SW) or trembling (TR) [17]. An almost similar feature as shown in Fig. 6 has been recently observed experimentally by Pommella et al. [18] for a surfactant

FIG. 4: Time evolution of the inclination angle $\theta_{\text{out}}$ (in degrees) and the apparent internal viscosity $\eta^*$ (in lattice units) of a tumbling BLV ($R_{in}/R_{out} = 0.75, \text{Ca} = 0.5$). The data corresponds to the snapshots in Fig. 1(d). The time evolution of the shear stress $\langle \sigma_{xy} \rangle$ and shear rate $\langle S_{xy} \rangle$ is also shown (in lattice units). The time dependence of $\eta^*$ suggests non-Newtonian fluid properties of the inner medium (fluid and inner vesicle). Note especially $\eta^* \rightarrow \infty$ (solid limit) when $\theta = \pm \pi/4$ (i.e. $\langle S_{xy} \rangle \rightarrow 0$).

FIG. 5: The time evolution of the outer inclination angle $\theta_{\text{out}}$ of the BLV for different values of Ca. The inner vesicle radius is sufficiently large ($R_{in}/R_{out} = 0.83$) and both vesicles have the same swelling degree $\Delta_{in} = \Delta_{out} = 0.9$. For the same structural parameters, only by varying the degree of deformation Ca from 1 to 15, the BLV ceases to tumble and transits to the undulating state.

FIG. 6: (a) Oscillations of the main axes of the inner ($\theta_{\text{in}}$) and outer ($\theta_{\text{out}}$) vesicles of a BLV during the undulating motion ($R_{in}/R_{out} = 0.85, \text{Ca} = 10$). (b) Snapshots, taken at equal time intervals, showing four lobes of the outer vesicle membrane and their rotation. The straight line denotes the consequent locations of two opposite lobes. The undulating regime replaces tumbling for larger $R_{in}/R_{out}$ and larger Ca.
multilamellar droplet subjected to strong shear. The authors describe the droplet motion as VB, but we disagree with this classification since the angle is found to oscillate about a non-zero mean value as it does for SW. Yet, SW can be ruled out as well since it is observed in the small deformation limit. The dynamic mode of the BLV can neither be described as TR which indeed is characterized by the formation of lobes \[17\] but also requires the shape to become perfectly elliptical at a certain moment. This is impossible for BLV due to the presence of the large inner obstacle. The appearance of this new unsteady motion (for larger Ca and larger \(R_{in}/R_{out}\)) that we name undulating motion cannot be explained solely based on the apparent viscosity contrast argument. The inner vesicle disturbs the motion of the outer one. By increasing its size, the thickness of the fluid layer between the membranes decreases to become a thin liquid film. The outer membrane tries to tank-tread under the external applied shear. However, the presence of the inner vesicle prevents this and thus it slides over the inner membrane, which plays the role of a nearly solid obstacle. Its shape is less deformable because i) the inner vesicle is smaller (\(Ca \propto R^3\)), and ii) the outer vesicle shields the inner one from the external flow. A thorough understanding of the appearance of the undulating motion is still missing. However, a relation to the Marangoni effect can be proposed: at large deformations, bending becomes less important than tension. We observe a non-uniform distribution of the surface tension for the BLV, \(\partial \sigma^*/\partial \delta \neq 0\). This is in line with the surfactant multilamellar droplets \[18\] and the instability of thin liquid films on a solid substrate \[19\].

So far we described how the inner vesicle alters the dynamics of the outer vesicle and so of the BLV. Next, we examine how the outer vesicle in turn influences the dynamics of the inner vesicle. This is controlled by the amount of the fluid between the two membranes. To understand its relevance we vary its amount by swelling (adding fluid) or deflating (removing fluid) the outer vesicle. We vary \(\Delta_{out}\) between 0.9 and 1, keeping all other parameters unchanged. Consequently, we observe another TT-TB transition, but this time for the inner vesicle as shown in Fig. 7(a)–(c). For \(\Delta_{out} = 0.9\), both vesicles tank-tread (Fig. 7(a)). Above a critical value of \(\Delta_{out} = 0.98\), the inner vesicle starts tumbling while the outer experiences a breathing-like motion (Fig. 7(b)). For \(\Delta_{out} = 1\) (Fig. 7(c)), the BLV behaves exactly as a solid body. The two vesicles rotate with the same angular velocity and also the fluids, between the membranes and within the inner membrane, behave like a solid medium. The dynamical transition observed here for the inner vesicle is expected to modify the apparent viscosity within the BLV. A similar link between the rheology and the micro-dynamics has been observed for red blood cells \[20\]. Although the BLV dynamics for the three cases (Fig. 7(a)–(c)) is apparently similar for an outside observer, their rheological properties may differ due to the dynamical state (TT or TB) of the inner vesicle. The dynamical transition induced by varying \(\Delta_{out}\) alone can be explained by the theory of Lebedev et al. \[21\] who predicted that even a non-viscous vesicle (\(\Lambda = 1\)) can undergo a TT-TB transition by increasing the rotational component of the external imposed flow. This was later confirmed experimentally by Deschamps et al. \[22\]. In our case, the inner vesicle is subjected to the flow induced by the tank-treading motion of the outer membrane. Fig. 7(d)–(f) depict the generated undisturbed flow (in the absence of the inner vesicle) for each \(\Delta_{out}\). A pure rotational flow is obtained for \(\Delta_{out} = 1\). For \(\Delta_{out} = 0.9\) and \(\Delta_{out} = 0.98\), a mixed flow is generated, i.e., a combination of pure shear and pure rotational flows. We quantify the relative importance of the rotational and the elongational components using the quantity \(\Gamma/S\), where \(\Gamma\) is the vorticity and \(S\) is the shear magnitude (\(\Gamma/S = 1\) for pure shear and \(\Gamma/S \rightarrow \infty\) for pure rotational flow). The computed values \(\Gamma/S\) for each \(\Delta_{out}\) are shown in Fig. 7(d)–(f). The TT-TB transition is induced by increasing \(\Gamma/S\) beyond the critical value of 27.62 (corresponding to the critical value \(\Delta_{out} = 0.98\)). Using Lebedev’s parameters, \(S = 7\pi Ca/\sqrt{3}\delta\) and \(\Lambda = 4(1 + 23\Lambda/32)\sqrt{\delta/\sqrt{3}\delta r}\) (\(\delta\) is the excess perimeter in 2D or excess area in 3D), the transition occurs in our case at \(S_{cr} = 0.42\) and \(\Lambda_{cr} = 11.41\). This \(\Lambda_{cr}\) is larger than expected when compared to the case of a viscous vesicle under shear flow (\(\Lambda_{cr} = 1.38\)) \[11\] or a non-viscous vesicle in general flow (\(\Lambda_{cr} = 1.2\)) \[22\]. At low deformation (\(S \rightarrow 0\)), \(\Lambda_{cr}\) is independent of \(S\) anyway \[21, 22\]. The discrepancy in \(\Lambda_{cr}\) or \(\Lambda\) can be explained by taking into account that Lebedev’s theory has been formulated for unbounded flows while, in our case,
the inner vesicle is strongly confined by the outer vesicle membrane. Confinement is found to shift the critical point of the TT-TB transition to larger values of \( \Lambda \) \cite{11}. Thus, Lebedev’s theory can explain only qualitatively the TT-TB transition observed for the inner vesicle in Fig. 7.

**IV. CONCLUSIONS**

We showed that a non-viscous vesicle exhibits rich complex dynamics when it encapsulates another non-viscous vesicle. Increasing the size of the inner vesicle triggers a dynamical transition from TT to TB or the newly found undulating motion. The BLV internal medium displays non-Newtonian behavior with a time-dependent apparent viscosity during unsteady motion: the same BLV behaves like a solid or a fluid depending on its orientation and the dynamical state of its inner vesicle. Our results suggest that a leukocyte cannot be simply mimicked with a solid spherical particle or with an inclusion-free vesicle enclosing a homogenous Newtonian fluid. The presence of an internal structure dictates its dynamical and rheological response to imposed flow. Our results suggest consequences on the margination and adhesion of leukocytes in the microcirculation, and its physiological and pathological implications. Along this study we approximated the two fluids, mimicking the cytoplasm and the nucleus, to be simple Newtonian with identical viscosity. This is a simplistic picture when compared to the actual complex nature of the internal structure of a leukocyte (presence of actine, microtubules, filaments). By considering the cytoplasm as a visco-elastic medium or a more viscous nucleus, we expect this to lead to different dynamical behaviors which are not captured by the present model because this would affect the way the outer and the inner membrane interact hydrodynamically. This will be the subject of future research. In the present model, the considered membranes have only bending properties, while in real 3D systems the membrane shear elasticity comes into play. This may lead to the appearance of new dynamical states, wrinkling of the membrane, or the formation of more than four lobes.

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