Complex Dynamics of Compound Vesicles in Linear Flow

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We report first experimental observations of dynamics of compound vesicles in linear flow realized in a microfluidic four-roll mill. We show that while a compound vesicle undergoes the same main tank-treading, trembling (TR), and tumbling regimes, its dynamics are far richer and more complex than that of unilamellar vesicles. A new swinging motion of the inner vesicle is found in TR in accord with simulations. The inner and outer vesicles can exist simultaneously in different dynamical regimes and can undergo either synchronized or unsynchronized motions depending on the filling factor. A compound vesicle can be used as a physical model to study white blood cell dynamics in flow similar to a unilamellar vesicle used successfully to model anucleate cells.

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Cellular components of blood consist mainly of red blood cells (RBC), leukocytes or white blood cells (WBC), and platelets. The concentration of RBCs (hematocrit) in a normal blood vastly exceeds the rest of the cellular components and reaches in average 45%, while WBC comprise only about 0.7% [1]. For this reason, RBCs have a decisive influence on viscosity of the normal whole blood, in particular on blood flow in large vessels, contrary to WBC. However, there are conditions, at which the WBC concentration becomes extremely high and comparable with the RBC hematocrit: the most prominent case is the leukemia. At these circumstances, WBC considerably affect large vessel flows, and so the understanding of their dynamics in the flow is crucial.

The giant unilamellar vesicle (GUV) encapsulating a fluid has been used successfully as a model to qualitatively reproduce RBC dynamics in an external flow and blood rheology. Contrary to RBC having essentially a lipid membrane with attached spectrin network encapsulating a concentrated hemoglobin solution, WBC has complicated internal structure, containing a nucleus, organelles, fibers etc. suspended in viscous cytoplasm [1]. So it is hard to imagine that the WBC dynamics could be modeled by GUV. Recently, compound vesicles have been suggested as a simple model of WBC [2-4], and their dynamics is expected to mimic the WBC dynamics. A compound vesicle considered further is a unilamellar vesicle encapsulating either a solid particle [3] or another unilamellar vesicle [4] suspended in a membrane-bounded fluid. The inclusion affects the interior flow, thus altering the dynamical and rheological properties of a compound vesicle in a nontrivial and, so far, poorly understood manner, depending on the occupied fraction of the interior volume, called the filling factor ϕ .

Dynamics of a single GUV in linear flow is governed by two constraints, namely the conservation of volume V and surface area A, which result in rather involved nonlinear

vesicle dynamics, characterized by three dynamical regimes: tank-treading (TT), trembling (TR), and tumbling (TU) [5,6]. A key feature of vesicle dynamics in the vicinity of the TT-TR transition and in the TR regime itself is a strong amplification of thermal noise [7,8]. Vesicle dynamics in a linear flow depends on the vesicle effective radius obtained via the volume $R = (3V/4\pi)^{1/3}$, and the excess area, $\Delta = A/R^2 - 4\pi$. The phase diagram of the vesicle dynamical states in a linear flow is discussed in terms of the dimensionless quantities that represent a competition between relevant time scales expressed via the above parameters, $S = 14\pi s \eta_0 R^3 / 3\sqrt{3}\kappa \Delta$ and $\Lambda = (32 + 23\lambda)\omega\sqrt{\Delta}/8s\sqrt{30\pi}$, where κ is the bending elasticity, $\lambda = \eta_i / \eta_o$ is the viscosity contrast, and ω and s are the strength of the rotational and extensional components of the linear flow, respectively [6].

Recently, the dynamics of compound vesicles were investigated numerically by focusing on the effect of an inclusion on the dynamics of a vesicle in shear flow, as a function of ϕ [3,4]. There is another parameter, except ϕ , the excess area of the internal vesicle Δ_{int} , which also affects the compound vesicle dynamics. It is fixed in the numerical simulations reported in [4], and in this way the number of the parameters in the problem is reduced to three instead of four: Λ , S, ϕ . Nevertheless, there exist four parameters in experiments, and one needs four-dimensional parameter space to describe dynamics of a compound vesicle. We are unaware a technique to proper produce such objects and moreover a capability to control geometrical and physical parameters of a compound vesicle Δ_{int} , $\Delta_{\text{ext}}, \phi, R_{\text{ext}}, \text{ and } \lambda$, except the control parameter of a linear flow ω/s .

In this Letter we report the first experimental results on the dynamics of the compound vesicles in a linear flow. We explore compound vesicle-in-vesicle structures with $\phi \in [0.35, 0.6], \Delta_{ext} \in [0.39, 1.92]$, and $\Delta_{int} \in [0.6, 1.2]$, by studying them in a wide range of the control parameters



FIG. 1 (color online). Experimental setup. (Top) Microfluidic four-roll mill. (Bottom left) Schematic drawing of the dynamical trap. The experimental control parameter is the pressure difference between pairs of inlets ΔP . Altering rapid and subtle variations of ΔP allows trapping and further manipulation of vesicles inside the dynamical trap. (Bottom right) Presentation of two components of a linear flow.

A, *S* and by comparing their dynamics to those of GUVs [9] and to numerical simulations of the compound vesicle dynamics [3,4]. For the comparison with the relevant numerical simulations, we use here the same definition of $\phi = R_{\text{int}}/R_{\text{ext}}$ as suggested in [4].

Vesicles were prepared via electroformation [7,10] in two cases: sucrose/water and glycerol/water solutions of different viscosities (38%, 5.3 and 60%, 10.66 mPa \cdot s, respectively, and $\lambda = 1$). In addition to GUVs, a small amount of various "exotic" structures is generated, which are further sorted out, as described below. This includes micelles, micelle tubes, and compound vesicles. Observations and measurements of vesicle dynamics were conducted inside a microfluidic four-roll mill [11,12]. The key component of this device is a dynamical trap, that allows controlled trapping of desirable objects and their investigation for a long time in a linear flow (Fig. 1). The flow inside the dynamical trap is generated by a hydrostatic pressure difference. The flow control parameter ω/s inside the trap is varied continuously by the single experimental control parameter ΔP , the pressure drop across the dynamical trap. Sorting out the products of the electroformation is carried out by method of "net fishing," i.e., trapping a group of vesicles and then subsequently sorting out the objects of interest. The net fishing effect is achieved by altering rapid and subtle variations of ΔP , thus changing the flow inside the trap from rotational to extensional, i.e., from closed to open streamlines, and vice versa. This method was previously used for simultaneous trapping of a pair of vesicles [13]. An object trapped inside the dynamical trap can be



FIG. 2. Comparison of time average spectra of vesicle shapes in TT of GUV with $\Delta = 0.66$ (from left inset) and compound one with $\Delta_{\text{ext}} = 0.67$, $\Delta_{\text{int}} = 0.63$, $\phi = 0.51$ (from right inset). Both vesicles are in TT at $\Lambda = 0.85$ and 0.98, respectively.

further observed for long observation time (compared to time scales of the flow and vesicle dynamics) in the controlled flow field. The vesicle dynamics was monitored using inverted fluorescence microscope (IMT-2, Olympus). The images of a vesicle at its largest cross section were collected with a Prosilica EC1380 CCD camera, aligned with the shear axis and synchronized with a mechanical chopper on the path of the excitation beam to reduce exposure time. We then applied image processing algorithm described in [7] for both the internal and external vesicles. Vesicle geometry was extracted from the twodimensional images, using c = (a + b)/2, where a, b, c are the principle semiaxes of the ellipsoidal approximation of the vesicle shape, c is perpendicular to the plane of the flow, $R = \sqrt[3]{3\pi abc/4}$, and $\Delta = 32\pi D^2/15$. Here D =(a-b)/(a+b) is the vesicle dimensionless aspect ratio. Geometry of the vesicles obtained by this method is a very good approximation to real geometry obtained by threedimensional scanning and is commonly used when threedimensional scanning is not possible [5,13,14]. The results were compared with previously obtained data on GUVs in water and water-sugar solutions [5,9,11].

We find the dynamical states of the compound vesicles, i.e., TT, TR, and TU, for the wide variations of Λ and *S* and in the range of ϕ and Δ_{int} exploited in the experiment in a good accord with those known for the GUVs [5,9,11]. In the TT regime, we notice slightly altered shapes of the external vesicle in the compound one, more parallelogram-like than ellipsoidal, as seen in the inset in Fig. 2. We quantitatively characterized its shape variations via Fourier transform of the vesicle dimensionless shape perturbations $u(\psi, t) = \sum_{q=1}^{n} u_q(t)e^{-iq\psi}$, where $u(\psi, t) = r(\psi, t)/R - 1$, $r(\psi, t)$ is the shape radial position at polar angle ψ at $0 \le \psi \le 2\pi$, and $R(t) = \langle r(\psi, t) \rangle_{\psi}$. As shown in



FIG. 3. Dependence of $\langle \theta \rangle / \pi$ on Λ for the external and internal vesicles compared with data on TT for GUVs [9]. The dash line is the fit to the data taken from [9] and the dash-dotted line is the same fit shifted up. The data presented are obtained for the whole ensemble of vesicles used in these studies with different Δ_{int} , Δ_{ext} , ϕ , R_{ext} .

Fig. 2, the even modes in the outer vesicle shape power spectra of the compound vesicle with $\phi = 0.51$, $\Delta_{ext} = 0.67$, $\Delta_{int} = 0.63$ are visibly amplified at $q \ge 6$ compared with those found for the GUV with a close value of $\Delta = 0.66$, in accordance with their shape difference notable in the inset in Fig. 2. And finally, at the close values of $\Delta_{ext} = 0.67$ and $\Delta_{int} = 0.63$ one can notice a visible difference in the inclination angles of the inner and outer vesicles.

Figure 3 shows a monotonic dependence of the average inclination angle $\langle \theta \rangle$ of outer vesicles on Λ in the TT regime similar to that observed for GUVs (see [5,9,11]). Thus, the effect of an inner vesicle on the external vesicle dynamics is little to none for $\phi \in [0.35, 0.6]$ (Fig. 3): indeed, we find the same quantitative dependence of $\langle \theta \rangle$ on Λ for both the compound (full circles) and GUVs (dash line) from [9]. Large scatter of the data occurs probably due to a wide range of the parameters of the compound vesicles Δ_{int} , Δ_{ext} , ϕ , R_{ext} used in the experiment. Contrary to our results, the numerical simulations [3,4] of a compound vesicle with both solid spherical inclusion and inner vesicle show a significant dependence of $\langle \theta \rangle$ on ϕ at given Λ , Δ_{ext} , Δ_{int} and the shifted value of Λ_c of the TT-TR transition in addition to the different dependence of $\langle \theta \rangle$ on Λ compared with the dependence for GUV in TT [5,9,11]. Moreover, the numerical simulations neglect thermal noise, which is known to play a key role in the vesicle dynamics, in particular in the vicinity of the TT-TR transition as well as in the TR regime [7,8]. Indeed, due to the amplification of thermal noise, Λ_c for the TT-TR transition was found to be lower for GUVs than predicted by "noiseless" models [7,11], and independent on other parameters such as the excess area [8].



FIG. 4 (color online). (a) Time evolution of both θ_{ext} and θ_{int} of compound vesicle with $\phi = 0.35$, $\Delta_{ext} = 1.04$, $\Delta_{int} = 0.73$ showing dynamics at various (Λ ; *S*): TT (1.03; 1270), TR (1.93; 675), TT (1.03; 1270), and TU (4.83; 270) regimes. (b) Zoom-in presentation of time evolution of θ_{ext} and θ_{int} to emphasize swinging (SW). (c)Snapshots of two subsequent cycles in TR of the same vesicle as in (a). Wrinkling in the external vesicle is clearly visible.

Significantly larger values of $\langle \theta \rangle$ and Λ_c are found for the inner vesicles (full squares) at the same dependence of $\langle \theta \rangle$ on Λ , so the dependence is shifted up, when the external flow parameters are used (upper dash-dotted line in Fig. 3). The latter means that the relative angle between the main axes of the external and internal vesicles $\Delta \alpha$ is nonzero and is independent on Λ and ϕ . Similar increase of the inclination angle of a GUV in TT is found in the simulations [15] due to confinement. It is also noticed in [4] that $\Delta \alpha$ differs from zero.

Upon a transition to TR and TU, the compound vesicle exhibits more complex and interesting dynamics. At small ϕ , the external and internal vesicle dynamics are weakly correlated, and both vesicles can be found simultaneously in different dynamical regimes [Fig. 4(b), (c)]. Indeed, unexpected behavior was observed for the external vesicle in TR that is distinguished by strong shape deformations, wrinkles, typical at high *S* [16,17], whereas the inner vesicle is found in a swinging (SW) motion, when its main axis oscillates around a positive $\langle \theta \rangle$ [Fig. 4(b), (c)]. Similar dynamics are observed in the numerical simulations for $\Delta_{int} > \Delta_{ext}$ and the solid inclusion in [3], where the external vesicle undergoes SW, while the inclusion



FIG. 5. Time evolution of both θ_{ext} and θ_{int} of the same compound vesicle as in Fig. 4 in TU. θ_{ext} : solid line and θ_{int} : dash line. Inset: Time dependence of $\Delta \alpha$ quantifies desynchronizing.

tumbles. The SW motion is also reported for the inner vesicle at large ϕ and S in [4]. The swinging was first observed for capsules and RBC in experiment, theory, and numerical simulations [18–21].

From Fig. 4(a) one finds that transitions to the new states due to (Λ, S) variations are simultaneous for both vesicles. Upon the transition to TU, we find that the TU velocity is slightly different for the outer and inner vesicles that leads to desynchronizing of $\theta_{int}(t)$ and $\theta_{ext}(t)$ (see Fig. 5), as quantitatively characterized in the inset in Fig. 5.

At sufficiently large $\phi = 0.6$, the dynamics of the inner and outer vesicles become synchronized in TR and TU [Fig. 6(a)]. Snapshots in the TR regime in Fig. 6(b) clearly show a pronounced wrinkling in the both inner and outer vesicles, similar to the images in Fig. 4(c) as well as for GUVs in TR at large S reported in Ref. [16]. In comparison with the latter case, the wrinkling of the outer and inner vesicles in Fig. 6(b) is much stronger even at smaller S but at larger Δ . It is revealed in strong variations, more than an order of magnitude, in amplitudes of high harmonics at $n \ge 5$ in the outer and inner vesicles shape spectra for compression and stretching moments during the TR cycle (for details see the vesicles' shape power spectra in [22]).

Here we would like to summarize our observations. First, we study typical behavior of the compound vesicles in TT and find discrepancies with the simulations [3,4]. The dependence of $\langle \theta \rangle$ on Λ in TT and the transition value of Λ_c to TR for compound vesicles in the wide range of S, ϕ , Δ_{int} agree within the scatter with that of GUVs. On the other hand, significantly larger values of $\langle \theta \rangle$ and Λ_c are found for the inner vesicles with the same dependence of $\langle \theta \rangle$ on Λ . Thus the whole dependence of $\langle \theta \rangle$ on Λ is shifted up, probably due to confinement, in a qualitative agreement with the simulations [4]. Second, we report rich and complex dynamical behavior depending on ϕ that manifests itself either in synchronizing at larger ϕ or



FIG. 6 (color online). (a) Time evolution of both θ_{ext} and θ_{int} of compound vesicle with $\phi = 0.6$, $\Delta_{ext} = 1.92$, $\Delta_{int} = 1.2$ showing dynamics at various Λ ; *S* in the TT (1.1; 530), TR (2.07; 282), TT (1.1; 530), and TU (5.19; 112) regimes. (b) Snapshots of the same vesicle as in (a) in TR. Wrinkling in both vesicles is clearly visible.

desynchronizing at smaller ϕ of the dynamics of the inner and outer vesicles. In the latter case, different dynamical states for the inner and outer vesicles at the same Λ , *S* are observed. Third, a new dynamical regime SW for the inner vesicle is observed at $\phi = 0.35$ in partial agreement with simulations [3,4]. Fourth, we present the evidence for the wrinkling of the outer vesicle in TR at small ϕ and of the both outer and inner vesicles at large ϕ , similar to the wrinkling in TR at large *S* recently reported for GUVs [16]. As shown recently, thermal noise plays a key role in describing GUV dynamics, particularly in TR and the TT-TR transition [7,8,16]. Thus we believe that the neglecting thermal fluctuations in theoretical models may be one of the main sources for the discrepancy with experiments.

To conclude, we present for the first time the results on the complex dynamics of the compound vesicles in a linear flow generated in the dynamical trap, which are determined by the four control parameters: Λ , S, ϕ , Δ_{int} . The inclusion of an inner vesicle leads to much richer vesicles dynamics than observed for GUVs but experimentally less traceable due to larger number of the control parameters. Currently we are not capable of controlling two of them, namely ϕ and Δ_{int} , which hinders us in reconstructing a phase diagram of all dynamical states of compound vesicles, though we find mostly the same main dynamical regimes and transitions between them as for GUVs [6,11]. Whether this complicated model can mimic the dynamical behavior of WBCs or one needs to further take into account a non-Newtonian nature of a WBC and its nucleus, consisting of fibers, organelles, cytoplasm, etc., remains an open question. We hope that this first experiment of its kind would stimulate further experimental and theoretical development towards modeling of complex biological structures.

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