JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS, Vol. 11, No. 9, September 2009, p. 1218 - 1221

Tumbling of viscous vesicles in a linear shear field near a wall♣

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The dynamics of lipid vesicles enclosing a viscous polymer solution is studied in a simple shear flow near a wall. The ratio between the internal fluid viscosity and the one of the outer aqueous solution is chosen to be above the tank-treading-to-tumbling transition for all analyzed vesicles. A clear influence of the presence of the wall on the tumbling motion of vesicles has been detected. In the entire range of applied shear rates, pure tumbling has not been observed due to the high density difference between the internal fluid and the suspending medium, keeping vesicles close to the wall. The strong deformability of their membranes, coupled to the high viscosity ratios, leads to a periodic modulation of the vesicle shape, with a periodicity correlated to the shear rate of the hydrodynamic flow and amplitudes depending on the vesicle's deflation. This shape modulation is coupled to a rolling motion of the vesicles.

(Received November 5, 2008; accepted December 15, 2008)

Keywords: Giant vesicles, White blood cells, Shear flow, Deformability

1. Introduction

The mechanisms of cell transmigration under flow have been an object of active experimental and theoretical research during recent years, due to their strong relevance to such important processes as the anti-inflammation response of the immune system and cancer metastasis formation in the organism [1]. The major role of white blood cells (WBCs, or leukocytes) is to protect the organism from the invasion of foreign bodies and infectious diseases. Being the principal participants in the innate immune response of the organism, WBCs are recruited to attack the infectious agents. After the chemoattraction, rolling and tight adhesion steps [2], leukocytes transmigrate the endothelial layer of the blood vessel (the so-called extravasation), in order to reach and eliminate the foreign cell.

Important progress in the understanding of the different steps of cell transmigration through the endothelial wall has been achieved recently. A special interest has been accorded to the leukocyte margination in the blood flow [3-5], favoured by WBC interactions with erythrocytes and the cell segregation in the flow. Red blood cell aggregation is reported to promote leukocyte adhesion to the vessel, by initiating and stabilizing attachments following leukocyte margination [6]. Evidence for the hydrodynamic recruitment of rolling WBCs [7] revealed the collective character of the leukocyte accumulation at inflammatory sites. Various types of leukocyte exist [8], but nevertheless the cells have some common morphological features – they are nearly spherical, with diameters of the order of 10 μm. These characteristics are common with those of giant lipid vesicles, which have been widely used during recent decades as a purely physical model of biological cells (see for example [9]).

Here, we present an experimental study of non-adherent viscous vesicles in a linear hydrodynamic flow near a wall. We attempt to model the rolling motion of leukocytes on the endothelium before the tight adhesion phase during the cell transmigration. In the absence of any specific adhesion in our study, vesicles are kept close to the bottom wall of the experimental flow chamber, due to the gravity and the positive density difference between the enclosed polymer solution and the surrounding fluid. So far, the dynamics of tank-treading vesicles, enclosing simple sugar solutions, has been studied in a wall-bounded shear flow [10]. In the following, we will focus on tumbling vesicles.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich Chem. (France). Giant unilamellar vesicles were electroformed [11] from dioleoyl-phosphatidylcholine in

♣ Paper presented at the International School on Condensed Matter Physics, Varna, Bulgaria, September 2008

aqueous solutions, containing dextran to increase the viscosity, and glucose to maintain its osmolarity. Electroformation chambers with ITO coated plates were used. Immediately after preparation, vesicle suspensions were taken from the electroformation chamber, and diluted with a slightly hyperosmotic sucrose solution, leading to the deflation of the vesicles (due to the non-zero membrane permeability towards water). Dextran, with high molecular weight ($\approx 5\cdot 10^5$ g/mol), was used. Its aqueous solutions show Newtonian behaviour over a wide range of shear rates. To attain the desired viscosity of dextran solutions, it is necessary to use relatively high weight concentrations of dextran. This makes the vesicles heavy compared to the outer solution, and thus promotes their rapid sedimentation during the flow experiment. Thus, approaching the bottom of the shear cell, vesicle tumbling will be affected by the proximity. The viscosities of all polymer solutions were measured with a controlled Bohlin Gemini 150 rheometer (Malvern Instruments, Germany) with cone-plate geometry (60 mm diameter, 2° angle). A capillary viscometer (Schott-Geräte GmbH – Mainz, Germany) was used to determine the viscosities of the sucrose solutions.

All experiments were performed at room temperature (22–25°C) on an Olympus IX71 inverted microscope, inclined at 90° with respect to its usual position, so that the direction of gravity was in the focal plane and perpendicular to the direction of flow. Two types of flow chamber were used: a Poiseuille flow chamber (a rectangular box with dimensions: height 1 mm, width 10 mm, and length 70 mm), in which the flow was created using a syringe pump; and a cylindrical Couette chamber operating at an imposed shear rate [12]. On the scale of a lipid vesicle of diameter 50 μm, the shear rate close to the wall in the Poiseuille flow chamber was constant. Vesicles were observed in a phase contrast mode, and acquired directly. Image processing yielded the relevant geometrical parameters.

3. Results and discussion

Viscous vesicles, at various shear rates (12 – 120 s^{-1}) covering the physiological values at vessel walls, have been studied in a linear hydrodynamic field in the proximity of the bottom of the experimental flow chamber.

For every vesicle studied, its volume V and membrane surface area S were determined from the vesicle contour at rest (assuming axial symmetry). In this way, each vesicle is characterised by a nominal radius $R_0 = \sqrt{\frac{S}{4\pi}}$ and its reduced volume is $\nu = \frac{3V}{4\pi R_0^3}$. In our experiments, vesicles were deflated with ν between 0.92 and 0.99, corresponding to prolate spheroidal shapes under equilibrium [13]. When the viscosity ratio $\lambda = \eta_{in}/\eta_{out}$ between the inner and the outer fluid is low enough, a deflated vesicle assumes a fixed orientation in an unbounded shear flow, and the vesicle's membrane undergoes a tank-treading motion. Above a critical value of the viscosity ratio (depending on ν), a transition to an unsteady periodic motion occurs, and vesicles tumble in the flow. In our study, λ was between 10 and 20, which is higher than the threshold value for the tank-treading-to-tumbling transition in the range of all reduced volumes we studied [14].

In their liquid-crystalline state, lipid membranes are characterised by a bending (curvature) modulus, k_c (~ 10^{-19} J), and a stretching modulus, k_s (~ 0.1 N/m). Roughly, we can imagine the lipid vesicle membrane as a non-extensible shell with very small resistance to bending. In this case, a dimensionless capillary number:

$$Ca = \frac{\eta_{out}\dot{\gamma}R_0^3}{k_c}, \qquad (1)$$

characterising the deformability of the vesicle into the hydrodynamic field can be defined, where $\dot{\gamma}$ is the shear rate. In our experiments, capillary numbers were in the range $3.10^3 \div 3.10^6$.

The rotation angle is defined as the angle between the major inertia axis of the vesicle and the shear flow direction.

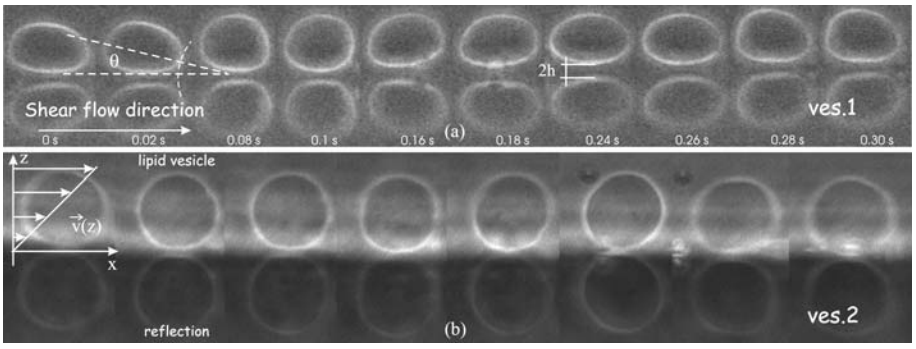

Fig. 1. Vesicles containing dextran-glucose aqueous solutions in a suspending fluid of 0.42 M sucrose in water, subjected to a linear shear flow near a wall: (a) Vesicle 1: $\nu = 0.92; \lambda = 10.3; R_0 = 30\ \mu m; \dot{\gamma} = 71.8\ s^{-1} \Delta\rho = 4\cdot 10^{-3}\ kg/l$, *internal solution: 7% Dextran and 0.38 M glucose; Poiseuille flow chamber; (b) Vesicle 2:* $\nu = 0.99; \lambda = 17; R_0 = 42\ \mu m; \dot{\gamma} = 50\ s^{-1}; \Delta\rho = 1.7\cdot 10^{-2}\ kg/l$ *internal solution: 10% Dextran and 0.38 M glucose;*

Couette chamber; time interval between two consecutive images 0.1 s.

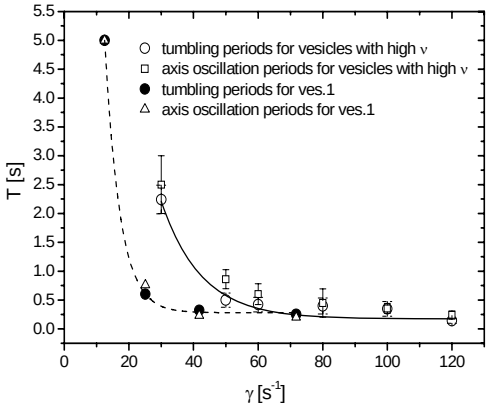

Fig. 2. Experimental data for the tumbling and the axis oscillation periods; the lines represent exponential decay fits of the type $T = T_0 + A \cdot e^{-\dot{\gamma}/\gamma_0}$ *: ves.1 (*$\upsilon = 0.92$*) – dashed line vesicles with high* $\upsilon \in [0.96, 0.99]$ *(the average period with its standard deviation is presented for at least three from an ensemble of ten vesicles)– solid line.*

The distance h between the vesicle and the bottom of the experimental cell is taken as half the distance between the vesicle's image and its reflection (see Fig. 1). In the range of shear rates studied, h was measured to be constant for a given vesicle (for vesicle 1 of Fig. 1 its value was $h = (7 \pm 1)$ μm). We were able to describe quantitatively the vesicle motion for different wall shear rates at viscosity ratios λ high enough to guarantee the tumbling of the vesicle far away from the wall.

In proximity to the wall, viscous vesicles roll in the flow direction with a translational velocity, depending on the shear rate of the flow. Their rolling motion is coupled to a shape modulation (measured through the variations of the two main axis lengths) with a periodicity correlated to $\dot{\gamma}$ (Fig. 2).

The tumbling and axis oscillation periods for a given vesicle were measured to be equal, within experimental error, for each shear rate, both for well deflated and nearly spherical vesicles (Fig. 2). The amplitudes of the shape fluctuations were higher for more deflated vesicles (Fig. 1). At small shear rates, the accumulation of the experimental data is accompanied by many technical difficulties (limited chamber length, restricting the maximum measurable distance of a vesicle's run, making impossible the recording of several periods for a given vesicle). When the shear rate increases, the tumbling and axis oscillation periods decrease down to a minimal value, which was found to be the same for deflated and more spherical vesicles (see the caption of Fig. 2). In particular, the tumbling period was not proportional to the inverse of the shear rate, contrary to the unbounded situation. In our case, the presence of a wall induced shape variations while the vesicle rolls. However, these deformations are limited by the membrane rigidity. Thus, when $\dot{\gamma}$ increases, the relevant time scale is no longer $\dot{\gamma}^{-1}$ but $\eta_{out} R_0^3 k_c^{-1}$, which is independent of the shear rate. Coherently, this asymptotic value is reached more quickly by deflated vesicles, for which the deformation amplitudes are more important (Fig. 1).

While they are rolling, vesicles also move in the flow direction. Goldman et al. [15] calculated the translational velocity U of rigid spheres of radius R_0 as a function of the applied wall shear rate $\dot{\gamma}$ in the limit $h << R_0$ (corresponding to our case, when vesicles are almost touching the bottom of the flow chamber):

$$U = \frac{0.743(1 + h/R_0)}{0.638 - 0.2\ln(h/R_0)} R_0 \dot{\gamma} \qquad (2)$$

We compare our measurements of the translational velocity of a deflated vesicle with this theoretical result. For the range of shear rates studied, the sphere's velocity calculated using (2) and the value of h experimentally measured is depicted in Fig. 3 (dashed line).

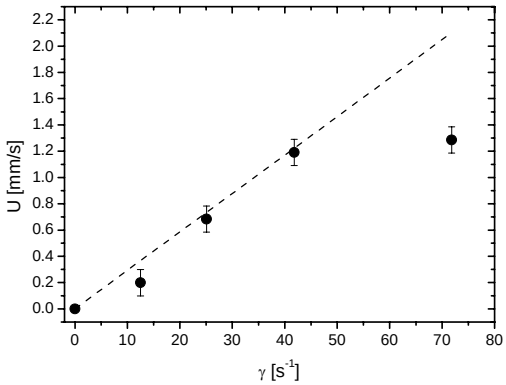

Fig. 3. The velocity of vesicle 1 as a function of the shear rate; dashed line: the theoretical results for the velocity of rigid spheres in proximity to the wall [15] (see text).

Besides the smaller apparent rotational velocity of deformable vesicles compared to that of rigid spheres ($T_{tumb} \approx T_0$ at high shear rates), lower translational velocities are measured, also for vesicles at high $\dot{\gamma}$ (in the range of the values, measured at the vessel wall). This can be related to saturation of the rotational velocity, which leads to an increase in the viscous friction between the vesicle and the wall. This observation may be relevant to the case of rolling WBC, which must slow down in order

to be able to adhere tightly, then to transmigrate the vessel wall and, afterwards, to reach the inflammatory site.

In the present study, a periodic rolling motion of deformable viscous vesicles in a linear shear flow near a wall has been observed and characterised. These deformable objects roll more slowly than rigid spheres in the flow at high values of shear rates, typical for those measured at a vessel wall in the blood flow. Our results may be helpful for the elucidation of the purely physical aspects of the cell rolling during the transmigration process through the blood vessel's wall.

Acknowledgements

V.V. acknowledges research grants from University "Joseph Fourier", Grenoble and CNRS (France). The contributions of CNES (France) and the Bulgarian National Science Fund (Contract FNI-1506) are acknowledged as well.

References

[1] C. Verdier, C. Couzon, A. Duperray, P. Singh, J. Math. Biol. (2008) (in press)
[2] C. A. Janeway, P. Travers, M. Walport,M.J. Shlomick, Immunobiology: The immune system in health and disease, Garland Science Publishing, New York (2005).
[3] L. L. Munn, M. M. Dupin, Ann. Biomed. Eng. **38**, 534 (2008).
[4] C. Magliorini, Y. Qian , H. Chen, E. B. Brown, R. K. Jain, L. L Munn, Biophys. J. **83**, 1834 (2002).
[5] V. Pappu, P. Bagchi, Biorheology **44**, 191 (2007).
[6] K. B. Abbitt, G. B. Nash, Am. J. Physiol. Heart Circ. Physiol. **285**, H229 (2003).
[7] M. R. King, D. A.Hammer, PNAS **98**, 14919 (2001).
[8] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson, Molecular Biology of the Cell, Carland Publishing, Inc. New York & London (1989).
[9] P. L. Luisi, P. Walde (editors), Giant Vesicles, John Wiley & Sons Ltd., Chichester (2000).
[10] M. Abkarian, A. Viallat, Biophys. J. **89**, 1055 (2005).
[11] M. Angelova, D. Dimitrov, Faraday Discuss. Chem. Soc. **81**, 303 (1986).
[12] M.-A. Mader, Dynamique de vésicules en écoulement, Ph.D. Thesis, University of Grenoble, France (2006).
[13] U. Seifert, K. Berndl, R. Lipowsky, Phys. Rev. A **44**, 1182 (1991).
[14] J. Beaucourt, F. Rioual, T. Seon, T. Biben, C. Misbah, Phys. Rev. E **69**, 011906 (2004).
[15] A. J. Goldman, R. G. Cox, and H. Brenner, Chem. Eng. Sci. **22**, 637 (1967).

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