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The Effects of Shear Force Transmission Across Vesicle Membranes

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Abstract

We report a comprehensive study on mechano-transmission of shear forces across lipid bilayer membranes of giant unilamellar vesicles (GUVs). GUVs containing fluorescent tracer particles were immobilized on a microfluidic platform and exposed to shear flows. A method was developed for the visualization of three-dimensional flows at high precision by defocusing microscopy. We quantify the symmetry of external flow around the GUV and show its effects on vortex flows and luminal dynamics. With increasing asymmetry, luminal vortices merged while liquid exchange in between them increased. The effect of membrane composition was studied through addition of cholesterol. Mechanotransmission efficacy, quantified by the ratio of luminal flow to external flow, ranged from $\epsilon = 0.094$ (0 mol % cholesterol) to $\epsilon = 0.043$ (16 mol % cholesterol). Our findings give new cues to the mechanisms underlying the sensing of strength and spatial distribution of shear forces by cells and the impact of membrane composition.

It is of great importance to understand the mechanical, chemical, and geometric aspects that govern the dynamics of shear force transmission across cell membranes. This mechanotransmission has diverse effects on cells, particularly endothelial cells (ECs), and enables them to determine their position, orientation, and migration direction.^{1,2} Due to the diversity of cellular response, cell models such as giant unilamellar vesicles (GUVs) with defined lipid compositions are frequently employed for studying shear forces in predefined environments and at reduced complexity.^{3–7} In the past, GUVs have been successfully employed to study effects of mechanotransmission such as changes in lipid order^{4,6} or membrane fluidity,^{8,9} which were originally observed in ECs.^{4,5,8,9}

External shear induces motion and deformation of the abluminal lipid monolayer, which is transmitted further into the luminal monolayer via intermonolayer friction,¹⁰ and finally into the vesicle lumen. Pioneering studies revealed shear flow speed-mediated changes in membrane flows and luminal flow,^{11–13} in agreement with theoretical predictions;¹⁴ however, they did not consider effects mediated by the spatial distribution of shear flow surrounding the GUVs. To also address the latter issue, we have developed a method for the

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quantification of mechanotransmission effects based on defocusing microscopy of fluorescent tracer particles enclosed inside GUVs, which are immobilized inside microfluidic channels. Our results show different luminal flow patterns and dynamics evolving inside GUVs as a consequence of different shear flow fields and flow speeds. The lipid composition of GUV membranes is another important aspect directly affecting mechanotransmission.^{6,7,12} Cell membranes contain a plethora of different proteins and molecules that modify its dynamic properties. Cholesterol is a natural constituent of cell membranes that decreases the fluidity of phosphatidylcholesterol (PC) membranes in a concentration-dependent manner.^{14–17} The effect of cholesterol concentration on the dynamic properties of lipid bilayer membranes is orders of magnitude smaller compared to the effect of lipid phase transition,¹⁵ and therefore difficult to detect. We quantify the effect of cholesterol on mechanotransmission in GUVs containing different concentrations of cholesterol above the phase transition temperature. Small differences in cholesterol concentration were detectable from bulk mechanotransmission measurements and thereby constitute a robust technique to quantify the dynamic properties of model cell membranes.

Defocusing microscopy takes advantage of the deterministic diffraction of highly regular objects, fluorescent micro spheres in this case, imaged outside of the focal plane (Figure 1). The principle has been applied to image normally invisible phase objects,^{18–22} two-dimensional flows by microparticle imaging velocimetry (μ PIV),²³ or for the detection of the three-dimensional orientation of single molecules.²⁴ An interesting approach employs 2D PIV of confocal z-slices and determines 3D movement of luminal flows by integration.¹³

Here, we use deterministic light diffraction to measure the bead's distance perpendicular to the focal plane (z-position) directly from each image. In combination with xy tracking of the bead's position in the image plane, we achieve highly quantitative 3D luminal flow tracing at great precision. The technique therefore allows direct observation of the time evolution of luminal flows, complementing the methods used in the seminal work in this field.^{12,13}

The setup of defocusing microscopy for 3D flow tracing requires correlation of the bead's diffraction patterns with the respective z-positions. A total of 646 beads were imaged at varying distances to the focal plane using a custom-built calibration device (Figures S1 and S2).

The data was aligned with the mathematical relation between diffraction ring diameter d_e in the microscopy image and the bead's z-position,^{25,26}

$$d_e = 1/\sqrt{2M[d_p^2 + 1.49\lambda^2(n_0^2/c_1NA^2 - 1) + 4(z - c_3)^2(n_0^2/c_1NA^2 - 1)^{-1}]^{1/2} + c_2} \quad (1)$$

where $M = 20$ is the magnification of the microscope optics, $d_p = 2 \mu\text{m}$ is the particle diameter, $\lambda = 515 \text{ nm}$ is the emission peak wavelength, $n_0 = 1$ is the optical density of the objective immersion medium (air), $NA = 0.45$ is the objective's numerical aperture, and c_1 ,

c_2 , c_3 are fit parameters. The tracking precision of our optical setup for the chosen bead size amounts to $\sigma_z = 0.791 \mu\text{m}$. Bead tracking in the xy plane is straightforwardly achieved by determining the center coordinates of the diffraction rings, resulting in $\sigma_x = 0.103 \mu\text{m}$ and $\sigma_y = 0.122 \mu\text{m}$. The herein presented combination of defocusing microscopy and 3D tracking yields 6-fold subresolution tracking precision in x , y , and z (details in the Supporting Information (SI)).

Validation of 3D tracing was done by evaluating bead movement inside the GUV lumen in the absence of flow through the channel. A freely diffusing bead, which is not attached to the lipid membrane, should display 3D Brownian motion. Bead movement was recorded over 10 000 images, and 3D positions were calculated as described in the SI. The anomalous coefficient α measures whether a tracked particle diffuses freely ($\alpha = 1$), experiences hindered diffusion ($\alpha < 1$), or active transport ($\alpha > 1$). Together with the diffusion coefficient D , it can be derived from the mean squared displacement as described in the SI (Figure S3). Results show indeed free diffusion inside the GUV ($\alpha = 0.987 \pm 0.018$), and the experimental diffusion coefficient $D = (3.75 \pm 4.777) \cdot 10^{-10} \text{ cm}^2/\text{s}$ was well within the limits of the theoretical diffusion coefficient $D_{\text{th}} = 6.548 \times 10^{-10} \text{ cm}^2/\text{s}$.

In the presence of external flow through the microchannels, a GUV experiences shear where its membrane is exposed to the bypassing liquid. Shear forces exert work on the GUV, which is transmitted into the membrane and further into the lumen, thereby inducing bead movement and vesicle deformation. Figure 2 shows two examples of luminal flow as a consequence of external shear flow of different strength and spatial distribution.

Luminal flow is generally organized in two neighboring vortices with respect to the x -axis. During one revolution, the flow follows along the vesicle apex in direction of the external flow, and back along the GUV equator antiparallel to external flow. This movement repeats with slightly varying paths, thereby allowing the bead to explore the entire GUV lumen over time (Movie 1 and Movie 2).

Figure 2 shows that flow speed inside the GUVs increases with external flow. Differences between the two GUVs A and B exist in the spatial organization of luminal flow, and we show here that these differences arise from the different flow fields surrounding the GUVs. Luminal flow in GUV A is symmetric with regard to the x -axis, and both vortices are of equal size. The black broken line indicates the division plane of luminal flow with respect to the x -axis and symmetrically divides luminal flow into left and right vortex (xy -view, Figure 2). Luminal flow speed is maximal near the division plane and decreases in the $\pm x$ direction until it becomes minimal at the stagnation points on the left and right side of vesicle. The positions of these stagnation points indicate the locations and orientations of the rotational axes of the two vortices, which is also symmetric (see black arrows in xz -views, Figure 2). The symmetry of luminal flow with respect to the x -axis, found inside GUV A, cannot be found in GUV B. Here, the flow pattern is asymmetric: the division plane is situated left of the geometric middle and divides luminal flow into a large vortex on the right and a small vortex on the left. Accordingly, the position and orientation of the rotational axes are different for left and right vortices.

The difference in spatial organization of luminal flow in GUVs A and B lies in the flow distribution generated by the different cross-sectional dimensions of the flow channels used, and the GUV positions therein. Figure 3 shows the cross sections of the flow channels used to investigate the GUVs in Figure 2, which were designed to generate symmetric (Figure 3A, $w_B = 1000 \mu\text{m}$, $h = 30 \mu\text{m}$) and asymmetric (Figure 3B, $w_A = 100 \mu\text{m}$, $h = 30 \mu\text{m}$) flows. The simulated 2D shear flow profile was calculated according to Bruus et al.²⁷

$$v_y(x, z) = \frac{4h^2 \Delta p}{\pi^3 \eta L} \sum_{n, \text{ odd}} \frac{1}{n^3} \left[1 - \frac{\cosh\left(n\pi \frac{y}{h}\right)}{\cosh\left(n\pi \frac{w}{2h}\right)} \right] \sin\left(n\pi \frac{z}{h}\right) \quad (2)$$

which shows highest velocities in the channel center and decreases toward channel walls, where it becomes zero. Depending on its lateral (x -)position inside the channel, a GUV experiences different shear acting on its membrane. In the channel center, the GUV experiences similar shear flow on left and right side and outside of the channel center it experiences a shear gradient between left and right. From the channel dimensions, GUV positions and sizes, we calculated the external flow speed at the vesicle apex, v_{apex} , as well as the external velocity difference between left and right side, v_{LR} for both GUVs. The ratio, $v_{\text{LR}}/v_{\text{apex}}$, is taken as a measure for flow symmetry and is independent of external flow speeds since velocities increase at the same rate throughout the channels. Thus, $v_{\text{LR}}/v_{\text{apex}} = 0.01$ for GUV A shows symmetry, while $v_{\text{LR}}/v_{\text{apex}} = 0.65$ for GUV B shows severe asymmetry despite being located closer to the channel center than GUV A. It is therefore important to consider the external flow speed at the location of the GUV rather than the bulk flow rate through the channel, as the latter does not account for the symmetric/asymmetric flow in and outside GUVs.

The effects of external shear symmetry go beyond division plane position and vortex size described above. We observed different luminal dynamics in the neighboring vortices, especially near the division plane. Left and right vortices seem spatially separated, but occasional bead movement across the division plane shows that they are connected. Figure 4 shows v_{lumen} , x , y , and z over time for GUV A ($v_{\text{ext}} = 694.4 \mu\text{m/s}$) and GUV B ($v_{\text{ext}} = 3472.2 \mu\text{m/s}$), which corresponds to Figure 2 (right columns of A and B, respectively). Oscillatory movement occurs on all axes; the bead is located in the right vortex for $x > \bar{x}(t)$ and in the left vortex for $x < \bar{x}(t)$, where the time average $\bar{x}(t)$ is the division plane position (red dashed line in $x(t)$ plot).

Oscillatory movement in GUV A shows that no significant differences in luminal dynamics exist between left and right vortex and thereby reflects the symmetry of external flow. Movement across the division plane does not affect luminal dynamics (zoom-ins, Figure 4A). During crossings from right to left (RL) vortex, the bead moves at a distance to the GUV membrane and at lower $\bar{v}_{\text{lumen}} = 20.2 \mu\text{m/s}$ than during left-to-right crossings (LR, $\bar{v}_{\text{lumen}} = 21.9 \mu\text{m/s}$). Bead movement across the division plane from left to right follows close to the GUV membrane.

Luminal dynamics in GUV B are distinctly different in each hemisphere and the movement across the division plane is accompanied by sharp transitions in luminal dynamics (zoom-ins, Figure 4B). During crossings from right to left vortices (RL), the bead is located far away from the GUV membrane in the center of the GUV while the movement becomes very slow ($\bar{v}_{\text{lumen}} = 33.4 \mu\text{m/s}$). Crossing back (LR) occurs when the bead reapproaches the GUV membrane and at much higher luminal velocity $\bar{v}_{\text{lumen}} = 104.8 \mu\text{m/s}$. Dynamics and spatial organization of vortex flows reflect the symmetry of external flow surrounding the GUV, quantified by $v_{\text{LR}}/v_{\text{apex}}$. These data suggest that the two neighboring vortices are de facto separated for symmetric external flow (vortex sizes and luminal dynamics are equal). For asymmetric external flow the vortices exchange liquid from right to left through the GUV center and back along the membrane, as if part of a single vortex spanning the GUV lumen. In other words, the liquid exchange between hemispheres is generally faster in GUVs exposed to asymmetric flows.

Shear forces exert work on the GUV by inducing bead movement and vesicle deformation. The deformation of the membrane can be derived from the luminal trajectories as well as by taking images of stained GUV membranes under flow. A detailed analysis of membrane deformation can be found in the SI (Figure S4).

In the vesicle lumen, the flow speed differs considerably between vesicle bottom and apex (SI Figure S5), which is largely in agreement with former observations by Honerkamp et al.¹³ The efficacy of mechanotransmission, ϵ , here defined by the ratio of $v_{\text{ext}}/v_{\text{bead}}$, can be best quantified at the vesicle apex, where luminal and external flow move in parallel. At this position, we determined the efficacy of mechanotransmission in dependence of the membrane composition (Figure 5). A total of 17 vesicles containing 0 mol % ($n = 5$), 5 mol % ($n = 4$), 10 mol % ($n = 3$), or 16 mol % ($n = 5$) cholesterol were exposed to up to five different flow rates ranging from 1 $\mu\text{L}/\text{min}$ to 50 $\mu\text{L}/\text{min}$, and luminal bead movement was analyzed.

The external flow speed v_{ext} was calculated at the apex of each GUV according to eq 2 and plotted against luminal flow speed measured at the apex. For this evaluation, we considered data within a z -range of $h_{\text{GUV}}/10$ under the apex for each vesicle. Luminal flow speed v_{lumen} scales linear with v_{apex} , and the slope defines the efficacy of mechanotransmission across the GUV membrane. In the absence of cholesterol ($\epsilon_0 = 0.094$), nearly 10% of the external flow is transmitted across the lipid bilayer. The addition of cholesterol impaired mechanotransmission already in small amounts (5 mol % cholesterol, $\epsilon_5 = 0.079$). Further increase of cholesterol content progressively decreased mechanotransmission efficacy, determined for 10 mol % cholesterol ($\epsilon_{10} = 0.061$) as well as 16 mol % cholesterol ($\epsilon_{16} = 0.043$), i.e., a drop of the mechanotransmission efficacy by more than 50% compared to GUVs lacking cholesterol. This concentration-dependent decrease of mechanotransmission complements former studies, where a decreased lateral diffusion of lipids was determined for increased cholesterol concentrations,¹⁵ suggesting a negative effect of cholesterol on membrane fluidity in GUVs.

Shear force transmission across lipid bilayers is one of the immediate effects of fluid flow on living cells. To investigate the force transmission in simple model cells, we developed a new method for the visualization of luminal flow patterns in 3D at high precision. The ability to observe bead movement over long periods lead to a full description of luminal flows. Importantly, our approach does not require labeling of the lipid membrane and is applicable to other studies on force transmission across interfaces, e.g., in droplets and emulsions.

We found that the external shear force profile given by the microchannel geometry is transmitted across the lipid bilayer and directly reflected in the spatial organization of the bihemispheric luminal flow (symmetric or asymmetric flow, which is determined by v_{LR}/v_{apex}). Symmetric flows lead to less liquid exchange in between hemispheres and near-equal velocities on either side: the hemispheres remain de facto separated. Asymmetric flows lead to intercalation of left and right vortices, increased liquid mixing between hemispheres, and great differences in luminal dynamics. In addition, we showed that small changes in the membrane composition affected the efficacy of mechanotransmission measurably in a concentration-dependent manner. We believe that our results give cues to how cells sense their environment, e.g., with regard to recent studies on the orientation of ECs in shear flows.^{1,2}

Experimental Methods

For details of the experimental methods see the [Supporting Information](#).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Defocusing microscopy for 3D particle tracking. (A) Schematic of the experimental setup. A GUV containing a fluorescent bead is immobilized at the bottom of a microfluidic channel and exposed to shear flow. The focal plane is set above the GUV so that the enclosed bead appears defocused at all times (see inset images). The bead appears increasingly diffracted as it moves away from the focal plane. Scale bars: $10 \mu\text{m}$. (B) Calibration curve for the z -position tracking. Particle image diameters d_e of all beads were plotted against their z -positions. Only beads with z -positions $< -5 \mu\text{m}$ (red circles) were considered for calibration due to the nonlinearity of the curve close to the focal plane ($z > -5 \mu\text{m}$).

Figure 2.

Luminal flow inside two GUVs experiencing symmetric (A) and asymmetric (B) shear at varying external flow speed. Each column depicts a GUV in different representations from top to bottom (3D, XY, XZ, YZ) as illustrated by the schematics on the left side. From left to right, each column shows the same GUV at increasing external flow. (A) The vesicle ($d_{\text{GUV}}^{\text{A}} = 9.1 \mu\text{m}$) is exposed to a symmetric external flow. (B) The vesicle ($d_{\text{GUV}}^{\text{B}} = 19.9 \mu\text{m}$) is exposed to asymmetric external flow. The two black arrows in the xz -representations of the

slowest external flow speed indicate the stagnation points of the luminal vortices. Schematics (left side): Black arrows and dots indicate direction and strength of external flow, while red arrows and dots display the luminal trajectory. Luminal flow velocity: color-coding according to legends at the bottom of each column.

Figure 3.

Flow simulation through different channel cross sections. GUVs are marked at their original positions. Due to $w \gg h$ in channel A, the GUV experiences nearly equal shear forces on its left and right side, whereas the shear forces acting on the GUV in channel B differ significantly on each side. Simulations were calculated according to eq 2 for a volumetric flow rate of $Q = 1 \mu\text{L}/\text{min}$ for both channels.

Figure 4.

Bead crossing between hemispheres. (A) Top: 1D time traces ($v(t)$, $x(t)$, $y(t)$, $z(t)$) of bead movement over 200 s at $v_{\text{ext}} = 694.4 \mu\text{m/s}$ show homogeneous oscillations. Bottom: 3D time traces of LR and RL hemisphere crossings occurring throughout the image sequence, which occurred at near-equal speeds. Schematics illustrate flow speed and location of hemisphere crossings. (B) 1D time traces ($v(t)$, $x(t)$, $y(t)$, $z(t)$) of bead movement over 100 s at $v_{\text{ext}} = 3472.2 \mu\text{m/s}$ show severe changes in oscillatory magnitude and frequency during crossing events. The red dashed lines in both $x(t)$ graphs of A and B indicate the division plane

positions. Bottom: 3D time traces of hemisphere crossings and schematics in the asymmetric GUV. While RL crossings occur near the GUV center and at low speed, LR crossings occur near the membrane and at much higher speeds as a consequence of the asymmetric external shear profile. Within each 1D time trace, solid lines at the bottom and dotted lines at the top indicate minimum and maximum GUV positions and velocities, respectively.

Figure 5.

Velocity transmission at the vesicle apex for different cholesterol concentrations. Near the vesicle apex, external and luminal flow are in parallel and therefore allow direct measurement of the transmission efficiency $\varepsilon = v_{\text{lumen}}/v_{\text{apex}}$. Shear transmission is highest in vesicles membranes lacking cholesterol and decreases with increasing cholesterol concentration: $\varepsilon_0 = 0.094$ (black, $n = 5$), $\varepsilon_{5\% \text{ Chol}} = 0.079$ (blue, $n = 4$), $\varepsilon_{10\% \text{ Chol}} = 0.061\%$ (red, $n = 3$), $\varepsilon_{16\% \text{ Chol}} = 0.043\%$ (green, $n = 5$). The inset shows the same graph with linear axes, highlighting the different slopes, hence mechanotransmission efficacies.