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The relative flow of the walls of phospholipid tether bilayers

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Abstract

Lipid nanotubes or "tethers" can be formed from liposomes or niosomes, pulled from the parent vesicles by micromanipulation. The tethers are cylindrical multibilayer tubes. Here, we describe the movement of these multilamellar walls, initiated by creating a surface tension gradient along the tether. The movement of lipid can give rise to a visible moving boundary. In the case of bilayer membranes, a tangential gradient in surface tension produces membrane bulk flow toward regions of higher surface tension. The flow of the bilayers comprising the tether nanotubes seems to be restricted to the inner bilayers, creating a velocity gradient in the bilayers. In this study, we discuss the implementation of tension-driven flows as a transport method in a tether-vesicle network. Interactions between fluid within the tether channels and the lipid layers are important, leading to anomalies in the transport of fluids and particles compared to bulk systems.

Keywords: Liposome; Tether; Nanotube; Soft-matter; Nanofluidics

In this note, we describe the behaviour of multilamellar tether nanotube walls. We hypothesise that the tension of these tethers may involve the selective movement of the tether bilayers. The work extends our study of the micromanipulation of liposomes and niosomes creating, inter alia, so-called vesicular shuttle systems (Nasseri and Florence, 2003a,b,c). A vesicular shuttle is a system in which a liposome or niosome moves within tethers pulled from its surface. The movement of vesicles or particles inside the tethers could involve interaction with the channel walls. The flexibility of the walls and the vesicles themselves may complicate the calculations of the frictional forces involved. In previous studies, we have presented evidence that a depleted vesicle, i.e. a vesicle whose outer bilayers have been extended from the tether, then suspended by its polar tethers can be set into motion, either by suction through micropipettes or by an elongation of one tether, creating an imbalance in the surface tension, subsequently propelling the vesicle in the opposite direction (Nasseri and Florence, 2003a,b,c). In the present paper, the subject of the investigation is the lipidic bilayers of the tether walls and their movement when only negative pressure is used to cause the vesicles to move.

Transformation of liposomes into satellite entities with protruding nanotubes (tethers) provides us with

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lipidic tubules capable of allowing solute or solid flow within their channels, a process, which exists in cells as a method of transport of content between cells (Onfelt and Davis, 2004). This behaviour in nature has been mimicked in liposomes (Karlsson et al., 2002; Nasseri and Florence, 2003a,b,c).

Multilamellar liposomes of cholesterol and distearoyl-phosphatidylcholine (DSPC) were prepared using the hand-shaking method. Borosilicate glass capillaries with tip sizes 1–2 μm were prepared and mounted on Narishige micromanipulators to handle the liposomes under an inverted microscope (×40 magnification). Multilamellar tethers were pulled with micropipettes from the surface bilayers of liposomes. The movement of tether wall bilayers was studied using marker microspheres or liposomes positioned to adhere to the tether surfaces. The flow behaviour of these adhered entities with respect to the flow of tether bilayers was investigated.

Photomicrographs (Fig. 1A) show the apparent flow of tether bilayers induced through a continuous deformation of the parent vesicle using a micromanipulator to apply negative pressure to the vesicle system. This deformation causes an increase in the surface to volume ratio and the membrane in the tether is stretched, resulting in an increased surface tension.

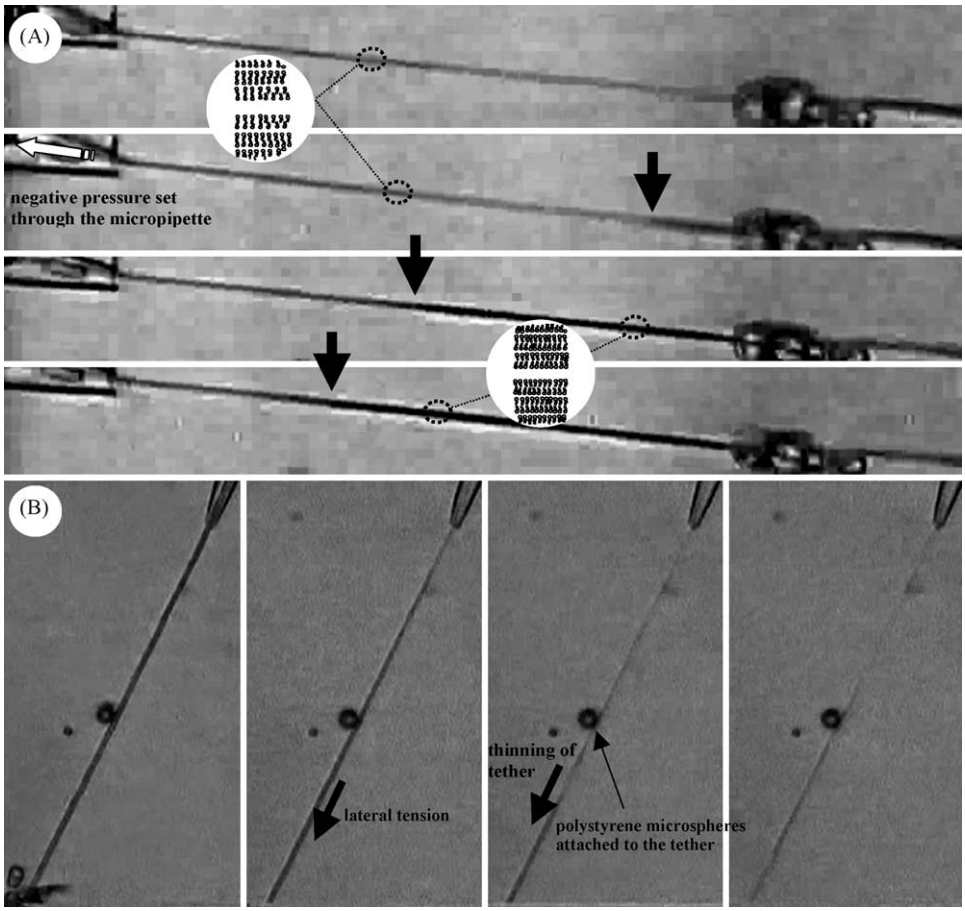

Fig. 1. Photomicrographs showing (A) a tether under tension, leading to the flow of bilayers evidenced by the dense areas moving along the tether. The insets show the state of a typical bilayer at various positions under different tensions. (B) The shift in the bilayers under tension (thinning) is shown, while adhered polystyrene microspheres remain at the same position. Arrows in (A) show the advancing front, which appears as a step.

To eliminate this tension gradient, lipid flows from regions of lower tension in the tether to regions of higher tension. The flow of the lipid membrane is evidenced by a lateral translation of a cylindrical wall, moving with a velocity, which depends on the balance of the tension forces applied. Bidirectional migration of lipid bilayers of tether walls advancing distances of up to 100 μm with speeds of up to 12.5 $\mu m\,s^{-1}$ can be achieved.

Polystyrene microspheres attached to the outside of the tether nanotubes acted as a marker of flow and lipid flow was observed by the thickening or thinning of the tether. Surprisingly, the attached beads did not move while the lipid flow in the tether bilayers continued, as shown in Fig. 1B. When a shuttle vesicle is set in motion within the tether channel, beads attached to the outside of the tether nanotube do not display any concurrent movement, as shown in Fig. 2.

The attachment of polystyrene beads to a tether seems to create a relatively strong fusion with the outer bilayers of the tether wall a process discussed in detail by Dietrich et al. (1997). Hence, this may help shed light on the relative movement of bilayers when the tether wall lipids are set to flow. While this attachment may only involve the outer bilayers of the tether wall, the fusion of a lipid vesicle to the tether undoubtedly

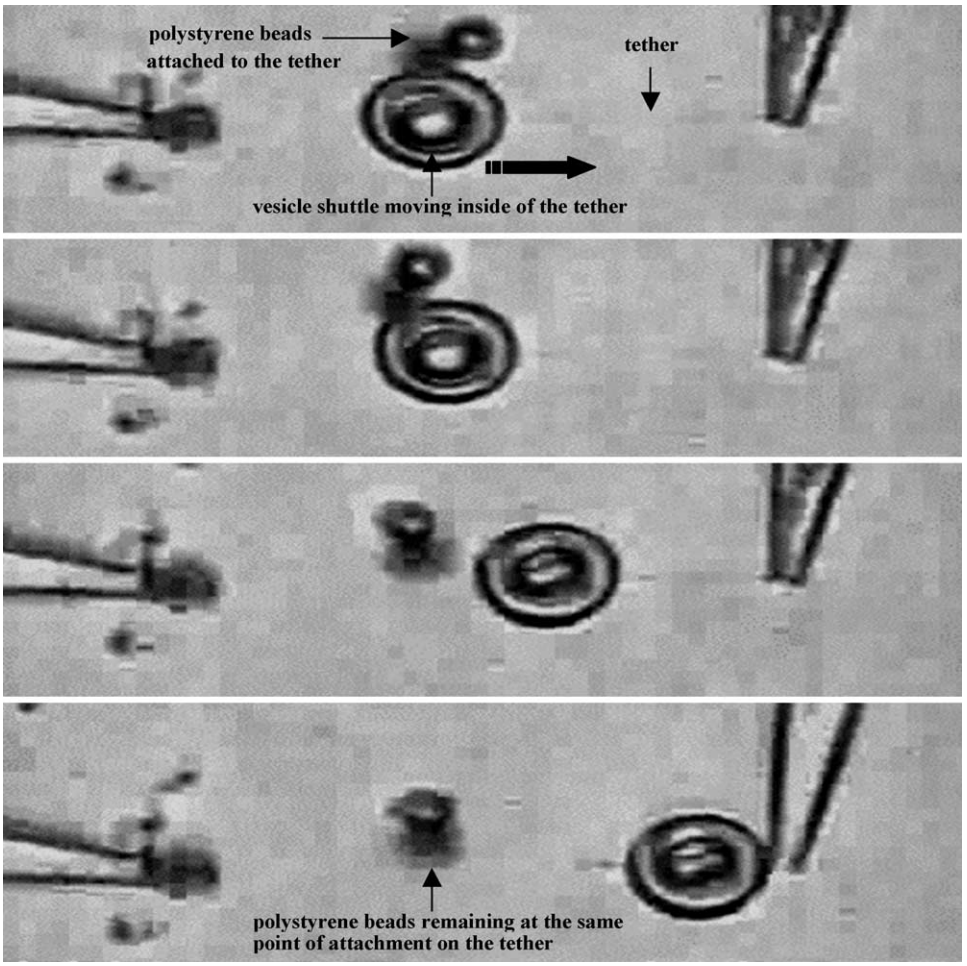

Fig. 2. Sequence showing the movement of a vesicular shuttle within a tether with a latex bead is attached to the surface of tether. The vesicle moves along the inside of the tether, while the bead remains at the same position riding the outer contours of the vesicle as it passes.

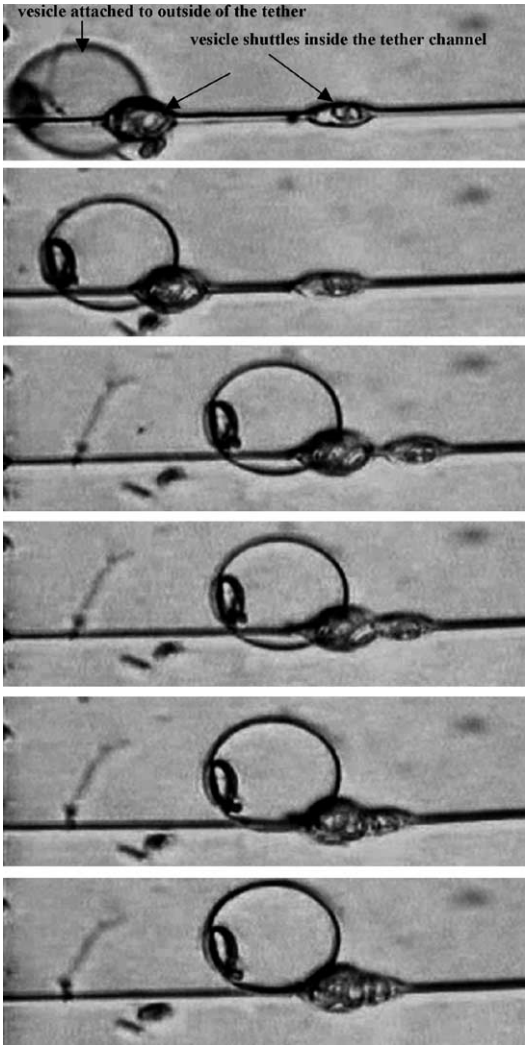

Fig. 3. Photomicrographs showing the movement of two vesicles inside a tether made to travel towards each other and fuse into one larger vesicle. In the process, the vesicle attached to the outside of the tether is dragged along (not seen with the attached microspheres). The latter remain at their positions when the tether bilayers move past the sphere (Fig. 1) or when a vesicle inside a tether is passing by (Fig. 2).

leads to different modes of interaction. In Fig. 3, a large vesicle is attached to the outside of a tether. The small vesicle inside the tether is set in motion. The adhered vesicle, as seen in Fig. 3, is dragged along with the moving inner shuttle vesicle; perhaps explainable if it is anchored to or interacting with a greater number of tether bilayers than the latex bead.

The schematic in Fig. 4A represents the multilamellar walls of a tether with the parent "depleted" vesicle (i.e. a vesicle having lost its outer bilayers to the pulled tether) now inside the tether channel (Nasseri and Florence, 2003a,b,c). The adhesion of a polystyrene microsphere and a liposome to the tether is also shown. Experiments showed that the adhesion of a liposome to a tether occurs more rapidly than a solid polystyrene microsphere. On average, keeping a liposome in contact with a tether for 30 s can ensure adhesion, while polystyrene microspheres required a longer period of contact, in some cases up to 3–5 min. In the case of liposomes, the evolution of secondary tethers attaching the liposome to the original tether was evident as shown schematically in Fig. 4B. The apparent reorientation of the amphiphilic molecules from both the tether and the liposome may provide possibly a strong adhesion, perhaps fusion. On retrieval of the liposome from the tether (Fig. 4B and C), the deformation of the tether following the path of the retrieving liposome may indicate the evolution of this secondary tether.

In Fig. 4B, only one bilayer is illustrated. However, the reorientation of the surfactants may involve more bilayers further inside the wall as these tethers are multibilayers. This may be the reason for the simultaneous movement of attached liposomes with the shifting tether wall bilayers, which is not seen with the attached polystyrene microspheres.

In microchannels with elastic membranes, the pressure field and the accompanying velocity are at their peak in the center of the channel. In the process of vesicle shape transformation (i.e. from leek shape to onion shape) the lamella bilayers have been shown to flow in relation to each other with their interabilayer interactions reducing as the movement takes place (Marlow and Olmsted, 2002). When fabricating polyhedral niosomes into microtubules by extrusion through microtipped pipettes, the inner bilayers of the microtubules were found to move faster so much that on reaching the closed end of the microtubule they would fill up any empty space or even shape the tubule into a spiral or "whorl" structures (Nasseri and Florence, 2003b). This would also explain the relatively low extent of movement of the multibilayer wall in the outermost part of the nanochannels. Results may suggest that under tension lipid bilayers in the walls of the tether network seem to show movement as seen in the thickening of the tether; however,

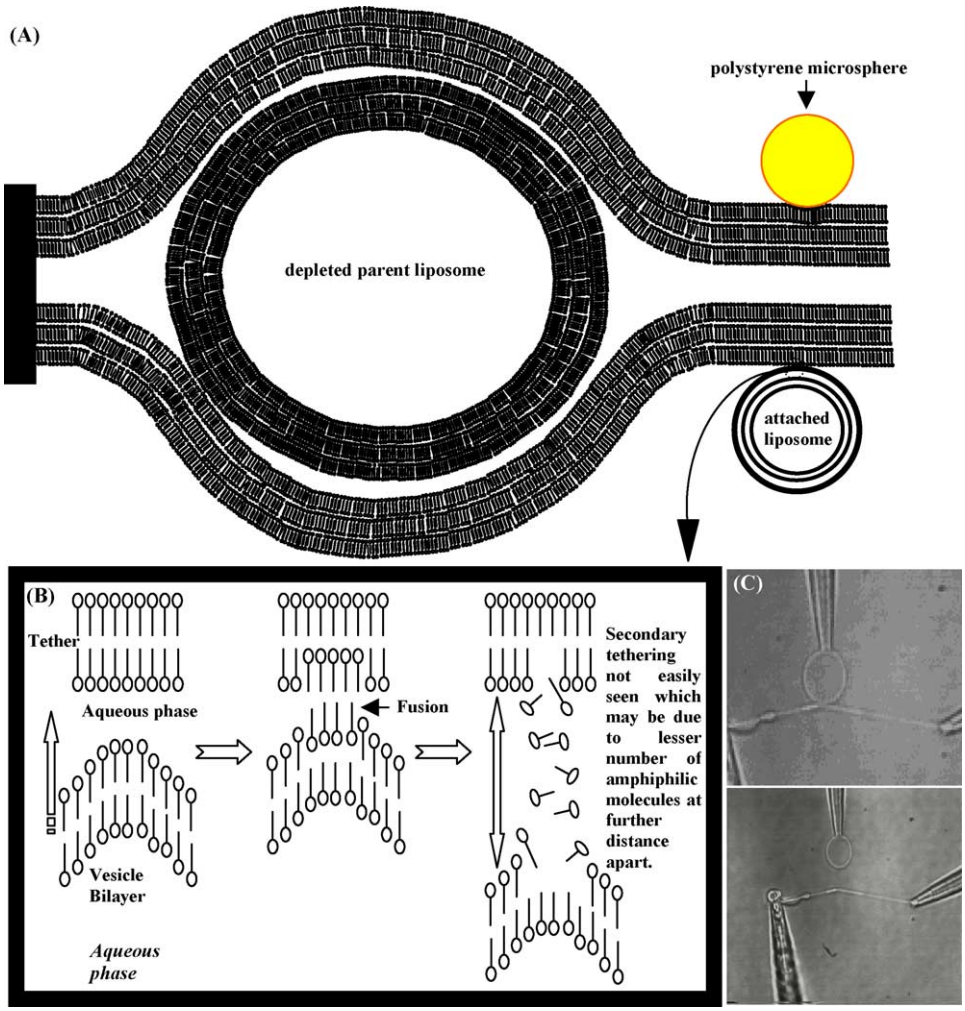

Fig. 4. Schematic showing (A) a depleted vesicle with its tether and a polystyrene bead and a vesicle attached to the outer part of the tether. (B) The depiction of the possible changes in bilayer structures involved in the attachment and detachment of a tether to a vesicle leading to evolution of a secondary tether as also shown by the photomicrographs (C) where, on withdrawal of the externally attached vesicle to the tether, the tether follows the path of the retrieving vesicle indicating the possible evolution of a secondary tether too thin to be visible.

this movement seems to be exclusive mostly to the innermost bilayers, leaving the outer bilayers almost motionless. This tension induced relative movement of bilayers may give rise to a phenomenon referred to as "necking" (Allison and Andrews, 1967), where, the surfactant molecules may be associated with a degree of reorientation and as the faster moving bilayers accumulate at the leading front and the need to expand the tether nanotube arises, a bottle neck effect is observed. The localized neck under yielding tension propagates through the whole length of the tether until the net surface tension across the tether is balanced out.

These findings suggest that, while it is possible to devise an ultra-flexible nanostructure capable of transporting large vesicles or particles, additional factors such as the selective movement of nanotube walls and their interaction with the inner moving particles will affect transport. It is therefore important to examine the behaviour of constitutional elements of these nanos-

tructures to obtain a better understanding of vesicle and wall effects.

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