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Microtubules formed by capillary extrusion and fusion of surfactant vesicles

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A paper dedicated to Professor Hans E. Junginger on the occasion of his 60th birthday

Abstract

Polyhedral non-ionic surfactant vesicles formed from mixtures of polyoxyethylene-5-cetyl ether ($C_{16}EO_5$) or polyoxethylene-5-stearyl ether ($C_{18}EO_5$) with poly-24-oxyethylene cholesteryl ether (Solulan C24) and low amounts of cholesterol, when extruded from microcapillaries under pressure fuse to form multi-lamellar tubules up to about 80 μm in length. The diameter of the extruded tubules depends on the exit diameter of the capillaries used, in this paper generally around 1 μm. Under some circumstances, instead of linear tubules, the tubules form as concentric whorls which can be unraveled into their constituent tubules, indicating the strength of the systems. Vesicles can be formed within these tubular structures to act as a model for vesicular flow in elastic capillaries. Microparticles encapsulated with the primary polyhedral vesicles after extrusion are seen within the tubules, promising the possibility of a model for the study of microparticle flow within vessels. Preliminary studies on the use of the tubules as templates for polymerisation are also presented.

Keywords: Polyhedral niosomes; Microfabrication; Microtubules; Surfactant tubules

1. Introduction

Non-ionic surfactant vesicles (niosomes) like liposomes, their phospholipid counterparts, are of interest as vectors for drug and gene delivery (Uchegbu, 2000). They can be formed as spherical unilamellar or multi-lamellar systems, as geodesic arrays (Sternberg et al., 1995) or as toroidal (Seifert, 1991) and polyhedral structures (Uchegbu and Florence, 1995) in aqueous media, in addition to the inverse structures apparent in non-aqueous solvents (Murdan et al., 1999). This morphological versatility is important as it allows exploration of carrier systems of different shape and properties and of optimal capacity for therapeutic agents, required for delivery to different sites in the body, such as the deeper layers of the skin where elasticity and flexibility are required for penetration (Cevc et al., 1998; van den Bergh et al., 1999).

The behaviour of drug delivery vesicles under stress in the capillary blood supply or in their movement through complex organs is of considerable importance but virtually unexplored. Extrusion through narrow glass capillaries of polyhedral forms of non-ionic surfactant vesicles formed with low levels of cholesterol can, as we describe here, result in the permanent loss of primary shape leading to the formation of stable surfactant tubules and related structures. The interest in such micromanipulation of vesicular systems, such

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as described by Karlsson et al. (2001) rests not only in creating new structures for drug and gene delivery, but also for templating to create new forms by polymerisation of entrapped monomers, or as we suggest here, creating systems for the study of particle flow in flexible capillaries, where models are scarce.

The manipulation and control of the morphology and properties of carriers suitable for drug, gene and vaccine delivery is increasingly a major theme in research. Extrusion of individual synthetic non-ionic surfactant or phospholipid vesicles intact from capillaries has been suggested by us to be a simple form of a biomimetic system (Arunothayanun et al., 1999a) allowing some simulation of natural vesicular delivery. However, our earlier work emphasised the problems faced in forcing an array of individual vesicles intact through capillaries, because of their tendency to deform, flocculate or assume new shapes. In this paper, we describe some of the morphologies that are possible under controlled conditions. A brief account of this work has been published elsewhere (Florence and Nasseri, 2001).

2. Materials and methods

2.1. Materials

Reagents and chemicals including 5(6)-carboxyfluorescein (CF), polyoxyethylene-5-cetyl ether ($C_{16}EO_5$) and polyoxethylene-5-stearyl ether ($C_{18}EO_5$) were obtained from Sigma. Poly-24-oxyethylene cholesteryl ether (Solulan C24) was donated by Ellis and Everald (UK). All materials were used as obtained from suppliers without further purification. Water was from an ultra high quality reverse osmosis water purifier (Elgast UHQPS-Elga, UK).

2.2. Preparation of polyhedral niosomes

Polyhedral vesicles were prepared from $C_{16}EO_5$ or $C_{18}EO_5$ and Solulan C24 in molar ratios of 91:9 and 98:2, respectively, using the hand shaking method (Arunothayanun et al., 1999b). Surfactant mixtures were dissolved in 5 ml chloroform, and the solvent removed under reduced pressure at 30 °C and 60 rpm, in a 250 ml round bottom flask (Buchi model Rotavapor-R). Residual organic solvent was removed by drying the surfactant film under a stream of nitrogen for 15 min. The dry surfactant film was then hydrated with 5 mM CF or 5 ml of de-ionised water incubated at 34 or 55 °C (slightly above the transition temperature of the surfactants used) for 1 h with constant mild shaking on a mechanical shaker (Griffin flask shaker). Niosome dispersions, with a final lipid/surfactant concentration of 150 μM, were left to cool at room temperature and kept overnight at 4 °C before use.

2.3. Niosomes with entrapped polystyrene latex particles

Niosomes entrapping 0.5 μm Fluoresbrite "plain" microspheres (Polysciences, UK) were prepared using Reverse Phase Evaporation (RVE) method (Szoka and Papahdjopoulos, 1978). Into 2 ml of surfactant solution, 0.1ml of distilled water was injected using a 23 gauge needle and the mixture probe-sonicated for 2 min using a Soniprep 150 (Sanyo MSE, UK) at setting 5 to form a water-in-oil emulsion. The emulsion was converted to a gel using the rotatory evaporator at room temperature, after which stream of nitrogen was used for 15 min. The gel was vortexed after the addition of 2.9 ml of a suspension of fluorescent particles in distilled water, which was then hydrated at 34 °C for 1 h. Niosome dispersions, with a final surfactant concentration of 150 μM, were left to cool at room temperature and then kept overnight at 4 °C before use.

2.4. Preparation of micropipettes

Borosilicate glass capillaries (inner diameter of 1.17 mm; outer diameter of 1.5 mm) were obtained from Harvard Instruments, UK. The glass capillaries were pulled into ∼1 μm exit diameter tips using a Narishige pipette puller (model PC-10). Other diameters could be made at will.

2.5. Extrusion process

Micropipettes were filled with niosome suspensions (150 μM) and connected to an electric pump which controlled the frequency and the duration of a nitrogen pulse (controllable from 68.95 to 2.76×10^4 Nm^{-2}) used to drive the vesicles from the micropipettes

into a Petri dish with the chosen medium. Minimum pressure of $3.45 \times 10^3\,Nm^{-2}$ was required for fusion to occur to form the tubular structures. Events were followed using a video camera attached to the Nikon-Microphot-FXA light microscope utilising a distant lens with Nikon UV light (model HB-10101AF). Individual frames were captured on computer using the Hauppage capturing software.

Phosphate-buffered saline (PBS), distilled water, NaCl and sucrose solutions, and sodium carboxymethyl cellulose (NaCMC) solutions, were used as media into which the vesicles were extruded, allowing variation of ionic strength, viscosity and tonicity.

3. Results and discussion

Polyhedral non-ionic surfactant vesicles (Fig. 1a) form in the presence of low amounts of cholesterol. As part of an investigation into the flow properties of

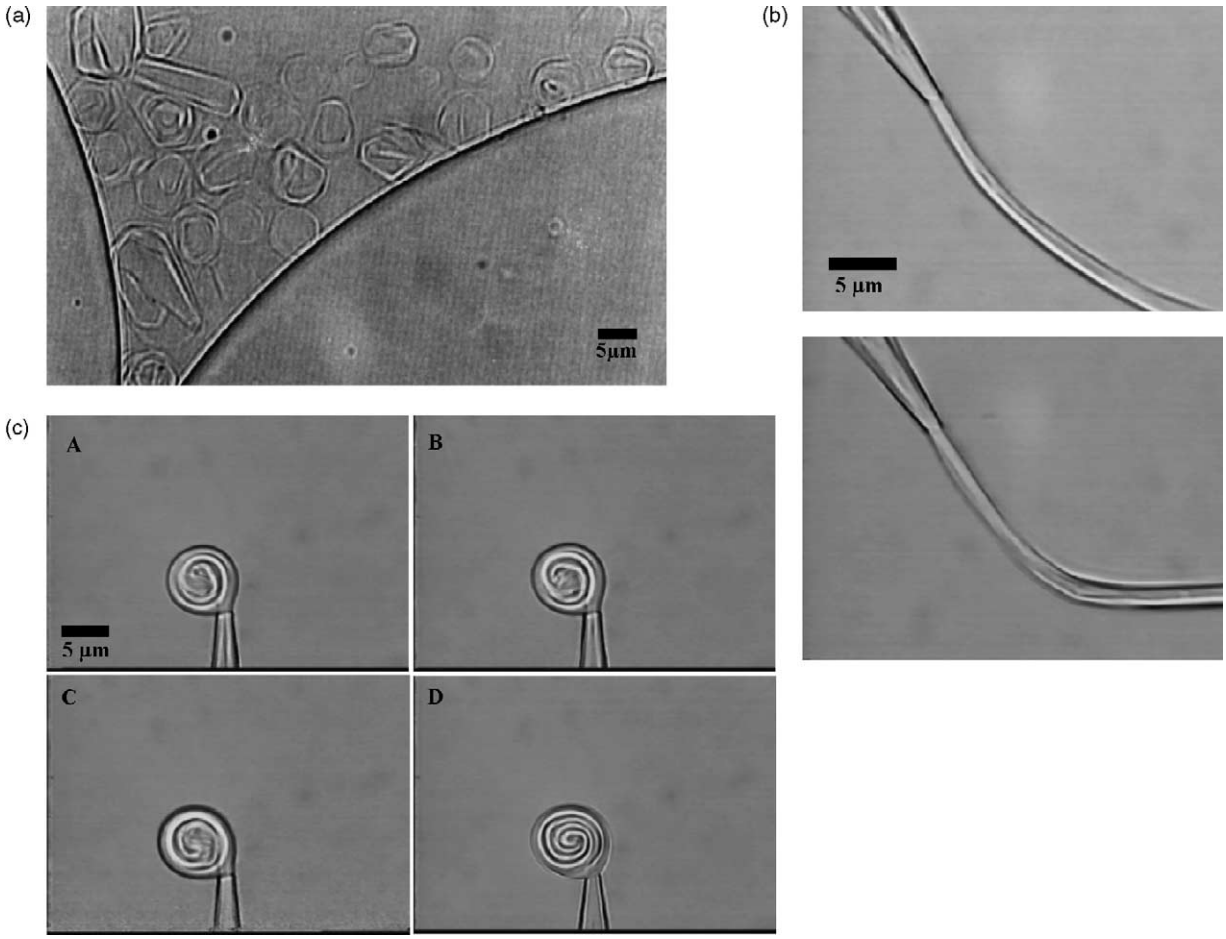

Fig. 1. (a) Photomicrograph of multi-lamellar polyhedral niosomes typical of those used in this work. (b) Elongated multi-lamellar tubules formed by extruding polyhedral niosomes through a glass capillary at pressures ranging from 3.45×10^3 to $2.41 \times 10^4\,Nm^{-2}$. These can grow to the length between 50 to 80 μm, hence each tubule is the result of the fusion of multiple polyhedral vesicles. (c) The production of concentric tubular whorls, arising from the extrusion of tubules into a primary spherical cap which guides the tubule. The typical structure shown here has a radius of 17 μm. Calculations show that this example is the result of fusion of around 20 vesicles. (d) The unravelling of the whorl by micromanipulation, illustrating the strength of the component tubule. These structures can also be drawn back into the capillary intact (not depicted).

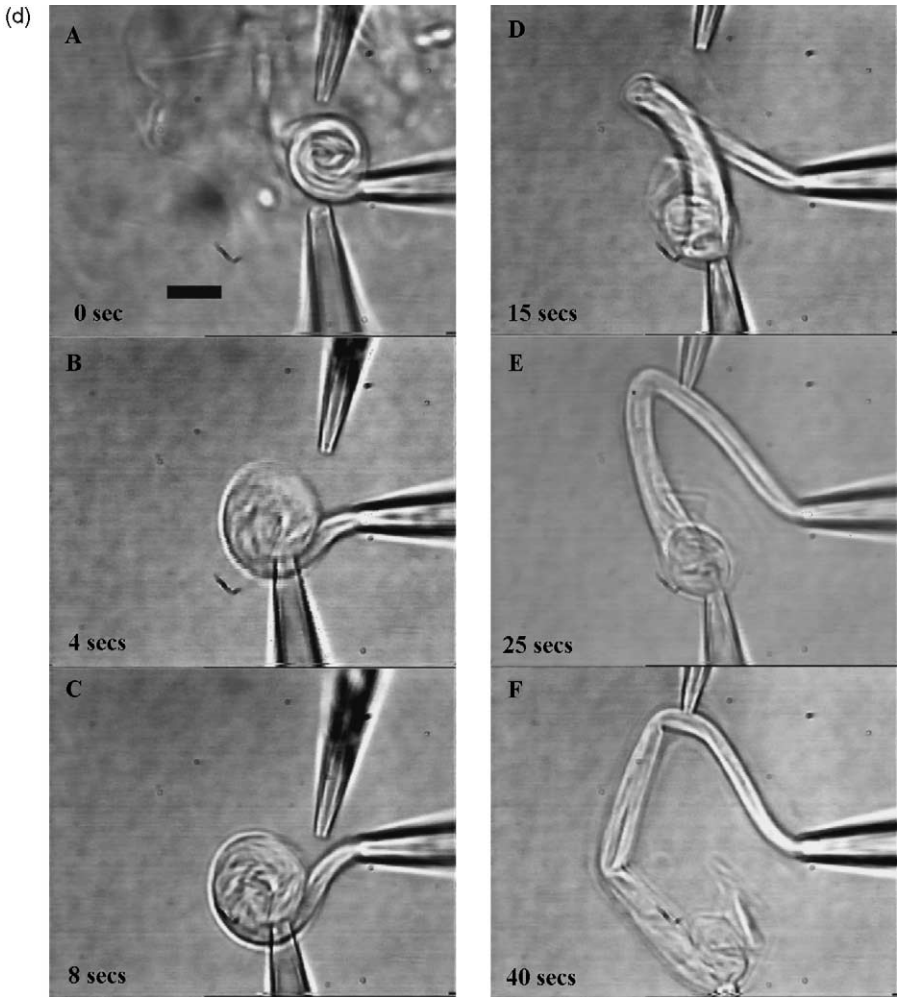

Fig. 1. (*Continued*).

spherical and polyhedral niosomes (Florence et al., 1999), we became interested in the ability of niosomes to deform when passing through capillaries with exit diameters smaller than the vesicle mean diameter. Spherical vesicles can be extruded as intact entities. However, we found, unexpectedly, that polyhedral vesicles when extruded under certain conditions into aqueous media, having lost the visco-elasticity of spherical niosomes and liposomes, fuse to produce long, continuous stable tubules with an approximate diameter of the exit capillary (1 μm). By controlling factors such as the pressure used to extrude the vesicles (in the range 0.35×10^4 to 2.5×10^4 Nm^{-2}) and both vesicle and medium composition, we can reproducibly form a range of structures, of which the most common are tubules (Fig. 1b) and tubule "whorls" (Fig. 1c). Fig. 1b shows clearly the long tubules extruded from the suspension of individual polyhedral surfactant vesicles. The tubule diameter is of the order of 1 μm; the wall thickness can be estimated to be of the order of 50–100 nm. Fig. 1c also shows whorl formation, caused by the extrusion of the exiting tubule into a primary spherical cap. The structures formed are stable; using micropipettes, these coiled structures can be unravelled, as illustrated in Fig. 1d, indicating the strength and flexibility of the primary systems.

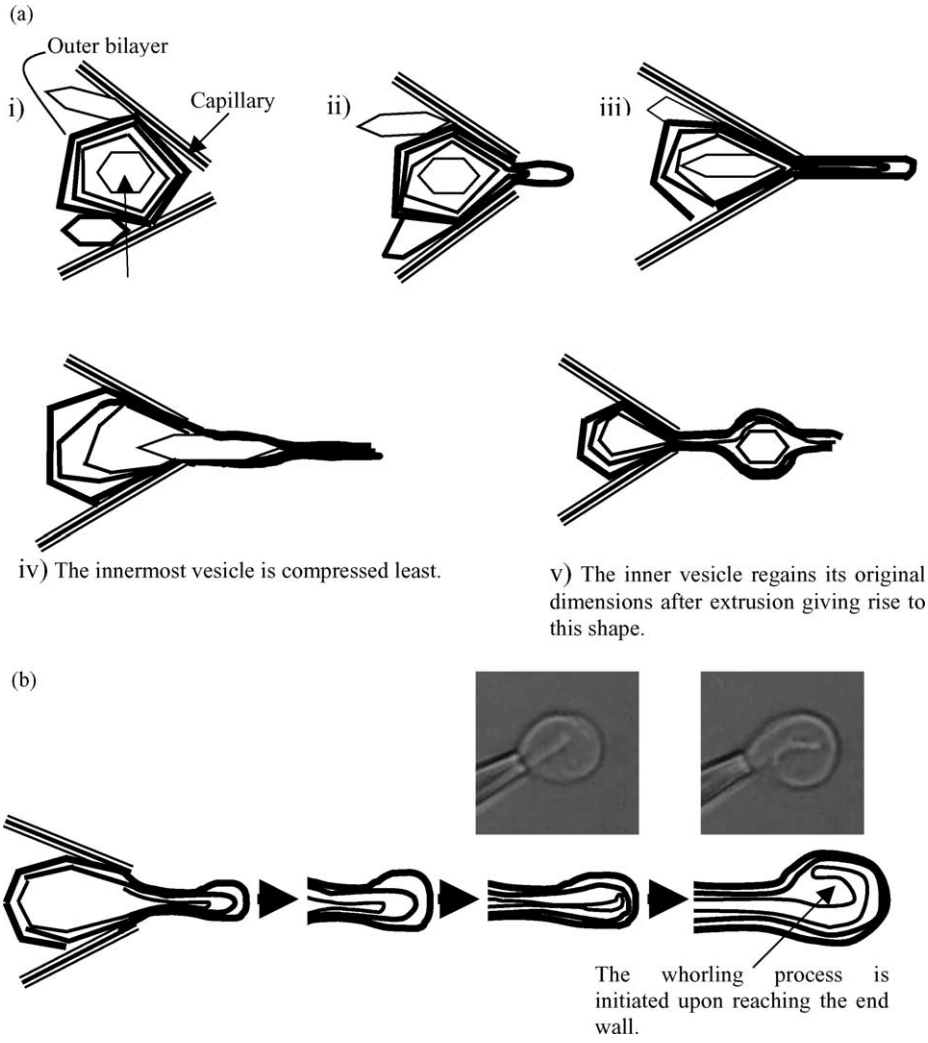

Fig. 2. (a and b) Diagrammatic representation of the processes involved in the formation of the structures depicted in Fig. 1, from detailed observation of video sequences. The multi-lamellar polyhedral vesicles under pressure form multi-lamellar tubules; the differential pressure within the capillary leads to the extrusion of vesicles into the tubule to provide the vesicle-in-tube or capillary model.

A diagrammatic representation of the events leading to the production of the elongated structures is shown in Fig. 2a and b, where it is assumed that there is fusion of the parent multi-lamellar structures. There is also differential flow of the multi-lamellar cylinders as they move through the constrained diameter of the capillary tube (Clerc and Thompson, 1994), perhaps leading to "stripping" of the outer lamellae of the original vesicles. This is most likely to be the explanation for the formation of vesicle in tubule structures (Fig. 3), diagrammatically represented in Fig. 2a, where the outer layers form the tubule walls and the inner lamellae form the vesicle flowing in the tube. The movement of vesicles along tethers pulled from the original vesicle involves structural processes. As the vesicle moves, the bilayers of the capillary or tubules are distorted, then reform to their original state once the vesicle has moved on. This combination provides a model for vesicle flow in flexible capillaries which will be discussed in later publica-

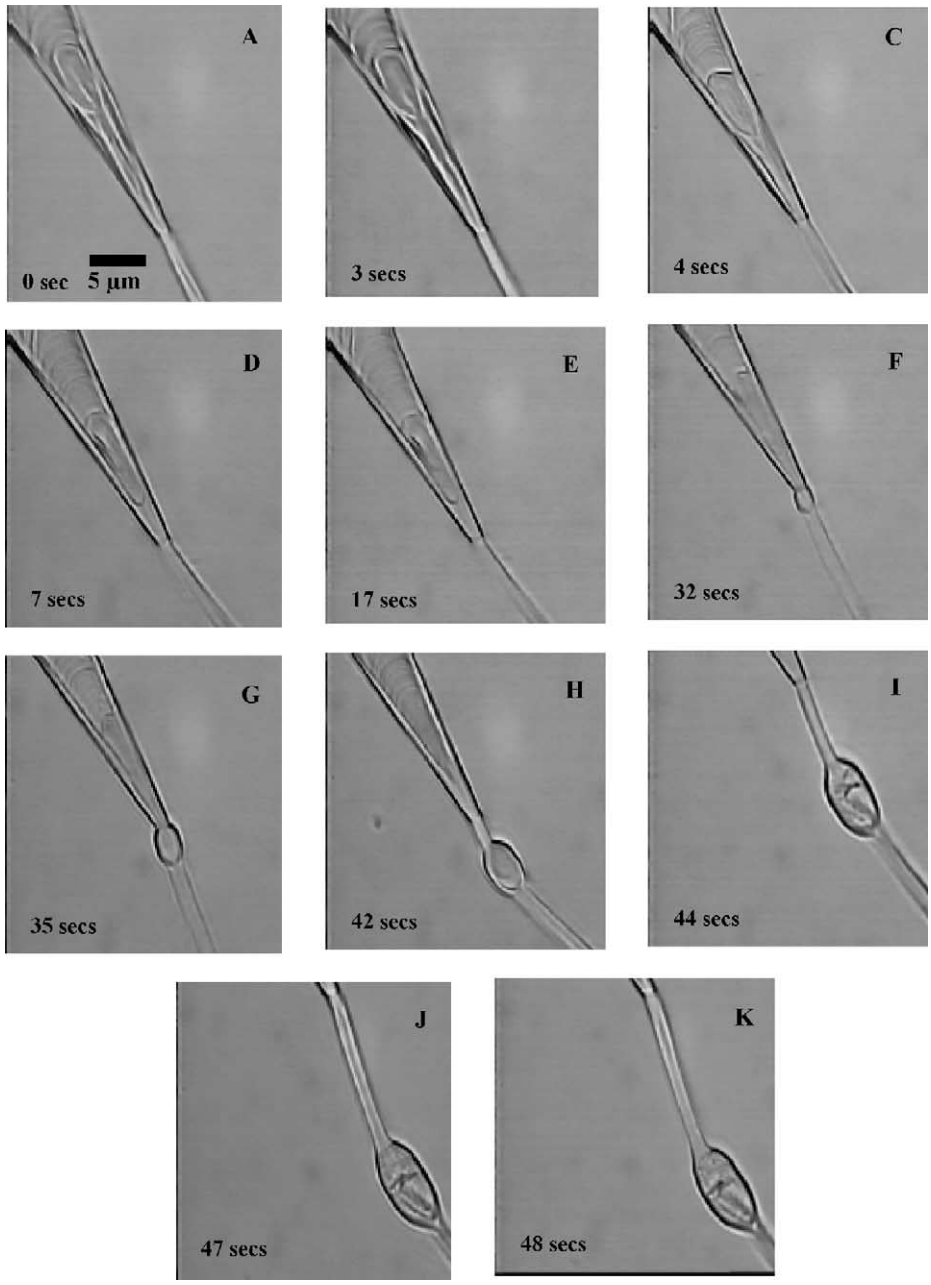

Fig. 3. Evolution and flow of a vesicle within a tubule system. The vesicle, which has an elongated shape due to its visco-elasticity, has its small axis greater than the radius of the tubule. It can be seen moving down the tubule at an average rate of $1.7\,\mu m\,s^{-1}$.

tions. There is little in the literature on the fate of lipid and surfactant vesicles following their encounters with capillary beds after administration; such constraints on their movement in vivo may be most important in relation to understanding the stress on vesicles in vivo and release of entrapped solutes by stresses placed on vesicles rather than purely by diffusional processes.

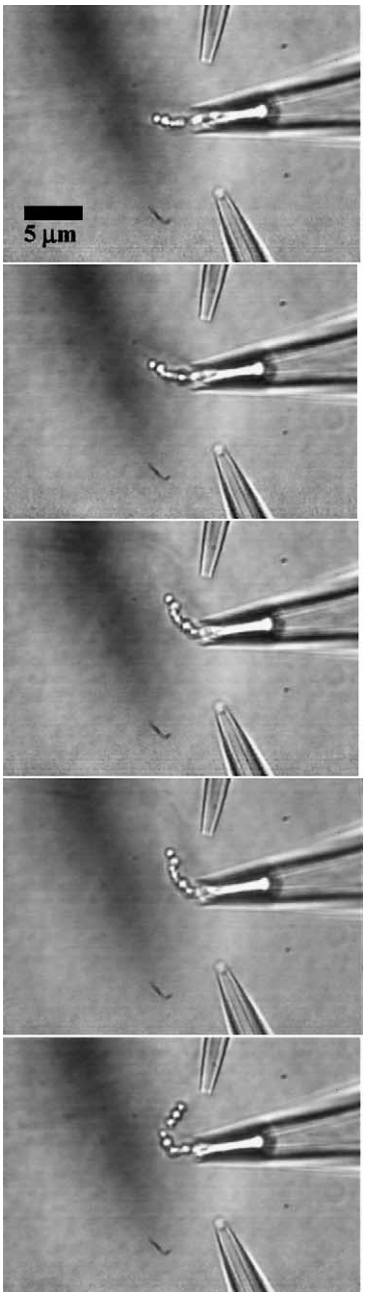

Fig. 4. The extrusion of 500 nm fluorescent polystyrene latex particles (originally entrapped in the polyhedral niosomes) within the surfactant tubules: a sequence showing the array of latex beads in the tube as extruded and then directed by micro-manipulator.

There is some theoretical work on the movement of spheres in capillaries where $d_{\text{sphere}} > d_{\text{capillary}}$ (Chen and Skalik, 1970), but to our knowledge, there is no experimental work in vivo, due to the difficult

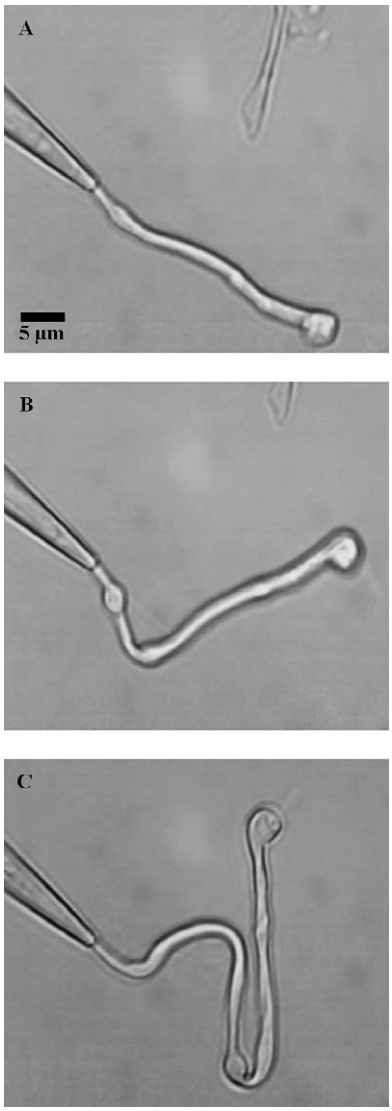

Fig. 5. Vesicles containing 0.1% w/v of acrylamide monomer were extruded into a Petri dish containing 5 M NaOH and the unentrapped monomer separated using centrifugation at 4000 rpm for 20 min. The figure depicts tubules that were formed during the interaction of NaOH with the acrylamide contained in the tubule. The curvature occurs perhaps because of the penetration of NaOH selectively into the tubule, polymerisation causing expansion of the acrylamide and hence bending.

task of visualisation and tracking. The incorporation of polystyrene latex beads of 0.5 μm diameter into our polyhedral vesicles prior to their extrusion provides an ordered array of beads within the tubular structures as can be seen in Fig. 4. Following the dynamics of bead transport in such a system may provide a model system for studying the capillary movement of microspheres such as those used in drug delivery and for chemoembolism. In Fig. 4, the individual nanospheres can be seen in a tubule which is being manipulated by a second micropipette. The particles are seen close packed as in the mathematical model described by Chen and Skalik (1970).

The possibility of using the extruded tubules not only as such models but as templates for polymerisation or biomineralisation presents itself. Fig. 5 shows structures formed from polyhedral niosomes containing 1% acrylamide. On contact with the external sodium hydroxide solution, the acrylamide polymerises. Differential access of the solvent causes unequal swelling of the polymer and leads to the contorted shapes seen.

4. Conclusions

Manipulation of polyhedral vesicles provides a range of new systems, which lend themselves not only as novel structures for drug delivery, e.g. in ophthalmic delivery but also as models for complex processes such as the movement of vesicles in flexible tubules, areas being explored in our laboratory.

Acknowledgements

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