generated liposomes, including size, size distribution, lamellarity, membrane composition, and encapsulation efficiency, form the basis for comparison. We hope that this critique will allow the reader to make an informed decision as to which method should be used for a given biological application.

1 Introduction

Liposomes, or lipid vesicles, are self-assembled lipid structures in the shape of closed membrane capsules. (See Figure 1.) They can act as biomimetic compartments with a membrane that closely resembles that of living cells, encapsulating materials such as DNA, proteins, drugs, or other chemicals.[1,2] They can be formed, manipulated, and modified in a variety of ways,[2] and due to their similarity to cells and naturally occurring vesicles, they have been extensively studied.[3–5] Tools for observing and characterising their properties are well developed,[6–14] and moreover basic molecular dynamics simulations of their structure and behaviour have been refined in recent years.[15–18]

First synthesised in a laboratory in the late 1960s,[19–22] liposomes have become a standard tool in lipid and membrane science,[23–27] drug delivery,[28–30] compartmentalisation of biomolecules,[31–34] as well as the formation of rudimentary artificial cells.[35–43] Liposome-based artificial cells can be used to study cellular systems in isolation and in a simpler physical environment than in living cells. For example, Sackmann and coworkers, as well as other research groups, have studied the reconstitution of cytoskeletal components inside liposomes,[44–50] while Noireaux *et al*. used liposomes to encapsulate an *in-vitro* transcription and translation reaction.[37,38]

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orders of magnitude, while simultaneously increasing throughput and analytical performance. This review will assess the merits of current techniques of liposome production and material encapsulation that are either entirely or partially performed within microfluidic devices, and appraise them according to application-specific criteria. As background for this discussion, a description of the desirable liposome and method characteristics follows.

1.1 Liposome and Method Specifications

Membrane composition and lamellarity. Vesicles have been made using many different surfactants besides phospholipids.[66] The choice or combination of surfactants greatly affects the properties of the membrane, such as shape, thickness, stability, elasticity, and permeability, as well as compatibility with biological materials.[67–70]

Naturally-occurring phospholipids such as L-α-phosphatidylcholine (Egg PC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) have the advantage that they are more likely to be compatible with membrane-bound proteins. This is important since pore forming peptides such as α-Hemolysin[71] allow for transport of molecules that would otherwise not be able to cross the membrane. This greatly extends the functionality of vesicles as bioreactors or artificial cells.[37]

One aspect of membrane composition that must be considered when producing vesicles is the presence of residue solvents or oil inside the lipid bilayer as a result of the formation process. Most vesicle preparation methods involve the dissolution of lipids in an organic solvent (typically chloroform), with subsequent removal of this solvent.[2] The solvent is usually removed by an evaporation, vacuum desiccation, or lyophilisation step prior to addition of the oil or buffer that will be used to prepare the liposomes. In some methods, water droplets are formed in oil and later transformed into liposomes by, for example, droplet

it is composed. A membrane composed of a single bilayer is unilamellar, while a membrane composed of many bilayers is multilamellar (see Figure 2). Unilamellar vesicles resemble living cells more closely both in structure and function, and are typically used in membrane protein studies.[78–81] Multilamellar vesicles, on the other hand, are primarily used for encapsulating substrates to be released upon disruption of the membrane at a later time, such as in drug delivery, or when membrane properties other than stability have no effect, such as the use of magnetic-particle containing vesicles as MRI contrast agents.[82] It is also worth noting that existing methods cannot control the number of bilayers in multilamellar vesicles.

Size and size distribution. Many vesicle preparation methods result in polydisperse vesicles — that is, a vesicle population containing vesicles of different sizes.[83] Although different sized vesicles may have different applications, most applications require vesicle populations to be monodisperse.[5] This is because variability in size causes variability in other application-critical factors such as stability, the amount of material encapsulated or bound to the membrane, membrane curvature, or rates of transport across the membrane.[5,84–86] As such, monodispersity is a key criterion in determining the usefulness of a technique, and one of the main motivations for using microfluidic systems to form liposomes.

Vesicles can range in diameter from a few tens of nanometres to a few hundred micrometres. A commonly used classification system is summarised in Figure 2.

Stability. The stability of vesicles depends not only on size and membrane composition, but also on properties of the internal and external phases such as osmolarity, pH, salinity, and temperature.[68,70,87] Since all of these properties are in some way affected by the method of formation, the method will also have a strong effect on vesicle stability. Physical instability of vesicles can manifest itself in the form of lysis, aggregation, coalescence, or budding (Figure 3).[83]

example, Angelini *et al*. improved stability by depositing PEGylated nanotubes on a membrane surface of charged multilamellar liposomes through charge interactions,[89] while Li and Palmer used actin polymerisation along the internal surface of the membrane.[6] The use of cross-polymerisable synthetic lipids to form the membrane, followed by initiation of polymerisation after liposome formation, have also been shown to greatly improve stability.[90–92] Although modifications like these are not ideal in many applications, they do result in a significant improvement in liposome stability. Indeed, Lee *et al*. carried out stability tests on vesicles prepared by electroformation, and found that they were strong enough to withstand electroporation and the mechanical stresses of microfluidic systems without reinforcing treatment after formation.[93]

Usability. It is important to consider usability when comparing technologies. All else being equal, it is often the simplicity or ease of use of a method that will decide how widely it is adopted. Microfluidic tools can often be difficult to set up or operate, especially those involving many fluid inputs, different fluid phases, or complicated flow control. In this review, we evaluate the ”usability” of each method by considering process setup and reusability, input-fluid handling, flow control, and whether the process can withstand fluctuations in operating conditions or alterations to operational parameters.

Encapsulation efficiency. Encapsulation efficiency is defined as the ratio of the concentration of material encapsulated to that found outside the vesicle after formation. Encapsulation efficiency can be inherently poor if the method does not separate the internal and external phases throughout the formation process. Stability also affects encapsulation efficiency, as ruptured or leaking vesicles release their contents into the external solution.[94,95] One of the main purposes of encapsulation of materials into vesicles is to isolate them from other components in an experiment. A process with a low encapsulation efficiency clearly defeats this purpose.

high shear stress, electric fields, or incompatible chemical environments may disrupt or inactivate fragile biological samples. Therefore, when choosing a method for a particular application, one should consider that descriptions of encapsulation efficiency do not necessarily account for the function of the material being enclosed.

The problem of low encapsulation efficiency can often be worked around by employing techniques such as dialysis, filtration, or column separation of the resulting vesicles after they are formed.[100–102] Such methods can remove most if not all of the material that has not been encapsulated.[103,104] However, this clearly adds to process complexity, wastes material, and may compromise vesicle stability.

Another option is to use micro-injection to introduce a desired material into an already-formed liposome. This requires the trapping of a liposome and careful disruption its membrane with a microneedle.[105,106] The reliance on individual trapping and disruption of a vesicle membrane for injection of material is a major drawback in terms of throughput, however. Furthermore, due to the highly sensitive nature of the trapping and injection operations, it is likely that liposomes of different sizes, composition, or with different contents will respond differently to the procedure.

2 Microfluidic Liposome Formation

2.1 Electroformation and hydration

Electroformation is probably the most common method for vesicle production. The process was first described by Angelova and Dimitrov in 1986.[53] It involves spreading lipids dissolved in an organic solvent such as chloroform on the surface of a planar electrode, evaporation of the solvent by vacuum desiccation to form a dry phospholipid film, immersing the coated electrode in

process was a simple modification of the conventional method in which they coated an electrode with a film of dry lipids and then placed two slits made of silicone rubber over it. The slits were then covered with a second electrode plate, thus forming microfluidic channels through which buffer could flow. The remainder of the process was identical to the conventional method, and resulted in a polydisperse population of liposomes.

In a further development, LeBerre *et al.* showed that vesicle size and size distribution could be controlled by artificially fragmenting the lipid film through patterning of the electrode surface with elevated microstructures (Figure 4).[117,118] This limited the film segments to the size similar to that of the microstructures. Since these were small, fragments did not break apart when peeling, resulting in vesicles with surface areas equal to that of the patterned structures. This was a significant improvement over the standard electroformation method because it allowed for the formation of monodisperse vesicles.

Hydration is another popular technique used for the formation of vesicles. The process is similar to that of electroformation, except that no electric field is applied. Instead, a vortex or controlled flow of aqueous buffer solution is passed over the coated surface. The shear stress acting against the lipid layers causes them to peel off, break, and self-assemble into liposomes. However, since there is no control over ”peeling” and ”fragmentation”, the resulting vesicles are polydisperse and multilamellar.[22,56,83] In addition, the hydration method is extremely sensitive to the type of phospholipids used and physical conditions such as the osmolarity of the buffer solution, temperature, and pH.[22,57,83] For this reason, its use in applications where encapsulation is intended is limited, because the materials being encapsulated will often affect the formation process.

The use of hydration in a microfluidic device has been described by Lin *et al.* in the formation of microtubes, and under certain conditions, connected networks of microtubes and vesicles.[119,120] This method was similar to that of Kuribayashi *et al.* in that the dry lipid film was formed on a glass slide before being covered by a block of polydimethylsiloxane (PDMS) with

followed by several hours of desiccation by vacuum before assembling the microfluidic channels. This prevents the continuous operation of the device, since lipids cannot be replenished during operation.

2.2 Extrusion

Extrusion of vesicles is typically carried out after their formation to modify properties such as lamellarity, size, and size distribution. The process was first described by Olson *et al*. in 1979.[121] It consists of passing a lipid or vesicle solution multiple times through the pores of a polycarbonate membrane or other mesh of small apertures. Extrusion makes vesicles unilamellar and monodisperse, with diameters controlled by the size of the pores, the pressure applied during the extrusion process, and the number of times the solution is passed through the membrane.[51,122] For example, Jousma *et al*. showed that a single pass through a membrane with a pore size of 200 nm resulted in liposomes with diameters of 320 ± 50 nm, while five passes yielded vesicles 143 ± 10 nm in diameter.[123]

The basic concept has been applied on a microfluidic chip by Dittrich *et al*. in the actual formation of liposomes (Figure 6).[124] The technique involved the use of a free-standing silicon nitride membrane, on which a lipid film coating was produced by desiccation, covering apertures with diameter of approximately 3.5 microns. Aqueous flow around the silicon-nitride membrane and through the apertures produced vesicles and microtubules, depending on flow speed. The resulting vesicles, however, were polydisperse. Nonetheless, this issue may be addressed if the method is modified such that the vesicles are passed through the membrane multiple times. Unfortunately, the authors provide no discussion of lamellarity or the encapsulation efficiency of their method. But since there is no safe isolation between the solution to be encapsulated and that in which the vesicles will be contained in, it is likely that the encapsulation efficiency is low. By way of comparison, the work by Hope *et al*. reported an encapsulation efficiency of only 30%.[51] Similar to the microfluidic implementations of the electroformation and hydration

Microfluidic tweezing, described by Lin *et al*., is somewhat similar to flow focusing, and is also only feasible within a microfluidic format. The technique was developed for the creation of membrane tubes, but it may be possible to adapt it for the formation of vesicles, or to form vesicles from the tubes.[128] The technique involves dissolving phospholipids in water inside a chamber that is connected to a T-junction through a small aperture. The centre channel of the T-junction is oriented away from the aperture. Two fast flows arrive into the T-junction from opposite directions, and turn into the central channel, flowing away from the aperture. As the flows converge and turn, they pull the phospholipid solution through the aperture, forming membrane tubes.[119,128] However, the vesicles resulting from this method are not monodisperse or unilamellar.

Importantly, the flow focusing method can be used for high-throughput vesicle production.[129,130] The reaction solutions are prepared separately and stocked, and can be loaded into containers mounted onto the microfluidic device, allowing for continuous operation over long periods of time. There is no discussion of encapsulation efficiency in the work by Jahn *et al*. However, since membrane formation depends on a continuous, diffusive exchange of solvent into an aqueous phase, it is likely that some of the material to be encapsulated escapes to the exterior of the membrane before it forms. Furthermore, the solution that ends up inside the liposome also contains alcohol, with alcohol molecules being partitioned into the membrane. This means that the former may affect the behaviour of the encapsulated material of interest, and the latter will affect membrane properties such as stability.

2.4 Pulsed jetting

Pulsed jetting is a very elegant technique, first described by Funakoshi *et al.*[77] It mimics the action of blowing soap bubbles through a loop supporting a thin soap film. The method consists of creating a bilayer membrane by carefully contacting two lipid-stabilised water droplets,[131] and using a micro-nozzle or micro-pipette to shoot small jets of aqueous solution across it. The jetted volumes are wrapped by the membrane as they pass through, and as their momentum carries them further from the

vided in regard to cell viability). More recently, Stachowiak *et al.* used the same technique to encapsulate 500 nm nanoparticles as well as the pore-forming protein α-hemolysin[132] and, in continuing work, refined the apparatus so that an inkjet system was used to control liposome formation (Figure 9).[133,134] One problem with this technique is that a residual amount of the solvent used in the phospholipid solution is found inside the membrane, in between the monolayers.[77] Kirchner *et al.* determined, using Raman spectroscopy, that the decane solvent used in membrane preparation is present throughout the membrane, forming a layer up to tens of nanometres thick.[135] This is almost certain to affect the behaviour of some membrane-bound proteins and the passive diffusion of other materials across the membrane. Stachowiak *et al.* do not address this issue in their work, so it is not clear whether this problem can be solved.[132–134] Although this problem has not been reported previously in other techniques, it is likely to be a factor in other vesicle formation methods.

Two major shortcomings of this method are that it is non-trivial to set up, and very sensitive to both the operating conditions and the materials being used. Indeed, a micromanipulator stage is needed to position the micro-nozzle in the right location with respect to the membrane. Since the droplets that form the interface bilayer are created by manual pipetting, it is very difficult to reproduce their exact location. This means that the micro-nozzle must be repositioned every time the apparatus is used. Also, since liposome formation depends on the membrane being deformed by the momentum of a fluid jet, the whole process is highly dependent on the viscosity of the solutions, operating temperature, and membrane composition. As a result, each time the materials being used are changed (lipids, and internal and external phases), it is likely that the protocol must be modified in some way. Another possible problem with the pulsed-jetting method is the high shear stress experienced by the sample during the jetting process. This may disrupt large or delicate proteins or other biomolecules, limiting the applications of this technique. However, to our knowledge, the consequences of this shear stress have not yet been investigated.

A very similar method was developed by Tan *et al*., who used a microfluidic device and oleic acid to produce a lipid-stabilised water-in-oil emulsion, and transferred it into an aqueous mixture of ethanol and water.[142] The oleic acid phase dissolved in the ethanol, forcing the phospholipids to rearrange into a bilayer membrane, thus forming a vesicle. Such vesicles are unilamellar, monodisperse, of controllable size, and very stable — they were shown to last for more than 26 days — and the procedure is simple and fast to implement. Figure 11 provides an illustration of the method. More recently, the same group demonstrated the entire process performed inside a microfluidic device.[143] Since in this case the solvent used for removing the oleic acid from the membrane is ethanol, the method is much more biocompatible than those previously described.

Although they did not discuss encapsulation efficiency in detail, Tan *et al*. demonstrated that their method can successfully encapsulate nano-sized proteins, microbeads, and HeLa cells.[142] They also stated that encapsulation efficiency is variable, and will depend on the flow rate during the emulsification process and on the concentration of alcohol in the external aqueous mixture when the droplets are transferred. Their method separates the internal aqueous solution from the external one throughout the initial stages of the process, and so it is likely that encapsulation efficiencies are good. The final stage is where leakage may occur, *i.e.*, when droplets are transferred to the aqueous mixture of water and ethanol and the phospholipids rearrange into a bilayer. At this point, rupture and mixing of droplets with the external phase may occur before the lipids rearrange into a bilayer membrane. Loss of efficiency due to the emulsification step is likely to occur because the size of vesicles affects their stability, while the formation rate affects the lipid concentration at the oil-water interface. Hence, if the droplets are too small or form too quickly (not giving enough time for lipids to stabilise the oil-water interface), the resulting vesicles are more likely to rupture and release their contents into the external medium, thus lowering the overall encapsulation efficiency.

An elegant method for forming vesicles that has only been demonstrated using microfluidic technology is that of transient membrane ejection, first demonstrated by Ota *et al* (Figure 13).[146] The technique consists of forming a lipid bilayer at a microchannel junction, and then disrupting that membrane to form vesicles. In this work, a laser is used to heat up a gas bubble in the aqueous solution on one side of the membrane. The resulting expansion of the bubble is enough to displace the fluid and push against the lipid bilayer to deform it. The bilayer then breaks off into liposomes in a way similar to the pulsed-jetting described in section 2.4. A modification of the technique by Kurakazu *et al*. uses pneumatic valves to decrease the volume of the channel instead.[147] The main advantages of this method are that it is fully integrated within a microfluidic platform, and results in monodisperse unilamellar vesicles with tunable size. However, the membrane used to form vesicles is eventually depleted, and needs to be replaced. This is achieved by pumping a lipid-containing oil phase into the junction, where a lipid-stabilised oil-water interface forms. The oil phase is then pumped out by a second aqueous phase. As the two oil-water interfaces come into contact, a bilayer membrane forms. This depletion and replacement of the membrane imposes a complication: the user must deal with intermittent oil phases separating batches of liposome samples. This membrane replacement action (and resulting two-phase output) along with the pneumatic or light actuation result in significant usability shortcomings in this method.

Ota *et al*. state that the lipid membrane produced by their method contains oil residue between the two lipid layers, though the discussion they provide on this topic claims that they did not experience problems related to this issue.[146] Nonetheless, the membrane pore protein α-hemolysin was successfully incorporated into the membrane, and an *in-vitro* gene-expression system based on E.*coli* cell-extract was encapsulated and used to express green fluorescent protein.

Finally, it is likely that encapsulation efficiencies are high, since the internal and external aqueous phases are kept separate by the lipid membrane or oil phase at all times.

formation technique eliminated the polydispersity issues experienced in the macroscale implementation.

Another droplet emulsion transfer method, this time fully integrated into a microfludic device, was described by Matosevic *et al.*[153] In this work, they formed lipid-stabilised water-in-oil droplets which were then displaced by a ramp-shaped obstacle into a co-flowing aqueous stream. When pushed from the organic into the aqueous phase, the droplets picked up a second monolayer of lipids at the oil-water interface (see Figure 15). This displacement stage, however, proved to be too disruptive, causing 95-99% of the droplets to burst as they crossed the interface. As a result, encapsulation efficiency (which should be close to 100% dropped to 83%), yield, and throughput were compromised. This method, however, seems very promising if the displacement step can be optimised to prevent droplets from bursting at the interface.

A major concern with the emulsion transfer method is the likely presence of oil residue inside the lipid bilayer after vesicle formation. Since the process involves the transfer of droplets through an oil phase, it is reasonable to suspect contamination of the final liposome membrane. As discussed in Section 1.1, the presence of oil in the lipid bilayer may have a significant impact in the behaviour of the membrane and associated proteins. It is therefore important to address this matter.

In their work first describing emulsion transfer, Pautot *et al.* performed a thin-layer chromatography assay on the final vesicles comparing them to pure lipids and pure oil samples, and could not detect the presence of oil in the vesicle sample.[59] This indicated that if any oil was present, it was less than 5% of the membrane (that being the sensitivity limit of their detection technique). They also suggested that squallene, a complex mixture of long-chain alkanes, is a good candidate for forming oil-free liposomes by emulsion transfer because it is immiscible in fully hydrated bilayers above the phase transition temperature.[59,72]

However, Kubatta *et al.* showed that a large amount of oil was clearly visible in the membrane of vesicles formed by emulsion transfer.[154] It should be noted, though, that the vesicles were between 3 and 5 millimeters in diameter — much larger than typical

3 Conclusions

In the last ten years there have been many new developments in liposome formation technology. Most notably, macroscale methods have been transposed to the microscale while entirely new methods that are only possible with microfluidic technologies were also developed. This happened because of the many benefits associated with reducing the dimensions of a fluidic process down to the microscale, such as improved throughput and analytical performance, and a high degree of control over operational parameters.

While progress has been rapid, a method that is capable of addressing all the requirements of the various applications of liposome technology has yet to be realised. For each method there is a trade-off. For example, hydration, electroformation, and extrusion result in excellent quality liposomes, but have very low encapsulation efficiency. Of those that achieve high encapsulation efficiencies, pulsed jetting and transient membrane ejection are difficult to implement, while double emulsion templates and ice droplet hydration are not very compatible with biological processes. Finally, droplet emulsion transfer, in spite of being a promising technique, has yet to be implemented in a reliable fashion.

Due to their qualities and drawbacks, different methods remain useful only in different application niches. For membrane protein biology membrane quality is paramount, while encapsulation efficiency or volume characteristics are less important. In such applications, electroformation and extrusion are well suited for vesicle production and hence their immense popularity. However, when liposomes are used as cell models, encapsulation efficiency and monodispersity are just as important as membrane quality. This demand has resulted in the invention of the other techniques, such as pulsed jetting, transient membrane ejection, and droplet emulsion transfer. Finally, the use of liposomes as enclosures for drugs or other chemical species tends to require

		Extrusion	Flow focusing	Pulsed jetting	Double emulsion templates	Ice droplet hydration	Transient membrane ejection	Droplet emulsion transfer
		Unilamellar	Unknown	Unilamellar	Unilamellar	Unilamellar	Unilamellar	Unilamellar
	Medium	Medium	Easy	Hard	Medium	Medium	Easy	Medium
	No	No	Unknown	Yes	Unknown	Unknown	Yes	Yes / Maybe
	Low*	Low	Low	High*	High	High	High	High
	LUV-GUV	SUV-LUV	SUV	GUV	GUV	LUV-GUV	GUV	GUV
	Polydisperse	Monodisperse	Monodisperse	Monodisperse	Monodisperse	Monodisperse	Monodisperse	Monodisperse
		124	119, 125–130	77, 132–134	136, 140–143	144, 145	146, 147	150–153

* As discussed in Sections 2.1 and 2.4, it is possible that the electroformation and pulsed-jetting processes may disrupt the function of encapsulated material.

demonstrated, as well as trapping,[164, 165] anchoring, and perfusing with reagents.[166] Notably, Robinson *et al*. have developed a microfluidic platform in which a population of liposomes can be mechanically trapped into individual chambers, allowing single-liposome observation and manipulation.[167]

In conclusion, we have seen that liposome formation methods can be fully integrated into planar microfluidic platforms, and that such methods show distinct advantages over macroscale or hybrid approaches. The challenge will be to create an on-chip method of forming bespoke liposomes in high-throughput with high encapsulation efficiencies.

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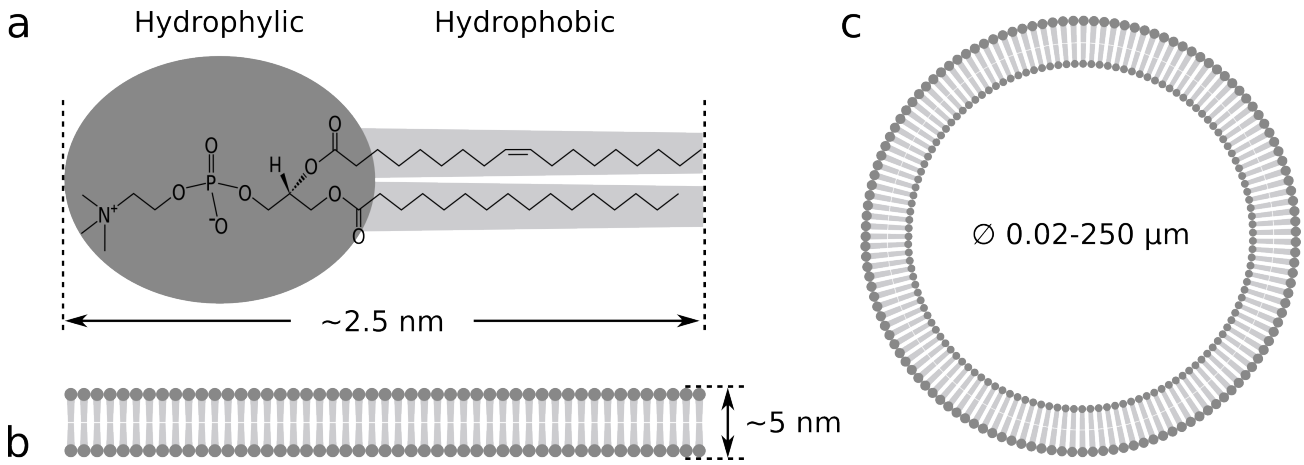

Fig. 1 (a) The structure of one POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) lipid molecule, showing the hydrophilic head and hydrophobic tail. POPC is a naturally-occurring lipid and is commonly used in the production of synthetic liposomes. (b) The heads and tails of the lipid interact to self-assemble into a membrane structure, and (c) a lipid vesicle.

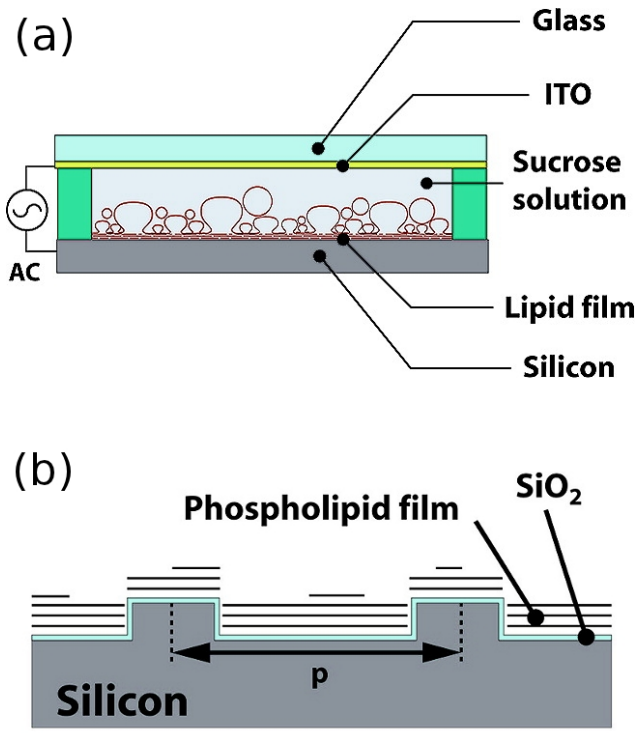

Fig. 4 The experimental setup for the electroformation of giant phospholipid vesicles on a silicon substrate (a), as demonstrated by Le Berre *et al*. (b) A section showing only the Silicon electrode, patterned with an array of micropillars 170 nm in height and with pitch sizes p of 7, 15, and 60 μm. Adapted with permission from 117. Copyright 2008, American Chemical Society.

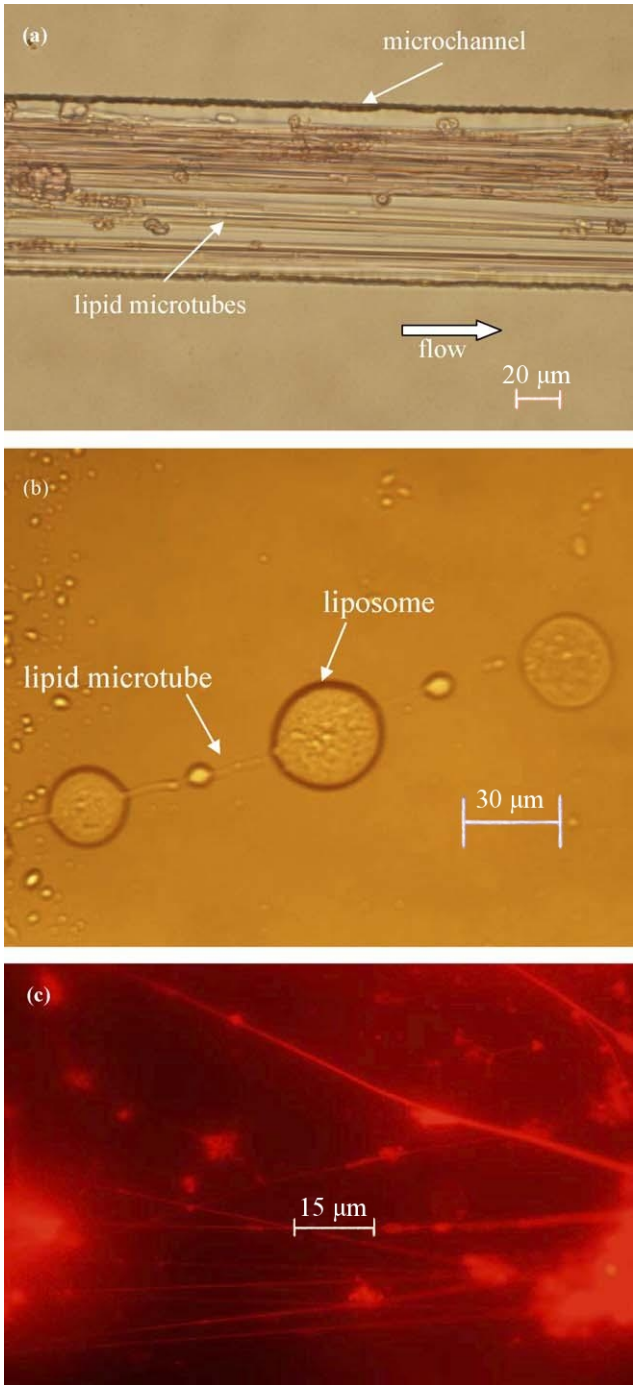

Fig. 5 Microtubule formation by hydration in a microfluidic channel, by Lin *et al*. (a) The DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) microtubules formed in a PDMS channel are stable and controllable. (b) The method sometimes also generated nanotube-vesicle networks. Vesicles are connected by nanotubules, allowing for material exchange between them. (c) A fluorescence image of a self-assembled network of lipid tubules and vesicles. Adapted with permission from reference 120. Copyright 2006, Elsevier.

apertures are fabricated on the silicon substrate in a rectangular area of approximately 3 mm in length. (b) A cross-section of the assembled device and one of its 3.5 μm diameter apertures. Lipids are coated over the aperture, from which vesicles and tubules are extruded. Reprinted with permission from reference 124. Copyright 2006, The Royal Society of Chemistry.

junction during vesicle formation. Reprinted from reference 125.

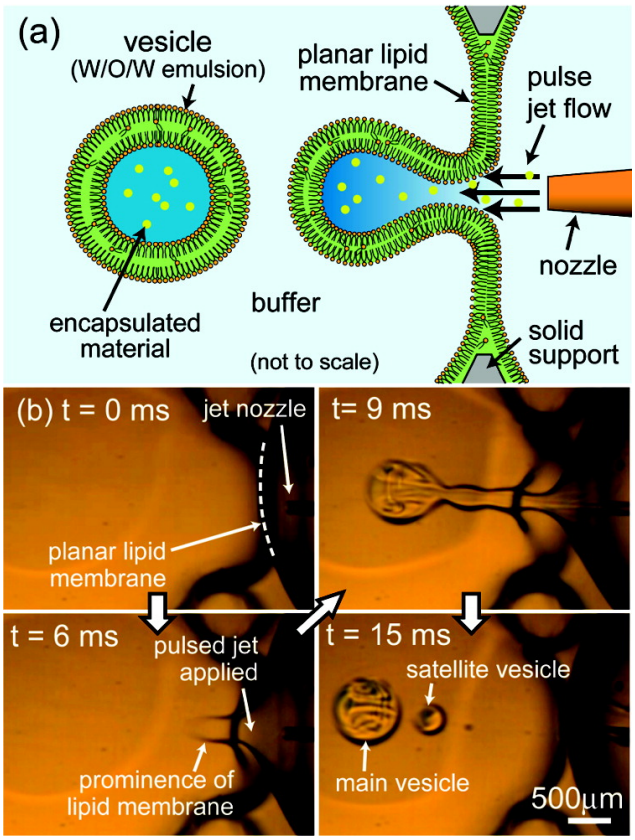

Fig. 8 Pulsed jetting, by Funakoshi *et al*. (a) A schematic of the vesicle formation method. The green area represents the organic solvent. (b) A sequence of images of vesicle formation captured by a high-speed camera. The planar membrane is stretched, forming a balloon that breaks out into spherical vesicles within 10 ms. Reprinted with permission from 77. Copyright 2007, American Chemical Society.

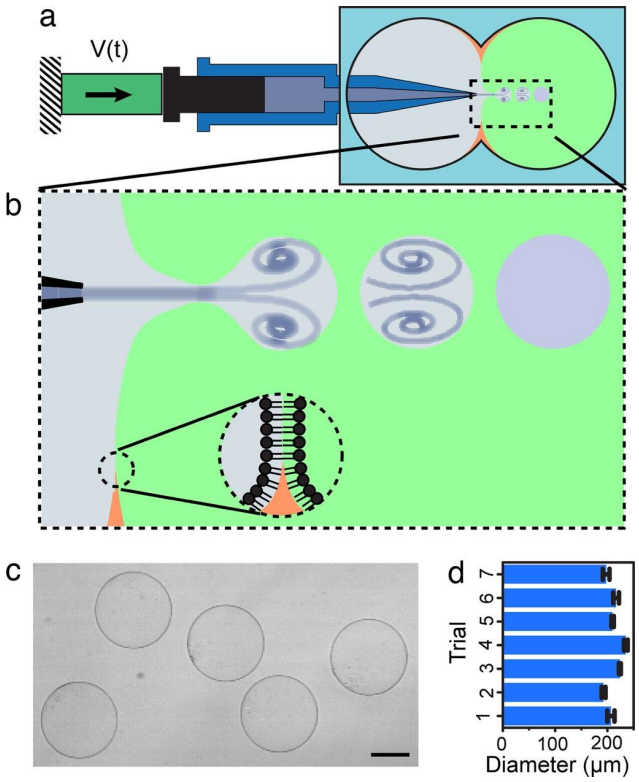

Fig. 9 Blowing vesicles by pulsed-jetting, after Stachowiak *et al.* (a) A schematic of the device and apparatus assembly. A micropipette is inserted close to a lipid membrane, and a piezo-electric actuator is used to generate pulses of fluid. (b) The pulsed jet crosses the membrane, forming vesicles. (c) A micrograph of the resulting liposomes (scale bar: 100 μm, and (d) the resulting diameters of vesicles formed in seven trials. Reprinted with permission from reference 132. Copyright 2008, The National Academy of Science.

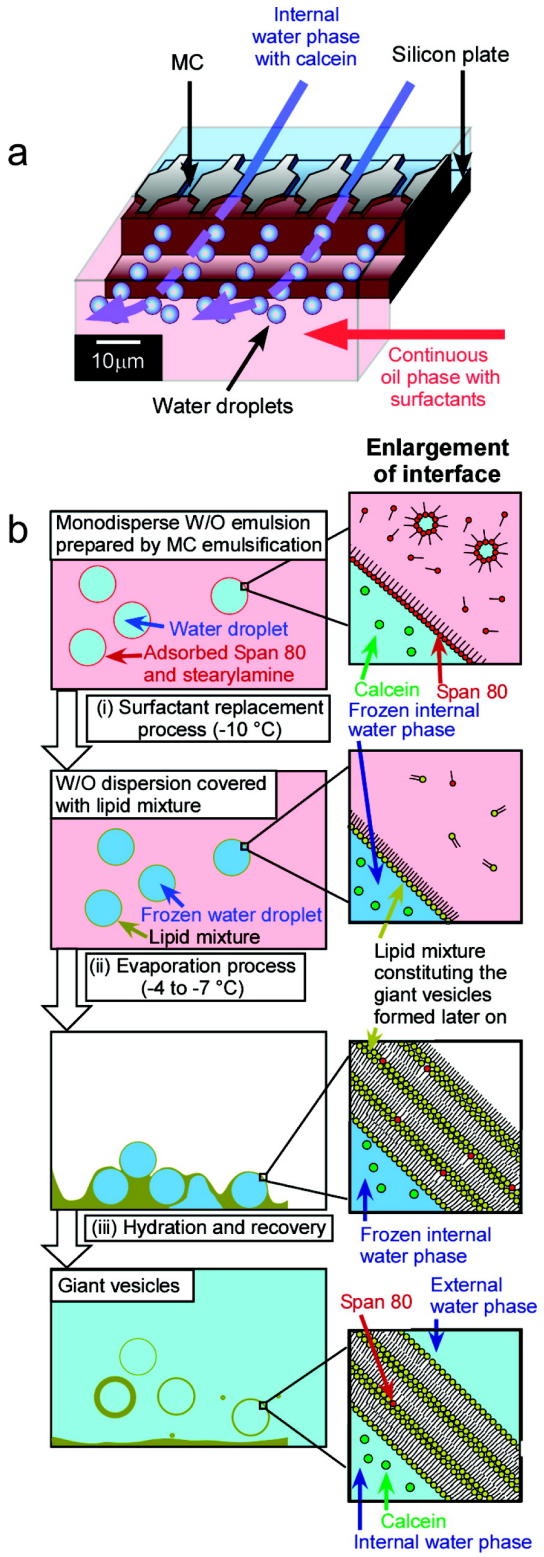

Fig. 12 Vesicle preparation by ice droplet hydration in a microfluidic device, by Sugiura *et al*. (a) A schematic of the microfluidic device, and (b) the process of ice droplet hydration for surfactant replacement. Reprinted with permission from reference 144. Copyright 2008, American Chemical Society.

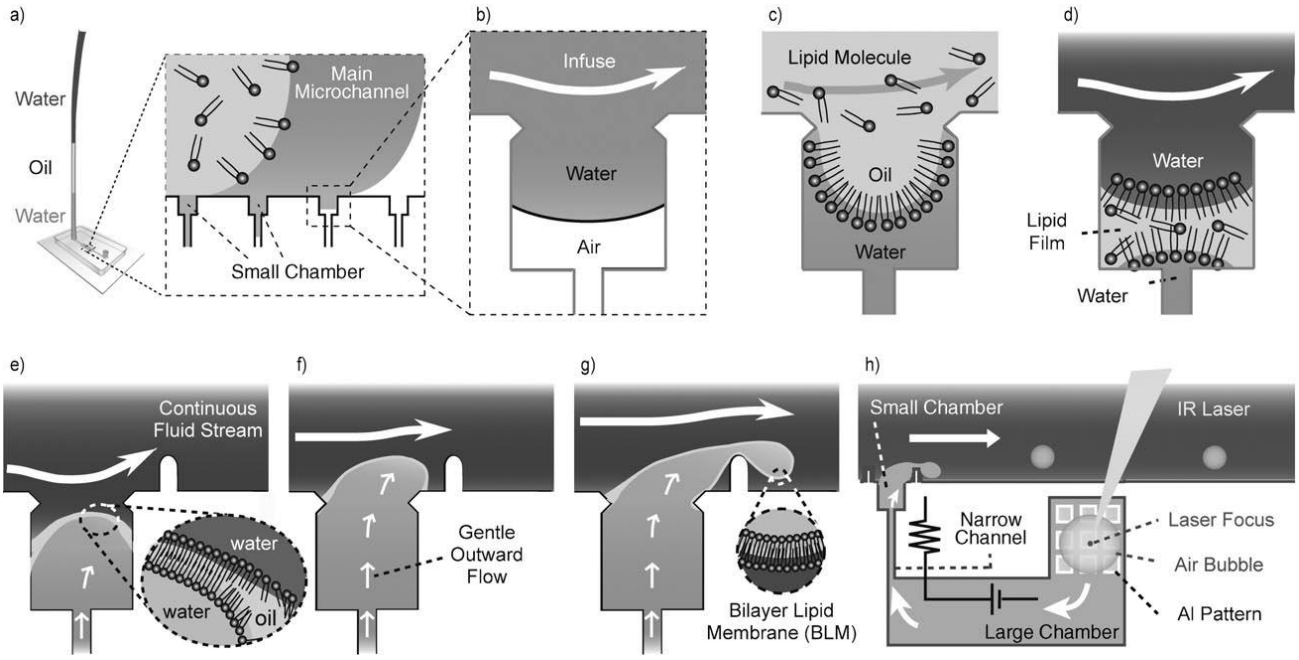

Fig. 13 The transient membrane ejection method invented by Ota et al. (a) Water, oil containing lipids, and water are sequentially passed through a main microfluidic channel flanked by many small chambers. (b) The first water phase enters the chambers, pushing the air out through the PDMS. (c) As the lipid-containg oil passes through the main channel, it forms a lipid-stabilised oil-water interface at the entrace of the chambers, enclosing small volumes of water in them. (d) As the second water phase displaces the oil, the two lipid-stabilised water-oil interfaces come into contact at the chamber entrance, forming a lipid bilayer membrane. (e-f) A gentle flow generated from the chamber out into the main channel displaces the membrane, causing it to bud off into vesicles. (h) This gentle outward flow is generated by the controlled expansion of an optically generated microbubble. Reprinted with permission from reference 146. Copyright 2009, WILEY-VCH Verlag GmbH.

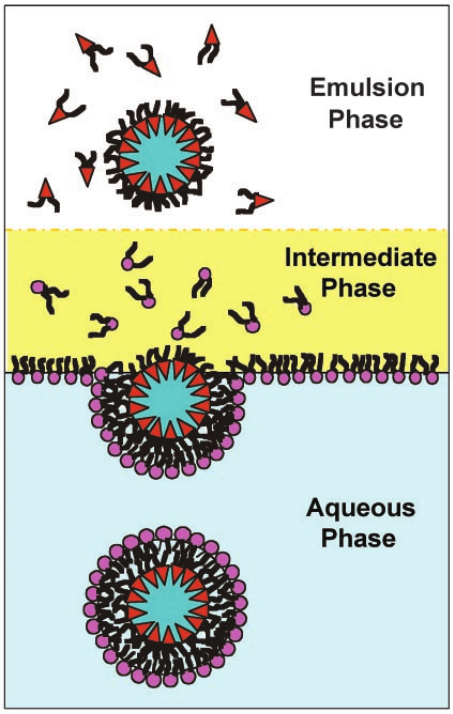

Fig. 14 Droplet emulsion transfer method for vesicle formation, developed by Pautot *et al*. Water droplets are formed in a lipid-in-oil suspension, and then passed through a oil-water interface which is itself stabilised by phospholipids. As the droplets cross the interface into the aqueous phase, they pick up a second layer of phospholipids, forming a bilayer membrane, and thus become vesicles. Reprinted with permission from reference 58. Copyright 2003, The National Academy of Sciences.

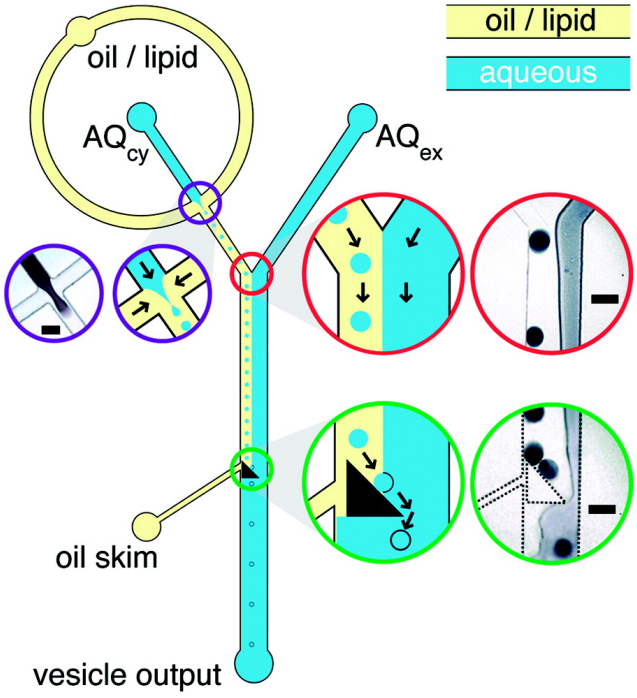

Fig. 15 Fully-integrated microfluidic droplet transfer method for vesicle formation, developed by Matosevic *et al*. Lipid-stabilised water-in-oil droplets are formed by flow-focusing of water into an oil stream at a microfluidic junction, and then forced across an oil-water interface by a ramp-shaped barrier. Similar to the macroscale method, the droplets pick up a second layer of lipids as they cross the interface, forming a bilayer membrane, and thus becoming liposomes. Scale bars: 100 μm. Reprinted with permission from reference 153. Copyright 2011, American Chemical Society.