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# Reinforced vesicles withstand rigors of microfluidic electroporation

David B. Robinson *, Eunice S. Lee [1], Zohora Iqbal, Judith L. Rognlien, Rafael V. Davalos [2]

*Sandia National Laboratories, 7011 East Avenue, Livermore, CA 94550, USA*

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**Abstract**

Living cells synthesize and utilize femtomole and picoliter amounts of material, and an important goal of analytical chemistry is to develop artificial interfaces to efficiently study substances on this scale. This could be achieved with a picoliter container that could be controllably loaded, transported, and unloaded, most desirably in a microfluidic environment. Phospholipid vesicles – surfactant multilayers that can form 10 μm spheres – have been studied for this purpose, but they suffer from fragility and high deformability, which have made them difficult to handle and have limited their application. We present an approach in which a gel is formed in vesicles shortly after they are created. Microfluidic mechanical testing of these vesicles shows that, in the absence of gel, vesicles are difficult to maintain in a trapped state, but the reinforced vesicles exhibit a wide window of pressures under which they can be trapped and manipulated. This improvement is likely to be an essential feature of practical applications of vesicles as microfluidic cargo containers.


*Keywords:* Vesicle; Microfluidic; Gel-filled; Electroporation; Pressure

## 1. Introduction

Most methods for establishing a chemical interface to living cells involve holding the cell with a macroscopic instrument such as a pipet and perforating the cell to cause exchange between it and an external environment. The concentration of material leaving the cell easily becomes much lower and less uniform, a problem that is exacerbated when attempts are made to transport the material to other locations [1,2]. In a much more desirable arrangement, substances extracted or exuded from a cell would be rapidly collected into a container of similar volume for transport and subsequent analysis.

The technology for making and handling containers in this volume range is still in its early development. Perhaps the most impressive approaches involve uniformly sized water droplets surrounded by a thick shell of an oil phase, stabilized by surfactants, formed using concentric microfluidic channels or other means [3–5]. These may prove useful in the transport of pre-concentrated analytes, but it may prove challenging to load and unload these in close proximity to cells. Further, analytes with hydrophobic character may partition into or be denatured by the oil phase. Block copolymer vesicles have many useful and tunable properties, but face similar challenges [6].

Another approach is the use of phospholipid vesicles. Natural phospholipids such as soy lecithin, and their synthetic analogs, readily form sheets of molecular bilayers or multilayers, and these sheets can form 10-μm-scale spheres in an aqueous environment [7–11]. While water can diffuse across the lipid layers, larger or charged molecules essentially cannot, so they can be used as closed containers [12–15]. To open a typical 10 μm diameter container in suspension, it is exposed to an electric field of about 100 mV/μm [16–18]. Under such conditions, pores form in the lipid membrane, allowing relatively large molecules to diffuse across it [19]. The same phenomenon can cause adjacent vesicles to fuse and combine their contents [20–22]. Such manipulations have been much more widely demonstrated for phospholipid vesicles than for the other methods described above.

Several disadvantages of phospholipid vesicles have limited their use, including challenges with efficient preparation of vesicles of uniform diameter and composition; property changes or instability as a function of salt concentration, temperature, pH, and other parameters; mechanical fragility and deforma-

* Corresponding author. Tel.: +1 925 294 6613; fax: +1 925 294 3410.
*E-mail address:* drobins@sandia.gov (D.B. Robinson).
[1] Current address: Department of Bioengineering, University of California, Berkeley, CA, USA.
[2] Current address: School of Biomedical Engineering and Sciences, Virginia Tech-Wake Forest University, Blacksburg, VA, USA.

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bility, making them difficult to handle [23–25]. The use of polymer-based or polymer-grafted membranes widens the range of solution environments that can be used, and the inclusion of charged polymers in the interior can improve mechanical properties by modulating the internal osmotic pressure and membrane tension [6,26]. In a microfluidic environment, design and cost constraints result in channels with sharp or rectangular edges, on which vesicles cannot be trapped as easily as they can with glass micropipets. Thus, even with recent improvements in vesicle formulations, microfluidic handling of vesicles has proven challenging. In a previous report, we were able to trap vesicles, but only with low yields, short durations, and careful handling, requiring hours of work for each experiment [27].

Even though vesicles are reasonably good approximations of cell membranes, living cells are much easier to handle using either micropipets or their microfluidic equivalents, being much more resistant to deformation and failure than vesicles [28–30]. This is because the interior of a cell is much more rigid due to the presence of structural proteins that form a cytoskeleton, as well as other cell contents—a fact suggesting that further improvements to vesicle mechanical properties could be obtained by adjusting the interior volume of the vesicle rather than just the membrane, in effect creating an artificial cytoskeleton.

A simple instance of this would be an aqueous polymer gel. Bulk gels can be made that resist shear despite consisting of more than 99% water and that can be easily loaded with small molecules as well as biological macromolecules [31]. A gel-filled vesicle would be much less likely to deform into the narrow channel of a microfluidic device or pipet than a vesicle containing higher concentrations of small-molecule or small-polymer additives. Several approaches to this have already been presented, including injection of gel precursors into a preformed vesicle, and release of gel precursors and lipids from electrodes. These formulations were not studied with respect to mechanical properties [32–34].

We present a convenient formulation in which vesicles form spontaneously from a surface (which could potentially be a microfluidic chamber) in the presence of aqueous buffer, with components that spontaneously crosslink to form a gel. Mechanical testing in a microfluidic trap shows that these vesicles are a dramatic improvement over those in which gelation is suppressed. This result shows that vesicles are much closer to being practical tools in the manipulation of substances on the picoliter scale than previously thought.

## 2. Preparation of vesicles

Our procedure builds upon the approach of Yamashita et al., in which vesicles form from a dry film of polymer-grafted phospholipids [26]. In our work, we modify not only the lipid membrane but also the vesicle interior in order to improve vesicle properties, and we handle materials in less hazardous solvents. Lipids were purchased from Avanti Polar Lipids: soy-derived L-α-phosphatidylcholine (lecithin) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000], ammonium salt (PEGDOPE). An approximately 100 mM lipid stock solution was made by adding 30 mg PEGDOPE and 155 mg lecithin (5 mol% PEGDOPE) to 2 mL ethanol. In another stock solution, a sub-milligram amount of fluorescein DHPE (Invitrogen) was added and dispersed with sonication. A stock solution of 0.4 M poly(allylamine) in ethanol was prepared by rotary evaporation of 285 μL 20% aqueous poly(allylamine) ($M_w$ 17 kDa, Aldrich) at 50 °C followed by sonication of the film into 2.5 mL ethanol. Also prepared were stock solutions of 1 M acetic acid in ethanol and 0.6 M sodium acetate in water. To gain an understanding of pH effects, 10 mL solutions of 25 mM poly(allylamine) in deionized water or 0.6 M sodium acetate were titrated by adding 5 μL drops of 4 M hydrochloric acid or acetic acid, respectively, and monitoring pH with an ISFET meter (IQ Scientific Instruments).

To a cylindrical vial with an inside diameter of about 12 mm was added 20 μL lipid stock solution, 60 μL poly(allylamine) stock solution, and 14–20 μL acetic acid stock solution, amounting to 0.6–0.8 equivalents of acetic acid per amine. The solution was mixed, and then the ethanol evaporated by rotating the vial under a gentle nitrogen stream to form a smooth film, followed by exposure to vacuum (less than 10 Torr) for 5 min.

To release vesicles from the film, it is first hydrated by placing a 2-μL water drop on the side of the vial and then heating the capped vial for 10 min at 37 °C. At the same time, a buffer vial composed of 1 mL 0.6 M sodium acetate, 15 μL poly (ethylene glycol) diglycidyl ether ($M_n$ 526, Aldrich) or poly (ethylene glycol) monomethyl ether ($M_w$ 550, Fluka) and 10 μL ethanol was warmed to 37 °C [35]. The buffer was added to the lipid film, which was agitated gently, forming vesicles in the 10–40 μm diameter range. These were kept at 37 °C overnight, during which time the crosslinking reaction occurs between the poly(allylamine) and poly(ethylene glycol) diglycidyl ether, as illustrated in Fig. 1.

Bulk gelation reactions were performed by preparing solutions consisting of poly(allylamine) as 1 M amine, varying amounts of acetic acid near 0.8 equiv., 0.6 M sodium acetate, and varying amounts of poly (ethylene glycol) diglycidyl ether below 1 vol%, and heating at 37 °C overnight.

### 2.1. Pressure and electrical measurement

The microfluidic test platform, outlined in Fig. 2, is built around a silicon chip (0.5 mm thick) with a 1 μm silicon nitride film, which is plasma etched to produce a single 6 μm diameter orifice. The silicon underlying the orifice is wet etched to expose a 1 mm × 1 mm membrane area [36]. The chip is clamped in a manifold that is open above the membrane and provides a fluidic connection to the closed chamber below the membrane. One platinum electrode and one Ag/AgCl electrode (In Vivo Metric, Healdsburg, CA) are in contact with liquid on each side of the membrane. Tubing connects the bottom chamber to a pressure transducer (Storm Series, Senstronic USA, San Francisco, CA) and a hand-controlled syringe pump, which is used to control pressure. The electrical signals are processed by op amps and connected to a National Instruments PCI-MIO-16E-4 card for computer control and measurement, as previously described [27]. Another previously described chip was used for the imaging in Fig. 3(d and e) [37].