MICROFLUIDIC ASSEMBLY OF CELL-LIKE SYSTEMS IN GIANT UNILAMELLAR LIPID VESICLES

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ABSTRACT

We employ microfluidic jet flows to deform lipid bilayers, forming giant unilamellar vesicles of controlled size and unrestricted content. By incorporating membrane proteins and cytoskeletal polymers within these vesicles, we demonstrate their potential use as building blocks for engineered cell-like systems.

KEYWORDS: Jet, Vortex, Drug delivery, Lipid, Vesicle

INTRODUCTION

The ability to assemble novel biological systems at the molecular level offers the potential to address challenges in healthcare, materials, and energy. However, a major challenge for 'bottom-up' design of biological systems is encapsulation of macromolecules within lipid bilayer vesicles. This paper reports the formation of synthetic cell-like systems created by direct encapsulation of macromolecules and particles in unilamellar lipid vesicles. We demonstrate (i) unrestricted solute encapsulation within unilamellar vesicles, (ii) high-throughput production of vesicle populations with controlled, highly-monodisperse size in the cellular range, and (iii) biological transport across vesicle boundaries using reconstituted pore proteins. Lipid bilayer membranes, which enable regulation of solute transport via channels and pores, are a fundamental requirement of living systems. However, previous methods for encapsulation of macromolecules in lipid bilayer vesicles have been unable to produce unilamellar vesicles of monodisperse size and unrestricted contents [1]. Recently, several groups have reported encapsulation within single and double emulsions [2]. While these structures have proven very useful in applications such as high-throughput diagnostics, their lack of unilamellar lipid bilayer boundaries prevents biomimetic transport and signaling through the emulsion interface, limiting their use in synthetic cell-like systems.

EXPERIMENT

We simultaneously form and encapsulate solutes within unilamellar lipid vesicles by controlling pulses from a microfluidic jet against a planar lipid bilayer (Fig. 1). This approach has been previously used to form double emulsions [3], and we have recently extended the technique to create unilamellar lipid vesicles [4]. We use a highly-controlled, piezoelectric-driven injector, enabling the high-throughput (burst rate up to 200 Hz, Fig. 2A) formation of true unilamellar vesicles free of contamination by solvents. Recently, through variation in the speed and volume of microfluidic pulses, we have controlled vesicle size over a broad range (30μm-300μm diameter, Fig. 2B). Tight control of vesicle size within this range, which

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encompasses three orders of magnitude in encapsulated volume and overlaps that of biological cells, represents an important step toward construction of synthetic biological systems within cell-like membranes.

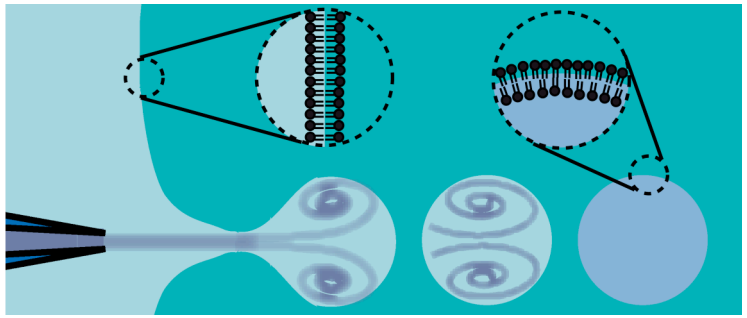

Figure 1. Schematic of vesicle formation by microfluidic jetting. A unilamellar planar lipid bilayer is deformed by a pulsed jet flow to form unilamellar lipid vesicles containing the jetted fluid.

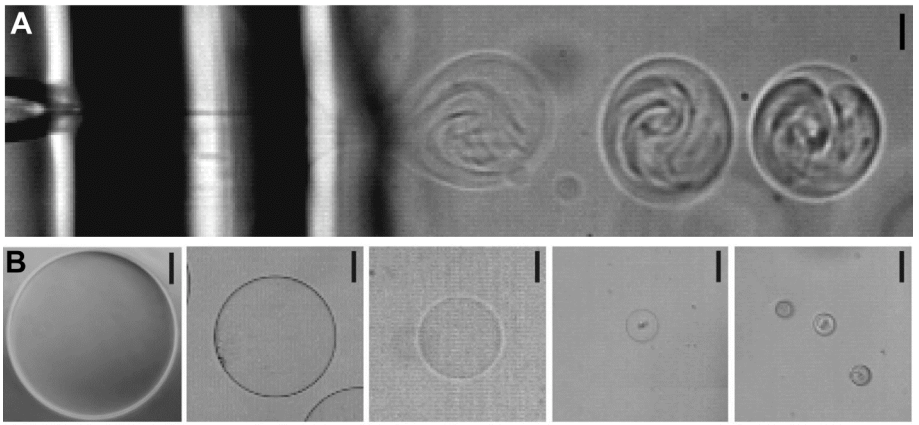

Figure 2. (A) High throughput (200 Hz) vesicle formation. (B) Vesicle diameter can be varied from 30 μm to 300 μm through control of the velocity and volume of the microfluidic pulse used to deform the lipid bilayer. Within each size population, vesicle diameter is highly monodisperse (<10% variation). Scale bars 50 μm.

RESULTS AND DISCUSSION

In order to show that our vesicles possess unilamellar boundaries capable of biological transport, we have inserted the membrane pore protein, alpha-hemolysin and observed subsequent transport of fluorescent dye into vesicles (Fig. 3A-B). This demonstration of biomimetic transport is an important step toward assembly of synthetic biological systems. Because we form vesicles using a jet containing the biological solutes to be encapsulated, encapsulation is unrestricted in terms of molecular weight and ionic charge of the solutes. Exploiting this property, we have recently demonstrated the encapsulation of latex beads (Fig. 3C), which are much

larger than biological macromolecules, showing that complex mixtures of high molecular weight solutes can be encapsulated in vesicles.

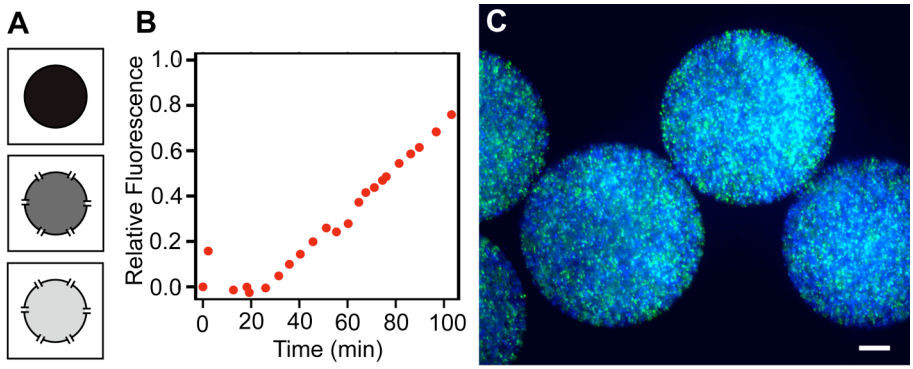

Figure 3. (A) Demonstration of protein-pore-mediated transport across unilamellar vesicle boundaries. Following vesicle formation, fluorescent dye was added to the solution that surrounded the vesicles. Pore protein monomers introduced in the surrounding solution enabled dye transport. (B) The ratio of fluorescent intensity inside the vesicle versus outside was measured as a function of time and observed to increase following introduction of the pore protein. (C) Encapsulation of high molecular weight mixtures (500 nm beads) in unilamellar vesicles. Scale bar 50 μm.

CONCLUSIONS

We have demonstrated formation of monodisperse, cell-sized, unilamellar lipid vesicles with unrestricted macromolecular content and the capacity for pore-mediated transport. Recently, we have begun to construct cell-like synthetic systems with the capacity for shape change by encapsulating and polymerizing cytoskeletal polymers inside vesicles. This work will further the development of engineered biological systems, possibly enabling biomimetic drug delivery systems capable of environmental sensing and active response.

ACKNOWLEGEMENTS

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