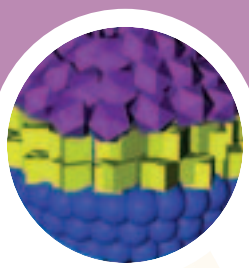


Vesicle making goes microfluidic

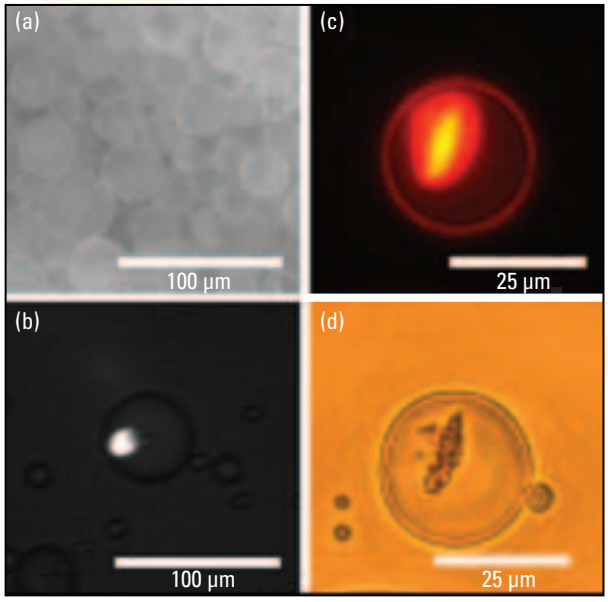
A microfluidic device can encapsulate beads, proteins, and cells inside large vesicles.

Researchers have a new way to package materials inside vesicles. Instead of performing the process in bulk solution, Abraham Lee, Yung-Chieh Tan, and colleagues at the University of California, Irvine, have encapsulated beads, proteins, and cells inside vesicles with the aid of microfluidics (*J. Am. Chem. Soc.* **2006**, *128*, 5656–5658). The technique could be used to create artificial cells, biosensors, and bioreactors.

Lee, Tan, and colleagues introduced two streams into a PDMS microfluidic device: an aqueous phase and a lipid phase. The aqueous phase contained the material to be encapsulated, such as proteins or cells. The lipid phase contained phospholipids dissolved in oleic acid. As the two phases came together at a T-junction, "shear stress created at that interface caused the breakup of the aqueous phase into droplets," explains Tan. "These droplets [were] coated with a layer of phospholipids" to form an emulsion.

The investigators took the emulsion out of the microfluidic device and placed it in a test tube containing an ethanol–water mixture. The oleic acid in the emulsion dissolved in the ethanol, forcing the phospholipids to rearrange themselves and form vesicles around the aqueous droplets. In this manner, green fluorescent protein (GFP), ~4-μm-diam beads, cervical carcinoma cells, and breast cancer cells were packaged into large (27–55-μm-diam) vesicles. The investigators demonstrated that the vesicles with GFP were stable for up to 26 d and that the encapsulated cervical carcinoma cells were alive for ≥2 h.

"It's hard to make large vesicles [in] general, because they are not very stable [and] they are usually not very monodispersed," explains Daniel Chiu at the University of Washington. "The nice thing about this [work] is that it combines fluidics with the formation of large, monodispersed vesicles." Darren Link of Raindance Technologies concurs: The investigators "have a very nice controlled way of getting objects inside droplets."



Fluorescence microscopy images of (a) GFP and (b) a cervical cancer cell encapsulated in vesicles. (c) Fluorescent microscopy and (d) light microscopy images of a breast cancer cell packaged in a vesicle.

However, as Chiu points out, it's hard to discern whether the vesicles are unilamellar (consisting of one lipid bilayer) or multilamellar. Large unilamellar vesicles tend to be difficult to make, so it would be interesting to see whether the technique can create such vesicles. Lee says that they have indirect evidence that the vesicles are unilamellar, and they are working toward confirming it.

Link adds that although the emulsion formation is controlled inside the microfluidic device, the organization of the phospholipids around the material to form vesicles in the ethanol–water mixture isn't controlled. Lee says that it should be possible to have the entire vesicle encapsulation process occur in a microfluidic format. However, the problem is that the addition of more channels downstream of the emulsion formation process affects the pressure drop needed to generate the droplets. "The whole system becomes a little bit more complicated," says Lee, "[but] we're pretty sure we can do it."

The efficiency of vesicle encapsulation currently depends on the size of the material being packaged. Smaller objects, such as GFP and beads, were ~85% encapsulated. "For the largest objects, the cells, the encapsulation efficiency, depending on the size of the cell, was 6–20%," says Tan. Lee suggests that the ethanol–water mixture step in bulk solution caused the loss in efficiency, because the materials had a tendency to break out of the vesicles.

The investigators envision that the vesicle encapsulation of biological molecules could lead to artificial cells. Lee says, "You're never going to get a complete cell, but you can have different functions of the cells compartmentalized" inside vesicles.

As an example, he imagines creating an artificial cell that contains the biological machinery for protein synthesis. "Protein synthesis, or other types of molecular biology kits that are proven to work on the macroscale in conventional biochemical processes, could simply be injected into these vesicles," he says. Chiu and Link agree that the encapsulated vesicles are suitable for artificial cells. Link says that for "making cell-like structures for basic studies in biology, this is perfect." ■

—Rajendrani Mukhopadhyay