

**[0196]** In this example, the use of drop-based microfluidic devices to encapsulate single mammalian cells in distinct pL-sized drops to isolate them in their own microenvironment is described. Because the volume of each drop is restricted, molecules secreted by an individual cell can rapidly attain detectable concentrations. In this example, distinct microfluidic devices are used for encapsulation, incubation, manipulation, and analysis, significantly enhancing robustness and flexibility. This example demonstrates the power of these devices by encapsulating individual mouse hybridoma cells in drops, where they remain viable for several hours while secreting antibodies at a rate similar to cells in bulk. Moreover the cells can be recovered from the drops and cultured.

**[0197]** Microfluidic flow chambers were fabricated by soft lithography. Negative photoresist (e.g., SU-8 2025 or SU-8 2100 from Micro-Chem, Newton, Mass.) was deposited onto clean silicon wafers to a thickness of 25  $\mu\text{m}$ , 40  $\mu\text{m}$ , or 100  $\mu\text{m}$ . The photoresist was patterned by exposure to UV light through a transparency photomask (CAD/Art Services, Bandon, Oreg.) and developed. Sylgard 184 poly(dimethylsiloxane) (PDMS) (Dow Corning, Midland, Mich.) was mixed with crosslinker (ratio 10:1), degassed thoroughly, poured onto the photoresist patterns, and cured for at least 1 hour at 65 degrees C. The PDMS replicas were peeled off the wafer and bonded to glass slides after oxygen-plasma activation of both surfaces. The microfluidic channels were treated with Aquapel (PPG Industries, Pittsburgh, Pa.) by filling the channels with the solution as received and subsequently flushing them with air prior to the experiments; this improved the wetting of the channels with fluorinated oil. Polyethylene tubing with an inner diameter of 0.38 mm and an outer diameter of 1.09 mm (Becton Dickinson, Franklin Lakes, N.J.) was used to connect the channels to syringes. Glass syringes were used to load the fluids into the devices. Flow rates were controlled by syringe pumps. Distinct devices were fabricated for encapsulation, incubation, and analysis. In some embodiments, devices for drop formation and cell encapsulation were 40 microns high with a 35-micron wide nozzle. To vary the drop size, varying nozzle widths were used with a channel height of 25 microns. Devices for cell incubation were 100 microns high, the channel width was 500 microns, and the length was 2.88 meters. Devices for analysis can include various on-chip functionalities, but in cases described in this example, require an interface between the incubation and analysis chips. This was accomplished with a nozzle to re-inject the drops into the channels. The reinjection nozzle was similar in geometry to the drop-formation nozzle, but was larger, with a 40-micron height and at least a 40-micron width, to facilitate the flow of drops into the devices. All inlet channels were equipped with patterned filters which prevented dust particles from clogging the channels downstream.

**[0198]** In this example, 2C6 hybridoma cells were grown. The 2C6 cells produced an anti-ovalbumin IgE (gift from Lester Kobzik/Lester Kobitz), in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L glucose, L-glutamine, and sodium pyruvate (Mediatec, Inc. Hemdon, Va.) supplemented with 10% (v/v) fetal bovine serum (FBS, SAFC Biosciences, Lenexa, Kans.) and 1% Penicil-lin/Streptomycin. The cells were split every 3 days under sterile conditions and incubated at 37° C. and 5% CO<sub>2</sub>.

**[0199]** Cells were grown on culture dishes to a density of 1.2 to 2.5 $\times 10^6$  cells/mL. Prior to the experiments, cells were washed at least once and resuspended in fresh media. The cell

density was adjusted to the desired value, which depended on the average density per drop and the drop size. Hybridoma cells were about 10 microns in diameter and the total volume of medium available to each cell was several times its own volume. Fluorinert FC40 fluorocarbon oil (3M, St. Paul, Minn.) was used to suspend the drops. To stabilize the drops a PFPE-PEG block-copolymer surfactant was added to the suspending oil at a concentration of 1.8% (w/w). This surfactant provided excellent drop stability against coalescence while ensuring good biocompatibility of the inner drop interface. For drop formation, the outer, carrier-oil flow rate was 300 microliters/hour and the inner, aqueous flow rate was 30 microliters/hour, leading to a drop production rate of 250 Hz. At this rate the incubation device was filled in 40 minutes. The cells were incubated by placing the whole device in a cell incubator at 37 degrees C. and 5% CO<sub>2</sub>.

**[0200]** Drop formation was imaged with a high-speed Phantom V5 camera (Vision Research, Inc., Wayne, N.J.), and individual frames were analyzed to determine the number of cells per drop and associated statistics. For each dilution, images of 350 drops at each of three different points in time were collected during the course of the experiment.

**[0201]** Cells were recovered from collected emulsions by diluting the emulsion with 10 $\times$  its fluid volume of fresh media and adding drop release reagent (RainDance Technologies, Inc., Lexington, Mass.) equivalent to 15% of its volume. The mixture was incubated for 2 minutes to allow the oil and release agent to settle. The supernatant containing the cells was transferred to a fresh vial. In separate tests of this procedure, no effect on cell viability was observed. To optimize the experimental conditions, cell viability was tested in each case using a live-dead assay. 1 micromolar calcein-AM (Invitrogen, Carlsbad, Calif., green fluorescence, live stain) and 1 micromolar ethidium-homodimer-1 (Invitrogen, red fluorescence, dead stain) in phosphate buffered saline (PBS) were used. The cells were incubated with the stains for 45 minutes at room temperature (RT) in the dark, and representative images of the sample were analyzed using fluorescence micrographs. Viability was determined from the fraction of live cells. This assay provided a means to compare viability under different experimental conditions.

**[0202]** The supernatant with the recovered cells was transferred into 96 well plates and incubated at 37° C. and 5% CO<sub>2</sub>.

**[0203]** Expression of anti-ovalbumin antibodies in bulk and in drops was determined by a kinetic enzyme-linked immunosorbent assay (ELISA). Cells were placed on ice prior to encapsulation for 30 minutes and maintained at 4° C. while being washed 2 times to remove any remaining antibodies from the suspension and to prevent premature antibody production. The supernatant from each wash was tested for antibody content. For comparison, one reference culture treated in an identical manner as the cells used for encapsulation was placed into a culture dish at the same high density (10 $\times 10^6$  cells/mL) and incubated in bulk for 6 h at 37 degrees C. and 5% CO<sub>2</sub>. Cells in drops were maintained at 37 degrees C. and 5% CO<sub>2</sub> on the incubation chip for 6 hours. Emulsions were broken and ELISAs were performed on culture supernatants after centrifugation to remove any remaining hybridomas. 50 microliters ovalbumin (Sigma, St. Louis, Mo.) (1 mg/mL) in PBS was added to separate wells of a 96-well plate (control wells contained only PBS) and incubated for at least 5 hours at room temperature. The antigen solution was removed, and the wells were washed 3 times with 1 $\times$  Tris-buffered saline (TBS) containing 0.2% Tween-20 (TBST) for 5 min each.