ESI

Materials and Methods

Chemicals and reagents: All lipids purchased from Avanti Polar Lipids (Avanti Polar Lipids, Alabama, USA) and used without further purification. 3 kDa dextrans tagged with AlexaFluor488 fluorescent dye purchased from Molecular Probes (Life Technologies Ltd., Paisley, UK). Melittin obtained from Sigma-Aldrich (Sigma-Aldrich Company Ltd., Dorset, UK). Sylgard 184 PDMS monomer and curing agent purchased from Dow Corning (Dow Corning, Massachusetts, USA).

Scanning electron microscopy (SEM): A PDMS device was coated with a 5 nm layer of gold-palladium alloy, using an automatic sputter coater (Agar Scientific, Essex, UK). The device was then visualised using a Hitachi S4700 SEM (Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

Electroformation of GUVs: The lipid mix was spin-coated at 600 rpm, onto an ITO-coated microscope slide. A PDMS device was plasma-bonded to a second ITO-coated microscope slide, defining the device's channels and electroformation chamber. The chip was filled with electroformation solution consisting of 100mM sucrose, 5 mM HEPES (pH adjusted to 7.4 using 25% KOH) and 10 µM 3kDa dextran-AlexaFluor488. The device was assembled as depicted in Figure 1, using a bespoke metal clamp to create a water-tight glass-PDMS-glass sandwich. Electrical contacts were made with each ITO-coated slide. An AC-field electroformation pulse sequence was applied across the fluid-filled electroformation chamber, as shown in Table 1. Electroformation was carried out, using an external Peltier heater to heat the device to 60°C, above the phase transition temperature of DPPC, ensuring thorough lipid mixing during the procedure. The temperature was then ramped down to room temperature at 1 ˚C/minute, during the 4 Hz square wave pulse sequence, see Table 1.

Electroformation pulse sequence

Pulse frequency (Hz)	Pulse voltage (Vpp)	Pulse shape	Duration (minutes)
10	0.1	Sine	10
10	0.5	Sine	20
10	1.0	Sine	30
10	1.6	Sine	60
4	2.0	Square	60

Table 1: Electroformation pulse applied across the electroformation chamber. The voltage was ramped from 0.1 to 1.6 Vpp over a 60 minute period, then maintained at 1.6 Vpp for a further 60 minutes, at the frequency of 10 Hz. This pulse sequence stimulated vesicle growth from the lipid-coated slide. The 2.0 Vpp pulse applied for 60 minutes, at a frequency of 4 Hz, aids GUV detachment form the slide.

Size distribution of GUVs: Figure 1 presents the size distributions recorded for mGUVs for both on- and off-chip electroformation procedures. The size distributions for both free and trapped on-chip vesicles are shown, to demonstrate the size filtering effect of the device. Trapped vesicles possessed larger average diameters than free mGUVs within the electroformation chamber, 19.74 ± 7.79 µm compared to 13.77 ± 8.15 µm respectively (see ESI). Vesicles manufactured off-chip, using similar electroformation conditions gave an average size of 13.29 ± 6.31 µm. The size-filtering effect therefore acts to remove unwanted smaller GUVs (<10 µm) from the population, enabling higher purity vesicle preparations. This phenomenon could easily be tuned to trap GUVs of varying sizes, by altering the thickness of the SU8-3050 resist layer, and the spacing of the microtrap array pillars.

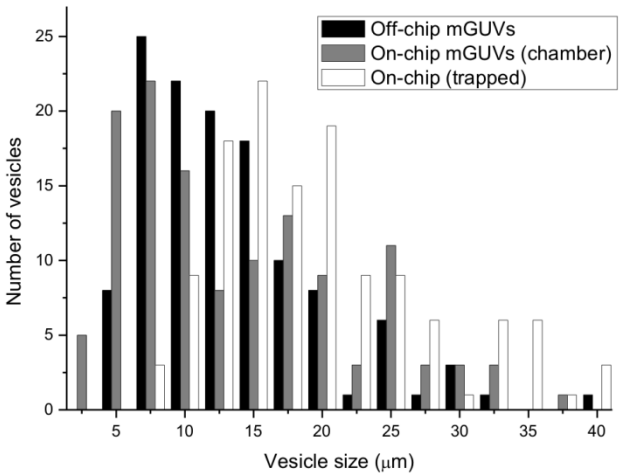

Figure 1: Size distributions of GUVs electroformed off-chip (124 vesicles), and on-chip, in both the electroformation chamber (127 vesicles) and contained within the microfabricated traps (121 vesicles). Off-chip and on-chip electroformed GUVs return similar size profiles, indicating electroformation results are comparable. The size distribution for the trapped mGUVs shifts towards larger diameters, due to the size-filtering effect of the microfluidic channel and microarray trap design.

Off-chip electroformation of GUVs: The device is compatible with electroformation of GUVs off chip, the vesicles can be transferred into the electroformation chamber, prior to device assembly and clamping. The GUVs can then be loaded into the microtrap analysis chamber as normal (figure 2). Figure 2 also demonstrates the high throughput achievable with the device, allowing dozens of leakage events to be simultaneously recorded.

Dye-leakage event: A video of the leakage of encapsulated 3 kDa dextran-AlexaFluor488, through peptide-induced pores, is presented as a compilation of fluorescent microscopy pictures.

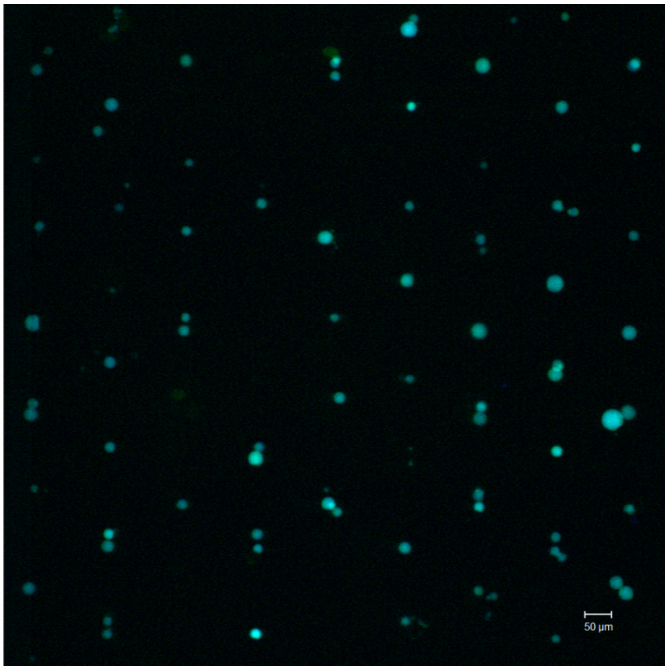

Figure 2: Mammalian biomimetic GUVs in the microtrap analysis chamber, after off-chip electroformation, and insertion into the device. These GUVs enclose two species of fluorescently tagged dextrans, 3 kDa AlexaFluor488 (green) and 10 kDa AlexaFluor635 (blue), to determine effect of size of the dye-leakage kinetics. Total number of trapped GUVs in the field of view is 89, and the trap occupancy is 87%. Of the occupied traps, 62% contain a single vesicle.