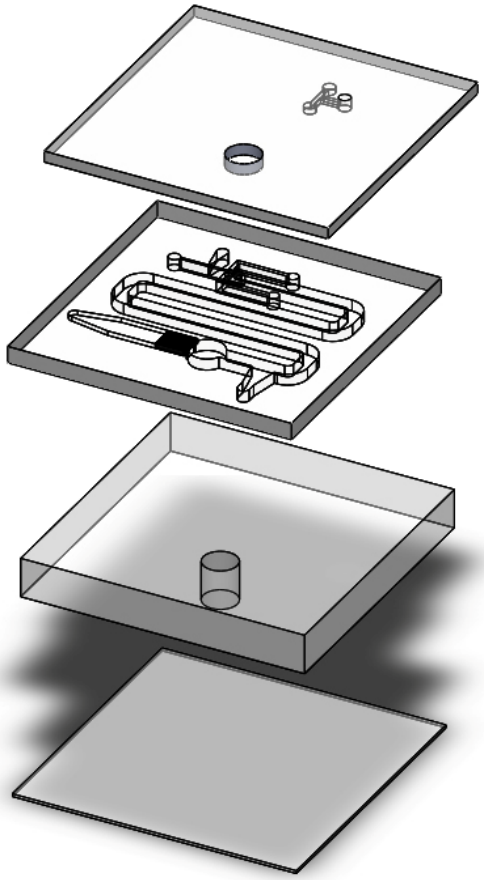
Electronic Supplementary Material (ESI) for Lab on a Chip

SUPPLEMENTAL DATA

Supplement 1: Microfluidic platform configure

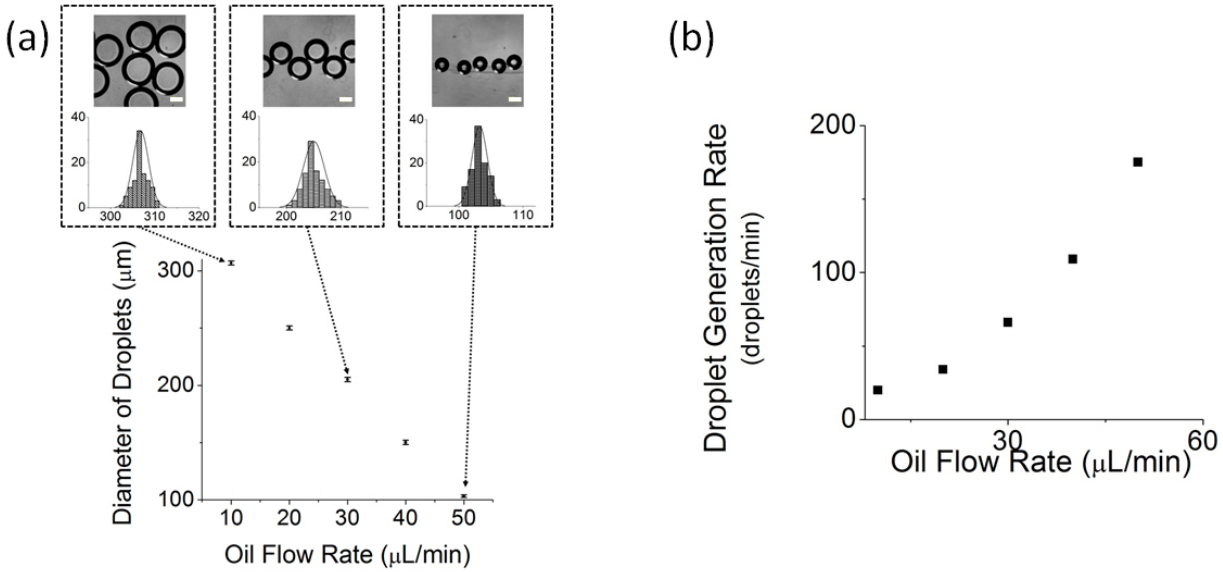
The microfluidic chip consists of three layers of PDMS substrate. The top PDMS layer contains the pre-warmed mineral oil delivery port and an extraction chamber. The main channel structure is located in the second PDMS layer, and third layer contains the collagen microsphere extraction chamber (Supplement Figure 1). These three PDMS layers and a bottom glass substrate were bonded after oxygen plasma treatment.



Sup. Fig. 1: The configuration of the microfluidic platform consisting of three PDMS and one glass substrates, all of which are bonded.

Supplement 2: Collagen microsphere diameter

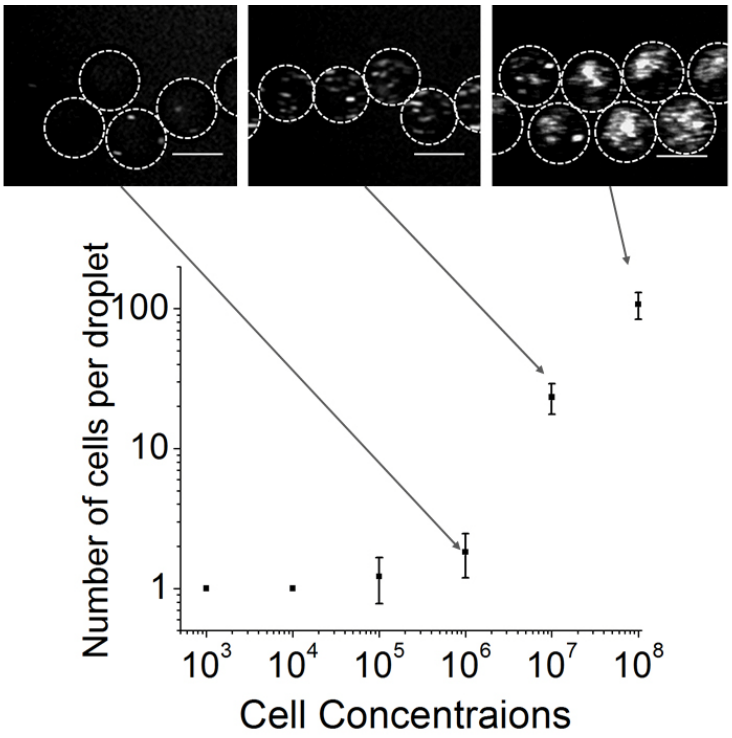
Sup. Fig. 2 summarizes the capabilities of the device to produce monodisperse collagen microdroplets with controlled sizes. To achieve different sizes, the collagen solution flow rate was fixed at 3 µL/min while mineral oil flow rate was varied. As shown in Sup. Fig. 2 (a), monodisperse droplets were formed at each oil flow rate with the diameter linearly decreasing from 306.82 ± 1.78 µm to 103.26 ± 1.2 µm as the oil flow rate was increased from 10 to 50 µL/min. In addition to decreasing droplet size, increasing the oil flow rate also increased the rate of droplet generation (Sup. Fig. 2 (b)).



Sup. Fig. 2: Collagen microsphere characterization. (a) Effect of oil flow rate on droplet diameter. Upper: optical images size distributions of droplets generated with mineral oil flow rate of 10, 30, and 50 µL/min (while collagen flow rate was fixed at 3 µL/min). Lower: droplet diameter decreases with increasing oil flow rate. (b) Droplet generation rate (droplets per minute) increases with increasing oil flow rate.

Supplement 3: The number of cells encapsulated in collagen microspheres

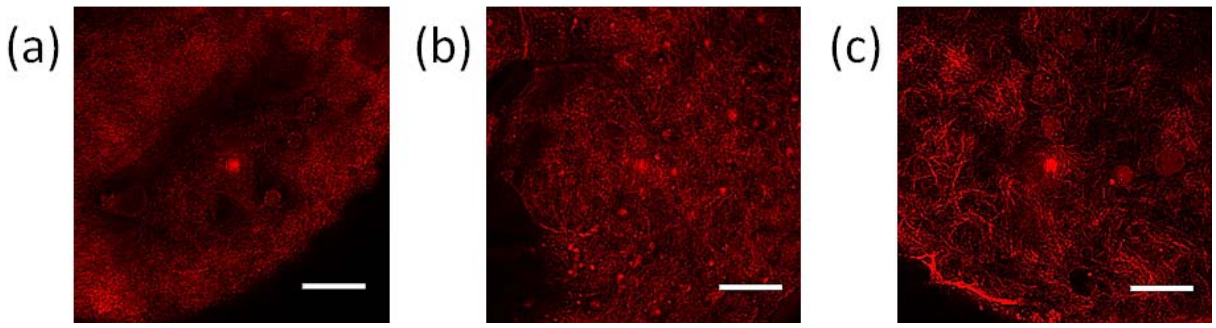
Collagen-cell solutions were prepared with a range of concentrations of MDA 231 cells. The flow rates of collagen and oil were set as 3 and 30μL/min, respectively, resulting in 200 μm diameter collagen droplets. The average number of cells encapsulated in collagen microspheres was 107±23 at a concentration of 1×10^8 cells/mL. When the cell concentration was lower than 1×10^5 cells/mL, single cell was encapsulated (some of the droplet had no cell and empty droplets were not counted), and multiple cells were captured at the higher concentration.



Sup. Fig. 3: The analysis of MDA 231 cell encapsulation rate. Upper: Fluorescence images of GFP-expressing cells in collagen microspheres (scale bar: 200 μm). Lower: Summary plot of encapsulated cell number per sphere at different cell concentrations in the cell-collagen solution.

Supplement 4: Collagen gelation at different incubation temperatures

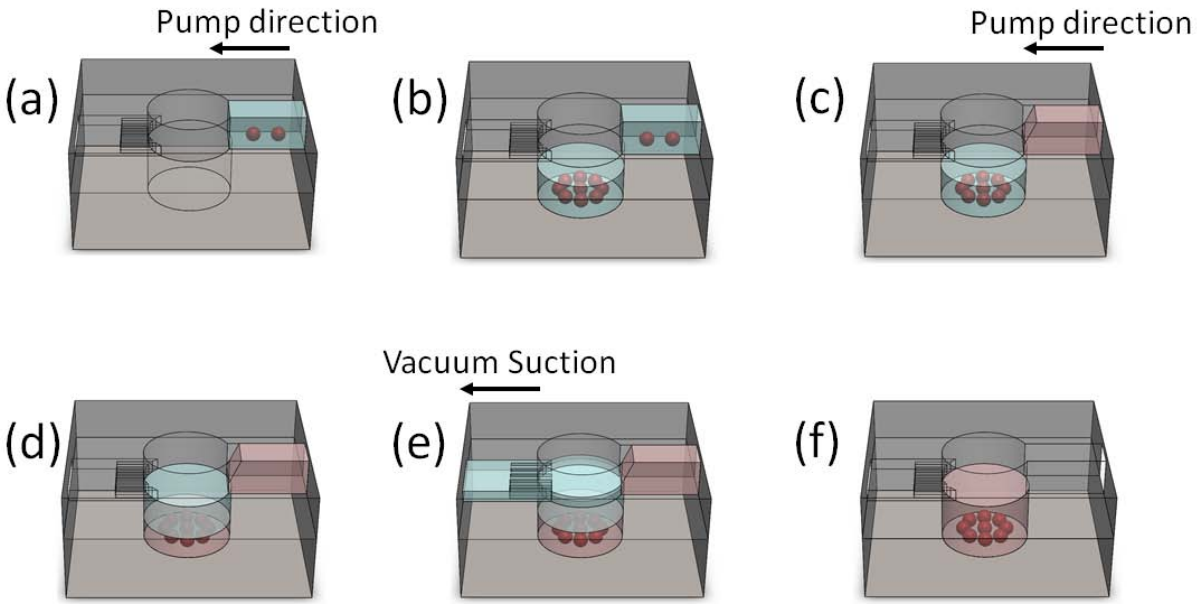
Collagen fibril configuration may affect incorporated cell viability and proliferation. In order to verify collagen gelation in a microfluidic chip, collagen droplets were gelled at different temperatures (4, 25, and 37°C). The collagen droplets generated with each condition were collected on a sterile petri-dish and collagen fibrils were imaged by confocal reflection microscope using a 60× water immersion lens. As shown in Sup. Fig. 3, no collagen fibrils were observed in microspheres gelled at 4°C, while only a small number of fibrils were observed when the gelation temperature was increased to 25 °C. At 37°C, collagen fibrils were readily apparent.



Sup. Fig. 4: Fluorescence images of collagen fibril at different incubation temperatures. (a) Incubation at 4°C. (b) Incubation at 25°C. (c) Incubation at 37°C. The bright dot in the center of the image is an artifact of confocal reflectance. Scale bar: 5 μm.

Supplement 5: Collagen microspheres extraction.

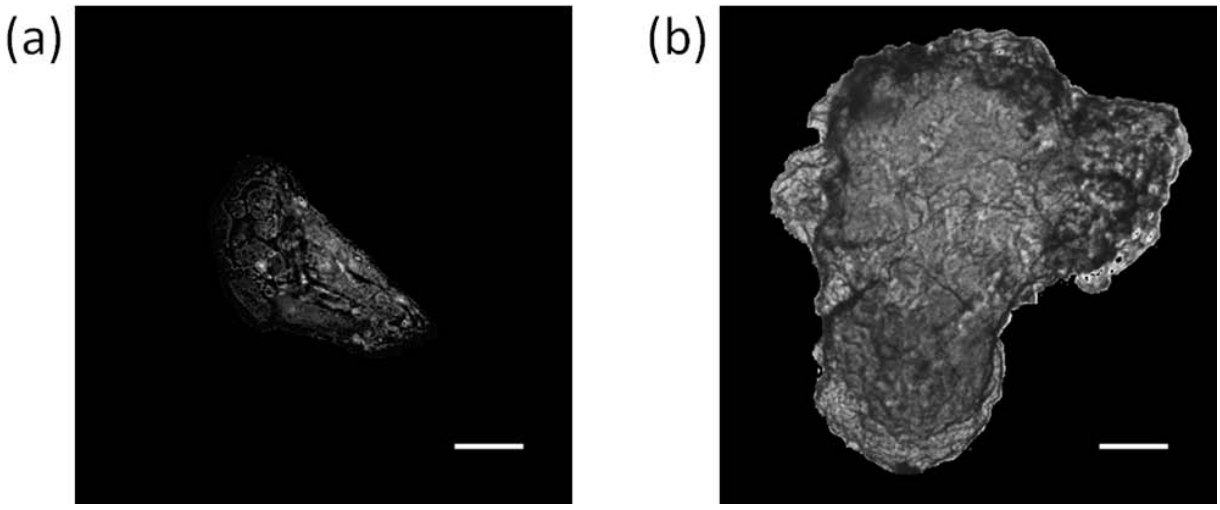
The detailed schematic diagram for the extraction of collagen microspheres from oil phase is shown in Sup. Fig. 5. Collagen microspheres are collected in the extraction chamber and incubated until the completion of droplet generations. After termination of droplet generations, culture media was pumped through the injection port. Culture media is filled from the bottom of the extraction chamber due to heavier density. The mineral oil is pimped to the top and sucked to the left side of chamber by the vacuum pump. Finally, all oil phase is replaced to aqueous media (culture media) and microspheres are collected by pipette.



Sup. Fig. 5: Collagen microspheres extraction. (a) Collagen microdroplets were generated. (b) Microspheres were collected in extraction chamber. (c) Culture media was injected. (d) Mineral oil separation by density. (e) Mineral oil removing by vacuum suction. (f) Collagen microspheres in extraction chamber.

Supplement 6: Collagen microdroplet deformations by centrifugation.

We have observed deformation and collapse of collagen microspheres when they are extracted by centrifugation. As shown in Sup. Fig. 6, deformed and agglomerated collagen microspheres were found after centrifugation process at the speed of 3000 rpm/min for 15 min, which is a typical speed and duration of centrifugation employed to separate collagen microspheres from the oil phase.



Sup. Fig. 6: Optical images of malformed collagen microspheres. (a) Shape deformation of a single microsphere. (b) Agglomeration of multiple microspheres.