Supplementary Materials

Green extraction of healthy and chemical free mitochondria with a conventional centrifuge

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Cell suspension container and cap design

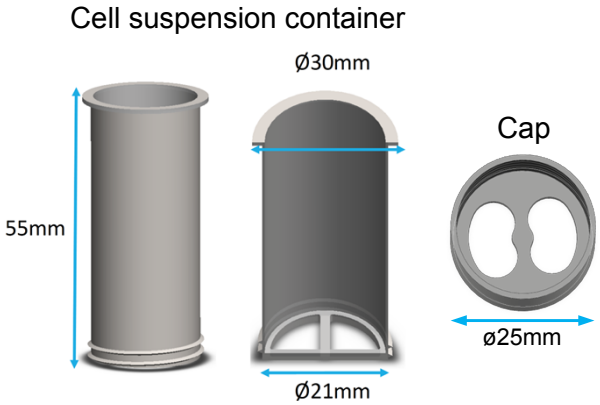

Figure S1. Cell suspension container and cap designed using SolidWorks

Microfluidic device fabrication details

(1) Photolithography

Photolithography, as shown in Figures S2(a) and S2(b), was used to build the original mold for successive nickel electroforming and PDMS casting molding. The process includes silicon wafer cleaning, spin coating of photoresist, exposure, and development.

(i) Silicon wafer cleaning and spin coating

A silicon wafer was sequentially soaked in acetone, alcohol, and deionized water and ultrasonically shaken for 15 min. After drying with nitrogen, the silicon wafer was placed on a hot plate and baked at 50 °C and 100 °C for 10 min.

(ii) Spin coating

In this study, the AZ-4620 positive photoresist was used. The desired photoresist thickness for our application is 8–12 µm. First, the silicon wafer was placed on a hot plate at 65 °C for 5 min. Then, the AZ-4620 positive photoresist was spin-coated (first stage: 500 rpm, 10 s; second stage: 3,500 rpm, 30 s). The sample was placed on a hot plate and soft-baked at 65 °C for 10 min to evaporate the solvent in the photoresist. Subsequently, the soft-baked sample is cooled down to room temperature to eliminate the thermal effect and enable curing of the photoresist. Finally, an 8 µm-thick photoresist layer is obtained.

(iii) Exposure

Exposure is performed using an OAI-500 mask aligner under an energy intensity of 18 mW/cm^2 for 15 s.

(iv) Development

The dedicated developer of the AZ-4620 positive photoresist, i.e., AZ-400K, is implemented for development for 45 s. Then, the sample is hard-backed at 100 °C for 10 min.

(2) Electroforming

The electroforming process used a microelectroforming system with bulk nickel as the anode and a patterned structure as the cathode. Then, electroforming was performed in nickel sulfamate tetrahydrate solution under the appropriate current density.

(3) PDMS casting molding

The casting molding approach, as illustrated in Figures S2(d) and S2(e), is adopted to transfer the silicon microchannel pattern fabricated by photolithography to PDMS.

(i) Fixing the nickel microchannel pattern (Figure S2(d))

A hollow acrylic mold that has an inner diameter of 2.2 cm, an outer diameter of 3 cm, and a thickness of 4 mm was cut using a laser cutting machine. This acrylic mold was placed on the silicon microchannel pattern to define the peripheral structure of the microchannels. At the center, a cylindrical acrylic with the diameter of 8 mm and thickness of 4 mm is used to define the cell injection hole. This mold can reduce the amount of PDMS, and the formed PDMS microchannel structure does not need further cutting.

(ii) PDMS solution preparation

Reagents A and B of PDMS are uniformly mixed in a 10:1 ratio. Then, a mixing solution is placed in a vacuum oven and evacuated for 30 min to remove air bubbles from the solution.

(iii) Casting molding

The PDMS solution is gradually cast onto the prepared acrylic mold until it is filled to capacity and dried in an oven overnight at 50 °C to finally form a 4 mm-thick microchannel structure (Figure S2(e)).

(4) Plasma treatment

Then, the PDMS microchannel structure is separated from the acrylic mold and cleaned with compressed air to remove particles. Next, plasma treatment was performed on the PDMS microchannel structure and the cover glass at 5 mTorr pressure for 1 min. Finally, the plasma-treated cover glass and the microchannel structure were bonded together to obtain the microfluidic device (Figure S2(f)).

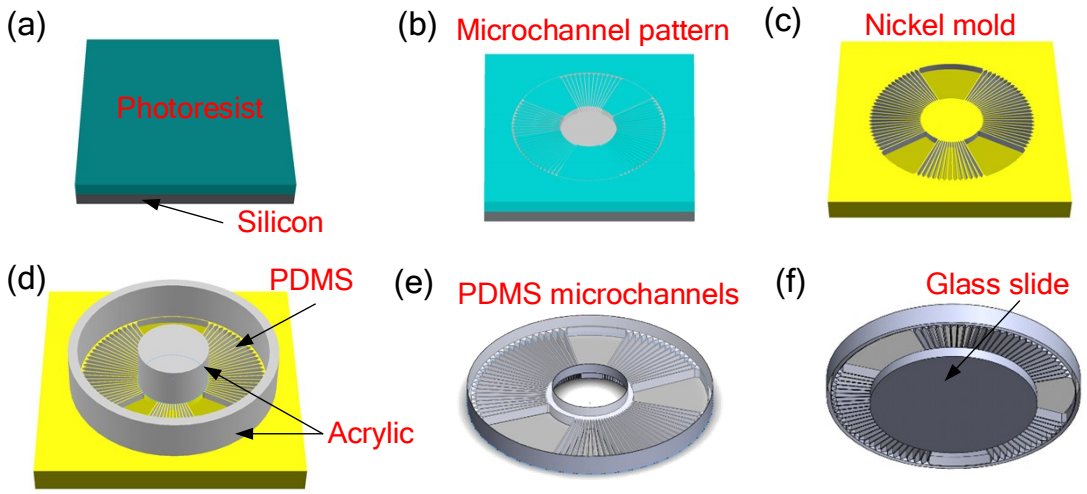

Figure S2. Schematic of the microfluidic device fabrication process: (a) silicon wafer cleaning and spin coating; (b) exposure and development; (c) nickel electroforming; (d) PDMS casting molding; (e) PDMS demolding; and (f) plasma treatment and packaging

PLA based centrifugal device by 3D printing

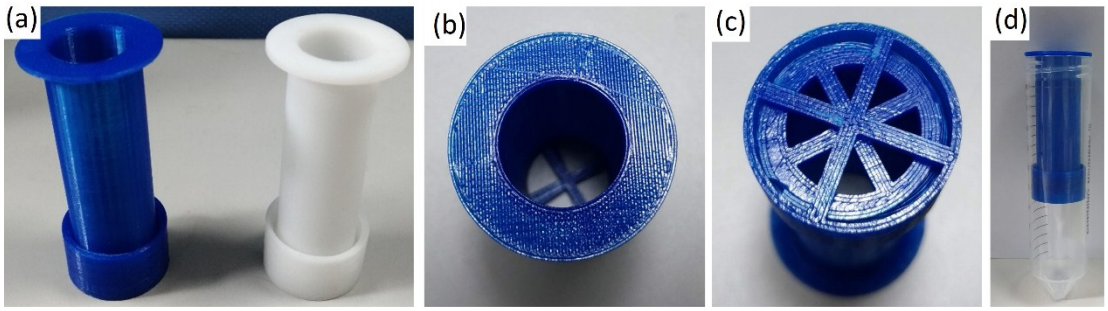

Figure S3. PLA based centrifugal device. (a)side view； (b)upper view； (c)bottom view； (d)centrifugal device inside a 50C.C. centrifugal tube

Western blot analysis of the mitochondria outer membrane protein Tom20

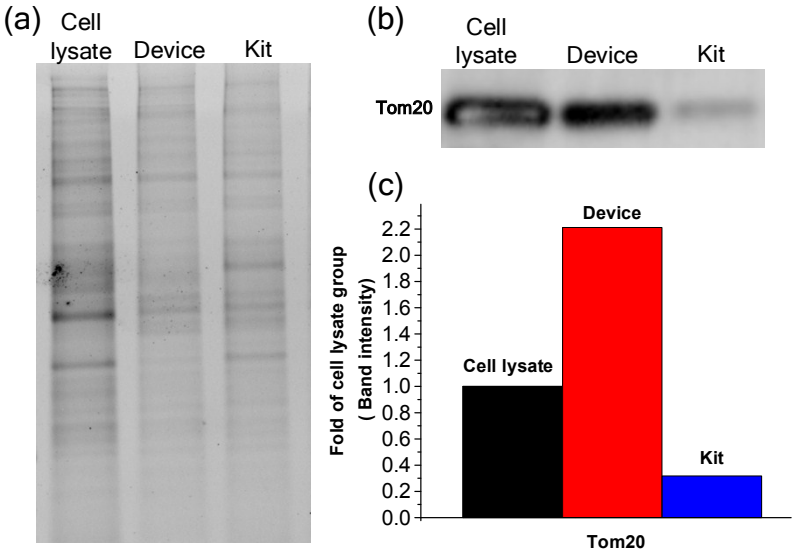

Figure S4. Western blot analysis of the mitochondria outer membrane protein Tom20 extracted by centrifugal microfluidic device and reagent based method. (a) total protein profile; (b) western blot results of Tom20 and (c) data quantified by normalizing the mitochondrial amount presented by the total protein profile (a). Device: centrifugal microfluidic device; Kit: mitochondria extraction kits; Cell lysate: IP lysis buffer.

CG1639 cocultures w/o transwell

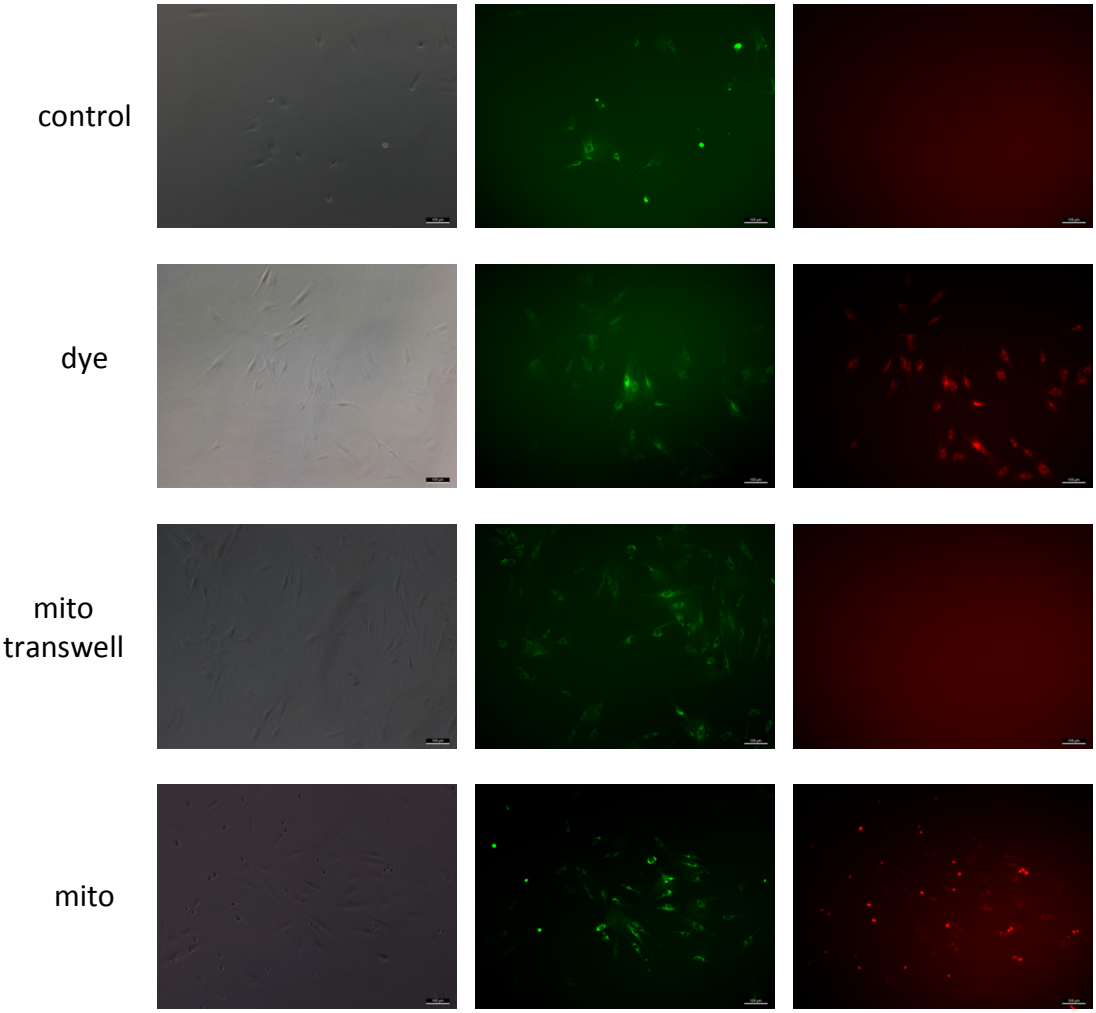

Figure S5. CG1639 cocultures w/o transwell.