Electronic Supplementary Material (ESI) for Lab on a Chip.

Viscosity-aided electromechanical poration of cells for transfecting molecules

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Supplementary method 1 Agarose gel electrophoresis

The samples were exposed to microbubbles induced by 30 trigger signals with electrical powers of 4, 12, and 20 W. Injection was conducted in medium (Opti-MEM) on the bottom of the microtube. Samples containing 200 ng of plasmid were electrophoresed using 1% agarose gel (prepared by dissolving 0.2 g of agarose in 20 mL of 1×Tris-borate EDTA (TBE) buffer) and visualized using GelGreen Nucleic Acid Stain (Biotium, USA).

Supplementary method 2 Preparation of the cell suspension on the bottom of the microtube

For NIH/3T3 and UMR-106 cells, the preparation process was similar to the adherent subculture protocol. After treatment with 2 mL of trypsin-EDTA with a final concentration of 0.025%, cells were transferred into several 1.5-mL Eppendorf tubes. Then, the cell suspension was centrifuged for 5 min at 200×*g*. After the supernatant was discarded, the cells were resuspended using Opti-MEM (ThermoFisher Scientific, USA) and centrifuged for 5 min at 200×*g*. Again, the supernatant was discarded. Then, 3 μL of plasmid with a concentration of 5 μg/μL was added to the cell pellet, and cells were resuspended gently. The volume was adjusted to appropriately 7 μL by adding Opti-MEM. A 7-μL suspension of cells with plasmid was used for the transfection experiments.

Chlamydomonas were prepared by centrifuging a *Chlamydomonas* suspension at 2,600×*g* for 5 min. 2000-kDa FITC-dextran (Sigma-Aldrich, USA) was used for transfection at a final concentration of 85 μg/μL.

Figure S1 The finite element method (FEM) simulation results of the electric field of the microbubble generator using COMSOL Multiphysics.

(a) The three-dimensional geometry of the microbubble generator system. The microbubble generator was made from a tungsten wire with a diameter = 100 μm and a Teflon tube as an insulating material with an inner diameter = 100 μm and an outer diameter = 300 μm. The generator and the negative electrode (a tungsten wire) were inserted into the water. 500 V was applied to the generator. The distance between the generator and the negative electrode was 1 mm. We meshed the model geometry through a physics-controlled mesh sequence type of the COMSOL Multiphysics. 4372 domain elements, 2044 boundary elements and 749 edge elements were generated. (b) The distribution of the electric field on the x-z surface (y = 0). The axes of the microbubble generator and the negative electrode were located on the surface. (c) Arrow plots of the electric field distribution on the surface vertical to the axis of the microbubble generator. The distance between the tip of the generator and the surface was h1 = 100 (upper) or h2 = 300 μm (lower).

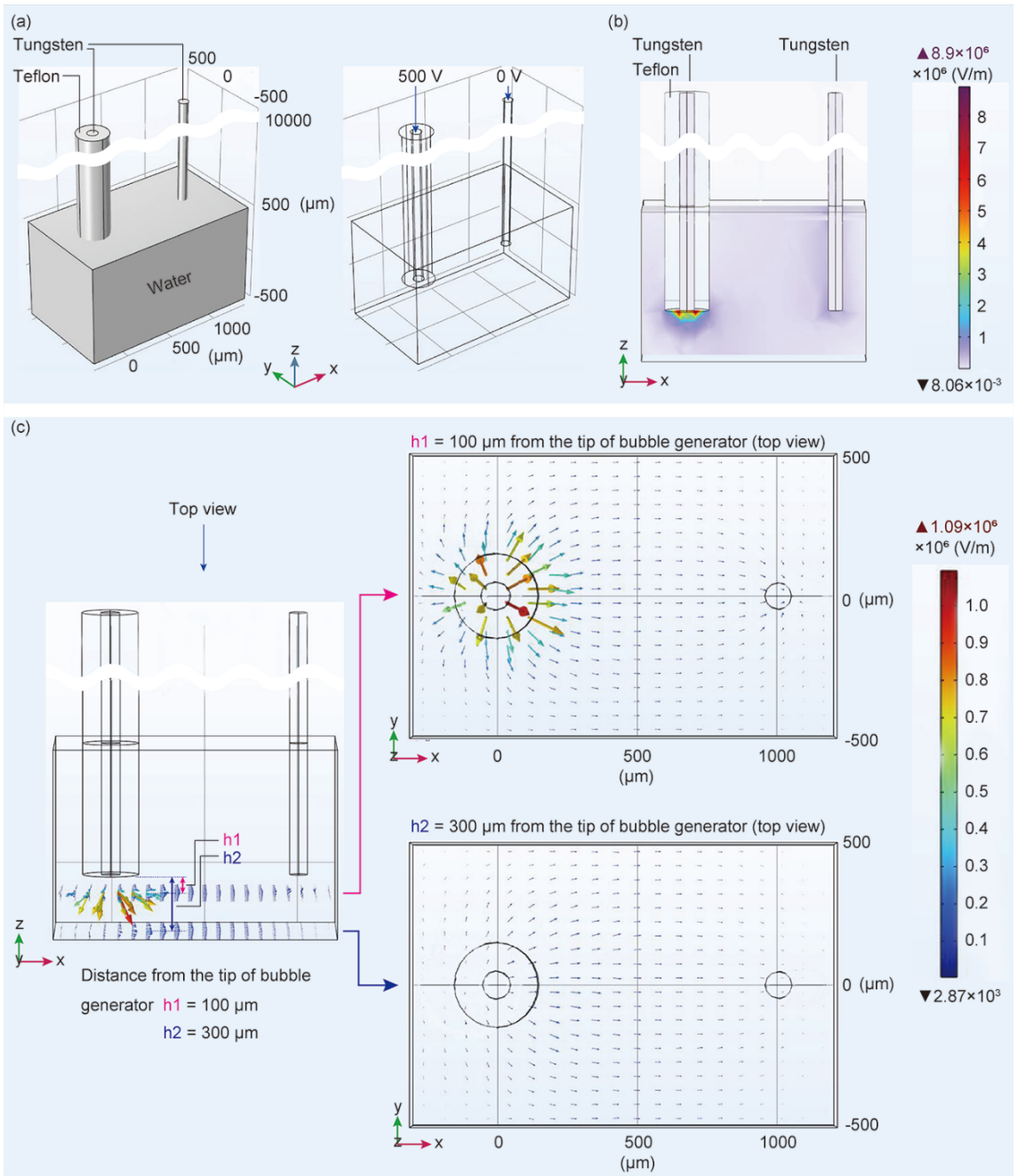
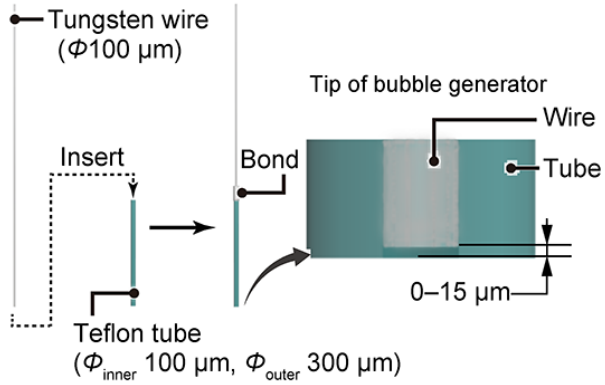

(a)
Tungsten
Teflon
500
0
-500
10000
500 (μm)
-500
1000
500
0
(μm)
Water
500 V
0 V
(b)
Tungsten
Teflon
Tungsten
▲8.9×10⁶
×10⁶ (V/m)
8
7
6
5
4
3
2
1
▼8.06×10⁻³
(c)
Top view
h1
h2
Distance from the tip of bubble generator h1 = 100 μm
h2 = 300 μm
h1 = 100 μm from the tip of bubble generator (top view)
500
0 (μm)
-500
0
500
1000
(μm)
h2 = 300 μm from the tip of bubble generator (top view)
500
0 (μm)
-500
0
500
1000
(μm)
▲1.09×10⁶
×10⁶ (V/m)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
▼2.87×10³

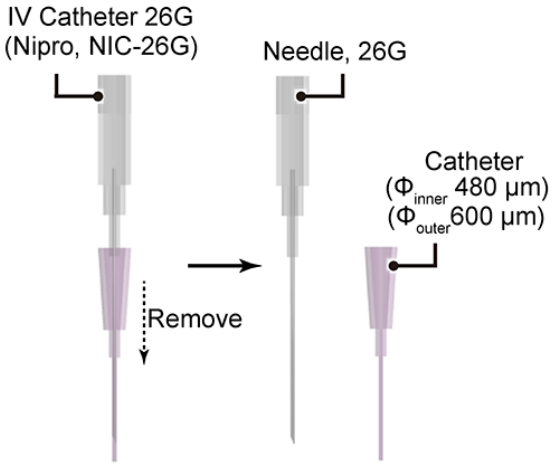
Figure S2 Fabrication of the bubble generator.

To fabricate the needle-free bubble generator, we used a tungsten wire with a cross-sectional diameter of 100 μm, a Teflon tube with a 100-μm inner diameter and a 300-μm outer diameter, an intravenous (IV) indwelling catheter and an electrode, as described in the following six steps. 1) The tungsten wire was inserted into the Teflon tube. Both materials are non-toxic to animal cells. The tip of the wire was 0–15 μm from the tube, which was confirmed using a stereo microscope. The small space between the wire and the tip (the 'bubble reservoir') is important for stabilizing the electric discharge and generating microbubbles. The end of the tube was bonded to the tungsten wire using a thin layer of LOCTITE 4305 (LOCTITE, Germany), which is UV-curable. 2) The IV indwelling catheter was disassembled into the needle and catheter parts. 3) The end of the injector tip was inserted into the tube of a conventional syringe needle. The tungsten wire was fixed to the needle by inserting a small amount of EPO-TEK-H20E electrical epoxy into the tube of the syringe needle and heating at 400°C for 10 s. 4) The catheter was replaced to cover the tungsten wire and the syringe needle. The tip of the catheter was fixed to the Teflon tube using LOCTITE 4305. It is important to confirm that all the metal parts are covered by this process. 5) The electrode was inserted into a 3D-printed holder. LOCTITE 4305 was added to bond the two parts. 6) A small volume of electroconductive DOTITE D-500 (Fujikura Kasei Co., Ltd, Japan) was pushed into the end of the needle. Finally, the electrode was inserted and connected to the end of the needle.

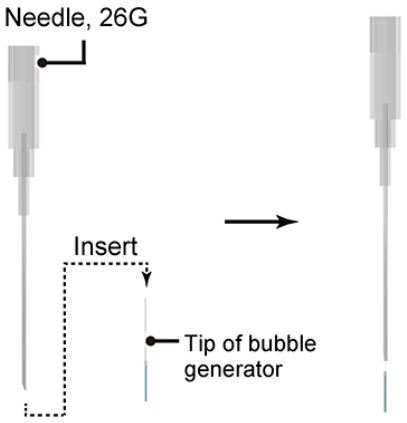
1) Preparation of the generator tip (enlarged view)



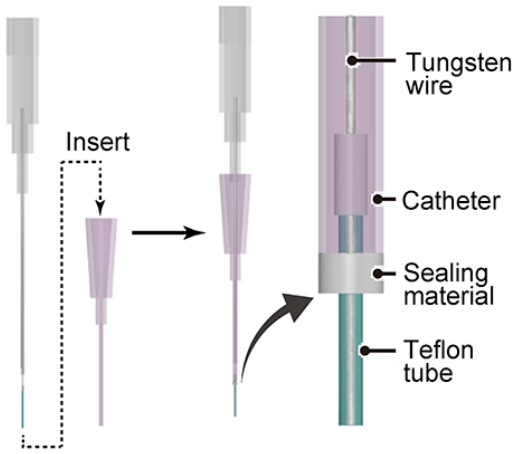
2) Disassembly of the intravenous (IV) indwelling catheter



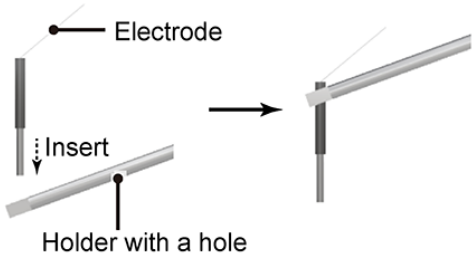
3) Insertion of the tip of bubble generator



4) Sealing the needle by the catheter



5) Assembly of the electrode and the holder



6) Assembly of the electrode and the generator

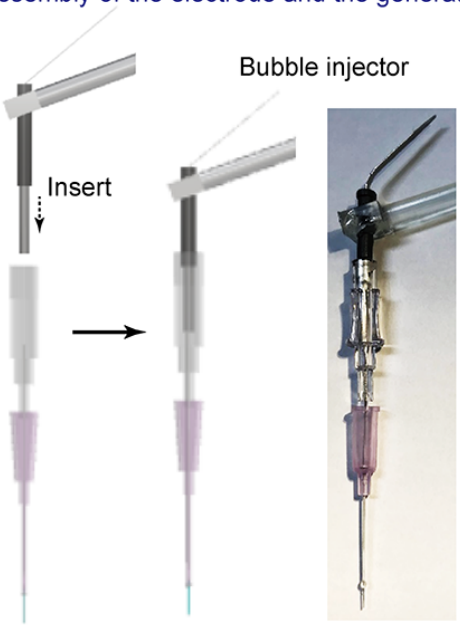


Figure S3 The representative images of NIH/3T3 cells for the calculation of cell viability after 24-h culture.

(a) Control. No microbubble exposure. (b) A sample exposed to microbubbles at 12 W. (c) A sample exposed to microbubbles at 15 W. Here, plasmid pEGFP-N1 with a final concentration of 2.14 μg/μL was added to the three samples.

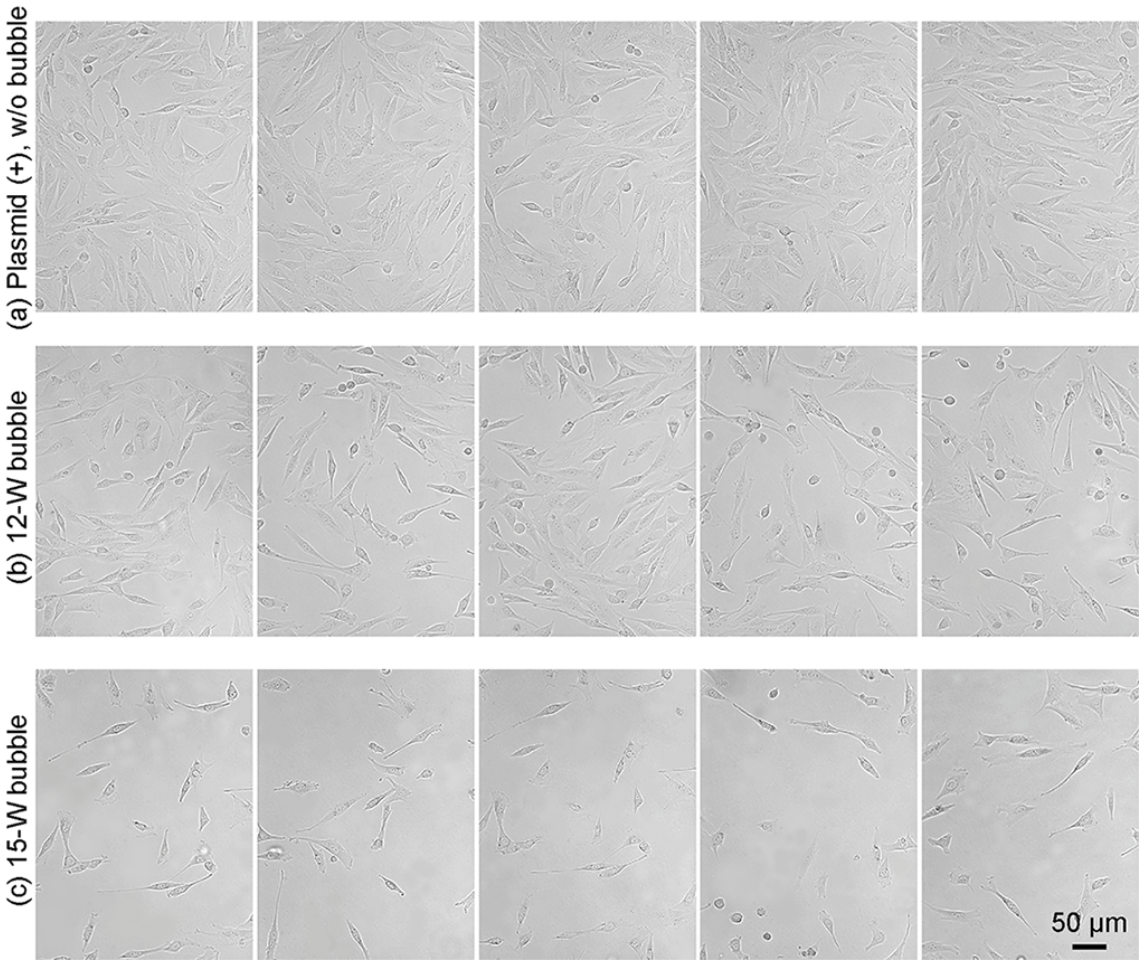


Figure S4 NIH/3T3 or UMR-106 cells after 24-h incubation with the addition of 21% LCNF or 12-W-bubble treatment.

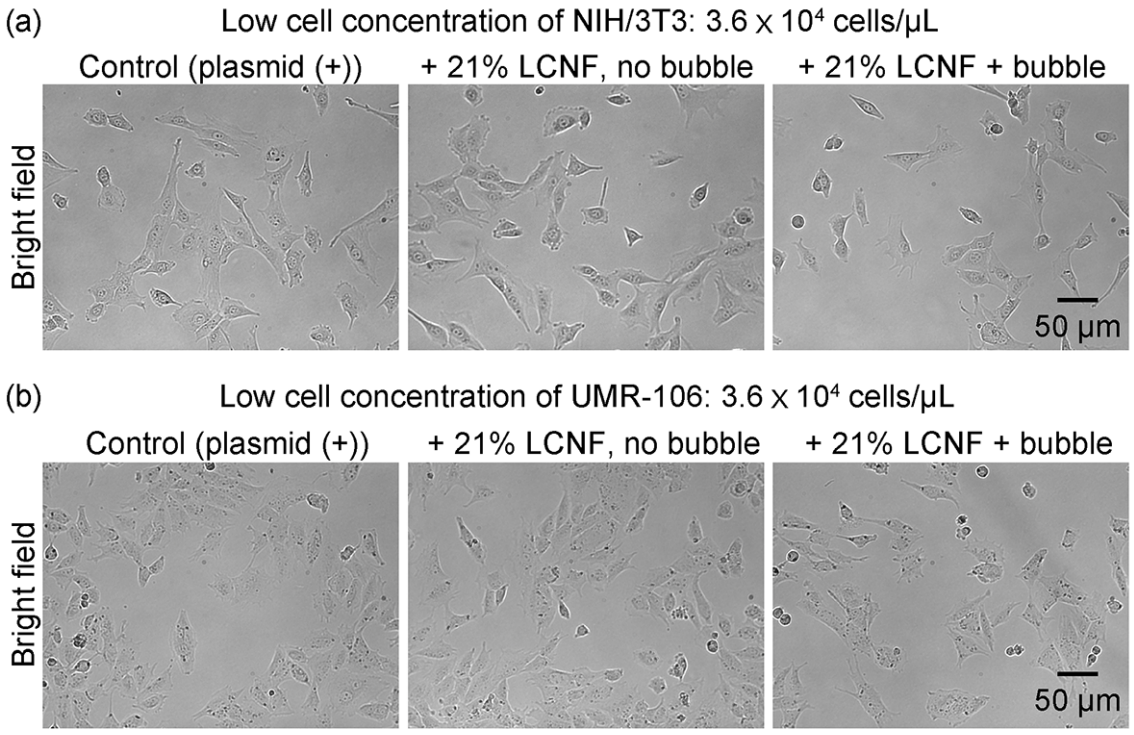


Figure S5 NIH/3T3 cells with the addition of 21% LCNF for the calculation of cell viability after 24-h culture.

(a) Control without microbubble exposure and LCNF addition. (b) A sample with the addition of 21% under the static condition. (c) A sample with the addition of 21% LCNF. Exposure of microbubbles was conducted at 12 W. Here, plasmid pEGFP-N1 with a final concentration of 2.14 μg/μL was added to the three samples.

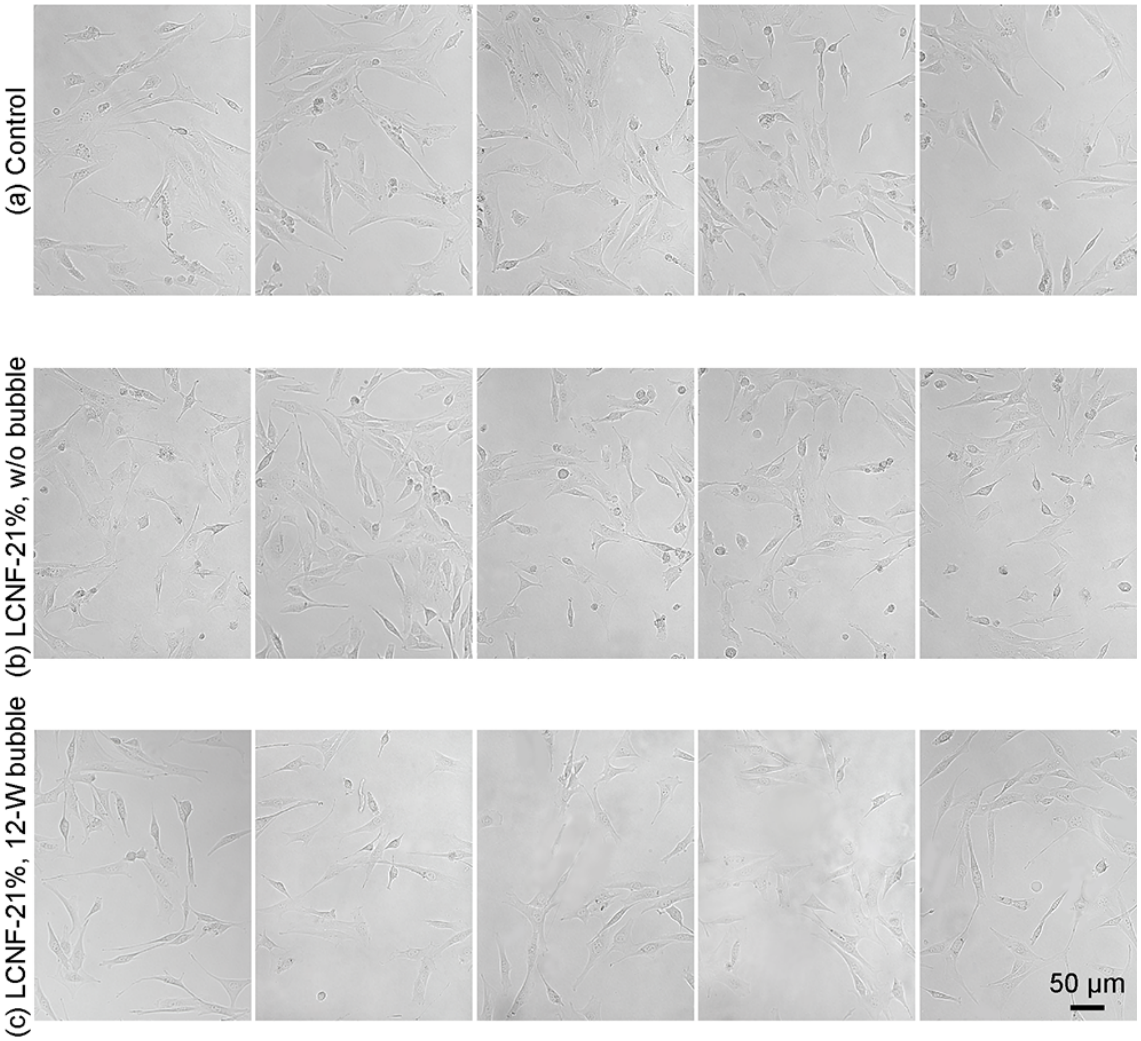


Figure S6 The representative images of UMR-106 cells with the addition of 21% LCNF for the calculation of cell viability after 24-h culture.

(a) Control without microbubble exposure and LCNF addition. (b) A sample with the addition of 21% under the static condition. (c) A sample with the addition of 21% LCNF. Exposure of microbubbles was conducted at 12 W. Here, plasmid pEGFP-N1 with a final concentration of 2.14 μg/μL was added to the three samples.

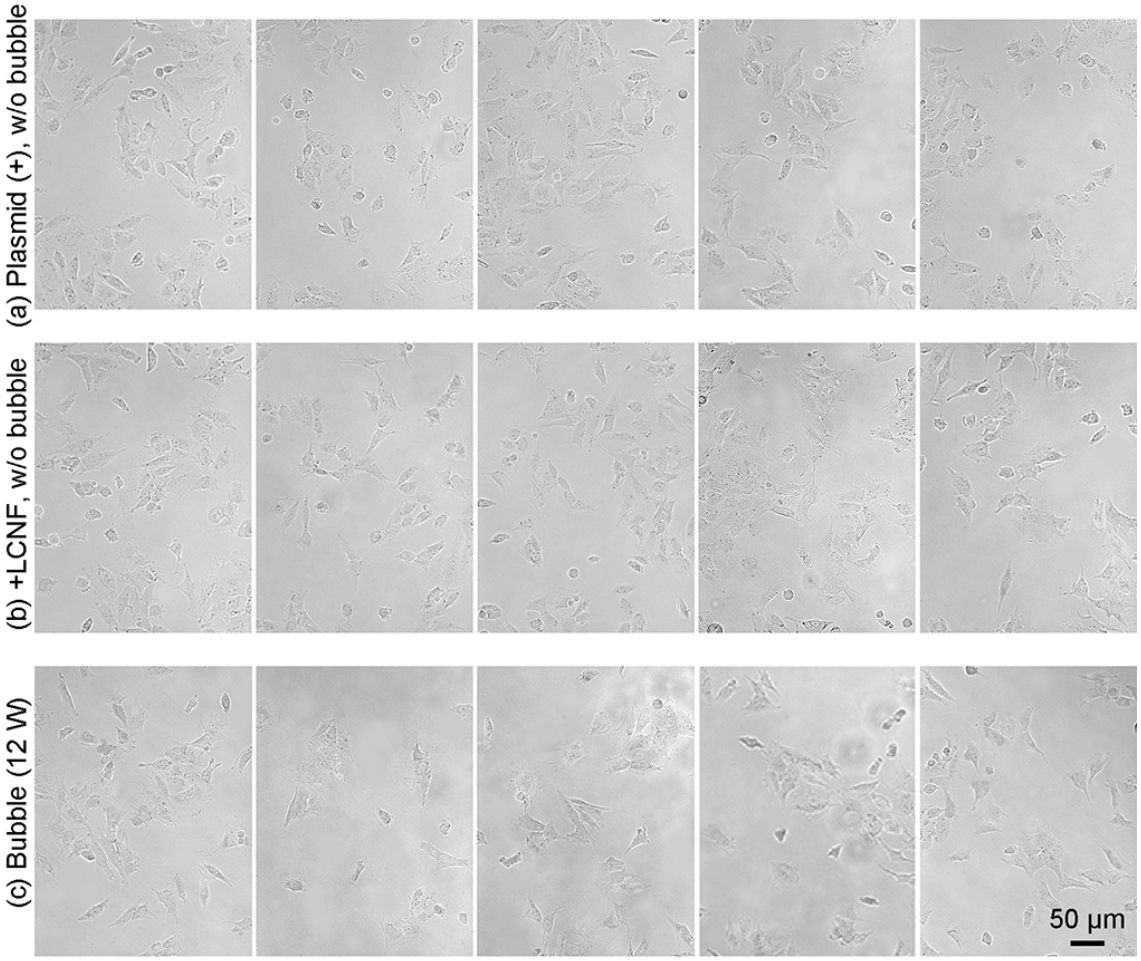


Figure S7 Delivery of plasmid pEGFP-N1 into UMR-106 cells for different plasmid concentrations.

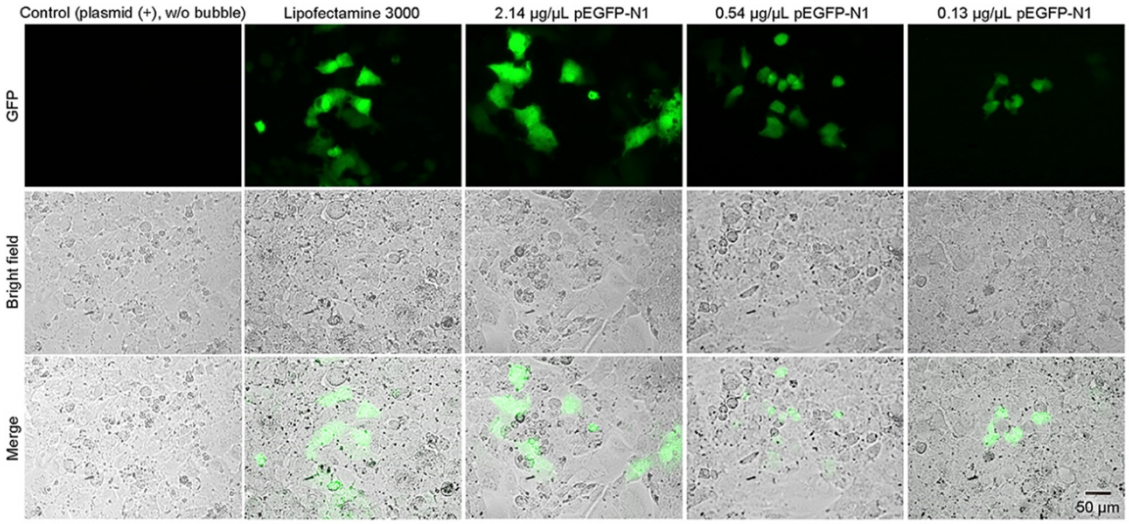
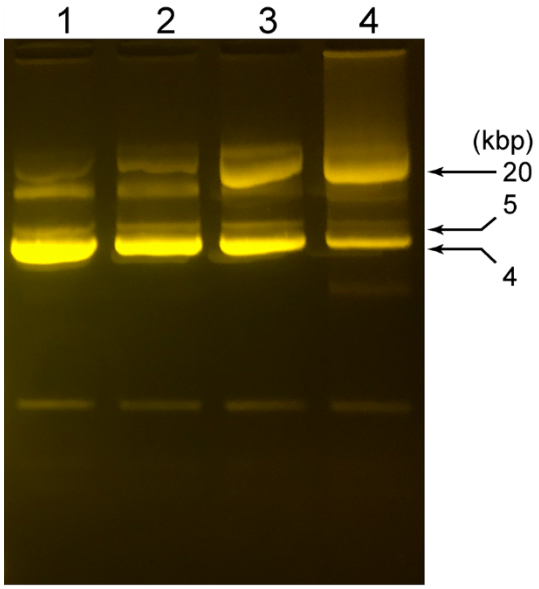


Figure S8 Plasmid DNA damage investigation after microbubble exposure using agarose gel electrophoresis. 200 ng of pEGFP-N1 (4.7 kbp) plasmid treated under conditions 1–4 were analyzed by agarose gel electrophoresis. Bands at 20 and 1.5 kbp were also observed, which may be related to impurities in the plasmids. No substantial damage to the plasmid pEGFP-N1 was observed at 4 or 12 W with 30 trigger signals.



Conditions
1: No bubble exposure (control)
2: 4-W bubble
3: 12-W bubble
4: 20-W bubble

Movie S1 Microbubbles in a suspension without the addition of LCNF (0% LCNF).

Movie S2 Microbubbles in a suspension with 5% LCNF.

Movie S3 Microbubbles in a suspension with 10% LCNF.

Movie S4 Microbubbles in a suspension with 15% LCNF.

Movie S5 Microbubbles in a suspension with 20% LCNF.

Movie S6 Microbubbles in a suspension with 25% LCNF.

Movie S7 Microbubbles at a cell concentration of 3.6×10^4 cells/μL (NIH/3T3) (low cell concentration).

Movie S8 Microbubbles at a cell concentration of 2.1×10^5 cells/μL (NIH/3T3) (high cell concentration).

Movie S9 Microbubbles at a cell concentration of 2.1×10^5 cells/μL (UMR-106) (high cell concentration).

Movie S10 The bubble injector and the growth, ejection, and collapse of microbubbles.