Supplementary Information (SI) for Lab on a Chip.

Supplementary information

Mechanical regulation of nerve stem cells' multiple behaviors via GHz acoustic streaming

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1. Methods

S1.1 The multiphysics simulation processes of gigahertz acoustic streaming field

Multiphysics simulations were conducted using COMSOL software to analyze gigahertz acoustic streaming field and shear force distribution. The specific simulation processes are as follows:

(1) Geometry and Material Construction:

2D and 3D geometric models were constructed, including the silicon substrate, Bragg reflector layer (composed of alternating layers of AlN and SiO_2), a bottom electrode, a piezoelectric layer, a top electrode and a liquid region (with different heights). Multiple semicircular solid structures, each with a diameter of 20 μm, were evenly spaced at the bottom of the liquid region to simulate adherent cells.

(2) Physical Study Module Setup:

The electrostatics and solid mechanics modules were utilized to couple the electric field and solid mechanics field through the piezoelectric effect, thus completing the modeling of bulk acoustic wave resonators. In the laminar flow module, the gigahertz acoustic field was integrated into the fluid domain through acoustic-structure boundary coupling and pressure acoustics-fluid dynamics coupling. These methods were used to simulate the gigahertz acoustic streaming field. The cell model forms a fluid-structure interaction interface with the liquid environment, and the Young's modulus and Poisson's ratio of the cells are set accordingly.[1]

(3) Mesh Generation and Result Computation:

Free triangular meshes were generated for the device, laminar flow region, and cell sections. Frequency domain calculations were performed on the physical model to obtain the characteristic frequency of 1.58 GHz, followed by steadystate calculations of the flow field.

2. Results and discussion

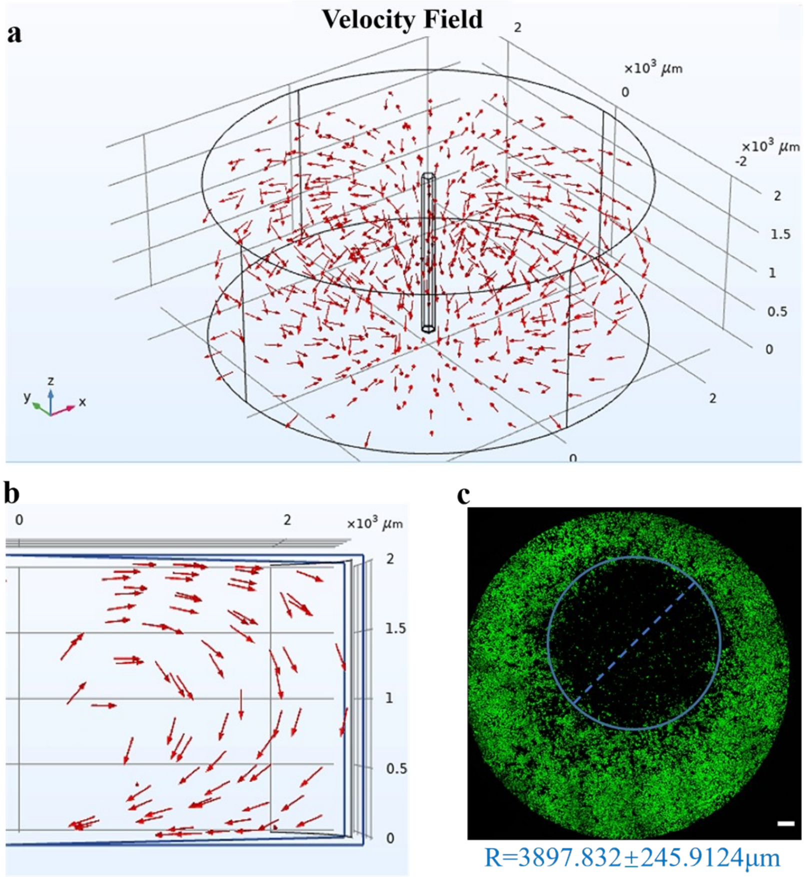

Fig. S1 (a) 3D FEM analysis of a hypersonic resonator at 1.58 GHz in the chamber and velocity field (b) simulation on x-z plane. Color bar indicates the distribution of fluid velocity, and red arrows describe flow direction (c) Under the AS with the chamber height of 2000 μm, the confocal image of the cell under the stimulation of AS. Green indicates the cell membrane. Blue circle and dotted line indicate the range of action and its diameter. Scale bar = 300 μm.

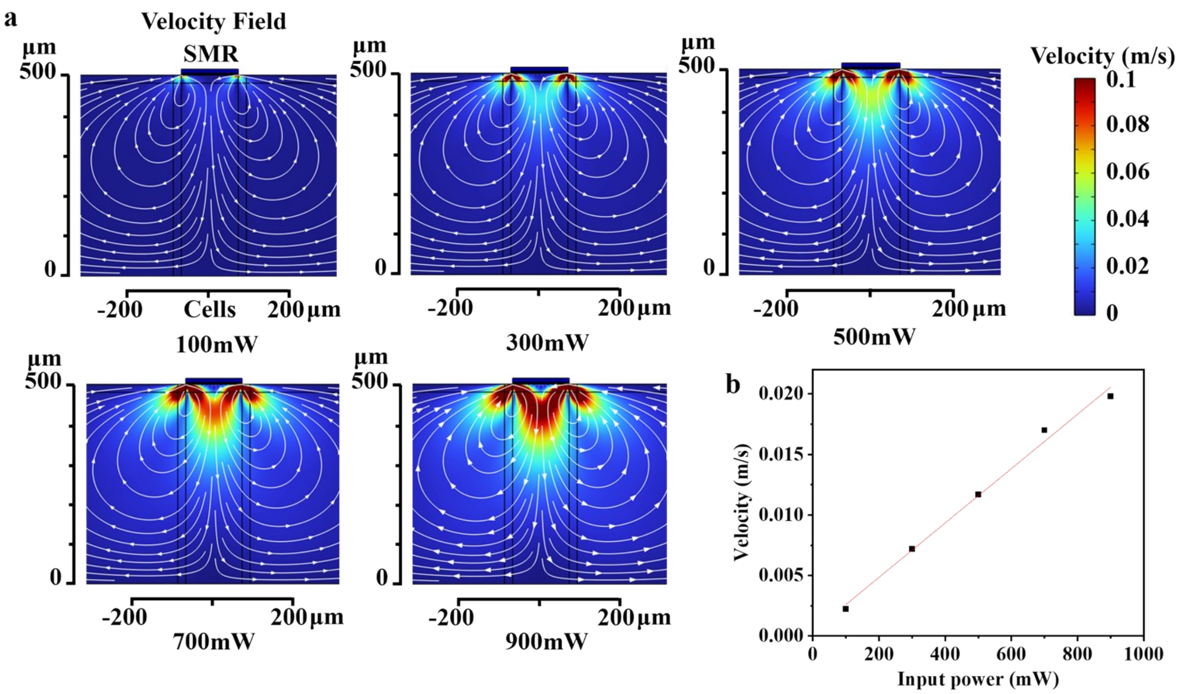

Fig. S2 (a) 2D FEM analysis of 1.58 GHz hypersonic resonator with different input power at the chamber height of 500 μm. (b) Data diagram of velocity and height of acoustic streaming.

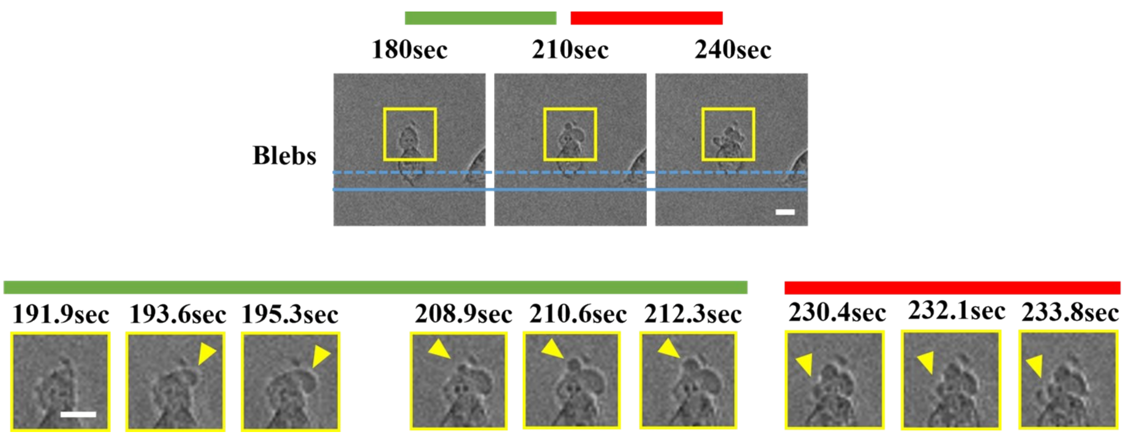

Fig. S3 Time course of AS inducing cells to move with blebs (between 180 and 240 seconds). The yellow arrow and yellow boxes indicate the blebs. Scale bar= 10 μm.

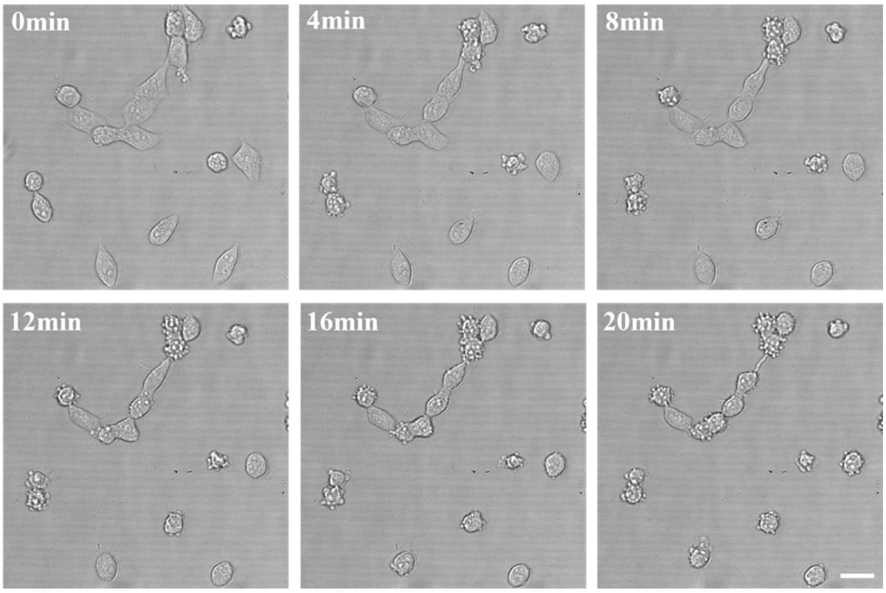

Fig. S4 The changes of NE-4C stem cells with time under ultra high AS stimulation. The data are expressed as the mean ± SD (n = 3). Scale bar = 25 μm.

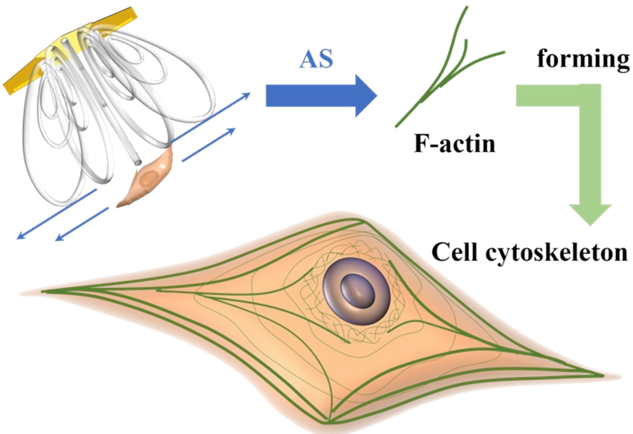

Fig. S5 Schematic diagram of AS affects the cellular cytoskeleton.

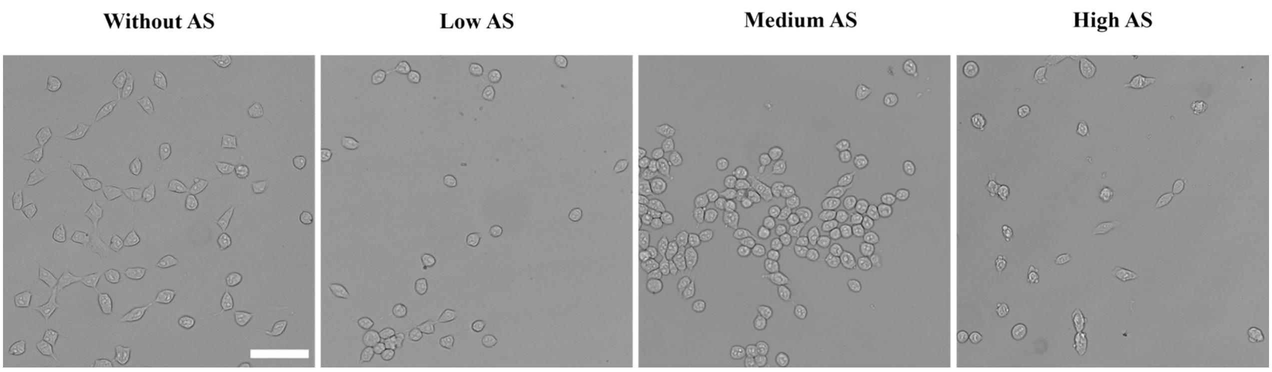

Fig. S6. The images of cells which untreated and stimulated by different intensities of AS for 8 min. Scale bar = 75 μm.

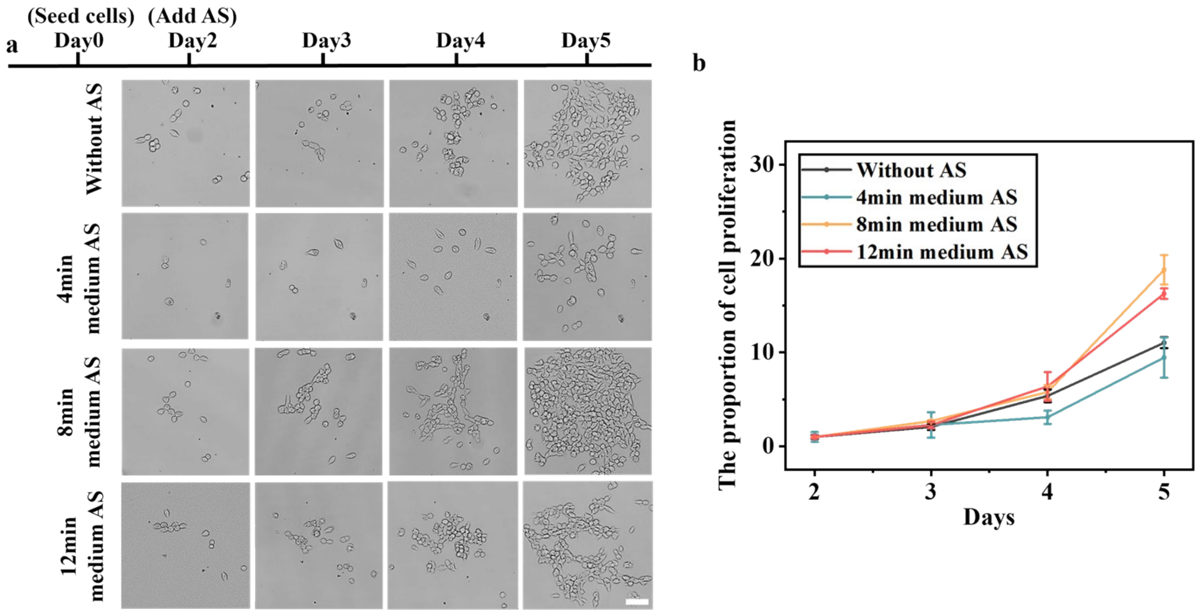

Fig. S7. (a) The image and data statistical chart (b) of proliferation cells which untreated and stimulated by medium AS once for different stimulation time. The data are expressed as mean ± SD (n = 3).

	Migration	Proliferation/ Differentiation	Blebs-driven	Apoptosis
Chamber height	2mm	0.5mm	0.5mm	0.25mm
Input power	500mW	500mW	700mW	700mW
Intensity of AS	Ultra low	Medium	High	Ultra high

Fig. S8. Corresponding system conditions for regulating cell behavior.

Table S1 Comparison of methods for inducing blebs-driven motion.

	Cell type	Simulation Method	Condition
1	Dictyostelium discoideum	Cell squasher (uniaxial compression)	Under the compression of 500 Pa (<30 s).[2]
2	Dictyostelium cells	Cell squasher (uniaxial compression)	Under the compression of as little as 100 Pa (<10 s).[3]
3	Dictyostelium discoideum	A confining microchannels with stable linear cAMP gradients.	In the microchannels with heights of 1.7 µm and 2.4 µm, under a 20 nM/µm cAMP gradient. (<1 s).[4]
4	Walker carcinosarcoma WC256 cells	Direct current electric fields (dcEF)	Under the dcEF of 3 V/cm.[5]
5	NE-4C stem cells	Acoustic Streaming (this study)	Under the shear force of high AS (<120 s)

References

1. W. Kim and A. Han, presented in part at the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences, Groningen, The Netherlands, October, 2010.
2. N. Srivastava, R. R. Kay and A. J. Kabla, *Mol. Biol. Cell*, 2017, **28**, 809-816.
3. N. Srivastava, D. Traynor, M. Piel, A. J. Kabla and R. R. Kay, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 2506-2512.
4. M. Ibo, V. Srivastava, D. N. Robinson and Z. R. Gagnon, *PLoS One*, 2016, **11**, e0163866.
5. J. Sroka, I. Krecioch, E. Zimolag, S. Lasota, M. Rak, S. Kedracka-Krok, P. Borowicz, M. Gajek and Z. Madeja, *PLoS One*, 2016, **11**, e0149133.