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

Supplementary Information

Ultra-rapid Modulation of Neurites Outgrowth under Gigahertz Acoustic Streaming System

Shan He, Zefang Wang , Wei Pang*, Chang Liu , Miaosen Zhang, Yang Yang, Xuexin Duan* and Yanyan Wang*

Email: yanyanwang@tju.edu.cn, xduan@tju.edu.cn,

weipang@tju.edu.cn

S1: Shear stress caused by acoustic streaming

With the gigahertz acoustic resonator mechanical vibration, the liquid was accelerated and moved upward from the center, then returned through the edge. When the uplifted streaming reached the interface of the liquid and solid (the coverslip with PC12 cells in our work), the flow direction was forced to change and the longitudinal component of streaming velocity was attenuated to zero in a short distance. The vertical gradient of the lateral streaming velocity caused the shear stress[1-4].

To modulate the intensitise of shear stress caused by acoustic streaming, different height of chamber and different input power of resonator were used. As described above, different height of chamber would induce different gradient of the fluid velocity, therefore generated different shear force on cells.

Meanwhile different input power of resonator could adjust the vibration intensity of the SMR resonator. In the composition of the SMR resonator, the highly c-axis oriented AlN was deposited as a piezoelectric layer between the top and bottom electrode. When an external electric signal was applied, geometric deformation was generated within the piezoelectric material because of the inverse piezoelectric effect, thus generated mechanical vibration. Different resonator power caused different vibration amplitude. The liquid above the pentagonal working area of the resonator was accelerated by device resonation[5]. According to this, different resonator power induced different initial acoustic streaming velocity v_0. Thus, the acoustic streaming velocity in the vicinity of the cells would change as the v_0 changes. As a result, the different saltation of fluid velocity caused different shear force on cells.

S2: Cells differentiation under resonator stimulation with different height of PDMS chamber and input power of resonator.

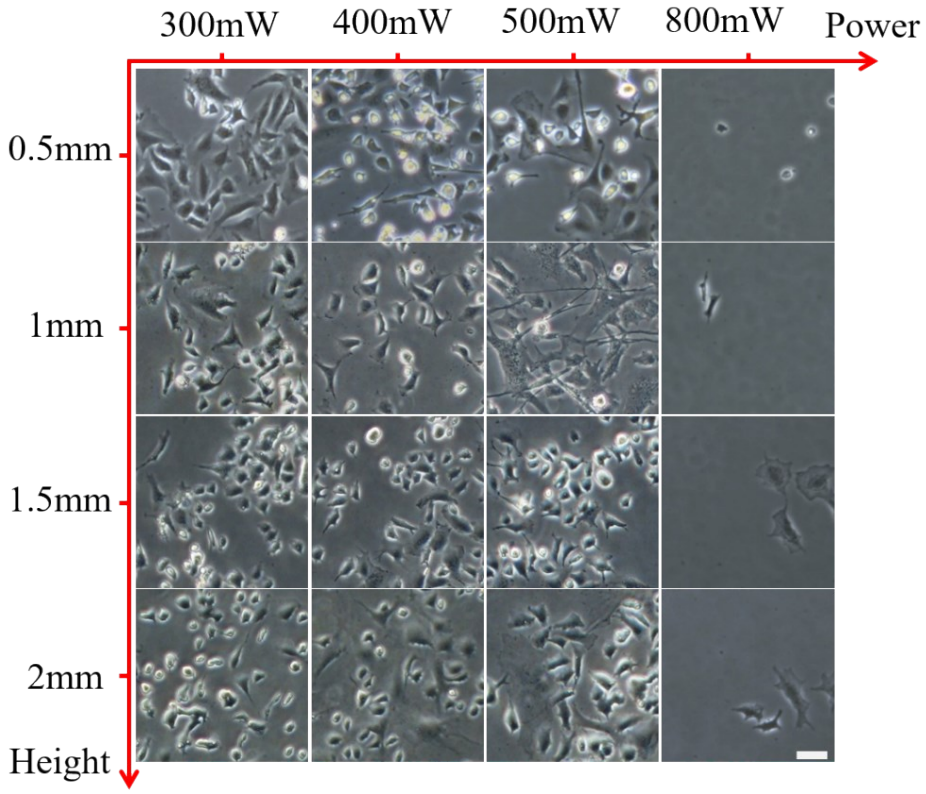

Figure. S1 Images of cells under resonator stimulation with different height of PDMS chamber and power of resonator. Scale bar = 50 µm.

S3: The temperature of liquid under the effect of resonator with different power applying.

The temperature of liquid under the acoustic effect was measured with miniature thermocouple with a distance of 1 mm away from the resonator. The temperature changes under different excitation power were shown in figure S2. The results demonstrated that the temperature increased quickly within the first 3 minutes, then remain consistent with further increasing time. When the resonator continuously working for 10 minutes at power of 500 mW (the optimized condition of the PC12 cells differentiation), the highest tempreture of the measurement was 43.7 ℃, which did not induce neurites outgrowth of PC12 cells according to previous

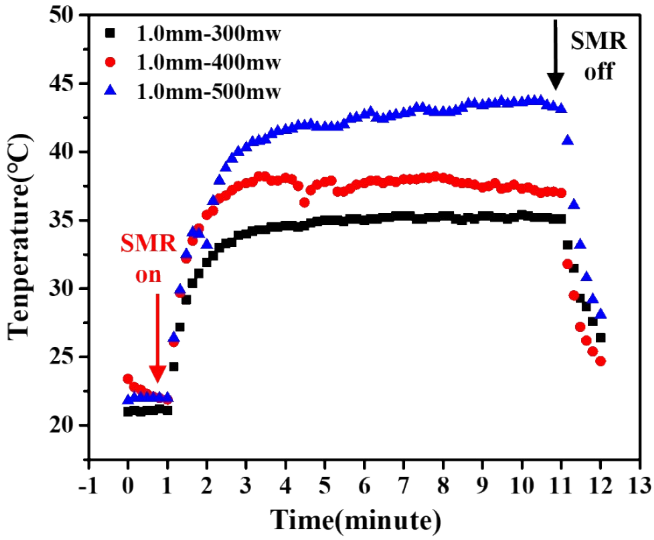

Figure. S2 The temperature of liquid under the effect of resonator with different power applying.

works[6, 7].

S4: The differentiation of cells pretreated with PD98059

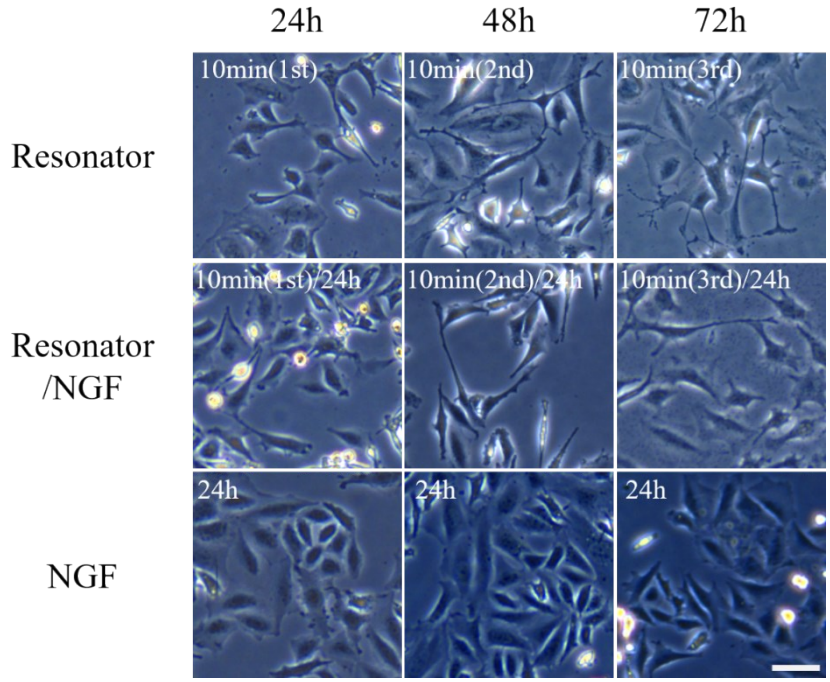

Figure. S3 Images of PC12 cells pretreated with PD98059 exposed to resonator (with chamber height of 1 mm and input power of 500 mW), resonator(with chamber height of 1 mm and input power of 500 mW) combined with NGF, or NGF. Scale bar = 50 μm.

S5: Cytoskeletal of cells with different stimulation method

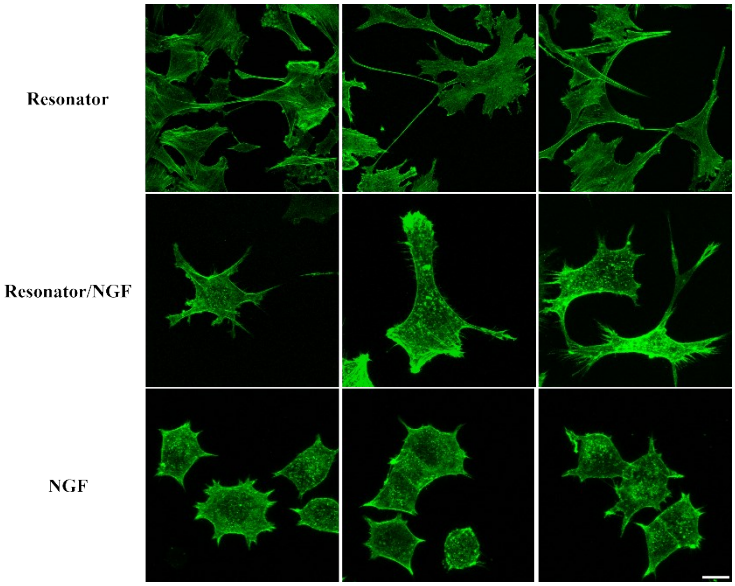

Figure. S4 Cytoskeletal characterization of cells after 72 h culture with three times resonator stimulation(with chamber height of 1 mm and input power of 500 mW), three times resonator combined with NGF stimulation (with chamber height of 1 mm and input power of 500 mW) and cells cultured for the same time period without any stimulation. Scale bar = 25 μm.

References

1. S. Pan, H. Zhang, W. Liu, Y. Wang, W. Pang and X. Duan, *ACS sensors*, 2017, **2**, 1175-1183.
2. X. Guo, H. Zhang, Y. Wang, W. Pang and X. Duan, *Journal of Nanobiotechnology*, 2019, **17**.
3. D. J. Collins, Z. Ma and Y. Ai, *Anal. Chem.*, 2016, **88**, 5513-5522.
4. M. B. Dentry, L. Y. Yeo and J. R. Friend, *Physical Review E*, 2014, **89**, 013203.
5. X. Guo, H. Zhang, Y. Wang, W. Pang and X. Duan, *Journal of nanobiotechnology*, 2019, **17**, 1-11.
6. D. E. Read, K. Reed Herbert and A. M. Gorman, *Brain Res.*, 2008, **1221**, 14-23.
7. Y. L. D S Dwyer, S Miao, R J Bradley, *Neurochem. Res.*, 1996, **21**, 659-666.