

Supplementary Information

A spatiotemporally controllable chemical gradient generator via acoustically oscillating sharp-edge structures

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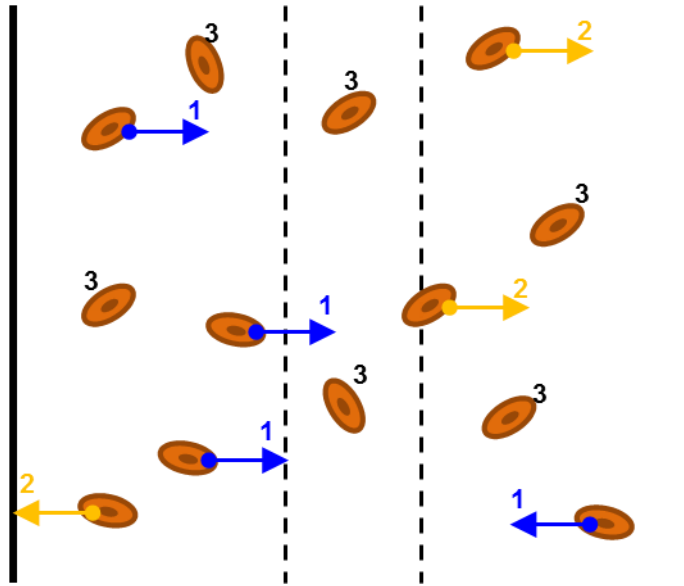


Figure S1. Schematic showing our approach to characterize cell movement. 1, 2, and 3 mark, respectively, those cells which moved toward the center (high VEGF concentration), away from the center (low VEGF concentration), and barely moved, after 6 hours. Tracking the position of each cell, we determined the type of movement for each cell. By doing so, we were able to determine the number of cells for different types of movement.

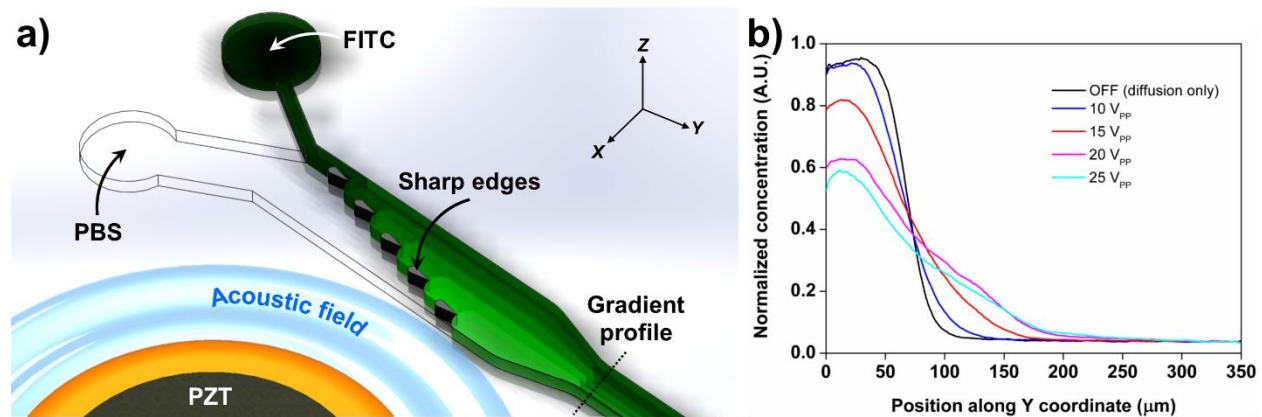


Figure S2. (a) Schematic showing the alternative design of our acoustofluidic gradient generator to generate different gradient profiles. (b) Plot showing the corresponding gradient profiles at region of interest (dashed line) under different input voltages of piezoelectric transducer. The results prove that by differently arranging the sharp-edge structures, various gradient profiles could be generated.

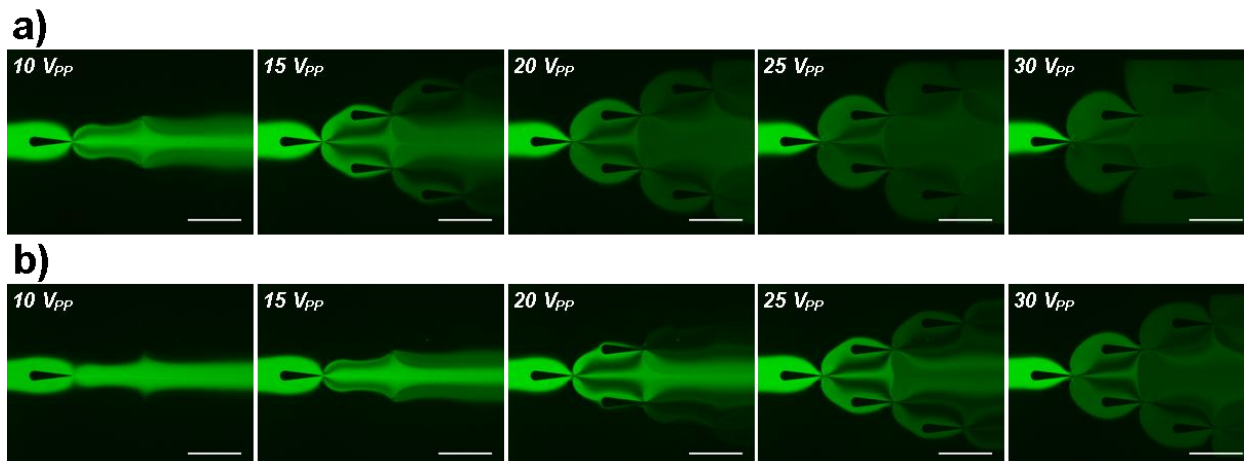
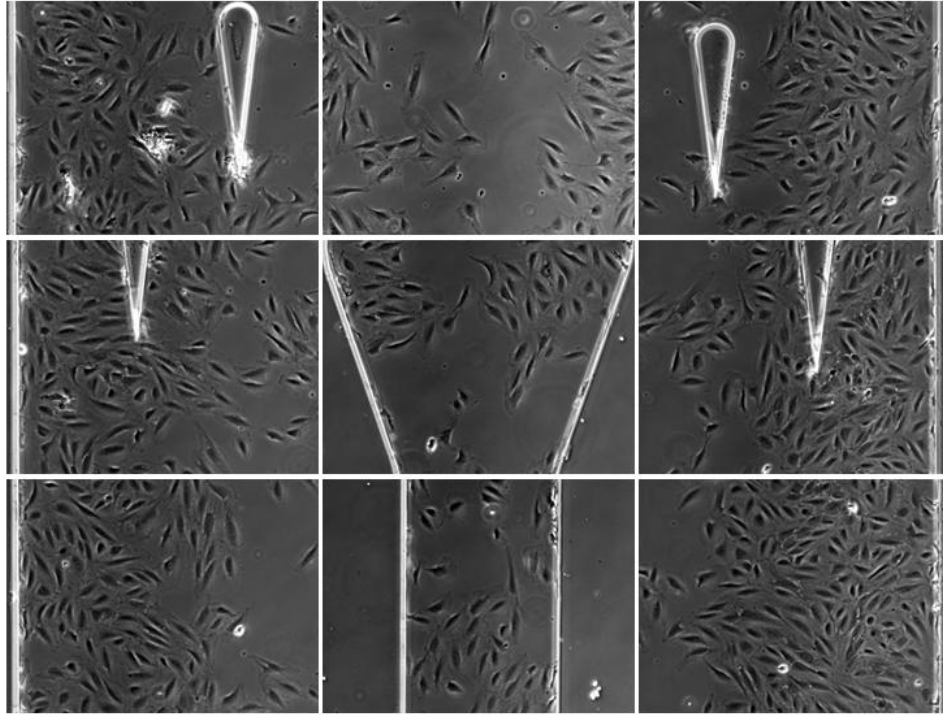


Figure S3. Fluorescence images of mixing behavior of FITC-dextran and DI water at sharp-edge region under different working voltages when the total flow rates of the fluids inside the channel is (a) 3 $\mu\text{L min}^{-1}$ and (b) 6 $\mu\text{L min}^{-1}$. The flow rate ratio remains constant, *i.e.*, $R_{\text{FLOW}} = 5$. These results clearly reveal that increasing the total flow rate inside the channel would significantly suppress the acoustic streaming and therefore, leads to different mixing behavior. On the other hand, however, various gradient profiles can be established by adjusting the total flow rates and the driving voltage. Scale bar: 200 μm .



Visually examined cell viability (after 16 hrs)

Figure S4. Representative bright field images taken at different regions in the channel showing the HMVEC-d cells morphology after 16 hours of the presence of acoustic field. The cells still appeared viable and active, demonstrating the biocompatibility of our acoustofluidic gradient generator for biological studies where long-term cell culture is needed.

Video Captions:

Video S1. Flow patterns and acoustic streaming patterns in the absence and presence of a flow field in the channel of our device.

Video S2. Generation of chemical gradients in the sharp-edge-structure region at a ON/OFF switching frequency of 0.25 Hz (T = 4 sec).

Video S3. Generation of chemical gradient monitored in the downstream regions.

Video S4. Generation of another type of gradient profile by arranging the sharp-edge structures differently.

Video S5. Spatiotemporally controllable generation of chemical gradients by sweeping the driving frequency from 13 kHz to 14 kHz with different time intervals.

Video S6. Time-lapse images showing the movement of HMVEC-d cell in response to a VEGF gradient generated with our gradient generator.