Electronic Supplementary Material (ESI) for RSC Advances.

An Ultrafast Enzyme-Free Acoustic Technique for Detaching Adhered Cells in Microchannels

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Supporting Information

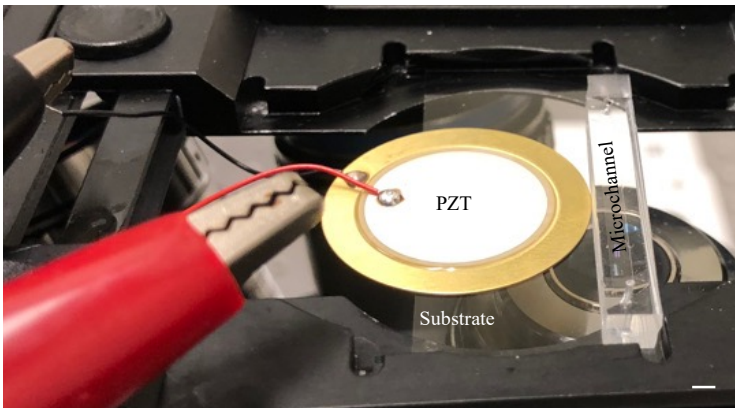

Figure S1 Photo of the experimental setup showing the acoustofluidic device mounted on a microscope stage. The scale bar represents 2 mm.

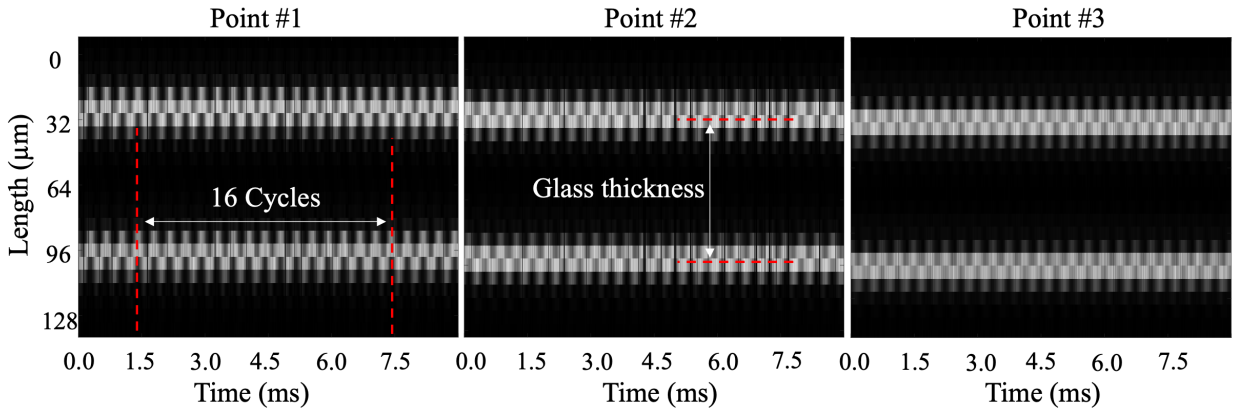

Figure S2 OCT measurements for three random points within the ROI, located near the PZT, inside the microfluidic channel. Displacements of the ~ 70 μm thick glass substrate were captured when the PZT was driven at 96 kHz and 10 V_{pp}. The vibration profile at these points shows a frequency of $\frac{16 \text{ cycles}}{\sim 6 \text{ ms}} = \sim 2.7$ kHz and an amplitude of ~ 13 μm.

Video S1 This video shows the detachment of MDA-MB-231 cells when the PZT is actuated at ~ 200 W input power for ~ 200 ms.

Note S1 Detachment efficiency

The detachment efficiency is calculated by counting the number of detached cells relative to the total number of cells (~ 300 cells per experiment), as follows: Detachment efficiency (%) = $\frac{\text{Number of fully detached cells after the exposure}}{\text{Total number of exposed cells}} \times 100$. The acousticaly-treated cells that can move freely from their initial (pre-exposure) location are considered as fully detached ones.

Note S2 Re-adhesion assay

In addition to the conventional PI staining, we measure the cellular viability indirectly by evaluating whether or not cells can re-adhere to the substrate surface post-exposure. For this purpose, a total of ~ 2,000 cells per experiment were monitored. This re-adhesion assay is conducted as follows:

- **Staining:** Before the acoustic exposure, cells were washed three times, and then their nuclei were stained with Hoechst 33342.

- **Post-exposure incubation:** When the exposure was over, the cells were incubated at 37 ºC overnight (i.e., ~ 16 hrs) to give the live cells enough time for re-adhering to the substrate surface.

- **Washout:** At 16 hrs post-exposure, we first took the microscope images of the cells (Figure S3a and c). Then, the cells were washed three times, so that all floating and loosely attached cells, which are assumed non-viable, were removed. Microscope images of the remaining cells, which were firmly adhered to the substrate (therefore considered to be viable), were taken.

- **Image analysis:** Next, Fiji[1] was used to analyze the fluorescence images (i.e., Figure S3c–d), specifically to measure the total area of the cells' nuclei before, A_1 (Figure S3e), and after, A_2 (Figure S3f), they were washed.

- **Calculation:** The cellular viability is then estimated as Viability (%) $= \frac{A_2}{A_1} \times 100$.

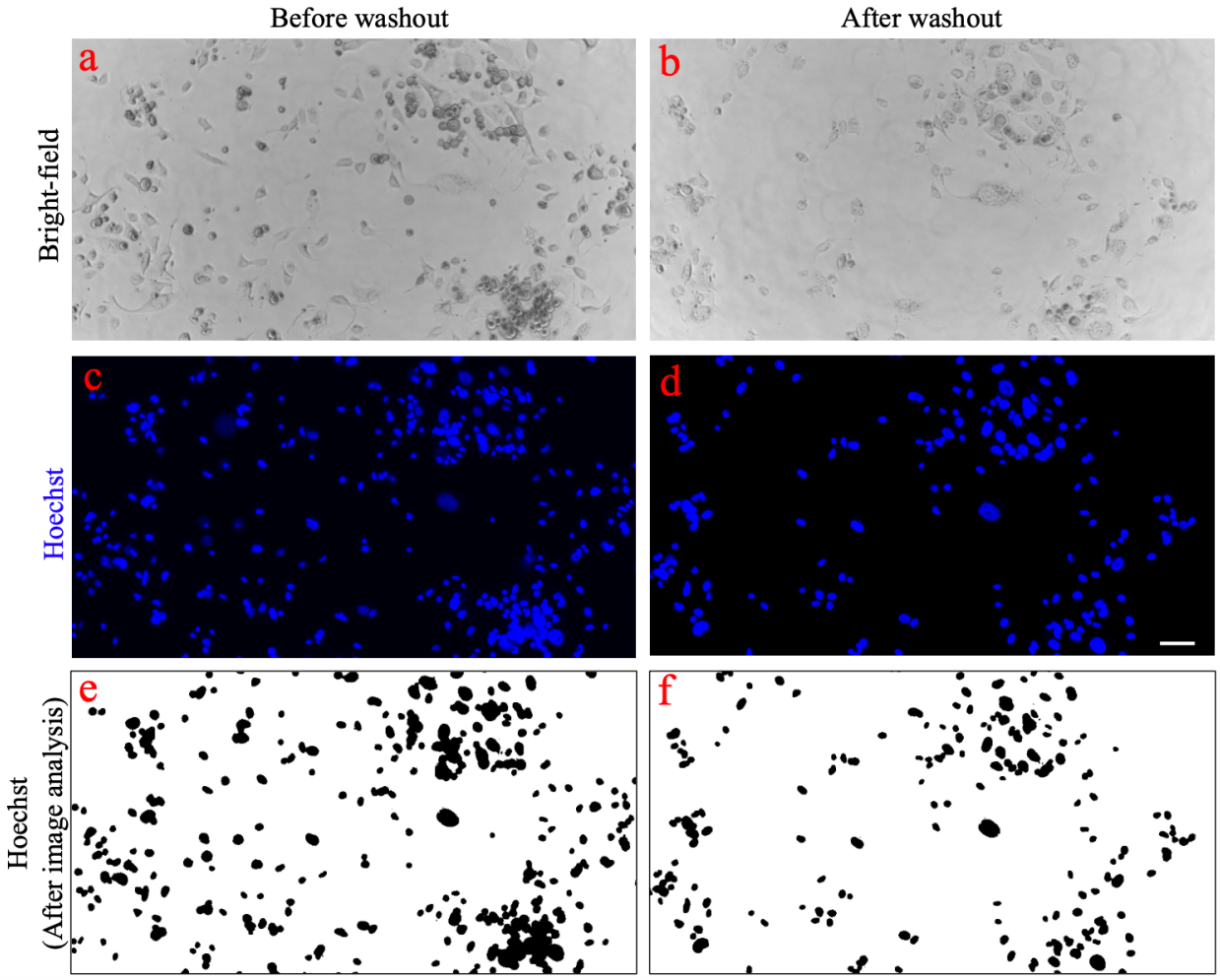

Figure S3 Fluorescence images are analysed to conduct the re-adhesion assay. The bright-field (a–b) and fluorescence images (c–d) are taken 16 hrs post-exposure. The images were taken before washing the cells (a and c) contained both viable and non-viable cells. However, once the cells were washed three times, only the viable cells are remained (b and d). It is assumed that the floating and loosely attached cells are non-viable and, therefore, will be washed out. (e–f) Fluorescence images analyzed using Fiji. The areas depicted in black in (e) and (f) represent the stained nuclei in (c) and (d), respectively. Cell nuclei are stained with Hoechst 33342. The scale bar represents 50 µm.

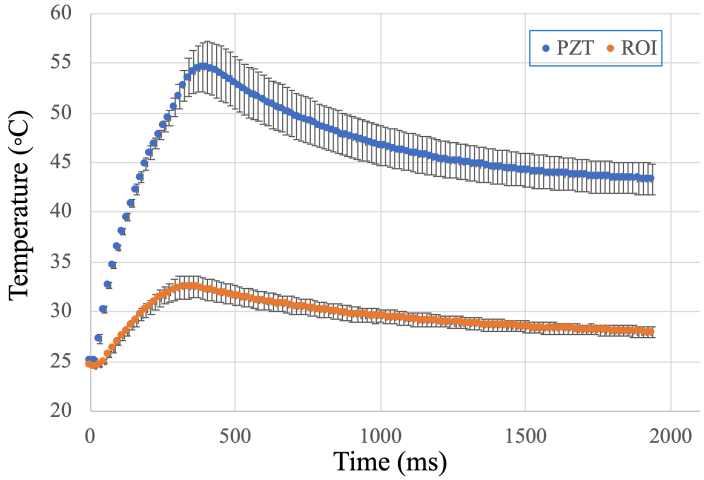

Figure S4 Maximum temperature measured on the surface of the PZT and within the ROI when the PZT is driven at ~ 200 W for ~ 200 ms. Even though the maximum temperature on the surface of the PZT increases to high values (close to ~ 55 °C), the highest temperature within the ROI increases to a maximum of only ~ 33 °C and then reduces to below 30 °C within one second. The error bars represent the standard deviation of four experiments.

References

1. Schindelin, J. *et al.* Fiji: An open-source platform for biological-image analysis. *Nature Methods* **9**, 676–682 (2012).