Enhanced immune capture of extracellular vesicles with gelatin nanoparticles and acoustic mixing

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1. The photograph of the acoustofludic device.

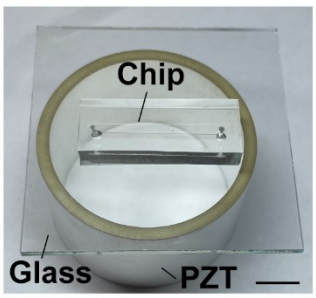

Fig. S1. The photograph of the device with the chip on the top and PZT at the bottom. The scale bar is 1 mm.

2. The simulation of flow velocity in the channel without sound.

When the device was not at the sound field, the flow would maintain laminar flow. The simulation result was shown in the Fig. S2, which indicated the vesicle was difficult to touch the substrate surface unless fluid mixing. Thus, it proves the importance of fluid mixing in increasing the probability of particle collision from the side.

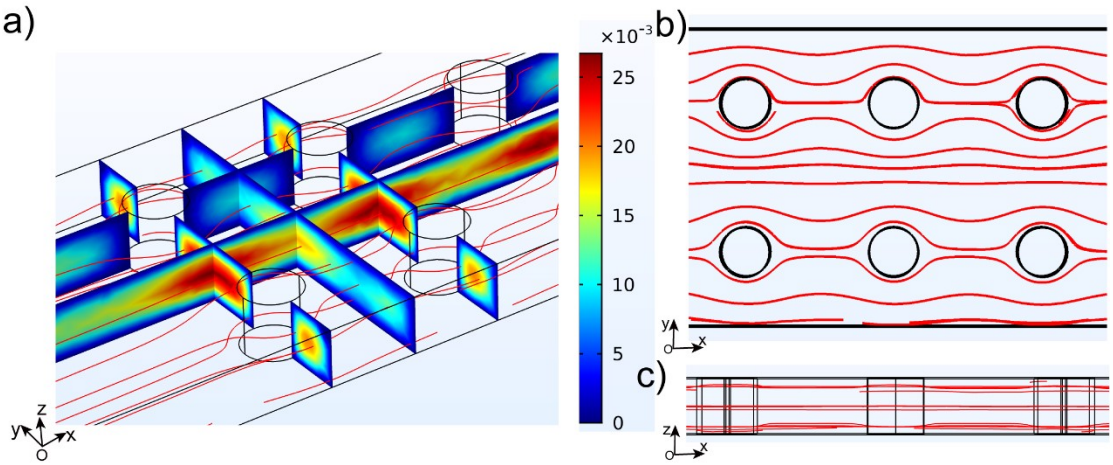

Fig. S2. The simulation of flow velocity in the channel without sound. (a) The 3D simulation result in the channel. (b) The simulation result at x- and y- direction. (c) The-simulation result at x- and z- direction.

3. The test of resonant frequency

In order to make the device work well, the test of the annual PZT is necessary, shown in Fig. S3.

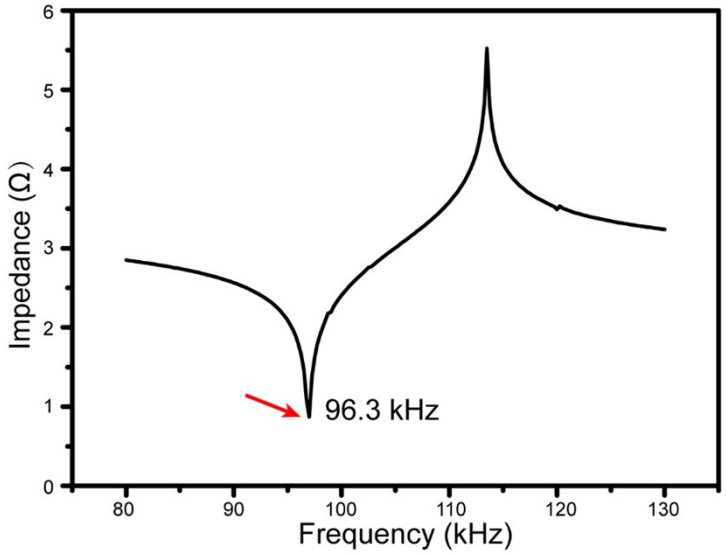

Fig. S3. The test of resonant frequency of the annual PZT.

4. The characterization of GNPs.

The size characterization of particles was shown in Fig. S4.a). The morphological characterization of particles was shown in Fig. S4.b). Fig. S4.c) showed the morphological characterization of the PDMS substrate modified with GNPs.

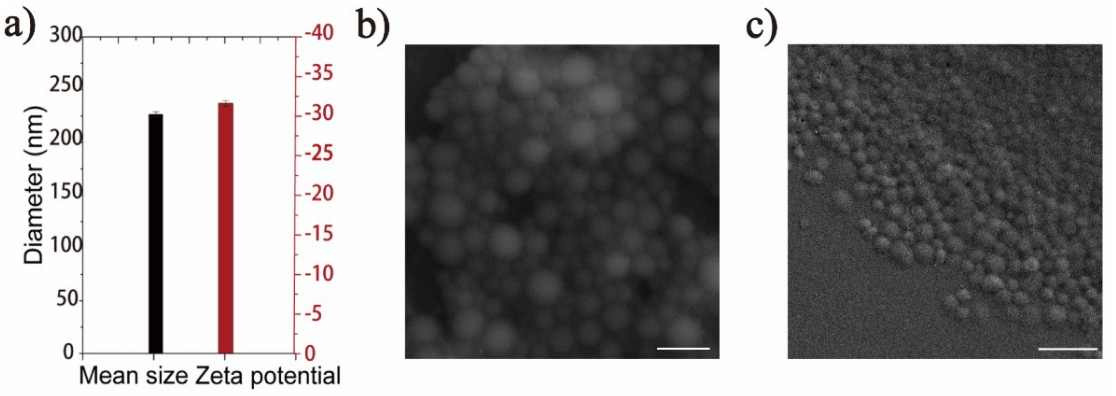

Fig. S4. a) The test of mean size and Zeta potential of GNPs. b) The SEM picture of GNPs. c) The SEM picture of the modified substrate. The scale bars are 1 μm.

5. Capture efficiency

Initially, the supernatant from the T25 cell culture bottle was centrifugated to

acquire the original sample of EVs, as outlined in Section 2.5. This sample was then divided into two equal parts, approximately 2 mL each. One served as the control, while the other part was assigned as the experimental group. The experimental group was subsequently injected into the chip to ensure the capture of vesicles, the collection of the sample at the outlet set as the treated sample. Both the control and treated sample underwent a process of ultra-centrifugation (15000 g, 30 min) to get the EVs precipitation. The obtained precipitation was then stained using Dio dye (30 min, in a dark place, 37℃) and washed with PBS. Following this, two groups of EVs suspension were prepared using the same volume of PBS, 1 mL of which was placed in the well of a 24-well plate. Fluorescence microscopy was employed to take photos (3 pictures per well), with subsequent calculation of the average fluorescence intensity. The whole experiment was conducted away from light. The capture efficiency was calculated using the following formula：

$$Capture\ Efficiency = \left(1 - \frac{Fluorescence\ Intensity\ of\ Treated\ Sample}{Fluorescence\ Intensity\ of\ Control}\right) \times 100\%$$

6. Western blot (WB)

The experiment of WB provide an approach to known about information of molecule through electrophoresis and the methods to analyze the proteins from extracellular vesicles were referenced from previous researchers, followed bellow simply[1,2,3].

1) Vesicle sample processing. The vesicles sample was treated with RIPA lysis buffer at ratio of 1: 1.5 and incubated on ice for 30 min. The mixture was then centrifuged and the resulting supernatant was stored at 4 °C for further analysis.
2) SDS-PAGE gel preparation. A 10 % SDS-PAGE gelatin was prepared using a mixture of DI water, 30 % ACr-Bis (29:1 ratio), 10 % SDS, 10% ammonium

persulfate and TEMED.

3) Gel perfusion. Approximately 7.5 ml of the gel mixture was poured into the center of the glass plate, and then a gentle layer of alcohol was added on top to facilitate a level surface.
4) Adding protein samples and electrophoresis. Protein samples were added to the precast wells in the gel. The added quality of each sample was 40 μg/60 μg. Electrophoresis was carried out at a voltage of 80 V for 30 minutes, followed by 120 V for 60-120 minutes to separate the proteins based on their molecular weight.
5) Membrane transfer and immune reaction. After electrophoresis, the proteins were transferred from the gel to a PVDF membrane. The transferred proteins were then subjected to an immune reaction.
6) Membrane exposure: The membrane was exposed to a specific primary antibody that recognizes the target proteins of interest. Following primary antibody incubation, a secondary antibody conjugated to an enzyme tag was applied to detect the presence of the target proteins on the membrane.

References:

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2. S. C. Taylor and A. Posch, *Biomed Res Int*, 2014, **2014**, 361590.
3. F. A. W. Coumans, E. L. Gool and R. Nieuwland, *Platelets*, 2017, **28**, 242-248.