# Supporting Information

## Ultrasensitive Microfluidic Analysis of Circulating Exosomes Using Nanostructured Graphene Oxide/Polydopamine Coating

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## Experimental Methods

### Reagents and materials

3-aminopropyl triethoxysilane (APTES), (3-Mercaptopropyl) trimethoxysilane (3-MPS), 4-Maleimidobutyric acid N-hydroxysuccinimide ester (GMBS), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxy-succinimide (NHS), Tris(hydroxymethyl) aminomethane (Tris), Dopamine hydrochloride and graphene oxide were purchased from Sigma-Aldrich; Recombinant Protein G, human block IgG were ordered from Thermo Scientific); Streptavidin conjugated β-Galactosidase (SβG), Fluorescein di-β-D-galactopyranoside (FDG) were purchased from Life Technology. The antibodies used in our experiments was listed in Table S1 below. 1× phosphate-buffered saline solution (PBS) was from Mediatech, Inc.; all other solutions were prepared with deionized water (18.2 MV-cm, Thermo Scientific). SβG and FDG were dissolved in the PBS working solution (PBSW) at pH 7.4 which contains 0.5 mM DL-dithiothreitol solution (Sigma-Aldrich), 2 mM $MgCl_2$ (Fluka Analytical), and 5% bovine serum albumin (BSA) (Sigma-Aldrich).

**Table S1.** The list of monoclonal antibodies used in this research.

| No. | Target | Vendor | Catalog No. | Clone |
|---|---|---|---|---|
| 1 | CD 9 (biotin, human) | Ancell | 156-030 /mono mouse | C3-3A2 |
| 2 | CD 63 (biotin, human) | Biolegend | 353018 /mono mouse | H5C6 |
| 3 | CD 81 (biotin, human) | Ancell | 302-030/mono mouse | 1.3.3.22 |
| 4 | CD 81 (human) | Ancell | 302-820 /mono mouse | 1.3.3.22 |
| 5 | CD 81 (FITC, human) | Ancell | 302-040 /mono mouse | 1.3.3.22 |
| 6 | EpCAM (biotin, human) | Abcam | ab187270 /mono mouse | MOC-31 |

**COMSOL Simulation**

Two-dimensional finite element simulations of fluid flow inside the Y-shaped micropost array were performed by using COMSOL Multiphysics 5.1 with the Microfluidics Module (Comsol Inc.). Navier-Stokes equations for incompressible fluid flow were used with an inlet flow rate of 1 μL/min. No slip boundary condition was applied for all walls.

**Preparation of Microchips for Control Experiments**

To modify the GO-coated chips with PEG, a published approach was adapted with slight modification[1]. With the assistance of EDC and NHS, the carboxyl groups of GO reacted with amine groups of $NH_2$-PEG-$NH_2$ (MW3400) (Laysan Bio Inc.). After PEG functionalization, Protein G was immobilized onto GO surface with glutaraldehyde as linker[2] for antibody immobilization. The control PDA only chip was prepared by coating a clean chip with PDA following the same protocol for PDA coating of the GO-modified chips. The flat microchips without the PDA-GO treatment were surface functionalized using a common silanization-based approach according to the previous report[3]. Briefly, the PDMS chips were treated first with (3-Mercaptopropyl) trimethoxysilane and then GMBS. At last Protein G (0.2 mg/mL) was immobilized through the reaction between amine group and thiol group.

**Raman Spectroscopic Characterization**

Raman spectrum measurements were performed with a Renishaw Invia Raman microscope system. A Spectra Physics argon-ion laser operating at 633 nm was used as the excitation source with a laser power of 3 mW. The Rayleigh line was removed from the collected Raman scattering using a holographic notch filter in the collection path. All SERS spectra reported here were the results of a single 10-s accumulation.

**SEM Imaging of Surface-Captured Exosomes**

For SEM measurements, the captured exosomes were fixed with 2.5% glutaraldehyde in PBS for 30 minutes and then rinsed for 3×5 minutes. The samples were post-fixed for 15 minutes in 1% osmium tetroxide and rinsed 10 minutes with water. The samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 95% and 100%) for 2×10 min at each step. The samples were then coated with gold using a high resolution ion beam coater and examined with FEI Versa 3D Dual Beam scanning electron microscope at the KU Microscopy and Analytical Imaging Laboratory.

# Supplementary Figures.

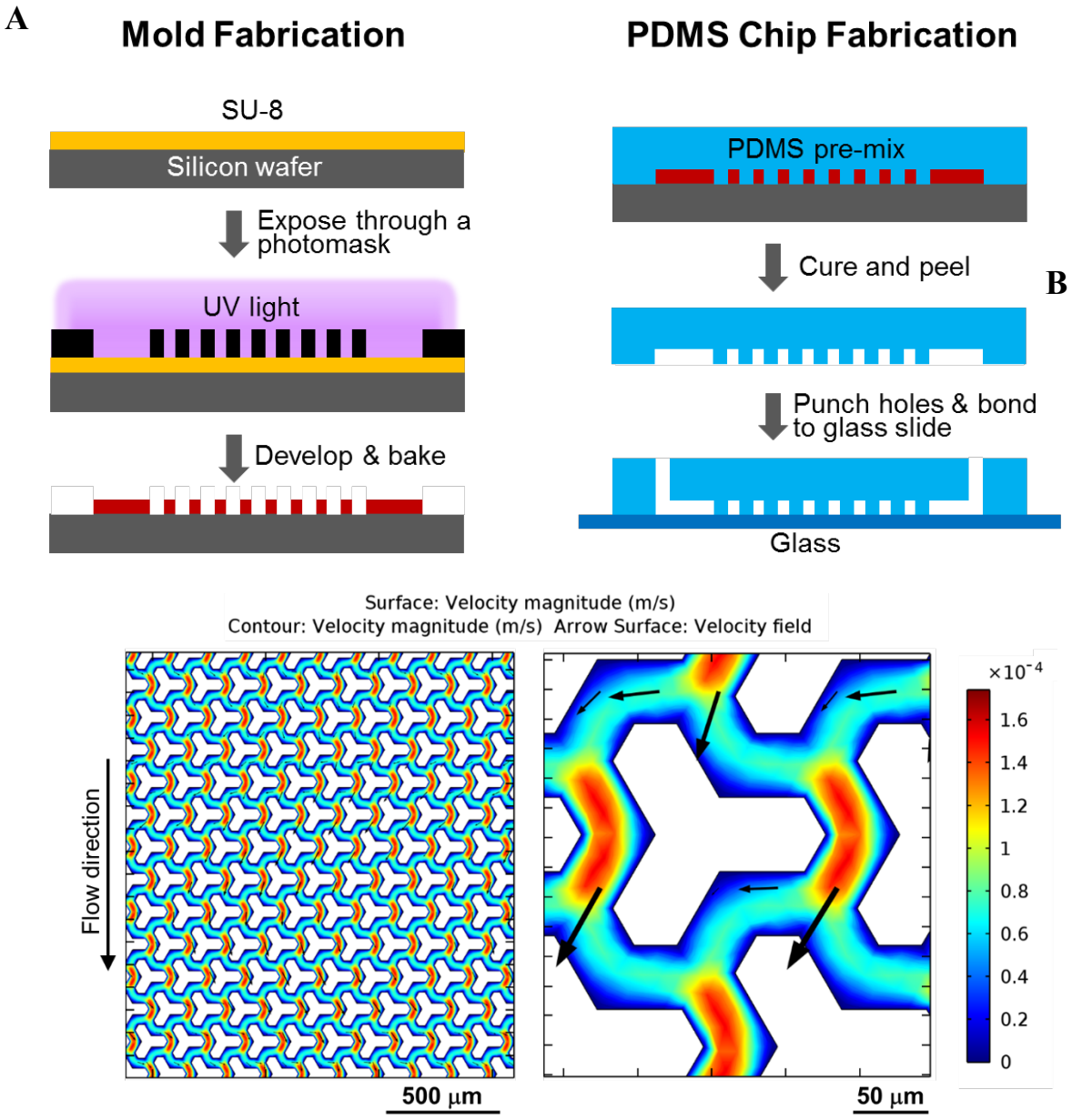


**Fig. S1**. (**A**) The procedure of soft lithography to fabricate the PDMS chip with the Y-shaped micropost structure. (**B**) Finite element simulations of flow velocity profile inside the Y-shaped micropost array. The enlarged view to the right shows asymmetric flow bifurcation at the upstream arms of the Y-shaped microposts, as indicated by the length of the arrows that represent overall fluid flow strength across this section of the channel. In addition, flow bifurcation enhances the mass transfer of exosomal vesicles to the surface for affinity capture, as indicated by the arrows in the channel center pointing to the downstream microposts.

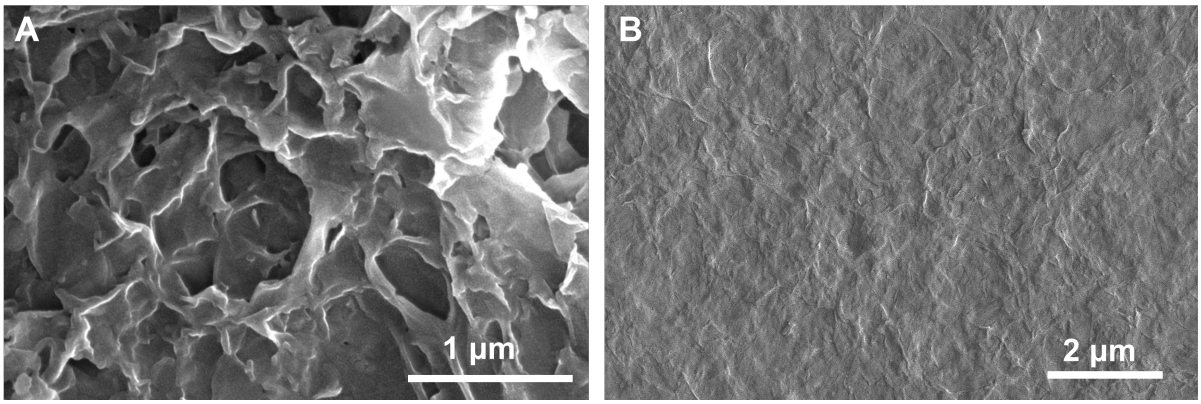


**Fig. S2.** PDA functionalization of GO-coated PDMS surfaces under different microfluidic conditions. (**A**) Continuous flow reaction for 3 hours. (**B**) Static incubation for 10 hrs.

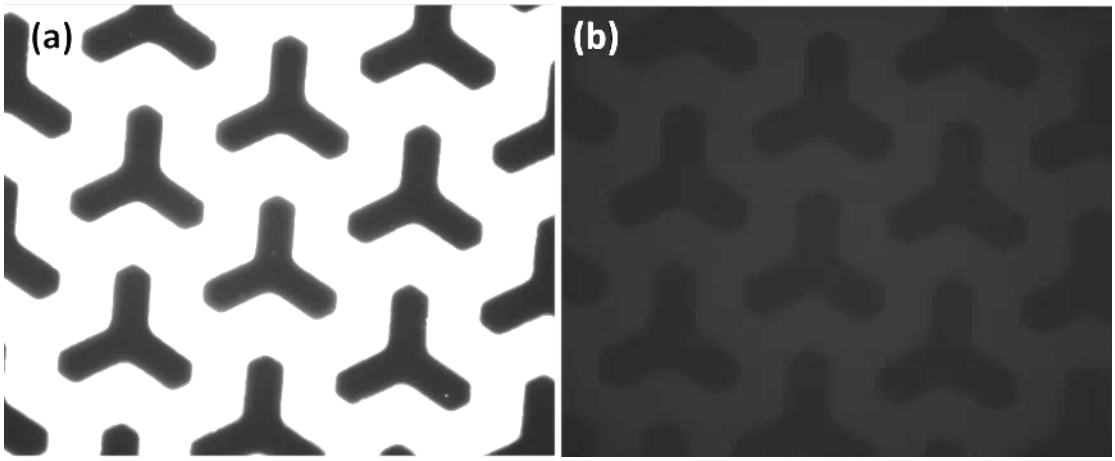


**Fig. S3.** Fluorescence images of antibody immobilization. (a) Protein G was first immobilized on the PDA-GO chip and FITC labelled anti-CD81 antibody was captured by Protein G. (b) For a PDA-GO chip without immobilized Protein G, very low background was observed after flowing the FITC-labelled CD81 antibody (50 μg/mL) through the channel. This comparison verified that the antibody was immobilized through the Protein G-IgG interaction.

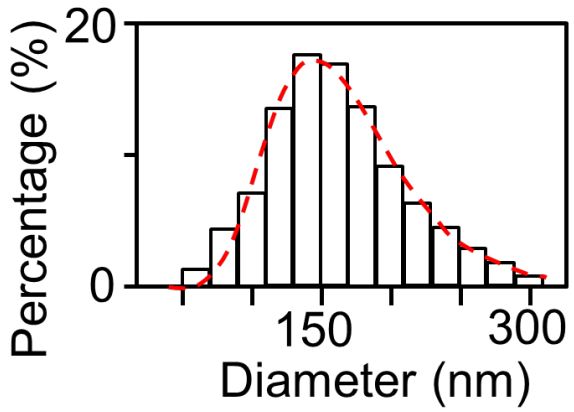


**Fig. S4.** Size distribution of ultracentrifugation-purified COLO-1 cell-derived exosomes measured by NTA.

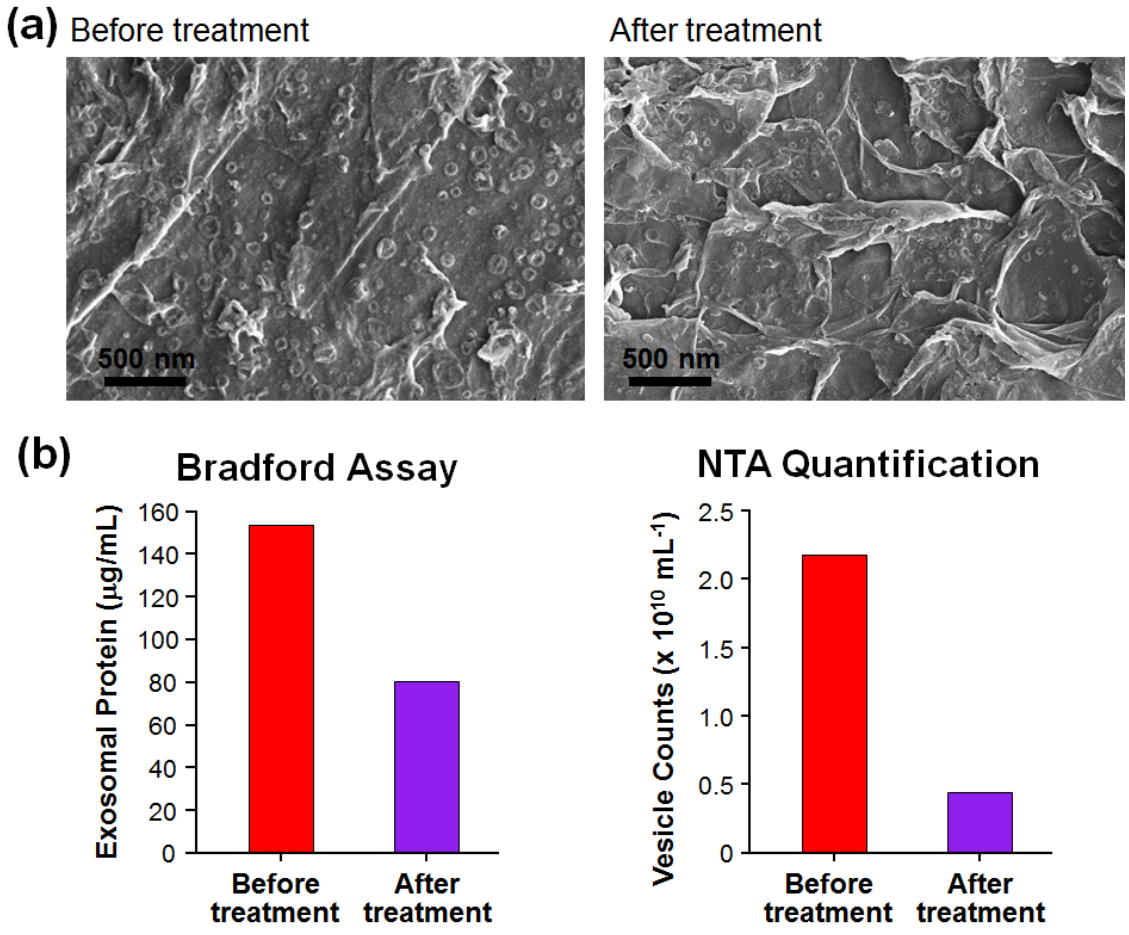


**Fig. S5.** Detection of plasma-borne exosomes in an OvCa patient before and after treatment. (**a**) SEM visualization of the chips right after the measurements shows a decreased density of captured exosomes from the post-treatment plasma. (**b**) Comparison of total exosomal protein level measured by the Bradford assay (left) and the exosome concentration quantified by NTA (right) between the plasma samples collected before and after treatment.

## Reference：

1. Liu, Z.; Robinson, J. T.; Sun, X.; Dai, H., PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. *Journal of the American Chemical Society* **2008,** *130* (33), 10876-+.

2. Li, Y.; Xu, X.; Deng, C.; Yang, P.; Zhang, X., Immobilization of trypsin on superparamagnetic nanoparticles for rapid and effective proteolysis. *Journal of proteome research* **2007,** *6* (9), 3849-55.

3. Kanwar, S. S.; Dunlay, C. J.; Simeone, D. M.; Nagrath, S., Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes. *Lab on a Chip* **2014,** *14* (11), 1891-1900.