Electronic Supplementary Information

Magnetic-Nanowaxberry-Based Microfluidic ExoSIC for Affinity and Continuous Separation of Circulating Exosomes Towards Cancer Diagnosis

Lihua Ding,[†ae] Xia Liu,[†a] Zhenzhong Zhang,[e] Li-e Liu,[a] Sitian He,[a] Yan Wu,[a] Clement Yaw Effah,[a] Ruiying Yang,[a] Aiai Zhang,[a] Wensi Chen,[a] Mubarak Yarmamat,[a] Lingbo Qu,*[bc] Xiaonan Yang,*[d] Yongjun Wu,*[a]

[a]*College of Public Health, Zhengzhou University, Zhengzhou 450001, China*

[b]*College of Chemistry, Zhengzhou University, Zhengzhou 450001, China*

[c]*Henan Joint International Research Laboratory of Green Construction of Functional Molecules and Their Bioanalytical Applications, Zhengzhou University, Zhengzhou, 450001, China*

[d]*School of Information Engineering, Zhengzhou University, Zhengzhou 450001, China*

[e]*School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China*

[†]*These authors contributed equally to this work.*

*Corresponding Author:

College of Chemistry, Zhengzhou University, Zhengzhou 450001, China. Henan Joint International Research Laboratory of Green Construction of Functional Molecules and Their Bioanalytical Applications, Zhengzhou University, Zhengzhou, 450001, China. E-mail: qlb@zzu.edu.cn (Lingbo Qu).

School of Information Engineering, Zhengzhou University, Zhengzhou 450001, China. E-mail: iexnyang@zzu.edu.cn (Xiaonan Yang).

College of Public Health, Zhengzhou University, Zhengzhou 450001, China. E-mail: wuyongjun@zzu.edu.cn (Yongjun Wu).

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1. Reagents and materials

Silica magnetic beads (MBs) were provided by BioMag Scientific Inc (Wuxi, China). The polydimethylsiloxane (PDMS) and SU-8 were obtained from Dow Corning (America). The Apt_{CD63} (COOH-TTTTTTCACCCCACCTCGCTCCCGTGACACTAATGCTA), were polymerized by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Fetal bovine serum (FBS) was procured from Lonsera (Uruguay). Total Exosome Isolation Reactant was supplemented by Invitrogen (America). Filtration membrane with pore diameter of 0.22 μm and Amicon Ultra-15 filter (100 kDa MWCO) were offered from Millipore (USA). Dulbecco's modified Eagle's medium (DMEM) were provided by Solarbio Life Sciences (Beijing, China). The rest of the reagents were analytical reagents grade or higher. This experiment made use of Milli-Q ultrapure water (18.2 MΩ·cm).

2. Instruments

Transmission electron microscope (TEM) of MNWB was completed by Talos F200X (Thermo, America). The hydrodynamic diameter and zeta potential were discovered by Zetasizer Nano-zs 90 (Malvern, England). The size distributions and concentrations of exosomes was measured on NanoSight NS300 (Malvern, England). The morphology of exosomes was observed by JEM-1400 plus (Japan). The fluorescence intensity was imaged by Multi-Mode Microplate Reader SpectraMax i3X (USA). The microwave digestion instrument was Mars6 (CEM, America). The picture of exosome labeled by fluorescence robes were captured by laser scanning confocal microscope (Leica, Germany). Aquilios was the scanning electron microscope (SEM) used in this research (Thermo, America).

3. The synthesis of MNWB

Initially, 5 mg MBs were added in 120 mL of 0.02 M $Zn(CH_3COO)_2$ ethanol solution. And 130 mL of 0.06 M NaOH ethanol was added drop wise into the aforementioned solution within 10 min. Then the mixture was heated at 60 ℃ for 30 min under ultrasonic irradiation to form ZnO nanocrystals encased MBs (ZnO seeded MBs). The particles were recovered by centrifugation at 7000 rpm for 5 min and then redispersed in 1 mL water. After that, 3.34 g PVA-1750, 1.86 g $Zn(NO_3)_2 \cdot 6H_2O$, and 2 mL ZnO seeded MBs were added in 250 mL water. After that, the mixture was stirred at 60 °C and 250 mL of 25 mM hexamethylenetetramine (HMTA) were added. Then, in order to produce MNWB, the solutions were put into a microwave digestion device and heated for 15 min at 90 °C.

4. Separation of MVs

The MVs in the A549 cell media were isolated using the procedures previously described.[1] We harvested the supernatant after the A549 cells had been grown in FBS-free media for 48 hours and then centrifuged at 300 g for 10 min to remove the cells. Centrifugation at 2000 g for 10 min was followed by centrifugation at 20,800 g for 15 min to enrich the MVs. The MVs were redissolved in PBS (0.01 M, pH 7.4) and stored at 80°C for future use and all the centrifugations were completed at 4°C. The hydrodynamic diameter and TEM were used to characterize the MVs.

5. TEM characterization of MBs and ZnO seeded MBs

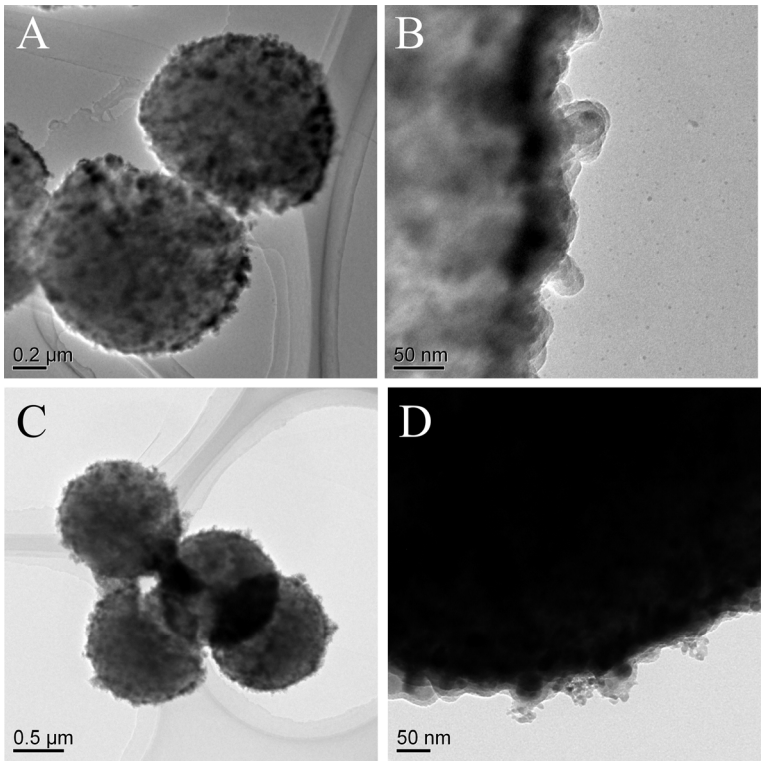

Fig. S1 The TEM characterization of MBs (A and B), and ZnO seeded MBs (C and D).

6. The characterization of exosome

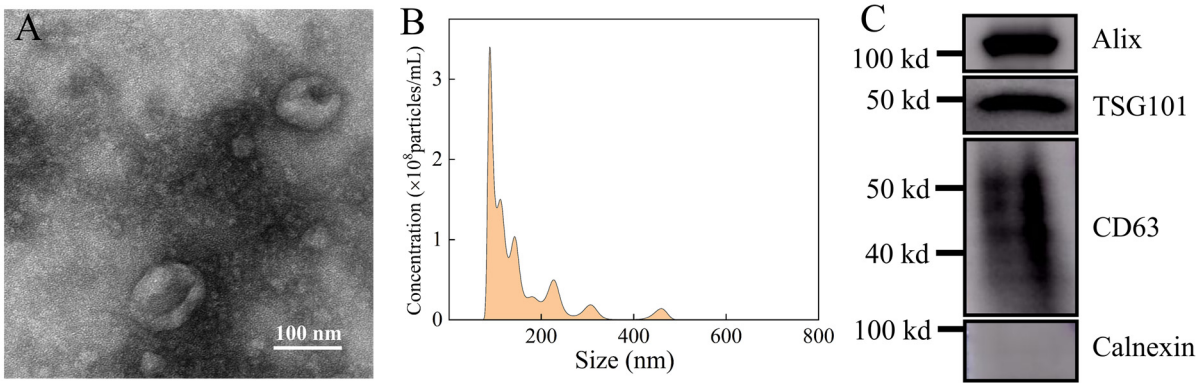

Fig. S2 The TEM (A), NTA (B) and western blotting (C) of exosomes isolated from cell supernatant.

7. Schematic diagram of the chips

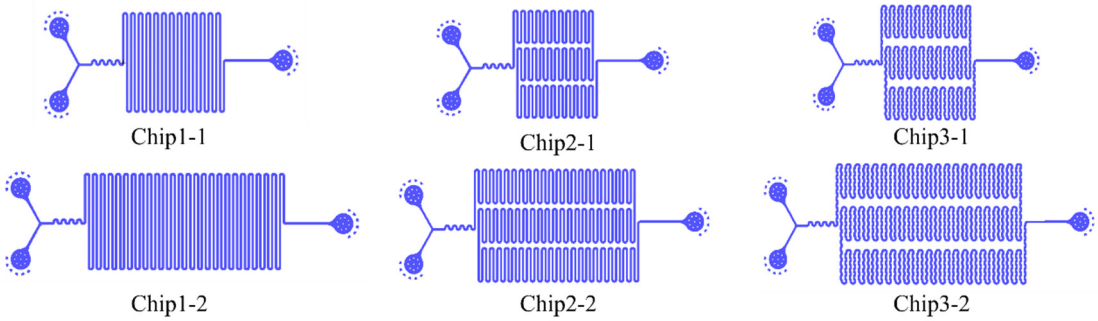

Fig. S3 The schematic diagram of the six types of chips. Each chip contains two inlets and one outlet. The microchannel length of Chip 1-1, Chip 2-1 and Chip 3-1 was about 25 cm. The microchannel length of Chip 1-2, Chip 2-2 and Chip 3-2 was nearly 50 cm.

8. Confocal microscopic images of PKH67 labeled exosomes

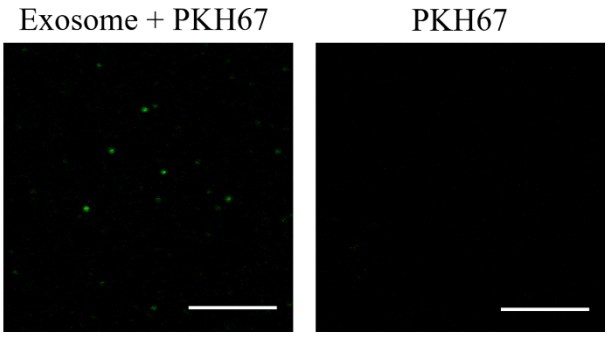

Fig. S4 Confocal microscopic images of PKH67 and exosomes. Excited at 488 nm. Scale bar is 5 μm.

9. Image of ExoSIC chip

Fig. S5 The image of the ExoSIC chip.

10. The UV-Vis absorption spectrum of Apt-MNWB

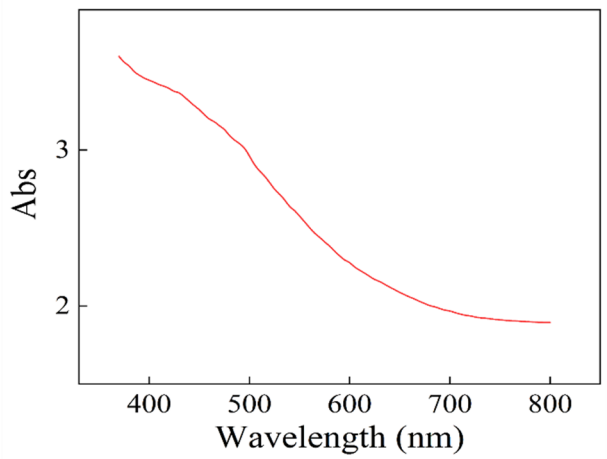

Fig. S6 The UV-Vis absorption spectrum of Apt-MNWB

11. The comparison of Apt-MNWB and Apt-MBs on exosome capture

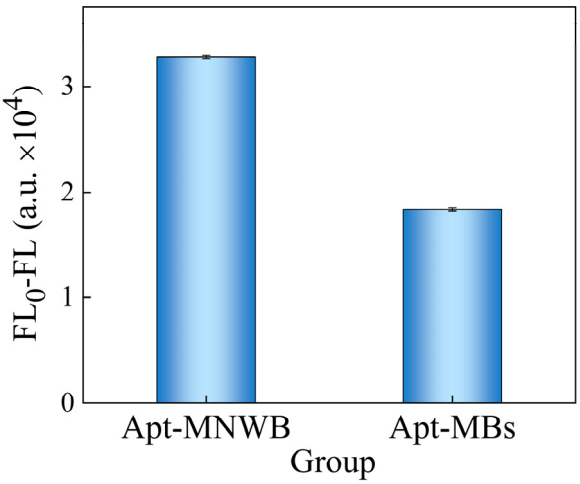

Fig. S7 The comparison of Apt-MNWB and Apt-MBs on exosome capture. The FL_0 and FL refer to the FL intensities of PKH67-labelled exosome before and after been captured by Apt-MNWB or Apt-MBs.

12. The polyacrylamide gel electrophoresis of Apt_{CD63} and its complementary nucleic acid

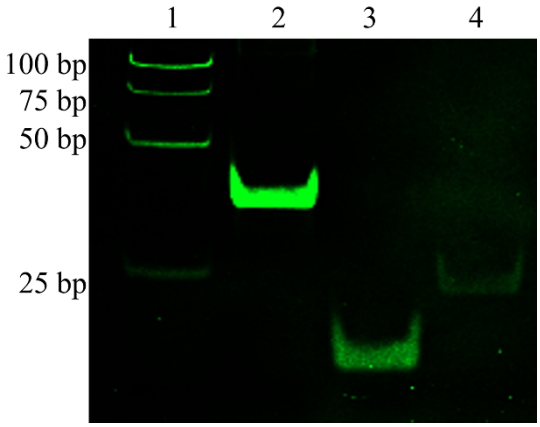

Fig. S8 The characterization of hybridization of Apt_{CD63} with its complementary nucleic acid by using polyacrylamide gel electrophoresis. Lane 1: DNA ladder; lane 2: Apt_{CD63} complementary nucleic acid + Apt_{CD63}; lane 3: Apt_{CD63} complementary nucleic acid; lane 4: Apt_{CD63}.

13. The characterization of MVs

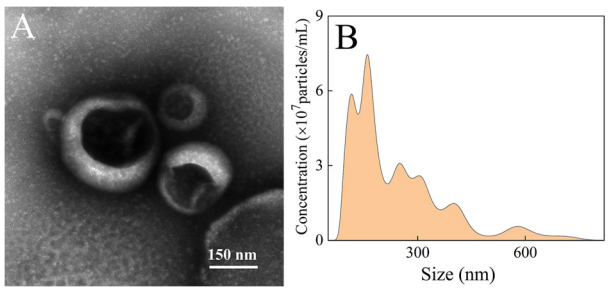

Fig. S9 The TEM (A) and NTA (B) of MVs isolated from cell supernatant of A549.

14. The effect of Apt_{CD63} on exosome capture based on MBs

First, APTES was used to couple amino groups on the surface of MBs (NH_2-MBs), and then EDC was used to couple Apt_{CD63} to the surface of NH_2-MBs. To explore the effect of Apt_{CD63} on exosome capture, the same amount of Apt-MBs and NH_2-MBs were incubated with exosome for 1 h. The uncaptured exosomes were removed by magnetic separation. After that, the PKH67 was used to label captured exosome and the fluorescence intensity was measured.

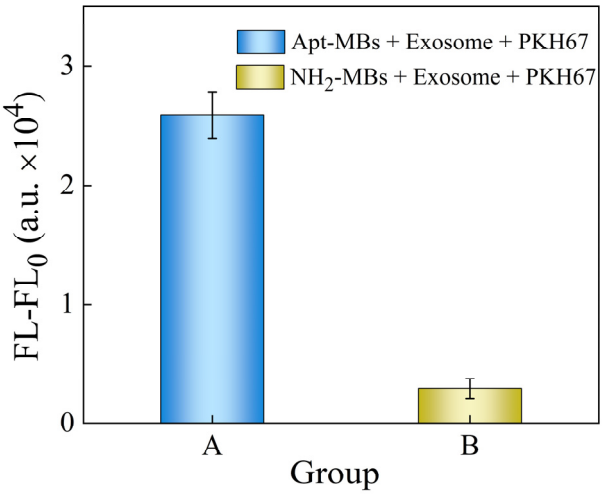

Fig. S10 The comparison of Apt-MBs and NH_2-MBs on exosome detection. FL and FL_0 refers to the signal intensity in the presence and absence of target exosomes.

15. Reference

1 K. P. O'Dea, Y. Y. Tan, S. Shah, V. P. B, C. T. K, M. R. Wilson, S. Soni and M. Takata. J Extracell Vesicles, 2020, 9, 1.