Electronic Supplementary Material (ESI) for Lab on a Chip.
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Electronic Supplementary information (ESI)

An all-in-one platform to deplete pathogenic bacteria for rapid and safe enrichment of plant-derived extracellular vesicles

Zhihao Wen‡[a], Jianning Yu‡[ab], Hyorim Jeong[c], Dong-Uk Kim[a], Ji Yeong Yang[ac], Kyung-A Hyun[a], Seoyeon Choi[*ac], Sunyoung Park[*ac], and Hyo-Il Jung[*ac]

[a] School of Mechanical Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul, 120-749, Republic of Korea

[b] Department of Biomedical Laboratory Science, Yonsei University, Republic of Korea

[c] The DABOM Incorporation, 50 Yonsei-ro, Seodaemun-gu, Seoul, 120-749, Republic of Korea

‡ These authors contributed equally to this work.

* Author to whom correspondence should be addressed: Corresponding authors at: School of Mechanical Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul, 03722, Republic of Korea

E-mail address: uridle7@yonsei.ac.kr (H. I. Jung), angelsy88@gmail.com (S. Park), sschoiyy@yonsei.ac.kr (S. Choi)

20 # Additional Experimental Section

21 **Design of retractable pen-like structure**

22 The structure of the push-type part is based on the principle of the original ballpoint pen. It is composed
23 of a 3D-printed shell, internal snap-in, and springs, and the whole structure is designed according to the
24 desired stop position. When force is used to press down on it, the card slot will be stuck.

25 **Preparation of Biotinylated Con A**

26 Con A was completely dispersed in PBS buffer, for the preparation of 1mg/ml Con A. The size of Con
27 A and biotin are calculated, and through spatial analysis, the maximum amount of biotin that can be
28 geometrically attached to Con A. 8.5mM NHS-PEG4 Biotin solution was added to a 1.5mL tube to make
29 100uL Biotin-Con A solution and leave at room temperature for 30min. The Desalting column is used
30 for the following centrifugation steps. 100uL Biotin-Con A solution was added and centrifuge again at
31 1500g for 2 min. Then it was kept refrigerated at 4C$^{\circ}$. And a Pierce Biotin Quantitation Kit (Thermo
32 Scientific, USA) was used to test the prepared Biotinylated Con A.

33 **Bacterial culture**

34 The *S.aureus* and *C.albicans* reference strains of were purchased from the American Type Culture
35 Collection (ATCC, Manassas, VA, USA) *S.aureus* and *C.albicans* were grown on nutrient agar plate (BD
36 Biosciences, Franklin Lakes, NJ, USA) under microaerophilic and 100 percent humidity conditions at
37 37°C and inspected after overnight

38 **Cell culture**

39 Human epidermal keratinocytes cells (HaCaT) were obtained from the American Type Culture
40 Collection (ATCC, Manassas, VA, USA), The cells were cultured in Dulbecco's Modified Eagle Medium
41 (DMEM) (Gibco, Thermos Fisher Scientific) containing 10% fetal bovine serum (Gibco, Thermos Fisher
42 Scientific), and 1% Phosphate buffered saline (tablet) (Sigma Aldrich). The cells were cultured at 37°C
43 in a humidified 5% CO_2 atmosphere.

44 **Nanoparticle tracking analysis (NTA)**

45 Extracellular Vesicles isolated from Fresh Licorice and Licorice extract and the Purified Fresh Licorice
46 were subjected to the Nanoparticle tracking analysis, using a Nano-Sight LM10 (Malvern Instruments
47 Ltd., UK). The basic data about the processed particles that can be acquired by this method include
48 average size, modal value, and size distribution. These parameters were analyzed by the NTA 3.3

49 analytical software according to the manufacture's protocol. Settings were optimized and kept constant
50 between samples. The Brownian movements of particles (Extracellular Vesicles) present in the sample
51 were subjected to a laser beam, recorded by a camera, and converted into size and concentration
52 parameters by NTA through the Stokes-Einstein equation. Every sample was measured in triplicate.

53 **Transmission electron microscopy (TEM)**

54 The morphological evaluation of the isolated Fresh Licorice EVs and Licorice extract EVs and the
55 Purified Fresh Licorice EVs were performed by JEM-2100plus TEM (JEOL Inc., Japan). Briefly, the
56 exosomes solution was dried in a copper grid with 200 mesh carbon films (CF200-Cu, Electron
57 Microscopy Sciences, USA). Excess fluid was carefully removed with filter paper and grids negatively
58 stained with 2 % (w/v) uranyl acetate for 5 min. The grids were air-dried, and TEM imaging was recorded.

59 **Cell viability assay**

60 HaCaT cells were seeded into flat-bottom 96-well plates at a concentration of 1×10^4 cells per well for
61 a cell viability assay. After overnight incubation, cells were treated with various concentrations of Fresh
62 Licorice-derived EVs, Licorice extract-derived EVs and Purified Fresh Licorice EVs. The growth and
63 viability of HaCaT cells were determined by the Cell Counting Kit-8 (CCK-8; GLPBIO, Montclair,
64 California). The cells were washed twice with PBS after treatment. Absorbance was measured using a
65 microplate reader (Molecular Devices, CA, USA) at 450 nm wavelength. The assay was repeated three
66 times.

67 **Quantitative real-time polymerase chain reaction (qRT-PCR)**

68 Total DNA was extracted from bacterial using MagListo™ 5M Genomic DNA Extraction Kit (Bioneer，
69 Korea). The Glyceraldehyde-phosphate dehydrogenase gene was used as the internal control to normalize
70 the sample differences. Real-time PCR was performed using the SYBR Green Lo-ROX qPCR kit
71 (Bioline, Cincinnati, OH, USA) and the QuantStudioTM 5 Platform (Thermos Fisher Scientific). Sample
72 quantification for mRNA was performed according to the threshold cycle using the ΔΔCt method. The
73 values presented in the graphs are mean ± SD values.

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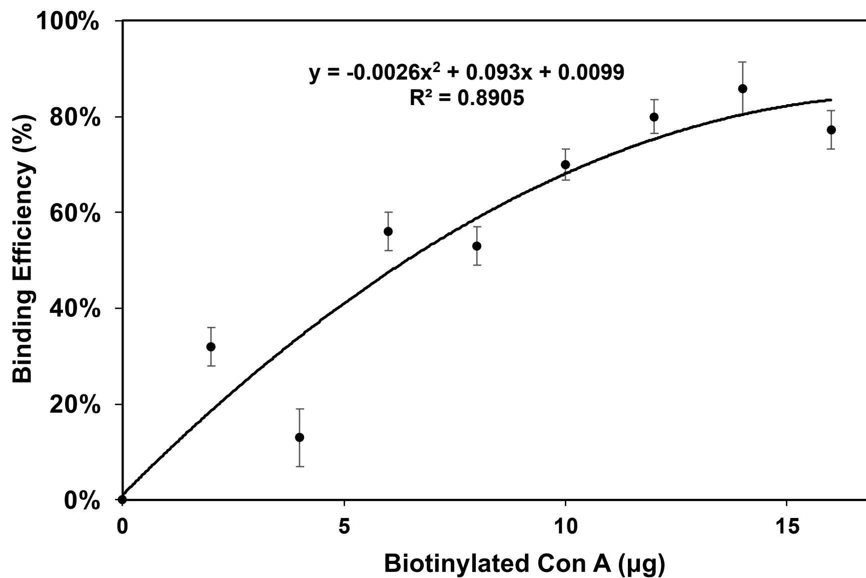
76 **Supplementary Figures:**



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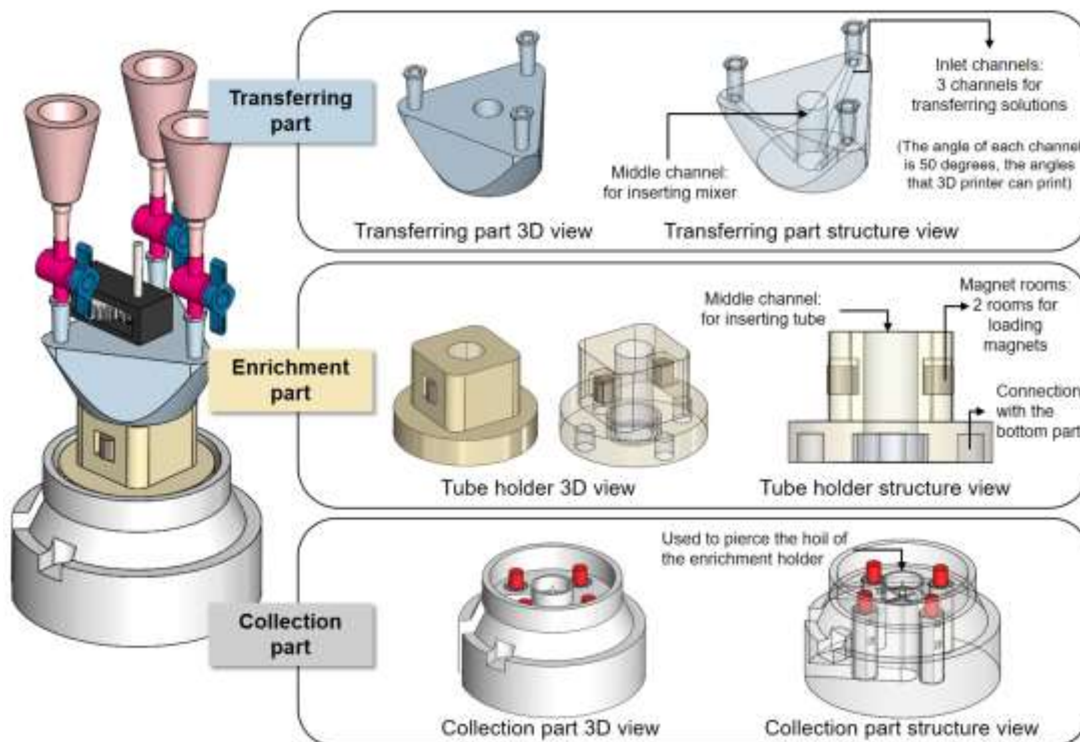
78 **Fig. S1** Binding efficiency of biotinylated Con A to magnetic beads.

79



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81 **Fig. S2** Structure design of core parts.

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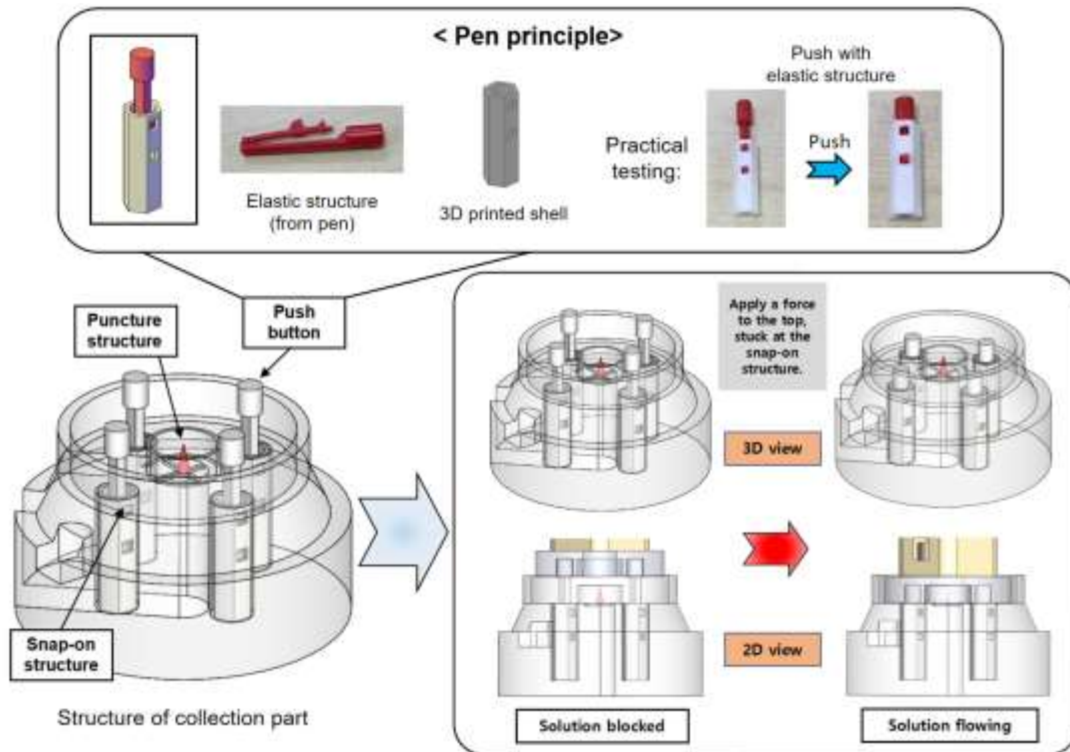
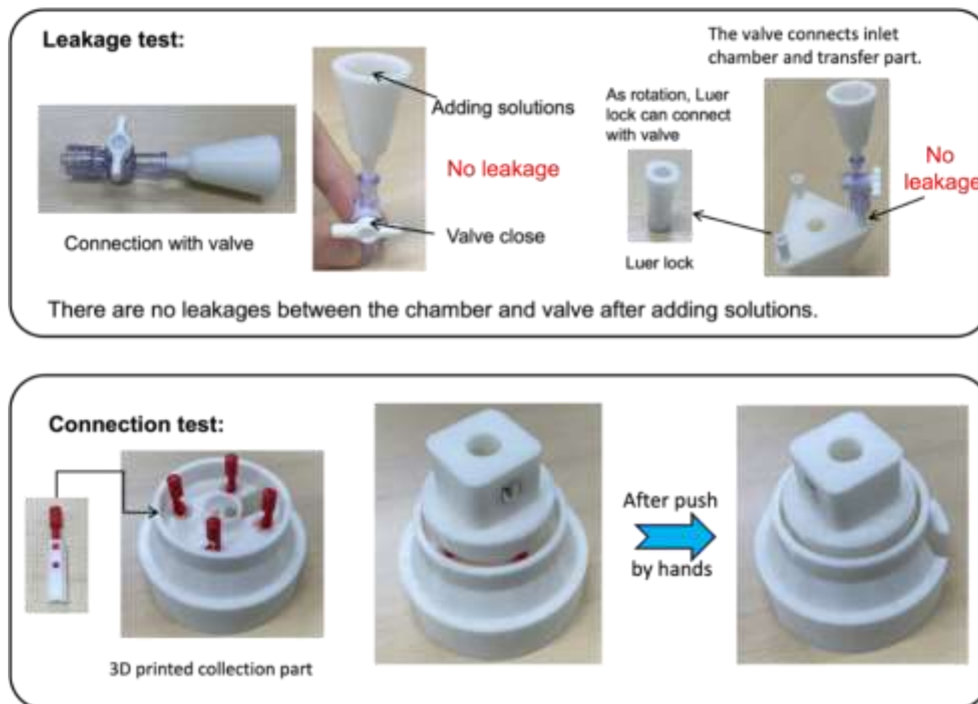
83



84 **Fig. S3** Working principle of the collection part.



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86 **Fig. S4** Leakage test of 3D-printed parts.

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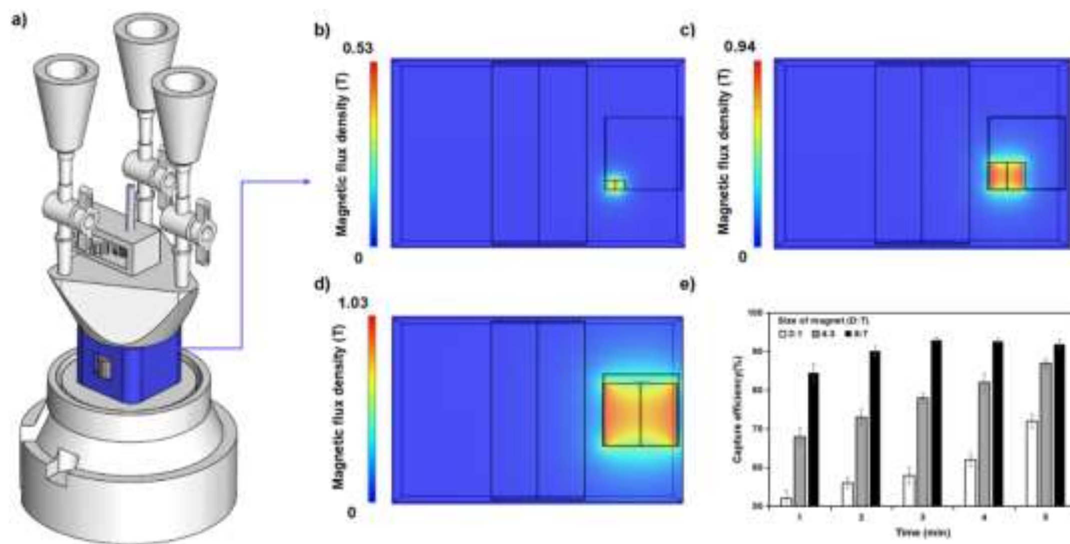

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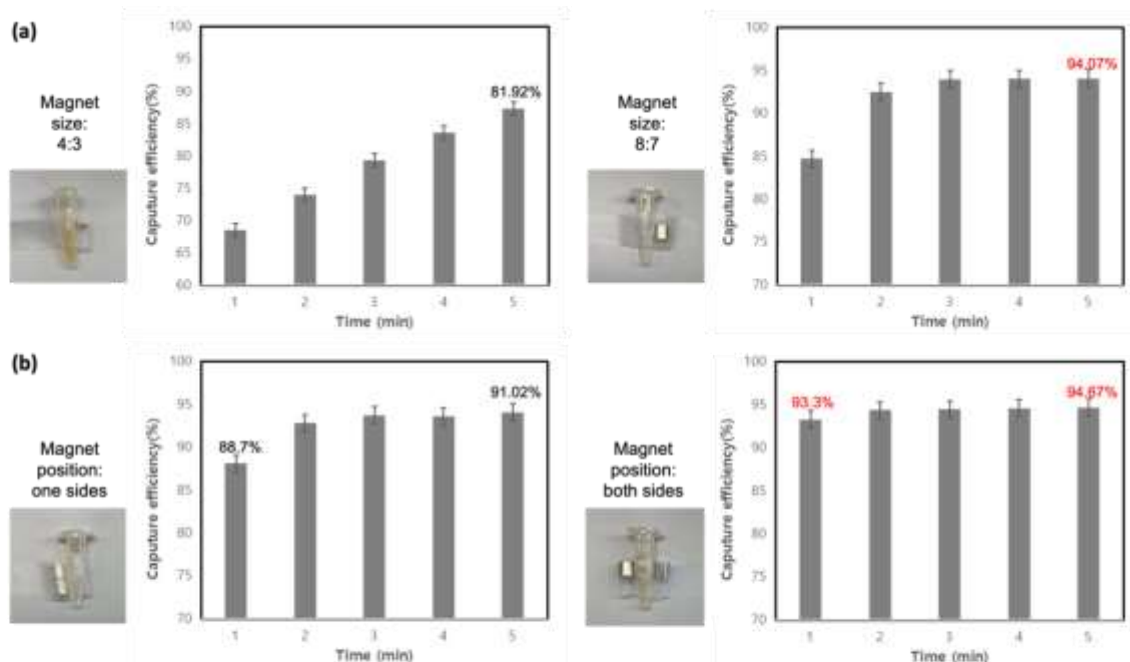
89 **Fig. S5** Schematic representation of enrichment holder for computational magnetic
90 fields. The magnetic fields were simulated for different magnetic sizes b) magnet ①
91 (Diameter: Thickness=2:1, pull force: 0.68N); c) magnet ② (Diameter: Thickness=4:3,
92 pull force: 3.4N); d) magnet ③ (Diameter: Thickness=8:7, pull force: 14.49N). And e)
93 the results of the simulation were confirmed by carrying out practical experiments.

94



95

96 **Fig. S6** Magnet capture efficiency of different magnet sizes and positions.

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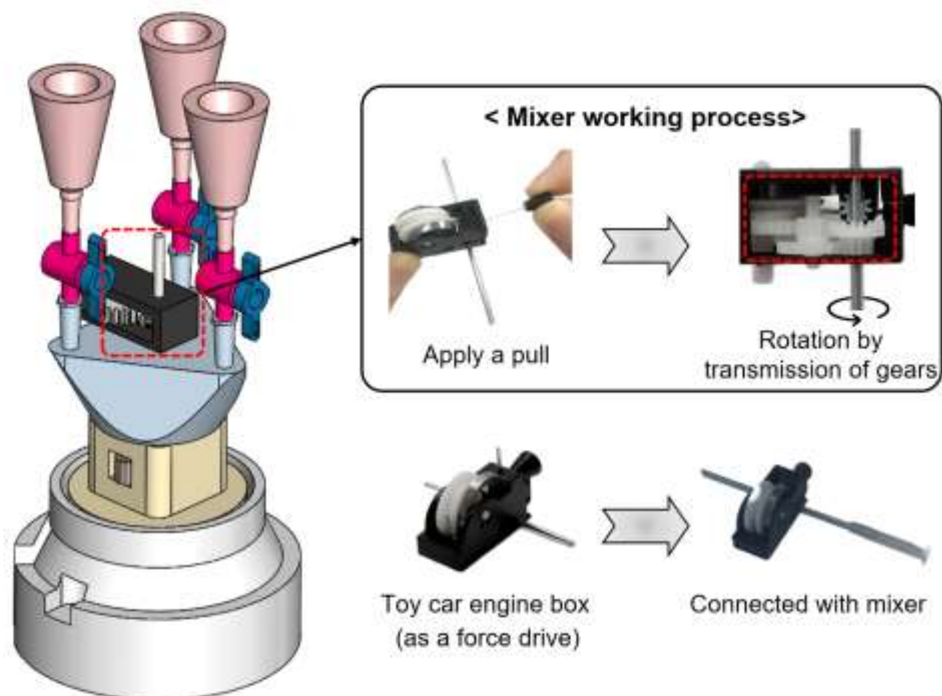

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99 **Fig. S7** Combination and operation principle of the manual mixer.

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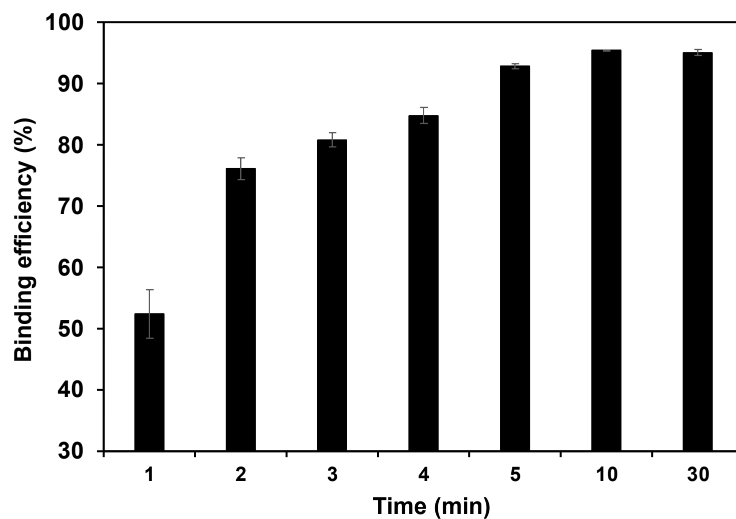

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102 **Fig. S8** Binding efficiency of bacteria captured by Con A-coated magnetic beads.

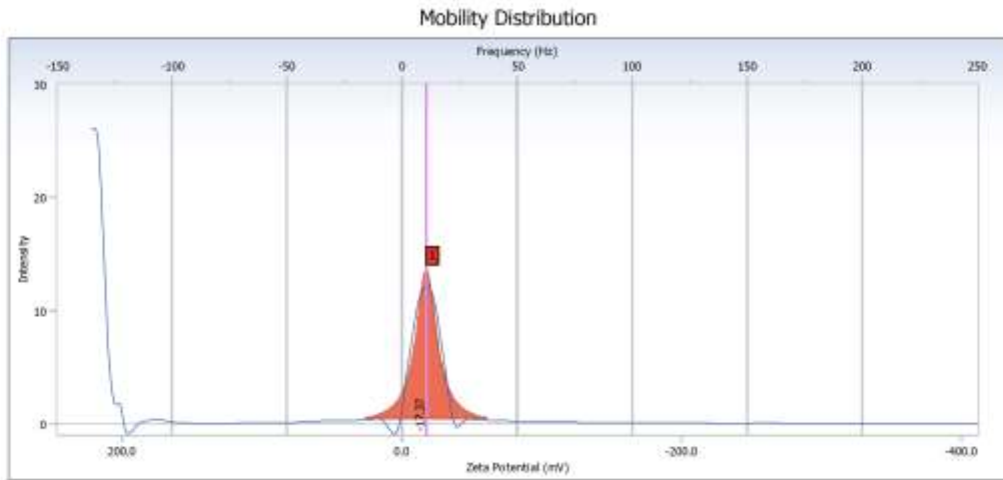

Fig. S9 The zeta potential for LdEVs - non dep.

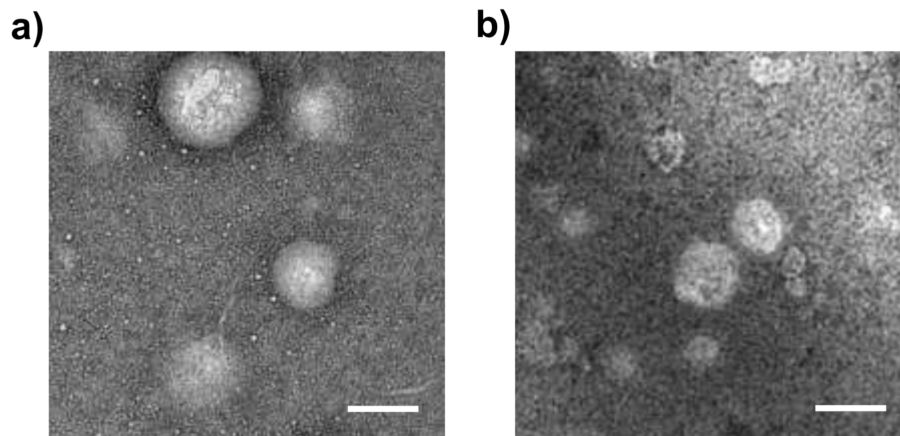

Fig. S10 TEM images of a) LdEV non-dep. and b) LdEV dep. (scale bar:100nm)

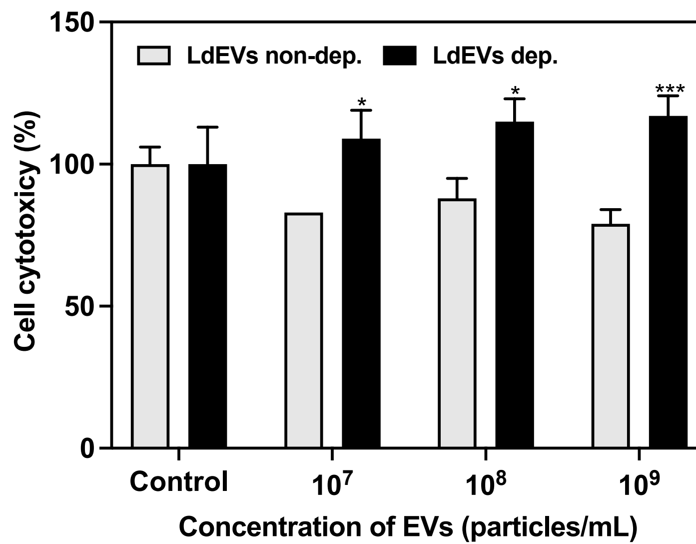

Fig. S11 Cell viability assay was used to detect cell viability in HaCaT cells with LdEV non-dep. and LdEV dep.

110 # Supplementary Tables

111 Table S1. Specification comparison table of different magnets

Magnet name	Magnet ①	Magnet ②	Magnet ③
Diameter (mm)	2	4	8
Thickness (mm)	1	3	7
Pull Force	0.68	3.40	14.49

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113 Table S2. Comparison of conventional method and all-in-one platform

Name	Conventional method	All-in-one platform
Total Time	120~180 min	15 min
Incubation Time	60~90 min	5 min
Required facilities	Complex infrastructure Rocker, Shaker, Pipette, etc.	No other equipment
Applicable environment	Specialized environments, Laboratory, etc.	Any environments
Complexity	High, Cumbersome manual operation, etc.	Easier to manufacture and operate
Preparation	Sample preparation process that adds complexity and cost	Little preparation
Power needs	Yes	No
Expertise	Highly need	No expertise or infrastructure required; suitable for field testing
Reference	Zheng, L., Wan, Y., Qi, P., et al. *Talanta*,2017 Li Y Q, Zhu B, Li Y, et al. *Angewandte Chemie International Edition*, 2014 Idil, N., Perçin, I., Karakoç, V., et al. *Colloids and Surfaces B: Biointerfaces*,2015	This work

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115 Table S3. Nucleic acid sequences required to prepare DNA tetrahedron.

Name	Sequence (5'→ 3')
C. albicans-F	TCT TTC TTG ATT TTG TGG GTG G
C. albicans-R	TCG ATA GTC CCT CTA AGA AGT G
S. aureus-F	CAC CTG AAA CAA AGC ATC CTA AA
S. aureus-R	CGC TAA GCC ACG TCC ATA TT
E. coli-F	CAT GCC GCG TGT ATG AAG AA
E. coli-R	CGG GTA ACG TCA ATG AGC AAA

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