Electronic Supplementary Material (ESI) for Analytical Methods.
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# Electronic Supplementary Information

## Construction of a cleavable linker chemistry-based $^{HB}$EXO-Chip to isolate circulating exosomes for breast cancer diagnosis

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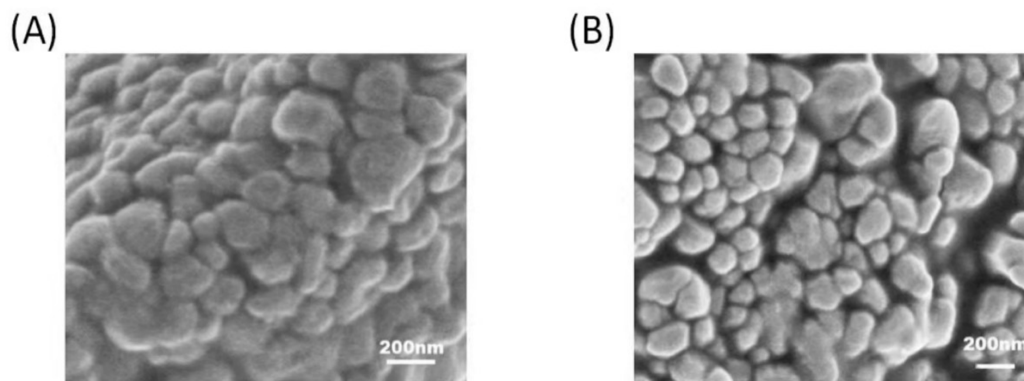
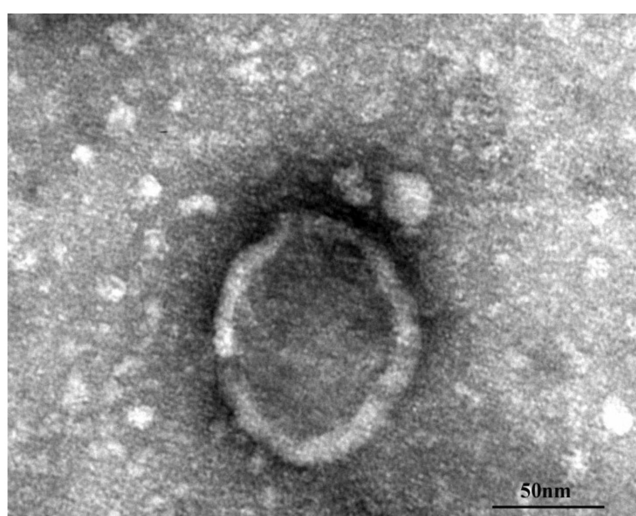
## S1: Quality control of $^{HB}$EXO-Chip

The quality control of our microfluidic chips encompasses several essential elements, such as bonding appearance, strength, chip surface appearance, microchannel structure, and microstructure deviation. After bonding, the $^{HB}$EXO-Chip should exhibit a defect-free and completed herringbone stitching appearance with no cracks, bonded areas under the couch, or unbonded spots. Visual inspection should indicate a clean surface free of fingerprints and impurities, while microscopic observation should reveal a transparent chip without residual glue. The structural integrity of the eight herringbone fluidic channels should exhibit less than 5% damage with no channel obstruction. Additionally, the internal herringbone structure collapse area should be less than 10%. Finally, the $^{HB}$EXO-Chip sealing strength should bear a pressure of 0.3Mpa. If all of these criteria are met, the $^{HB}$EXO-Chip is suitable for subsequent experimental use.

## S2：Supporting Figures




FigS1.Scanning Electron Microscopy image of exosomes




FigS2.Transmission Electron Microscope image of exosomes

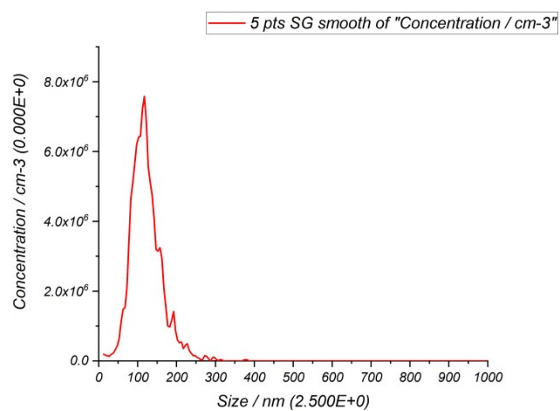



Fig.S3 Nanoparticle Tracking Analysis images of exosomes

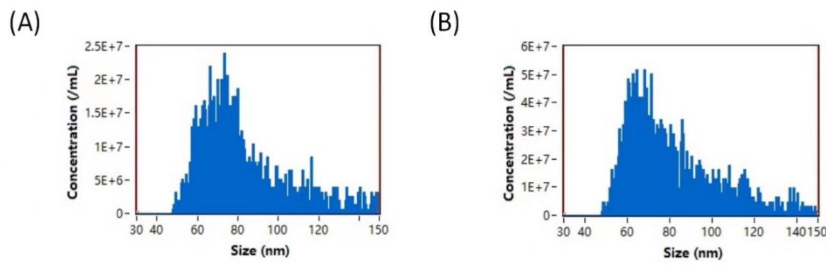



Fig.S4 Nanoflow analysis image of exosomes

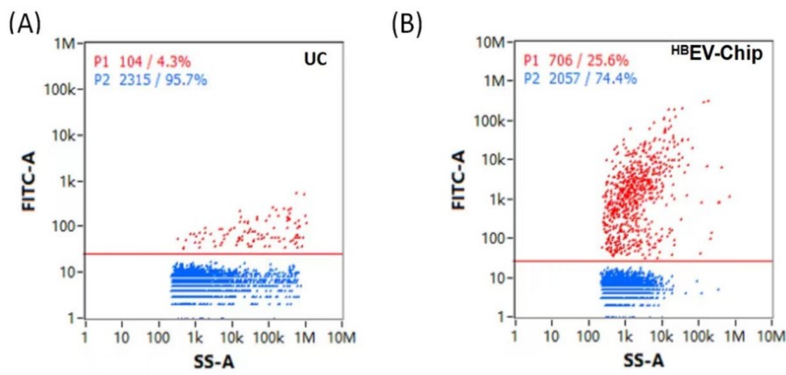



Fig.S5 Nanoflow analysis images of exosomes on different platforms

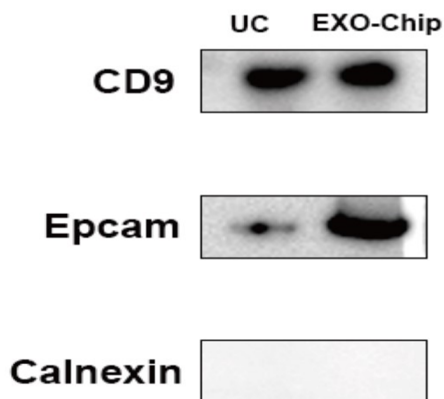



Fig.S6 Comparison of exosome contents isolated from different platforms (UC/$^{HB}$EXO-Chip)

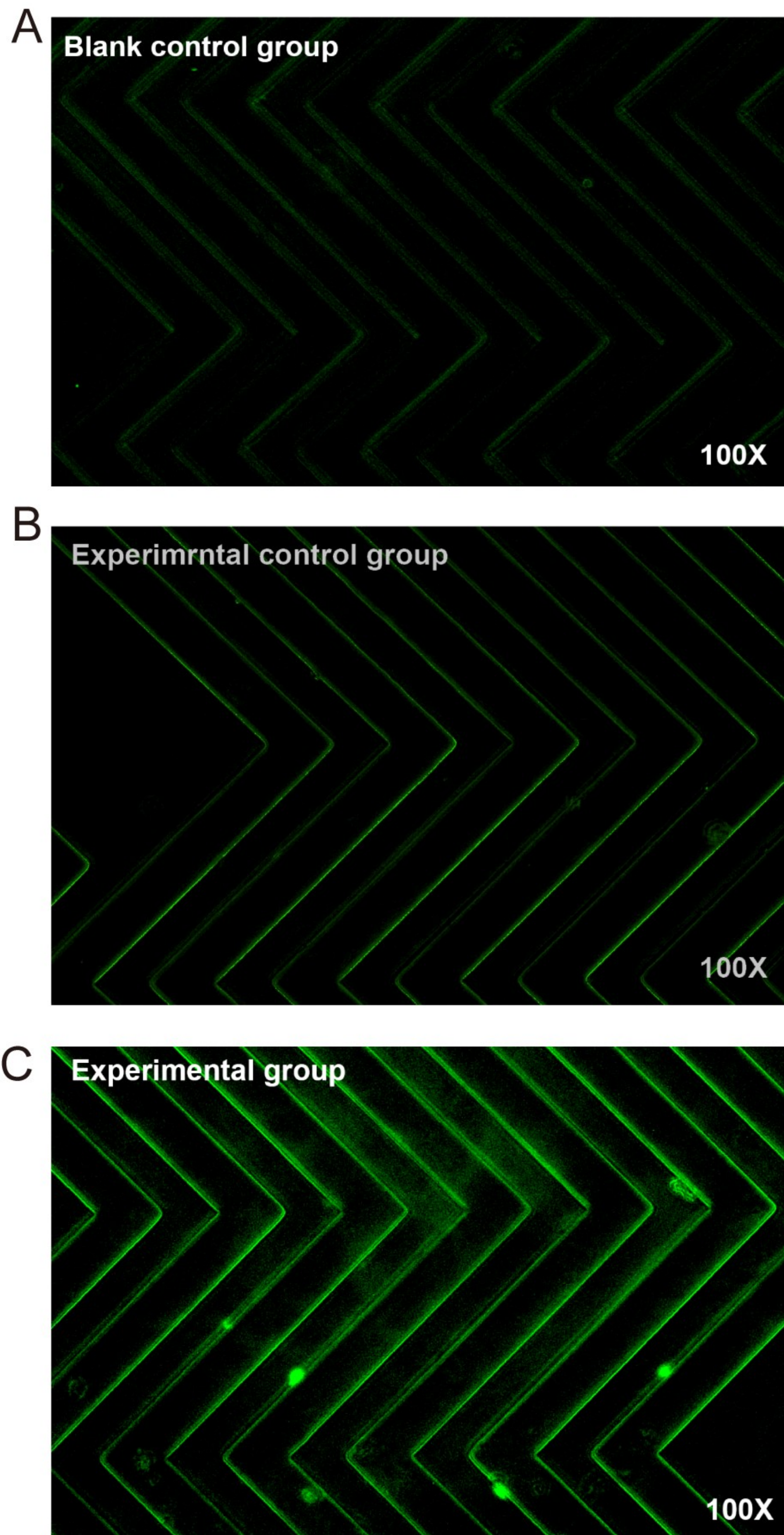



Fig.S7 Fixation of biotinylated Epcam antibodies in a herringbone channel under fluorescence microscopy

**S3：Supporting Tables**

TableS1：Clinical Patient Information

**Clinical patient sample information(N=40)**

| Group | Healthy People(n=10) | Benign breast lesions(n=14) | Breast Cancer(n=16) |
|---|---|---|---|
| Age (Mean) | 43.5 | 39.1 | 50.6 |
| Disease | Age (20-60) | Mammary gland Hyperplasia(n=2) | Carcinoma in situ of breast(n=4) |
| Classification | No disease(n=10) | Breast fibroids(n=12) | Invasive carcinoma of breast(n=12) |
| WHO | | | Carcinoma in situ of breast(n=4) |
| classification | | | Mixed invasive carcinoma(n=2) |
| Level Ⅰ 5 | | | n=1 |
| Level Ⅱ 6 points | | | n=1 |
| Level Ⅱ 7 points | | | n=4 |
| Level Ⅲ 8 points | | | n=4 |
| CA153(Mean) | 8.25 | 9.52 | 11.38 |
| CA199(Mean) | 13.66 | 13.85 | 11.27 |

TableS2: Summary of assays for exosomes and their contained biomarkers

Summary of assays for exosomes and their contained biomarkers

| Cancer | Target | Methods | Capture efficiency | Time | Medium | Ref |
|---|---|---|---|---|---|---|
| Breast cancer | Epcam | immuno-capture | 82% | 10min | plasma | HBEXO-Chip |
| Breast cancer | CD9, CD63, GGT1 | immuno-capture | | 3h | serum/plasma | [1] |
| Breast cancer | CD63, miR-126, miR-21 | magnetic separation | 54.3% | 5h | serum | [2] |
| pancreatic cancer | GPC-1, mRNA, | ultracentrifuge-based Separation | | >6h | serum | [3] |
| breast cancer | CD63, CD81 | size-based separation | | 2h | serum | [4] |
| ovarian cancer | CD81, CD24, Epcam, FRα | immuno-capture | >81% | >5h | plasma | [5] |
| Breast cancer | GPC1, mRNA | rough surface immuno-capture | 70% | | | [6] |

TableS3: Exosomes isolation strategies

| Exosomes isolation technique | Advantages | Disadvantages | Sample Volume | Ref |
|---|---|---|---|---|
| Ultrafiltration | Fast, high exosome purity, No specialized equipment required | Exosome deformation | μL-mL | 7 |
| Acoustic Purification | High specificity and sensitivity | Expensive equipment and complex setup | 10μl | 8 |
| Wavy microchannel structures within viscoelastic fluids sorting | Simple operation and low cost | Limited throughput and potential for clogging | | 9 |
| Vesicle trapping on array of ciliated (nanowires) micropillars | High efficiency and scalability | Requires specialized materials and fabrication processes | 1000μL | 10 |

## S4: Evaluation Criteria for $^{HB}$EXO-Chip and Definitions

Capture efficiency is defined as the ratio of the number of exosomes isolated by the $^{HB}$EXO-Chip to the number of exosomes in the initial feed. In this study, we evaluated the number of isolated exosomes by subtracting the number of exosomes in the effluent from the number of exosomes in the initial feed. The Nanoparticle tracking analysis (NTA) technique was employed to measure the number of exosomes within the range of 30-150 nm. The calculation for capture efficiency is as follows:

***Capture Efficiency* (%)**

= [(Number of exosomes in Initial-Num. of exosomes in Effluent)/Num. of exosomes in Initial]X100%

Release efficiency is defined as the ratio of the number of exosomes released from the $^{HB}$EXO-Chip to the number of isolated exosomes. To assess this, we measured the quantity of exosomes in the release resultant. The NTA technique was utilized to measure the number of exosomes within the range of 30-150 nm. The calculation for release efficiency is as follows:

***Release Efficiency*** (%)

= [ (Number of Exosomes in Release Resultant)/ (Num. of Exosomes in Initial-Num. of Exosomes in Effluent)]x100%

## S5: Supplemental references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