Rapid Capture of Cancer Extracellular Vesicles by Lipid Patch Microarrays

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Supporting Information

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Figure S1. Control experiment for unspecific PKH dye adhesion. Fluorescent microscopy image of a lipid microarray after incubation with PKH26 stained MCF7 EVs. Only the EpCAM antibody-functionalized array columns on the right light up in fluorescence, while the non-functionalized (DOPC only) columns on the left remain dark. This indicates that no unspecific adhesion of PKH dye or unspecific EV fusion with non-functionalized lipid patches occurs. The non-functionalized patches even appear darker compared to the substrate background, indicating the non-fouling properties of DOPC which even suppresses unspecific adhesion compared to the naked substrate. Scale bar equals 50 µm.

Figure S2. Control experiment with other purification fractions. Incubation of the other (non-EV) fractions of the purification process on AB functionalized lipid microarrays does not lead to any significant fluorescence signals from the lipid patches. Scale bars equal 50 µm.
Figure S3. FACS analysis of MCF7 and HT1080 cells. a) Both cell lines are CD63 positive. 
b) Only MCF7 cells are strongly EpCAM positive with only minor interaction in HT1080. 
c) Both cell lines show negligible interaction with the isotype control IgG.
Figure S4. STEM images of Au-NPs bound to a lipid patch. a) The original STEM images (magnification of 115,000×, scale bars equal 100 nm) of the 2 nm sized Au-NPs on the lipid patch. b) Pictures after identification of Au-NPs by ImageJ. The area of each image is 666 × 666 nm² or 0.443 µm². The average number of Au-NPs in each image is 2530 ± 101. Extrapolating from these images, around 5.712 × 10³ ± 228 Au-NPs are bound per µm². This translates to ~5.1 × 10⁶ ± 0.2 × 10⁶ ABs on a lipid patch of 30 × 30 µm². As consistency check, the analysis was repeated with c) even higher magnification images (160,000×, scale bars equal 50 nm), d) thresholded in the same way. Here, 1269 ± 35 particles are counted per area of 471 × 471 nm², yielding 5.723 × 10³ ± 159 Au-NPs bound per µm². Extrapolating onto a whole patch, ~5.1 × 10⁶ ± 0.2 × 10⁶ ABs are expected, in agreement with the analysis of the lower magnification images.
Figure S5. Direct surface immobilization vs. lipid patch based immobilization. a) Fluorescent image of a click-chemistry bound CD63 AB microarray after incubation with fluorescently labelled MCF7 EVs (PKH67 dye, green fluorescent). To visualize also the microarray itself, fluorescently labelled streptavidin (streptavidin-Cy3, red fluorescent) was used for building up the AB sandwich structure. While the microarray itself is clearly visible (left), only random attachment of EVs is observed (middle) with no correlation between array features and EV attachment visible in the overlay (right). b) The parallel experiment on the lipid microarray bound ABs shows a perfect correlation between the lipid microarray (left), captured EVs (middle) in the overlay image (right). Scale bars equal 50 µm.
**Figure S6.** Control experiments for EV capture from unpurified conditional medium. a) To exclude unspecific binding of EVs or other interfering components of the conditional medium to the lipid microarrays were coated only with streptavidin, not carrying any anti-CD63 AB. After incubation with MCF7 EVs from unpurified conditional medium and subsequent staining against EpCAM (rabbit anti-EpCAM AB, Alexa-647 conjugated anti-rabbit secondary AB), no signal rises on the lipid patches, indicating a successful negative control (no unspecific binding). b) To exclude unspecific interactions of the secondary detection AB, lipid microarrays with anti-CD63 AB were incubated with MCF7 EVs as before, but then directly incubated with the secondary detection AB (Alexa-647 conjugated anti-rabbit AB) without prior incubation with the primary detection AB (rabbit anti-EpCAM AB). No signal is visible on the lipid patches, showing that the secondary detection AB has no significant unspecific interaction with the lipid microarrays and EVs. c) Lipid microarrays carrying IgG1 isotype control AB show no detection signal after MCF7 EV incubation and subsequent staining, indicating no unspecific interaction of EVs and the lipid microarrays. d) When anti-CD63 AB carrying lipid microarrays, after incubation with MCF7 EVs, are incubated with rabbit IgG isotype control AB (instead of rabbit anti-EpCAM detection AB), subsequent incubation with the fluorescently labelled anti-rabbit secondary AB also induced no signal. This indicates that there is also no unspecific interaction between non-target detection ABs and the lipid microarrays or the bound EVs. The scale bars in all images equal 50 µm.
**Figure S7.** EV capture from patient samples. The image shows CD63 antibody-functionalized lipid patches after EV capture from patient sera and staining against EpCAM (rabbit anti-EpCAM AB, Alexa-647 conjugated anti-rabbit secondary AB). The columns correspond to the different patients, the lines then give the results for different fractions and the negative control (Void). All scale bars equal 50 µm.
Figure S8. RNA retention from patient samples. For two of the patients, additional staining for RNA detection was performed. The image shows CD63 antibody-functionalized lipid patches after EV capture from patient sera and staining for nucleic acid with SYTO confirming RNA cargo retention. The columns correspond to the different patients, the lines then give the results for different fractions and the negative control (Void). All scale bars equal 50 µm.
Table S1. Comparison of EV studies.

| Capture Approach | Detection Method | Sample Volume | Detection Time | Limit of Detection | Retrieval of EV cargo | Clinical Samples |
|------------------|------------------|---------------|----------------|--------------------|-----------------------|------------------|
| Flat PDMS channel functionalized with specific capture ABs | Membrane staining dye, fluorescence plate reader | 400 µL | 100 min | n/a | Lysis and RNA extraction | from serum |
| Graphene oxide/polydopamine nano-interface functionalized with specific capture ABs | Secondary AB fluorescence / ELISA | 20 µL | 120 min | 5 x 10^4 / mL | Potentially by elution or lysis | from plasma |
| Magnetic beads functionalized with specific capture ABs, immobilization by magnet | Secondary AB fluorescence | 20 µL | 40 min | n/a | Magnet release | from plasma |
| Herringbone microstructures functionalized with specific capture ABs | Secondary AB fluorescence / RNA detection in lysate | 200 – 2000 µL | 180 min | 10^5 / mL | Elution or lysis and RNA | from plasma and serum |
| Magnetic beads functionalized with specific capture antibody, immobilization by magnet | Electrochemical, differential pulse voltammetry | 30 µL | 210 min | 4.39 x 10^5 / mL | Magnet release | from serum |
| Magnetic beads functionalized with specific capture antibody, immobilization by magnet | Secondary AB colorimetry / ELISA | 2 µL | 90 min | n/a | Magnet release | from plasma |
| Magnetic beads functionalized with specific capture antibody, immobilization by magnet | Secondary AB fluorescence | 20 – 100 µL | 40-200 min | 10^4 / mL | Elution* | from serum |
| Magnetic beads functionalized with specific capture antibody, immobilization by magnet | Raman-active beads functionalized by specific ABs | 20 µL | 60 min | 1.6 x 10^5 / mL | Magnet release | from serum |
| Arrays of supported lipid membranes functionalized with specific capture antibody | Secondary AB fluorescence | 50 – 80 µL | 60 min | 4 x 10^7 – 4 x 10^9 / mL depending on AB | Potentially picking of lipid patches, lysis** | from serum |

* needs higher concentration of EVs (10^5 / mL) or larger sample volumes (10mL) to obtain enough material for downstream analysis

** retainment of RNA on the lipid arrays shown by fluorescence staining

[1] S.S. Kanwar, C.J. Dunlay, D.M. Simeone, and S. Nagrath, Lab Chip 14, 1891 (2014).
[2] P. Zhang, M. He, and Y. Zeng, Lab Chip 16, 3033 (2016).
[3] Z. Zhao, Y. Yang, Y. Zeng, and M. He, Lab Chip 16, 489 (2016).
[4] E. Reátegui, K.E. van der Vos, C.P. Lai, M. Zeinali, N.A. Atai, B. Aldikacti, F.P. Floyd, A. H. Khankhel, V. Thapar, F.H. Hochberg, L. V. Sequist, B. V. Nahed, B. S. Carter, M. Toner, L. Balaj, D. T. Ting, X.O. Breakefield, and S.L. Stott, Nat. Commun. 9, 175 (2018).
[5] H. Xu, C. Liao, P. Zuo, Z. Liu, and B.-C. Ye, Anal. Chem. 90, 13451 (2018).
[6] W. Chen, H. Li, W. Su, and J. Qun, Biomicrofluidics 13, 054113 (2019).
[7] P. Zhang, X. Zhou, M. He, Y. Shang, A.L. Tetlow, A.K. Godwin, and Y. Zeng, Nat. Biomed. Eng. 3, 438 (2019).
[8] Y. Wang, Q. Li, H. Shi, K. Tang, L. Qiao, G. Yu, C. Ding, and S. Yu, Lab Chip 20, 4632 (2020).