Electronic Supplementary Information

Single Microbead-based Fluorescent Aptasensor (SMFA) for direct isolation and in situ quantification of exosomes from plasma

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1. The sequences of DNA aptamers and fluorescent DNA probe

Table S1. The sequences of DNA aptamers and fluorescent DNA probe

Name	Sequence(5′→3′)
CD63-aptamer	CACCCCACCTCGCTCCCGTGACACTAATGCTATTTTTTTT TT-Biotin
EpCAM-aptamer	CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGG GTTGGCCTGTTTT-Biotin
FAM-T_{25}-cholesterol	FAM-TTTTTTTTTTTTTTTTTTTTTTTTT- cholesterol

2. Optimization of FAM-T_{25}-cholesterol (FAM-Chol) anchor concentration

For sensitive detection of exosomes, the FAM-Chol anchor concentration was the key factor which could affect the anchoring efficiency to exosome-bound single MB in the fluorescence imaging. So the FAM-Chol anchor concentration was optimized. In this work, to evaluate the anchoring efficiency of FAM-Chol anchor towards exosomes, a series of FAM-Chol anchor at different concentrations (50 nM, 100 nM, 500 nM, 1 μM and 5 μM) were used to react with a certain concentration of exosomes (4.9×10^6 particles/μL). Other experiment conditions were the same as described standard procedures. Meanwhile, MBs treated with only such varying concentrations of FAM-Chol anchor but without exosomes were used as the blank control, respectively.

As shown in Fig. S1, in the presence of exosomes, the fluorescence signals gradually increased with increasing FAM-Chol anchor from 50 to 500 nM. However, no obvious fluorescence increase was observed when further elevating the FAM-Chol anchor from 500 nM to 5 μM. For the blank controls, the fluorescence signal was slightly enhanced with the increasing of FAM-Chol anchor concentration (Fig. S1b). And when 500 nM of FAM-Chol anchor was used, the ratio of the signal produced by the exosomes to the blank control reached the highest (Fig. S1c). Therefore, we selected 500 nM as the optimal concentration of FAM-Chol anchor for further analytical applications.

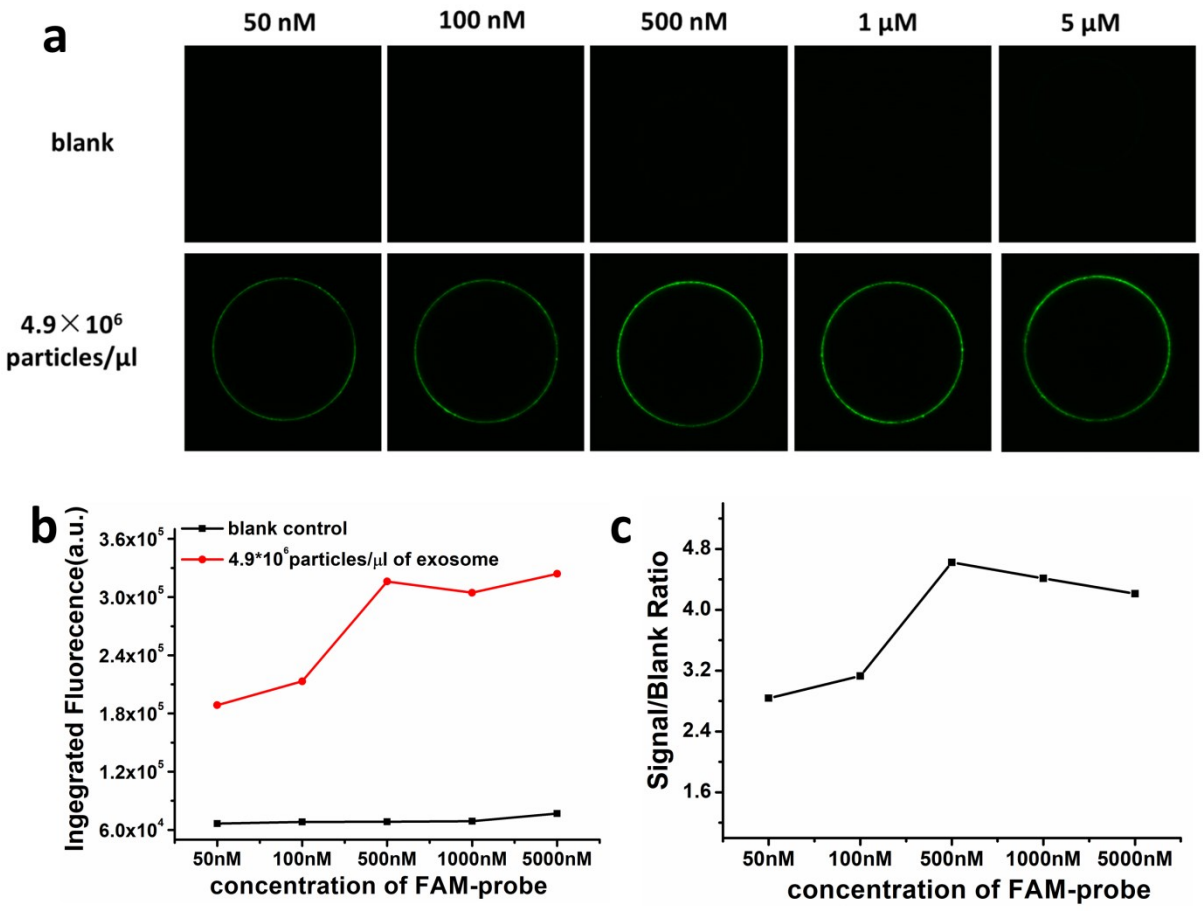

Fig. S1 Optimization of FAM-Chol anchor concentration in the reaction system. a). The fluorescence images of the MBs treated with FAM-Chol anchor at different concentrations (50 nM, 100 nM, 500 nM, 1 μM, and 5 μM) in the presence and absence of exosomes. b). Plot of the integrated fluorescence intensity of the MBs with different concentrations of FAM-Chol anchor. c). Plot for the ratios of the signals produced by exosomes to the blank control. The concentration of exosomes was 4.9×10^6 particles/μL

3. Optimization of incubation time of FAM-Chol anchor with exosomes

In order to further improve the anchoring efficiency of FAM-Chol anchor towards exosomes, the incubation time of the FAM-Chol anchor and exosomes was also explored. In this study, FAM-Chol anchored towards exosomes (4.9×10^6 particles/μL) with different incubation times (15, 30, 60, 90, and 120 min), other experiment conditions were the same as described in the standard procedures. The results were shown in Fig. S2. As increasing the incubation time, the fluorescence signals of samples and blank controls both gradually increased. While, when the incubation time was 30 min, the signal ratio of the exosomes to the blank control reached the highest. Therefore, we selected 30 min as the optimal incubation time for further analytical applications.

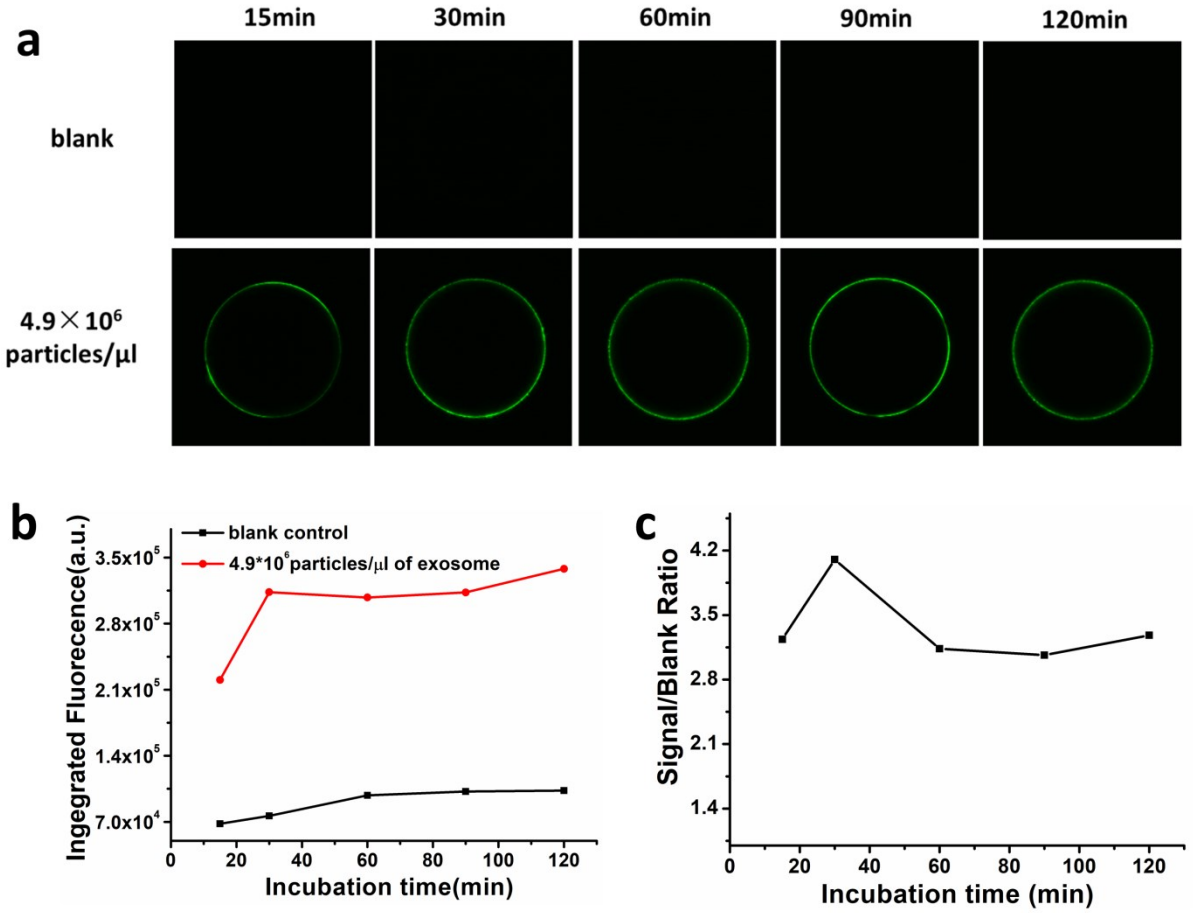

Fig. S2 Optimization of the incubation time of FAM-Chol anchor with exosomes in the reaction system. a). The fluorescence images of the FAM-Chol anchored towards exosomes with different incubation times (15 min, 30 min, 60 min, 90 min, 120 min) in the presence and absence of exosomes. b). Plot of the integrated fluorescence intensity of the MBs at different incubation times. c). Plot for the ratios of the signals produced by exosomes to the blank control.

4. Spike-in experiment for exosome detection in plasma samples

Table S2 Recovery results of exosomes in plasma by the proposed assay

Spiked amount (particles/μL)	Detected amount (particles/μL)	Recovery (%)	Relative Standard Deviation (RSD %)
2.45×10^6	2.17×10^6	88.7	4.2
3.43×10^6	2.98×10^6	86.9	0.6

5. The confocal fluorescence images of individual single MBs functionalized by EpCAM-aptamer in the presence of different concentrations of exosomes

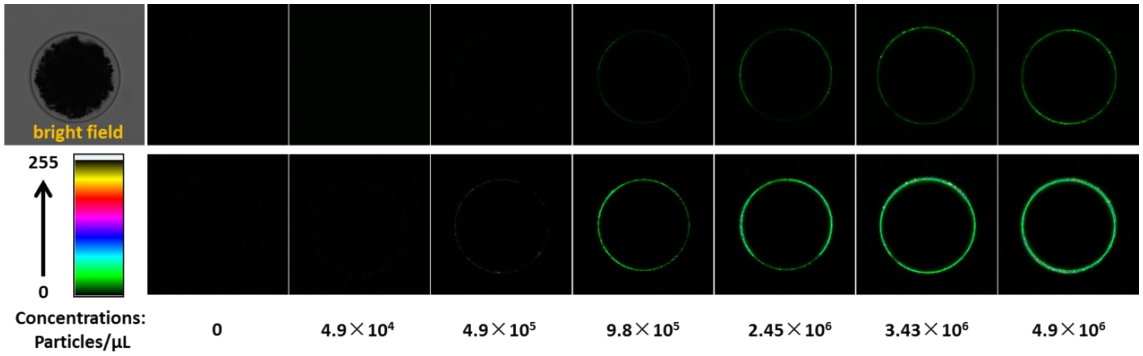

Fig. S3 Top panel: fluorescence images of the MBs treated with different concentrations of exosomes from 0 (blank) to 4.9×10^6 particles/μL for the detection of EpCAM-exosomes (PMT voltage for the imaging: 600 V); Bottom panel: illustration of the corresponding MB images by using pseudocolor bars in which different intensities were indicated by different colors. The sizes of all MBs are 80 ± 5 μm.

6. Verification of fluorescence intensity of MB correlated with exosomes

To further verify the correlation of fluorescence intensity of MB-aptamer with exosomes from plasma, the exosomes in plasma was firstly removed by using a filtration concentrator (100 kDa). Different concentrations of exosomes were spiked into the exosomes-depleted plasma and analyzed by the proposed method. The results were also compared with the exosomes analyzed in PBS buffer. As shown in Fig.S4, the fluorescence intensity of the MB was gradually enhanced with the increasing of exosome concentration in the exosomes-depleted plasma, and the fluorescence intensity obtained from the exosomes in exosome-depleted plasma were almost the same as that in PBS. These

results indicated that the fluorescence intensity was well correlated with the concentration of exosomes from plasma and the proposed method can work well in the plasma sample.

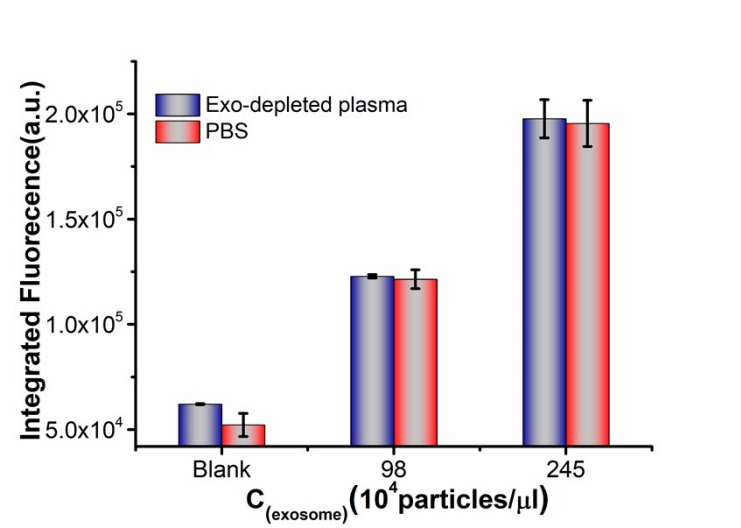

Fig. S4 Detection of the exosomes in exosomes-depleted plasma and PBS by the proposed method