Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


# Multifunctional Luminescent Immuo-magnetic Nanoparticles: Toward Fast, Efficient, Cell-friendly Capture and Recovery of Circulating Tumor Cells

*Xiaoxi Zhou,* † *Bin Luo,* † *Ke Kang,* † *Shaohua Ma,* † *Xiaohui Sun,* ‡ *Fang Lan,* †*Qiangying Yi,* *† *Wu Yao**†

† National Engineering Research Center for Biomaterials, Sichuan University, 29 Wangjiang Rd., Chengdu 610064, Sichuan, PR China.

‡ West china hospital, Sichuan University, 17 third section, People's south Rd., Chengdu 610041, Sichuan, PR China.

*Email - wuyao@scu.edu.cn

## METHODS

*Magnetic Separation Rates of MLNs.* The magnetic separation rates test was carried out according to reference 1. Proportional relationship of MLNs absorbance at 600 nm versus MLNs concentration was firstly analyzed. The capture efficiency of MLNs at different attraction time was measured and calculated as follows: series of centrifuge tubes containing same concentration MLNs were put onto the same magnetic scaffold for different time. After that, suspensions in each tube were discard, pellets attracted were re-dispersed in water and their absorbance at 600 nm was measured. Capture efficiency of MLNs = (attracted pellets' absorbance/ MLNs' original absorbance) ×100%.

*Confirmation of PEGylation and Antibody Conjunction onto PIMNs.* To prove the successful PEGylation, RB-$PEG_{2000}$-$NH_2$ bearing fluorescent component (RB) was used instead of the $CH_3$-$PEG_{2000}$-$NH_2$ during the preparation, and as gained PIMNs-RB were also observed with the CLSM.

To prove that rabbit anti-EpCAM antibody was successfully conjugated, MLNs and PIMNs were separately incubated with FITC-labeled goat anti-rabbit IgG for 1 h, washed with PBS to remove surplus IgG and observed under a fluorescence microscope.

*Estimation of the Amount of Antibody on PIMNs.* To start with, the relationship of FITC-labeled secondary antibody emission intensity at about 520 nm versus its concentration was firstly analyzed. It's worthy to note that certain amount of MLNs were added in this solution since black magnetic particles may have some influence on absorbance. Then fluorescence intensity of PIMNs after incubation with five serial dilutions of rabbit anti-EpCAM antibody and excessive amount of FITC-labeled secondary antibody were also measured.

### 1. Characterization of $Fe_3O_4$ Nanoparticles and MLNs

As shown in Fig. S1 A, the original $Fe_3O_4$ nanoparticles had a negative surface potential of -20 mV. The adsorbed PAH on the $Fe_3O_4$ nanoparticles caused a reversal in zeta potential (+37.9 mV), and subsequent deposition of QDs and HA reversed the zeta potential (-28 mV, -35 mV separately). The successive surface charge changes proved the successful stepwise deposition of polyelectrolytes layer and QDs. Besides, comparing TEM image of $Fe_3O_4$ nanoparticles with MLNs (Fig.1 and Fig. S1), the building components (polyelectrolytes and QDs) with lower contrast were adsorbed onto the surface of the MLNs, and diameter increased from 301.2±29.9 to 336.6±22.4 nm (Fig. S1D-E). Fig. S1F showed the proportional relationship of absorbance at 600 nm versus MLNs concentration, so the capture efficiency of MLNs at different attraction time can be measured and calculated (Fig. 1H).

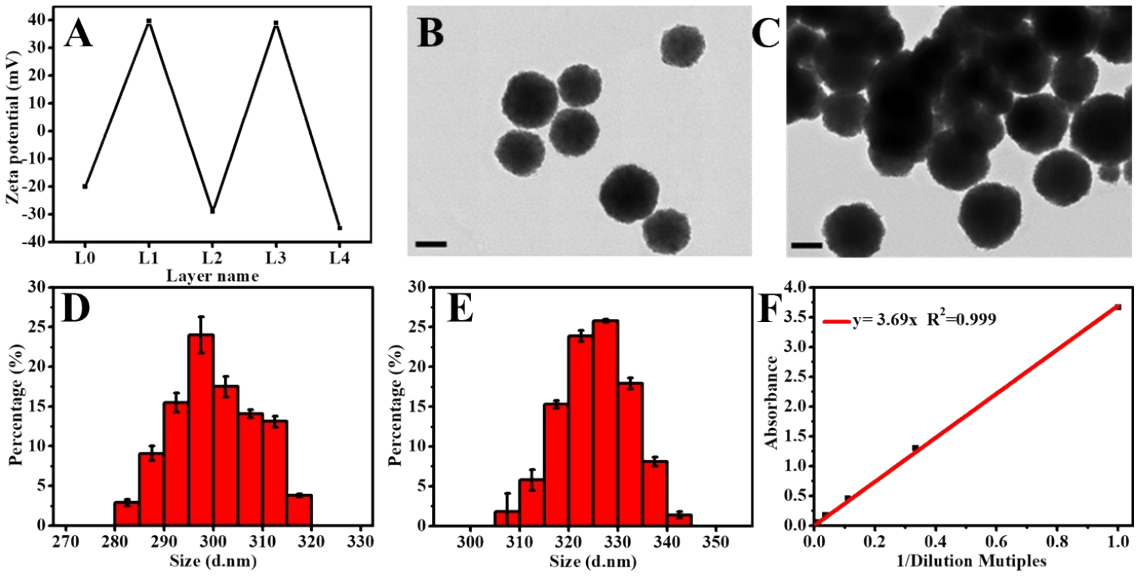


**Fig. S1** (A) Zeta potential changes of nanoparticles during the LbL assembly process, TEM images for $Fe_3O_4$ nanoparticles (B) and MLNs (C). The scale bar is 200 μm. Particle size distribution histograms of $Fe_3O_4$ nanoparticles (D) and MLNs (E). (F) Standard curve of MLNs of different concentrations.

## 2. Characterization of PIMNs

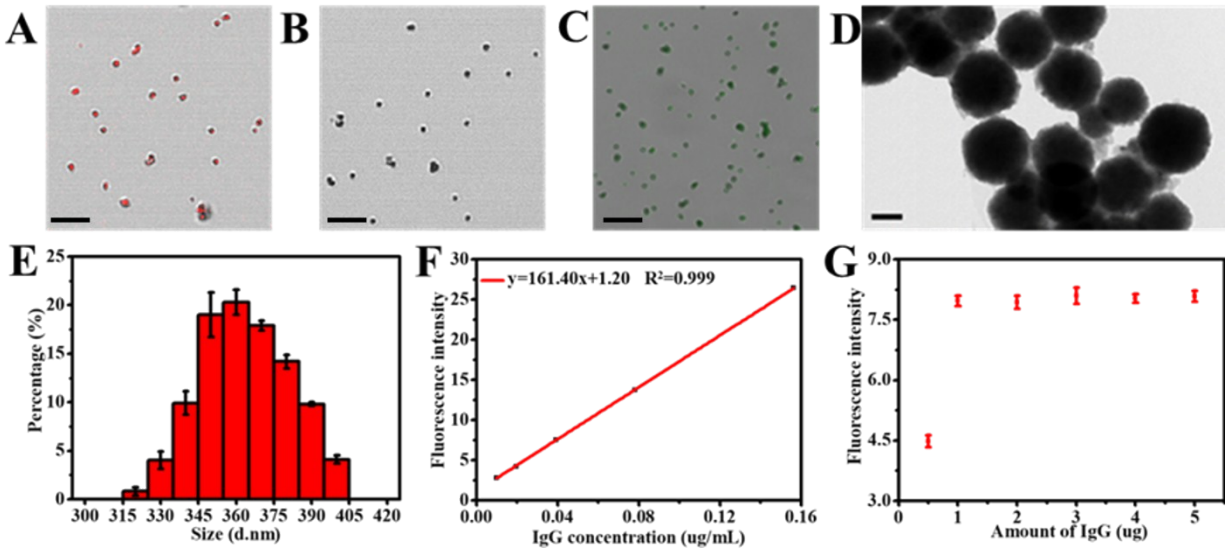


**Fig. S2** (A) CLSM images of PIMNs-RB, (B) MLNs incubated with FITC-labeled secondary antibody and (C) PIMNs incubated with FITC-labeled secondary antibody. The scale bar for A-C is 10 μm. (D) TEM image of PIMNs, (E) Particle size distribution of PIMNs. The scale bar for D is 200 nm. (F) Standard curve of fluorescent intensity of FITC-labeled rabbit IgG at different concentrations in the presence of MLNs, (G) Fluorescent intensity of PIMNs incubated with different amount of anti-EpCAM antibody.

From Fig. S2A, PIMNs-RB showed strong red fluorescence signal, suggesting that the PEGylation was successful accomplished. Besides, the green fluorescence of PIMNs incubated with FITC-labeled secondary antibody was obvious (Fig. 1E and Fig. S2C), while no green signal was observed on the MLNs (Fig. S2B). Those results indicated that anti-EpCAM antibody was successfully conjugated to PIMNs. Fig. S2D showed the proportional relationship of fluorescence intensity versus FITC-labeled rabbit IgG of different concentrations in the presence of 100 μg MLNs. From Fig. S2E, the fluorescence intensity remained nearly unchanged when the amount of

IgG reached 1μg, this result indicated that 1μg antibody was enough for 100 μg PIMNs. TEM image of PIMNs was in Fig. S2F and the diameter of PIMNs was 365.2±29.6 nm (Fig. S2G).

**3. Capture Efficiency Evaluation of PMNs and MNs**

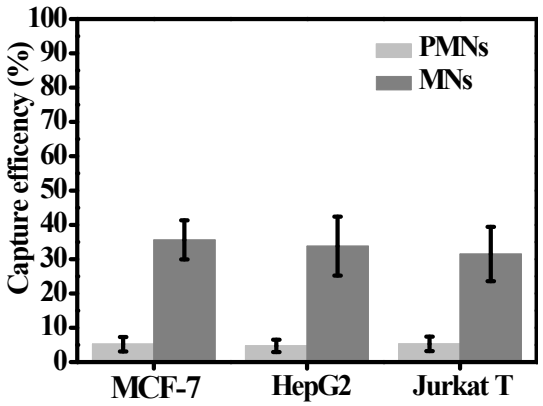


**Fig. S3** Capture efficiency of PEGylated magnetic nanospheres (PMNs, without EpCAM modification) and magnetic nanospheres (MNs, without PEGylation or EpCAM modification) towards MCF-7, HepG2 and Jurkat T cells under the optimized conditions.

**4. Evaluation of PIMNs' Capture Specificity in Synthetic Samples**

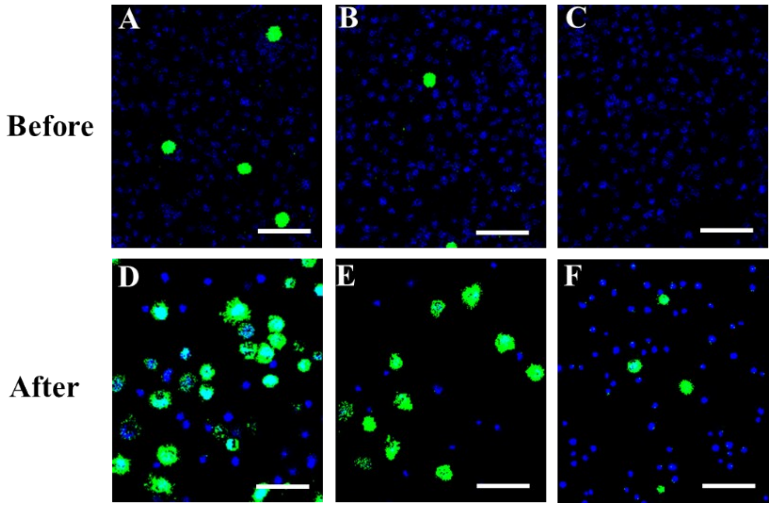


**Fig. S4** CLSM images for cell mixtures of GFP-MCF-7 and Jurkat T cells with proportions: 1:40, 1:100 and $1:10^4$ before capture (A-C) and after enrichment (D-F). Both cells are prestained with Hochest 23324 and the scale bar is 50 μm.

## 5. Migration and Invasion Assays of Tumor cells Captured

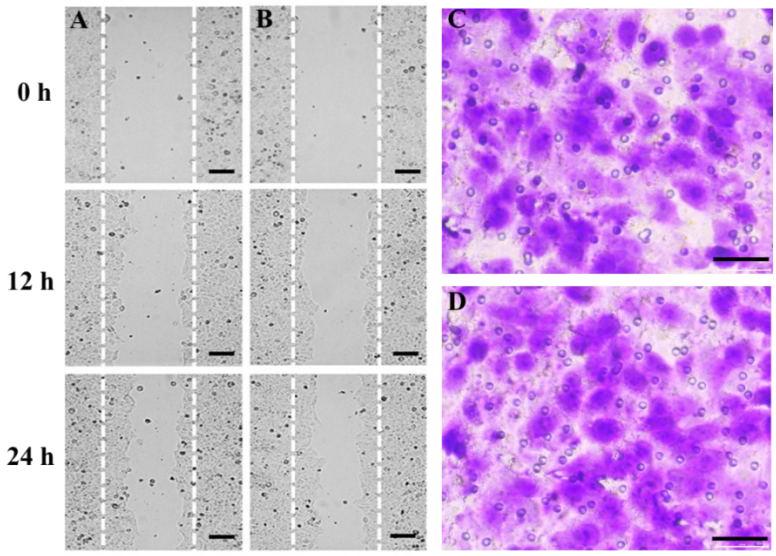


**Fig. S5** Migration and invasion ability comparison between untreated tumor cells (A, C) and recovered tumor cells (B, D) determined by wound healing assay and Transwell assay. The scale bar for A-B is 100 μm, and 50 μm for C.

## 6. Typical CLSM images of CTCs from Patients

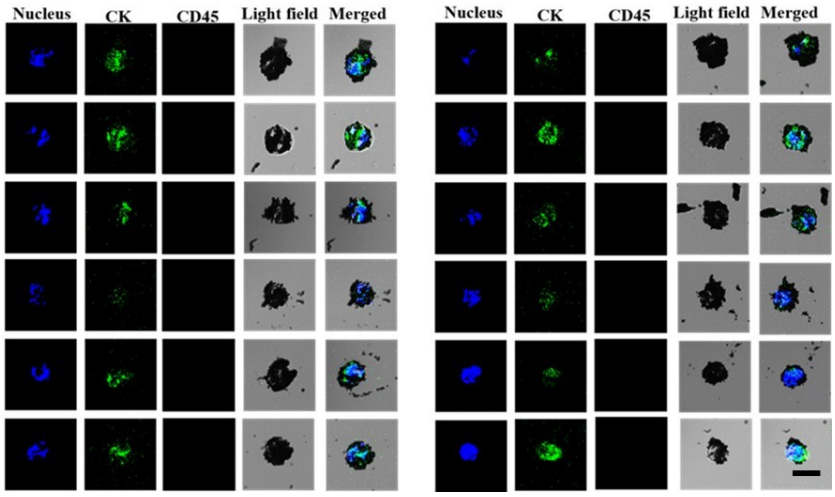


**Fig. S6** Three-color ICC of CTCs captured from one patient's blood. The scale bar is 20 μm.

**Table S1**. CTCs quantification of blood samples from cancer patients with an epithelial type.

| Sample No. | Cancer Type | Gender | Age | Volume processed/mL | CTCs |
|---|---|---|---|---|---|
| 1 | thyroid | M | 62 | 1.5 | 12 |
| 2 | thyroid | F | 27 | 1.5 | 4 |
| 3 | thyroid | F | 42 | 1.5 | 19 |
| 4 | stomach | M | 70 | 1.5 | 1 |
| 5 | esophagus | M | 59 | 1.5 | 2 |
| 6 | thyroid | M | 38 | 1.5 | 8 |
| 7 | kidney | M | 67 | 1.5 | 18 |
| 8 | prostate | M | 53 | 1.5 | 12 |
| 9 | thyroid | F | 36 | 1.5 | 8 |
| 10 | prostate | M | 72 | 1.5 | 8 |
| 11 | prostate | M | 77 | 1.5 | 3 |

**Table S2.** CTCs quantification of blood samples from healthy volunteers.

| Sample No. | Gender | Age | Volume processed/mL | CTCs |
|---|---|---|---|---|
| 1 | F | 33 | 1.5 | 0 |
| 2 | M | 28 | 1.5 | 0 |
| 3 | M | 29 | 1.5 | 0 |

| | | | | |
|---|---|---|---|---|
| 4 | F | 27 | 1.5 | 0 |
| 5 | F | 27 | 1.5 | 0 |
| 6 | F | 23 | 1.5 | 0 |
| 7 | M | 25 | 1.5 | 0 |
| 8 | M | 23 | 1.5 | 0 |
| 9 | M | 24 | 1.5 | 0 |

**References**

1 C. Y. Wen, L. L. Wu, and Z. L. Zhang, et al., *ACS Nano*, **2014**, *8*, 941-949.