Supporting Information

Two-Dimensional Immunomagnetic Nano-Net for the Efficient Isolation of Circulating Tumor Cells in Whole Blood

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Theory calculation for magnetic nano particle's size in consideration of magnetic force.

(1) *Magnetic Particle Force*[2]

$$F_m = \mathrm{N}_{number} V_{volume} (\frac{\Delta\chi}{\mu_0})(\mathrm{B}\cdot\nabla)\mathrm{B} \quad \text{..... (Eq. S1),}$$

where,

N is the number of particle

V is the volume of the particle (m^3)

$\Delta\chi$ is the difference in magnetic susceptibilities between the particle and the surrounding medium (dimensionless)

$\mu_0 = 4\pi \times 10^{-7}$ is the permeability of vacuum (TmA^{-1})

B is the applied magnetic field (T).

(2) *Drag Force in solution*[2-3]

$$F_d = -6\pi\eta r V \quad \text{[2a] ..... (Eq. S2),}$$

where,

η is the dynamic viscosity of the suspending medium (kg m^{-1} s^{-1})

r is the radius of the bead (m)

V is the velocity of the superparamagnetic bead (m s^{-1})

In consideration of magnetic force and drag force in whole blood. The desired MNP size should be suitable in the range of 200 nm to 500 nm.

(3) *The formula used to estimate the binding force, Fc, between CTCs and designed scaffold.*

The binding force Fc can be estimate in following equation, [4]

$$\frac{F_C}{R_C} = \frac{2k_B\theta}{l_b}\ln\left\{1+\frac{N_L}{\eta k_D}\right\} \text{ or}$$

$$F_C = \frac{2A_C N_R k_B\theta}{l_b}\ln\left\{1+\frac{N_L}{\eta k_D}\right\} \quad \text{..... (Eq. S3),}$$

Where,

R_C is the receptor number per bead in the contact region

A_c is effective contact area (m^2)

N_R is cell receptor density (#/m^2, 1×10^4 to 1×10^6 EpCAM antigen/ CTC. It corresponds to $3.2 \times 10^{13} \sim 10^{15}$ #/m^2 when cell size is 10 micrometer in diameter)

k_B is Boltzman's constant ($JK^{-1} = 1.38\times10^{-23}$)

θ is absolute temperature (K = 298)

l_b is the extent of stretch to reach critical force to break single bond (m, 8.8×10^{-10})

N_L is substratum ligand density on bead (if 1 nm^2)

η is fitting specificity (conversion parameter for K_D, $M^{-1}m^{-2}$,)

k_D is equilibrium dissociation constant (M, ~ 1×10^{-9} for antibody to antigen)

From the equation clearly shows the Fc, the binding force between CTCs and designed capture scaffold is proportion to effective contact area. Therefore, the large and fixable GO surface can provide much higher Fc than micro-size magnetic beads in CellSearch® system.

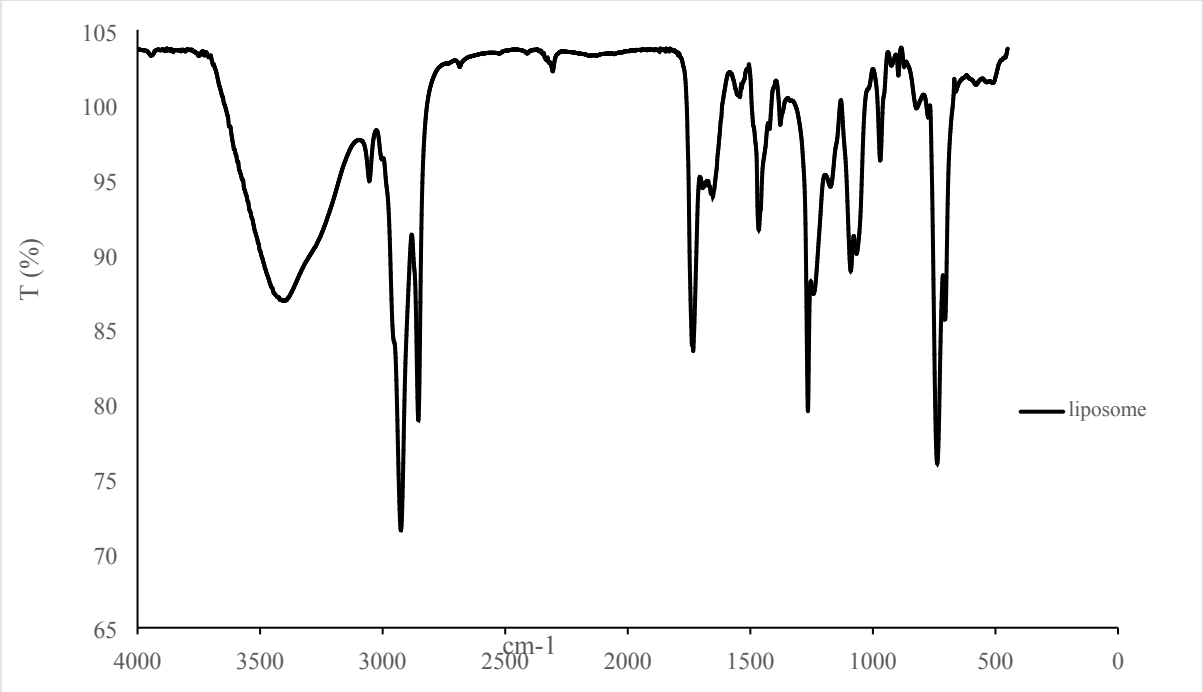

Figure S1. The FTIR spectrum of biotin-contained liposome (15% b-PE and 85% POPC).

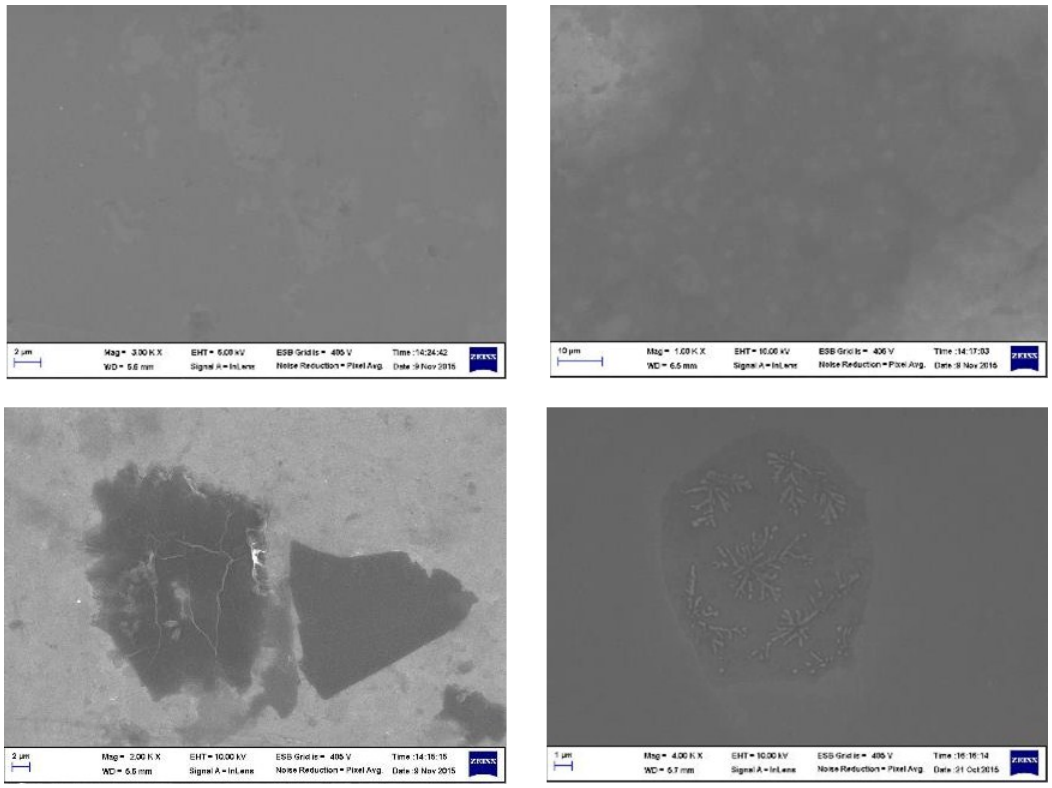

Figure S2. The SEM of GO. GO has a very broad size distribution.

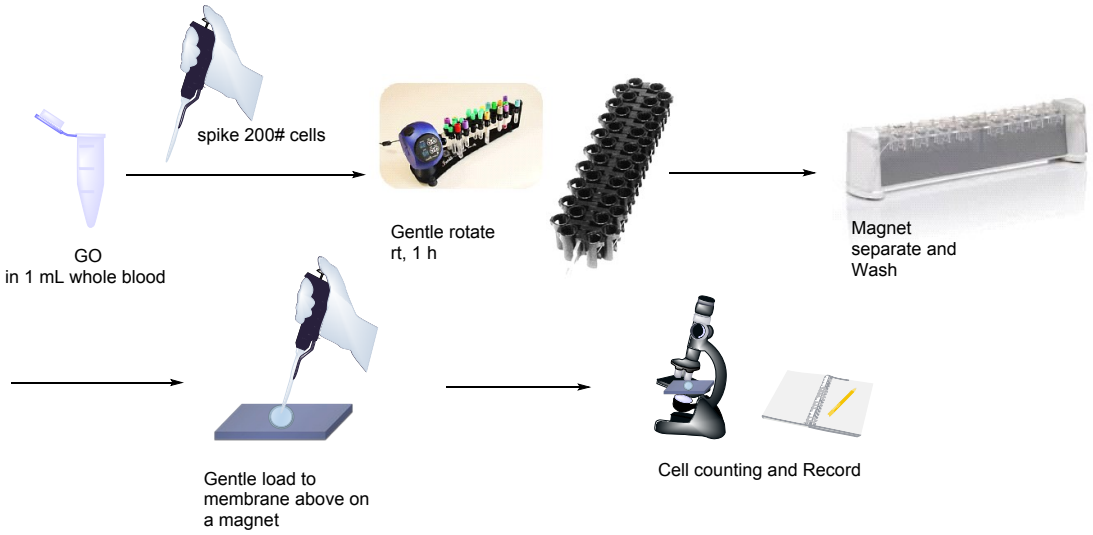

Figure S3. Ab@beads or Ab@Lipo-MNP-GO's CTCs capture procedure.

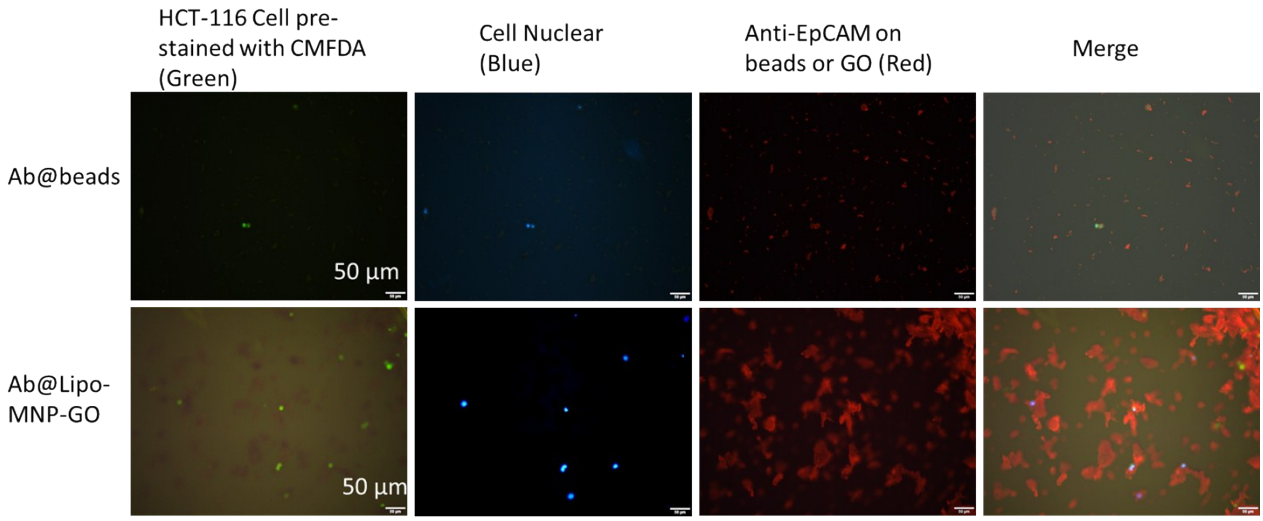

Figure S4. Experiments were performed in an identical condition with #200 CMFDA pre-stained HCT-116 spiked into cell culture medium. 20 ug of Ab@beads or Ab@Lipo-MNP-GO was applied to capture HCT-116 cells, respectively. From the image data only in 12-well plate, already can clear show Ab@Lipo-MNP-GO having much better capture ability. (The anti-EpCAM on Ab@beads or Ab@Lipo-MNP-GO was stained by Alexa 568-labeled goat anti-mouse IgG antibody. The scale bar is 50 µm.)

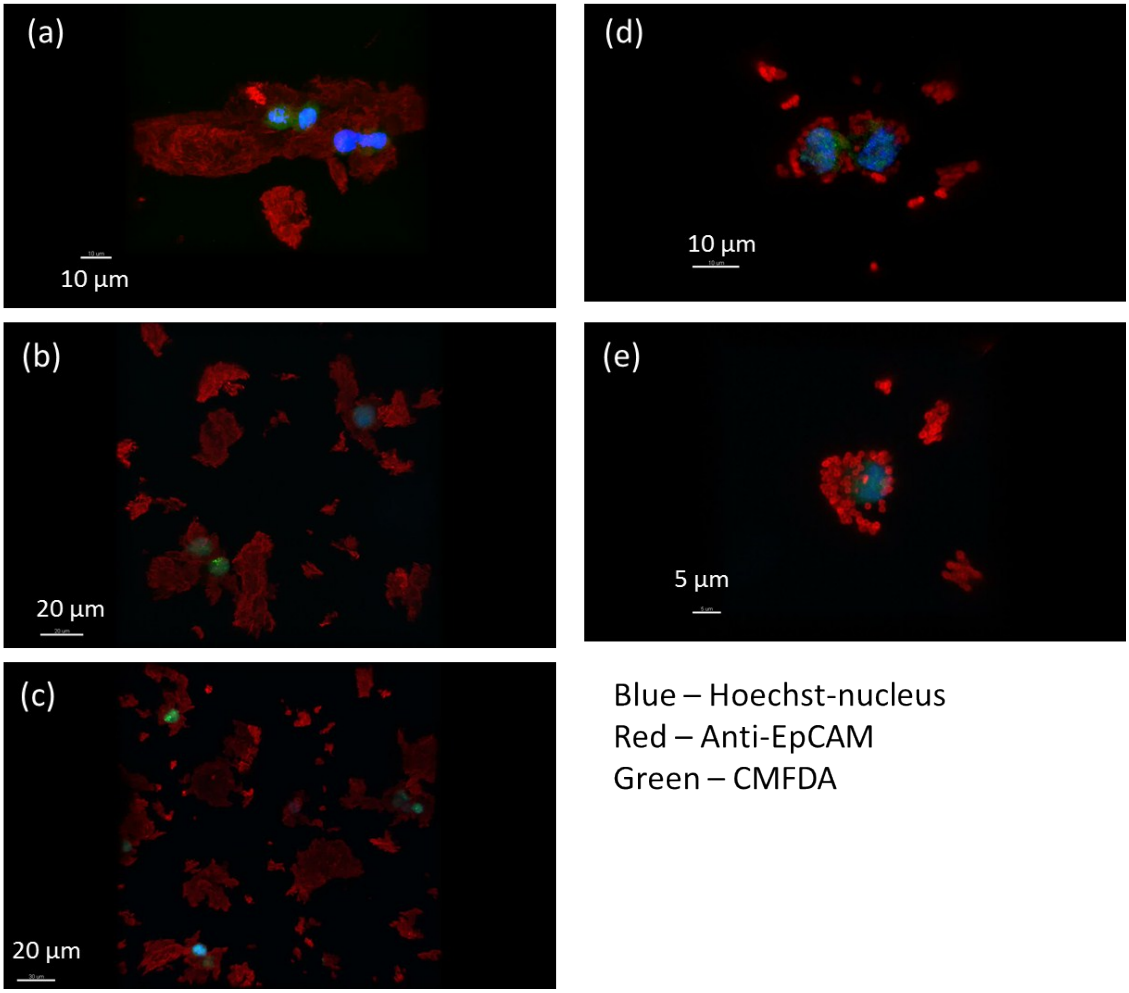

Figure S5. More confocal data. HCT-116 cells were capture by (a, b, c) **Ab@Lipo-MNP-GO**, or (d,e) **Ab@beads**, respectively. Confocal images clearly shown HCT-116 cells were wrapped by several species of **Ab@Lipo-MNP-GO**. By contracts, each of **Ab@beads** only had individual adhesion on the cell surface. There are also 3D-movies in additional supporting information.

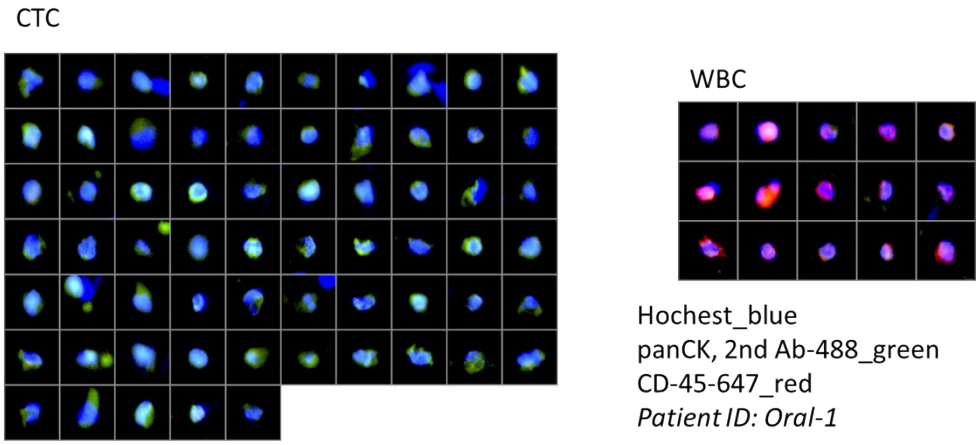

Figure S6. Example of whole CTCs captured images by using Ab@Lipo-MNP-GO from 1.0 mL blood of Oral cancer patient (ID: Oral-1).

Table S1. The comparison CTC capture number by Ab@Lipo-NMP-GO or Ab@beads.

Patient's ID	Type of magnetic scaffold	Scaffold amount	Patient's blood volume	CTC number
healthy	Ab@Lipo-NMP-GO	20 μg	1.0 mL	0
	Ab@beads	20 μg	1.0 mL	0
Oral-1	Ab@Lipo-NMP-GO	20 μg	1.0 mL	65
	Ab@beads	20 μg	1.0 mL	0
Oral-2	Ab@Lipo-NMP-GO	40 μg	1.0 mL	63
	Ab@Lipo-NMP-GO	20 μg	1.0 mL	59
	Ab@Lipo-NMP-GO	20 μg	0.5 mL	34
	Ab@beads	100 μg	1.0 mL	0
	Ab@beads	20 μg	1.0 mL	0
CRC-S1	Ab@Lipo-NMP-GO	20 μg	0.9 mL	67
	Ab@beads	100 μg	0.9 mL	3

CRC-S2	Ab@Lipo-NMP-GO	10 μg	0.5 mL	88
CRC-1	Ab@Lipo-NMP-GO	20 μg	1.0 mL	43
	Ab@beads	20 μg	1.0 mL	2
	Ab@beads	100 μg	1.0 mL	3
CRC-2	Ab@Lipo-NMP-GO	20 μg	1.0 mL	189
	Ab@beads	20 μg	1.0 mL	1
	Ab@beads	100 μg	1.0 mL	2
CRC-3	Ab@Lipo-NMP-GO	20 μg	1.0 mL	207
	Ab@beads	20 μg	1.0 mL	3
CRC-4	Ab@Lipo-NMP-GO	20 μg	0.5 mL	316
	Ab@beads	20 μg	0.5 mL	5
Lung -1	Ab@Lipo-NMP-GO	20 μg	1.0 mL	558
	Ab@beads	20 μg	1.0 mL	69

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