ACS APPLIED MATERIALS & INTERFACES

Research Article

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In Vivo Capture of Circulating Tumor Cells Based on Transfusion with a Vein Indwelling Needle

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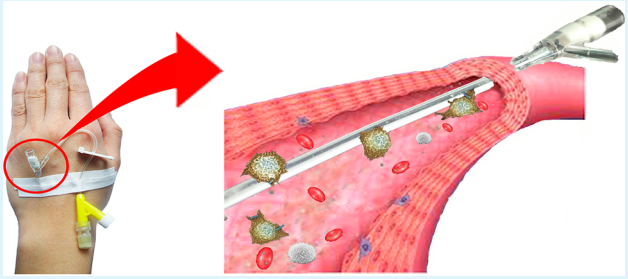
Supporting Information

ABSTRACT: Detection of circulating tumor cells (CTCs) could be used as a "liquid biopsy" for tracking the spread of cancer. *In vitro* detection methods based on blood sampling and *in vitro* CTC capture often suffer from the small sampling volume and sampling error. Here, the *in vivo* capture of CTCs based on transfusion with a surface-modified vein indwelling needle is proposed. When the needle was applied to transfusion in the vein, the simultaneous capture of CTCs was performed. To investigate the actual capture efficiency of the *in vivo* capture method, labeled MCF-7 cells were directly injected into the veins of rabbits, wild type mice, and nude mice and could be successfully captured. Two of 5 MCF-7 cells injected into the veins of nude mice were successfully captured. To investigate the CTC capture of mouse tumor model and compare with the *in vitro* method, mice were subcutaneous inoculated with metastatic 4T1 cells. Seven and 21 days after inoculation, CTCs were captured for the first time using *in vivo* and *in vitro* methods, respectively. This predicted that the *in vivo* method could be more suitable for use of early diagnosis of cancer than the *in vitro* method. As CTC capture can be performed at the same time as transfusion and does not cause further bodily harm, it would be easily accepted by patients. This efficient, simple, and less damaging method involving the use of a vein indwelling needle could be popularized easily in the clinic.



KEYWORDS: *cancer diagnosis, circulating tumor cell, in vivo, vein indwelling needle, transfusion*

1. INTRODUCTION

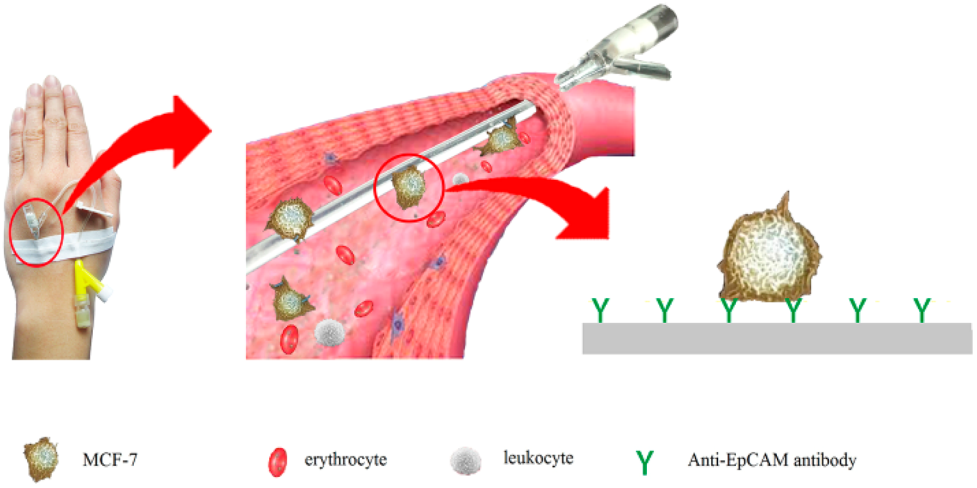
It has been recognized for decades that cancers metastasize because primary tumors shed cells into the blood, carrying them to other organs, where they seed new tumors.[1,2] It has recently been determined that the capture and counting of circulating tumor cells (CTCs) can be used as a new "liquid biopsy" technique for tracking the spread of cancer or predicting the survival benefit of treatments.[3−7] Currently, most CTC detection methods are based on the sampling of several milliliters of human blood and *in vitro* capture of CTCs.[8−14] We believe that the fundamental sampling principle of analytical chemistry is that the samples should be representative. Due to the irregular shedding of CTCs, a cluster of cells may be shed into the blood vessel erratically. As such, the distribution of CTCs in the blood is nonuniform. Accordingly, the random sampling of several milliliters of human blood from the vein is not representative and may lead to false-negative results.[15−18] Even if the established analytical methods could detect one cell in several milliliters of human blood, the result is not exact due to the nonuniform distribution of CTCs in the blood throughout the body.

To avoid sampling errors, schemes for assessing a large blood volume *in vivo* using the principles of *in vivo* flow cytometry with photothermal,[19] photoacoustic,[20] or fluorescent detection[18,21] have been proposed. These *in vivo* tests have the capability to monitor the dynamics of CTCs continuously and noninvasively. However, the CTCs can not be captured for further *in vitro* characterization and component analysis by the various molecular profiling techniques currently under development. Saucedo-zeni et al.[15] functionalized a structured medical Seldinger guidewire (FSMW) with antiepithelial cell adhesion molecule (EpCAM) antibody to capture and enrich CTCs *in vivo*. The FSMW was inserted into the cubital veins of patients to capture CTCs. After removal, the CTCs were identified by immuno-cytochemical staining of EpCAM and/or cytokeratins. Wang et al.[16] used a nylon wire modified with an anti-EpCAM antibody for the *in vivo* capture of CTCs. The capture ability was demonstrated in a nude mouse tumor model, but the

Received: July 28, 2015
Accepted: August 28, 2015
Published: August 28, 2015

DOI: 10.1021/acsami.5b06874
ACS Appl. Mater. Interfaces 2015, 7, 20477−20484

Scheme 1. Schematic of the *in Vivo* CTC Capture



capture efficiency was not exactly tested and compared with *in vitro* capture methods. Additionally, these types of *in vivo* capture methods are independent processes that would cause further damage and may result in some obstacles for acceptance by patients.

Here, the *in vivo* capture of CTCs based on transfusion with a vein indwelling needle is proposed (Scheme 1). CTC capture was performed at the same time as transfusion, and there was no additional bodily harm. As such, it would be easily accepted by patients. The vein indwelling needle was modified with an anti-EpCAM antibody, which could specifically recognize the EpCAM antigen on the surface of CTCs. When this surface-modified vein indwelling needle was applied to transfusion in the vein, the simultaneous capture of CTCs was performed. When the vein indwelling needle was drawn out, the captured CTCs were cleared from the vein and could be eluted for further microscope counting or component analysis.

The vein indwelling needle could be kept in the vein for 3 days, and the constant contact of blood passing the needle would help to improve the capture efficiency. With the advantages of being efficient, simple, and less damaging and given the widespread use of vein indwelling needles, this *in vivo* capture method of CTCs based on current transfusion protocols could be popularized easily and shows good application prospects.

2. EXPERIMENTAL SECTION

2.1. Materials and Reagents. Vein indwelling needles (Vialon and Teflon; 24 G × 0.75, 0.7 mm × 19 mm) were separately purchased from Jiangxi San Xin Medical Polytron Technologies Inc. (Jiangxi, China) and Suzhou Bi Di Medical Devices Co. Ltd. (Suzhou, China). Polydimethylsiloxane (PDMS) prepolymer and its curing agent, Sylgard 184, were obtained from Dow Corning (Midland, U.S.). PVA (poly(vinyl alcohol), MW = 72 000 g/mol) was purchased from Sangon Biotech (Shanghai, China). Anti-EpCAM antibody was purchased from Beijing Boosen Biological Technology Co. Ltd. (Beijing, China). Alexa Fluor 488-anti-cytokeratin8 antibodies and fluorescein isothiocyanate (FITC)-anti-CD45 antibodies were purchased from abcam and eBioscience. MCF-7 cells were purchased from KeyGEN Biotech (Nanjing, China). Dulbecco's modification of Eagle's medium Dulbecco (DMEM) culture media was purchased from Thermo Fisher biochemical products Co., Ltd. (Beijing, China). 4T1 cells and RPMI-1640 culture media were purchased from Cell bank of Chinese Academy of Sciences (Shanghai, China). Glutaraldehyde, Irgacure 2959 (2-hydroxy-4′-(2-hydroxyethoxy)-2-methyl-propiophenone), PEGda (poly(ethylene glycol) diacrylateaverage, Mn 575), FITC, Janus Green B, and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St. Louis, U.S.). BALB/c nude mice were purchased from Changzhou Cawensi Lab Animal Co. Ltd. (Changzhou, China). Rabbits were purchased from Shizhong Yunxiang poultry farms (Jinan, China). Mice were purchased from the laboratory animal center of Shandong University (Jinan, China).

2.2. Cell Culture, Staining, Observation, and Counting. The commercially available breast carcinoma cell lines, MCF-7 cells and 4T1 cells, were separately maintained in DMEM and RPMI-1640 culture media supplemented with 10% fetal bovine serum, cultivated at 37 °C and incubated in 5% CO_2 conditions. Adherent cells were harvested by trypsinization. Either 200 μL of 0.2 mg·mL^{-1} Janus Green B or 20 μL of 1 mg·mL^{-1} DAPI was added to 200 μL of cell suspension (approximately 5 × 10^5 cells·mL^{-1}), followed by a 15 min incubation at room temperature and washing three times with PBS.

The stained cells used for the spiking or injection experiments were accurately counted on the ELISA plate under an inverted fluorescence microscope (Leica Microsystems Ltd., Wetzlar, Germany). Then, the counted cells were washed three times with physiological saline to ensure their complete transfer to the syringe.

The cells captured on the vein indwelling needle were eluted with pH 2.7, 0.1 mol·L^{-1} Gly-HCl to an ELISA plate hole, immediately neutralized with NaOH solution, placed for 10 min, and then observed under an inverted fluorescence microscope. The cell survival rate was determined by MTT assay.

2.3. Human Blood Samples. The human blood used to simulate blood circulation was obtained from healthy participants at Shandong Normal University (Shandong, China). In all cases, informed written consent was obtained from all participants. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the academic committee of Shandong Normal University approved the experiments.

2.4. Animal Experiments. Adult (female and male, 10-week-old, 20 ± 2 g) BALB/c nude mice were fed and maintained under specific pathogen-free conditions at constant temperature (24−26 °C) and humidity (30−50%). Sterilized food and tap water were given *ad libitum*. Adult rabbits (female and male, 2000 ± 100 g) or wild type mice (female and male, 7-week-old, 25 ± 2 g) were fed and maintained under general animal breeding conditions. All animal experiments were conducted at the laboratory animal center of Shandong Normal University in compliance with the Guidelines of Shandong Normal University for the Care and Use of Laboratory Animals.

2.5. Surface Modification of Vein Indwelling Needle. The surface modification procedure of the vein indwelling needle included coating it with PDMS/PVA/PEGda and the adsorption of the anti-EpCAM antibody. To coat the vein indwelling needle with PDMS, the

DOI: 10.1021/acsami.5b06874
ACS Appl. Mater. Interfaces 2015, 7, 20477−20484

needle was immersed into a mixture of PDMS prepolymer and its curing agent at ratios of 5:1, 8:1, and 10:1. The needle was rotated clockwise to ensure better coating, after which it was withdrawn and cured for 3 h in a convection oven at 65 °C.[22,23] To coat the needle with PVA, it was immersed into the PVA solution (5, 10, and 15 wt % PVA dissolved in distilled water at 95 °C). It was then taken out and immersed in a solution of 50% (v/v) glutaraldehyde and 50% (v/v) hydrochloric acid at room temperature for 3 h.[24] For coating with PEGda, the needle was immersed into a solution of 750 mg·mL^{-1} Irgacure 2959 in PEGda and cross-linked at a wavelength of 365 nm with a UV light.[25]

To adsorb antibody, the coated needles described above were then immersed into a solution of anti-EpCAM antibody, gently shaken for 2 h, taken out, washed 3 times with PBS, and then placed at 4 °C for cell capture. To test the amount of antibody adsorbed on the needle, anti-EpCAM antibody was labeled with FITC by first adjusting the pH of the antibody solution (1 mg·mL^{-1}) to 8.5−9.0 with 1 mol·L^{-1} Na_2CO_3. Then, 1 mg of FITC was added per milligram of antibody, and the mixture was incubated for 10 min at room temperature with intermittent shaking. The sample was dialyzed at 4 °C for 3 days with PBS, which was changed three times daily and then placed at 4 °C for cell incubation. The nude or PDMS coated needles were immersed into the FITC-conjugated antibody solution, gently shaken for 2 h, taken out, and washed with eluents, after which the fluorescence intensities were assessed with a FLS-920 Edinburgh Fluorescence Spectrometer.

2.6. *In Vitro* Capture Method Based on Immune-Magnetic Microbead. According to the sampling volume of 7.5 mL relative to the 4000−5000 mL of blood throughout the body of an adult, not less than 4 μL of blood should be sampled for the 2 mL of blood throughout the body of a mouse. Thus, a 5 μL blood sample of each tumor mouse was collected and then mixed with anti-EpCAM antibody conjugated magnetic microbeads for 4 h at room temperature. Magnetic separation was carried out. Separated cells were fixed, permeabilized, and identified as the following method described in Identification of CTCs Captured from the Vein of a Mouse Tumor Model.

2.7. Mouse Tumor Model and CTC Capture. We subcutaneously inoculated the armpit of the right anterior limb of some BALB/c mice with 2 × 10^5 4T1 tumor cells.[26] On the scheduled days after subcutaneous inoculation of 4T1 cells, CTCs were simultaneously captured using *in vivo* and *in vitro* methods.

2.8. Identification of CTCs Captured from the Vein of a Mouse Tumor Model. According to the report of Nagrath et al.,[8] CTCs captured from the vein of a mouse tumor model were fixed in paraformaldehyde (4 wt %) for 15 min, permeabilized with 0.5% PBS-Triton X-100 for 30 min, and then identified using a comprehensive image analysis algorithm, consisting of staining with DAPI for DNA content, using Alexa Fluor 488-anti-cytokeratin8 antibodies for epithelial cells and FITC-anti-CD45 antibodies for hematologic cells. Cells captured and stained with cytokeratin8 were scored as CTCs, whereas CD45-positive cells were scored as normal hematologic cells.

3. RESULTS AND DISCUSSION

3.1. Surface Modification and Characterization of the Vein Indwelling Needle. To specifically capture CTCs, the vein indwelling needle should be modified with anti-EpCAM antibody. The direct adsorption of the anti-EpCAM antibody onto vein indwelling needles by physical action was compared among the needle treatments. The results showed that the nude needles adsorbed very few anti-EpCAM antibodies and captured almost no cells (Figure 1a). As the Vialon (polyurethane) and Teflon (polytetrafluoroethylene) needles were all chemically inert and difficult to modify chemically, physical coatings were used to change the needles' surface adsorption performance. Vialon needles, which are most commonly used in the clinic, were selected for further coating experiments. PDMS, PVA, and PEGda were further selected to coat the

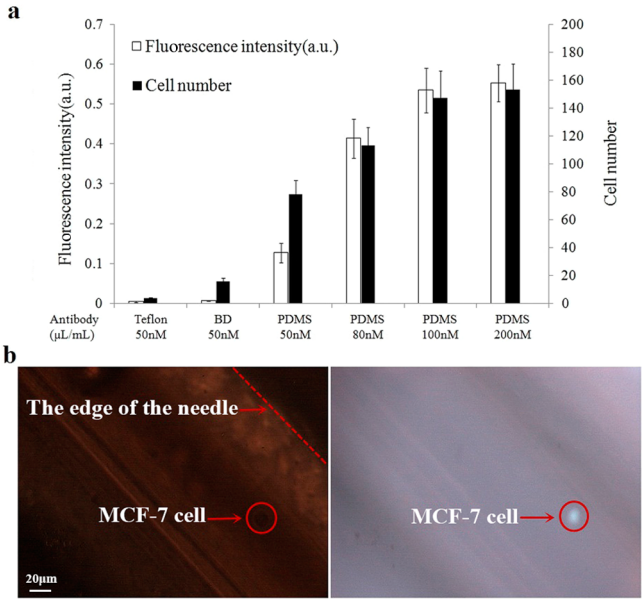


Figure 1. (a) Optimization of the antibody adsorbing and cell capture. (b) Microscope images of the DAPI stained MCF-7 cells captured on the needle. The error bars were estimated from three replicate measurements.

needles due to their good biocompatibility, high flexibility, low toxicity, and easy synthesis.[22−25] PEGda solution was more prone to forming droplets and could not adhere evenly to the surface of the needle. As such, it was rejected (Figure 2a). Visible roughness and uneven color were observed on the surfaces of the needles coated with PVA (5%, 10%, and 15%) (Figure 2a). Cross sections of the needles coated with different materials (5:1, 8:1, and 10:1 mixing ratios of PDMS prepolymer and its curing agent; 5%, 10%, and 15% of PVA) were observed using SEM to investigate whether the synthesized materials and needles fitted together closely. The SEM images, shown in Figure 2b, demonstrated obvious delamination and gaps between PVA (10%), PDMS (8:1 and 5:1), and the corresponding coated needles. The gauges of the PDMS (5:1) and PVA (15%) coatings were uneven, and PVA (5%) coating exhibited desquamation from the needle. Thus, PDMS coating, which was smooth and flat, had uniform thickness, and exhibited well meshing with the needle when synthesized at a mixing ratio of 10:1, was selected for further experiments (Figure 2a,b).

To investigate the changes of the PDMS coated needles following penetration and maintenance in the vein for a much longer time, 10 PDMS coated needles were inserted into the veins of 5 rabbits' ears and kept for 1, 2, 3, 4, and 5 days, respectively. The SEM images in Figure 2c showed the delamination and gaps observed between the PDMS and the corresponding coated needles after 4 days indwelling and the partial desquamation of the PDMS from the needle after 5 days indwelling in the vein. As such, 3 days indwelling in the vein, which resulted in no obvious changes, was suggested for clinical applications.

PDMS coated on the surface of the needle could directly adsorb the anti-EpCAM antibody, and the adsorbed concentration of anti-EpCAM antibody was optimized. Anti-EpCAM antibody was labeled with FITC, so that the amount of antibody adsorbed could be determined according to the FITC fluorescence intensity eluted from the needle. The fluorescence

DOI: 10.1021/acsami.5b06874
ACS Appl. Mater. Interfaces 2015, 7, 20477−20484

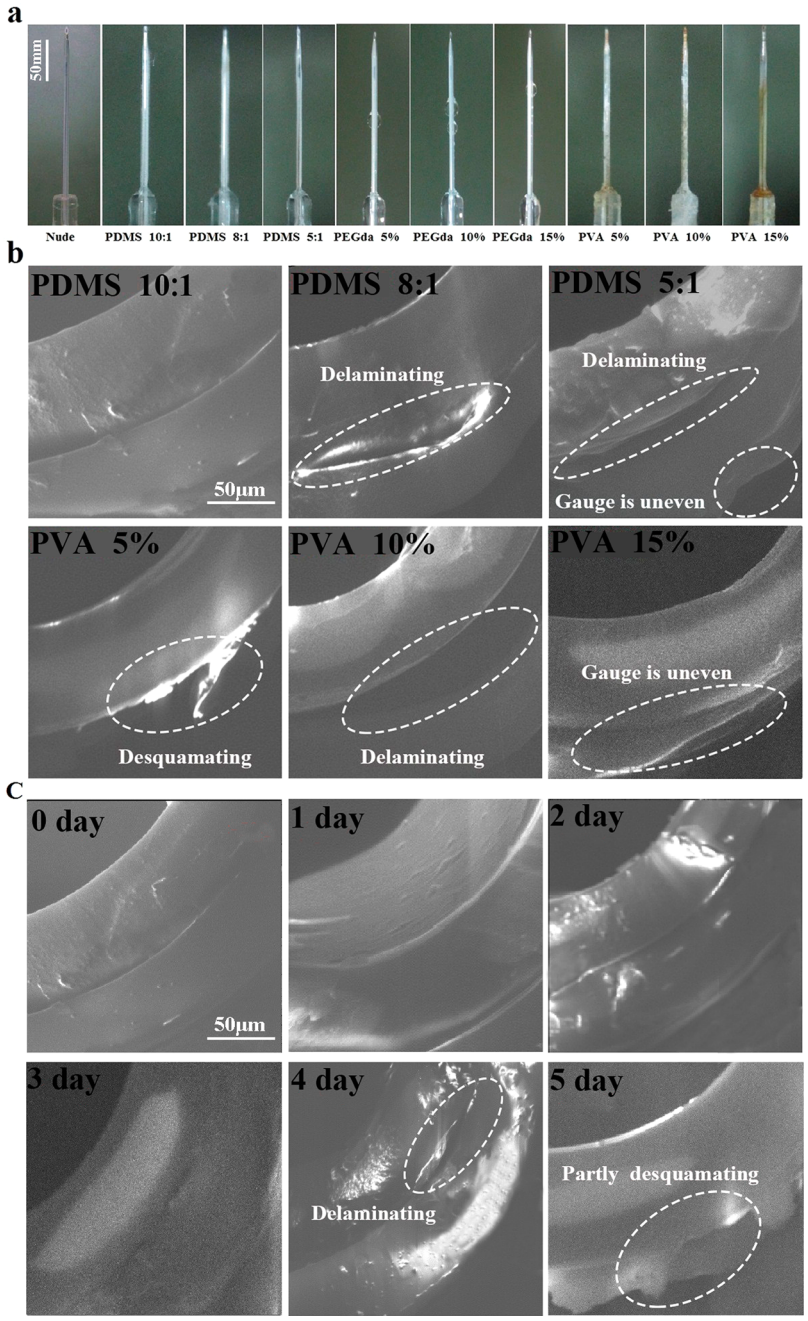


Figure 2. (a) Appearance of the coated needles. (b) SEM images of the cross sections of the coated needles. (c) Influence of vein indwelling time on the coated needle.

intensities increased with the concentration of the FITC-anti-EpCAM antibody (Figure 1a). The efficiency of CTC capture by the antibody adsorbed needles were also investigated. When adsorbed with 100 $\mu g \cdot mL^{-1}$ antibody, the captured cell numbers reached their maximum (Figure 1a). To assess the nonspecific adsorption of blood cells, the modified needles were immersed in human blood for 30 min. After washing with physiological saline to remove the physical adsorption, no blood cells were eluted with Gly-HCl (pH 2.7, 0.1 $mol \cdot L^{-1}$).

3.2. Cell Staining for Tracking the Injected MCF-7 Cells. To test the *in vivo* capture efficiency, MCF-7 cells were first injected into a vein and captured with a vein indwelling needle in another vein. To ensure that the cells captured and counted were those injected into the vein, the MCF-7 cells were labeled by staining, with as few cells as possible damaged in the process. Furthermore, due to the long time required for the maintenance of the vein indwelling needle *in vivo*, it was necessary to maintain the color or fluorescence of the stained cells for as long as possible. Two types of living-cell staining methods were tested. Cells stained with Janus Green B were difficult to distinguish from impure substances and destained within an hour of being in the blood circulation. Those stained with DAPI could be easily distinguished and maintained observable fluorescence after 3 h in circulation in simulated human blood circulation (Figure 3c) or rabbit blood circulation (Figure S1). Considering the factors involved in the staining method, the animal tolerance duration of the needle, and the cell survival time in blood circulation, the subsequent *in vivo*

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ACS Appl. Mater. Interfaces 2015, 7, 20477−20484

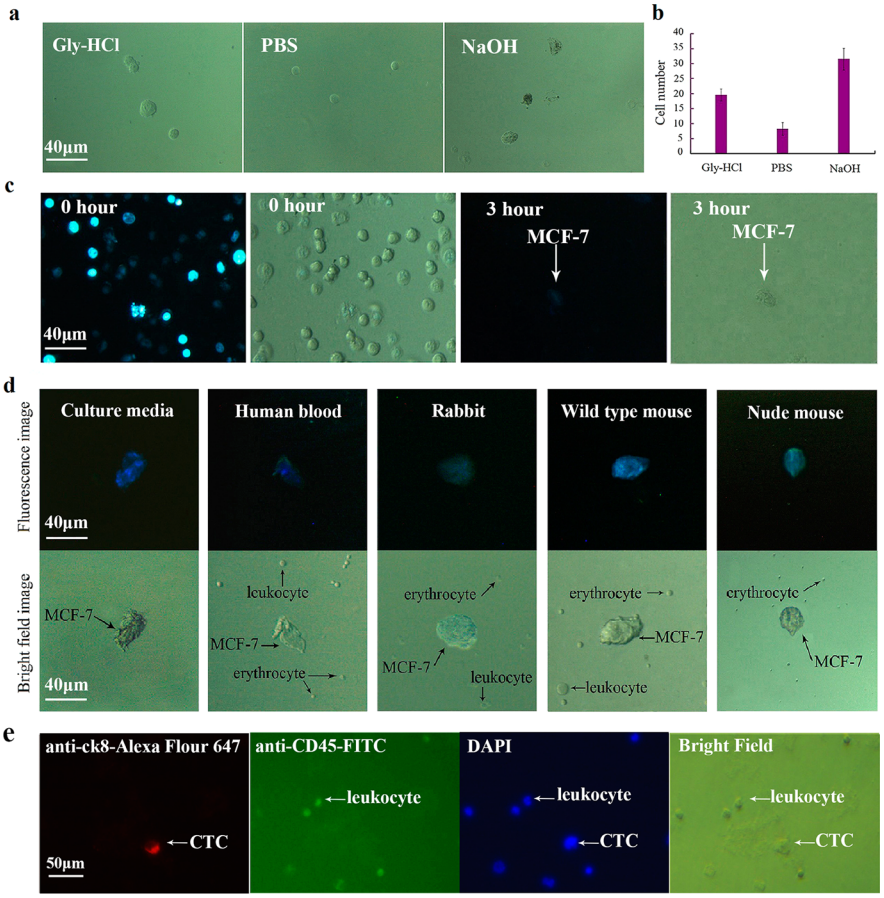

Figure 3. (a) Cellular morphology following elution with different eluting solutions. (b) The number of eluted cells with different eluting solutions. The error bars were estimated from three replicate measurements. (c) Microscope images of DAPI stained MCF-7 cells before and after 3 h of circulation in human blood (simulated blood circulation): (1, 3) fluorescence image; (2, 4) bright field image. (d) Microscope images of DAPI stained MCF-7 cells captured from culture media, human blood, rabbits, wild type mice, and nude mice. (e) Microscope images of the identification of CTCs captured from the mouse tumor model.

test experiments lasted for 3 h. It is predicted that the capture efficiency will be higher when the vein indwelling needles are applied clinically during transfusion and CTC capture, which will last for 3 days.

3.3. Elution of the Captured Cells from the Needle. Figure 1b shows that the needle could successfully capture the labeled cells, but it was difficult to directly count the cells on the surface of the needle under the microscope. As such, eluting the captured cells from the needle, while maintaining good cellular morphology and ensuring high elution efficiency, is required. To optimize the elution conditions, three types of eluents (PBS, pH 2.7, 0.1 mol·L^{-1} Gly-HCl, and 0.1 mol·L^{-1} NaOH) were tested. The highest elution efficency was obtained when eluting with NaOH (Figure 3b), but this also resulted in serious cell damage (Figure 3a). Cells eluted with Gly-HCl and PBS maintained good cellular morphology (Figure 3a); the elution efficiency of Gly-HCl was higher than PBS (Figure 3b), so Gly-HCl was selected as the eluent.

3.4. Evaluation of Capture Efficiency. Considering that the MCF-7 cells injected into the vein will be partly damaged or cleared, to accurately investigate the capture efficiency of the vein indwelling needle, veins and blood circulation were simulated with perfusion tubes and a constant-flow pump (Supplementary Movies 1 and 2). The diameter of the perfusion tube (4 mm) and the flow velocity (20 mL/min) were set according to the diameter (3−5 mm) and blood flow velocity (<90 mL/min) of the point of intravenous injection on the hand veins. The MCF-7 cells, which were suspended in culture media, were drawn into the perfusion tube and circulated using a constant-flow pump. The vein indwelling needle was inserted into the perfusion tube, so that the circulating cells would be recognized by the antibody on the surface of the needle. This configuration was used to test the actual capture efficiency. The results shown in Table 1 and Figure 3d indicated that the vein indwelling needle could successfully capture the MCF-7 cells in the simulated blood circulation. Owing to the limited surface area and capture capacity of the vein indwelling needle, a greater number of

Table 1. Capture Efficiencies of the Spiked MCF-7 Cells in Simulated Blood Circulation

	spiked cell numbers	mean numbers of the captured cells (n = 3)	capture efficiencies (%)
DMEM culture media	50	14	27.3 ± 14.5
	200	67	27.8 ± 9.5
	500	86	17.2 ± 6.8
human blood	50	8	16.0 ± 5.7
	500	47	9.3 ± 2.1
	1000	55	5.5 ± 1.1
	5000	81	1.6 ± 0.7

DOI: 10.1021/acsami.5b06874
ACS Appl. Mater. Interfaces 2015, 7, 20477−20484

spiked cells led to lower capture efficiencies. In fact, as the number of CTCs in human blood circulation is minuscule, the above efficiency loss should not have any tangible impact. Due to the lack of clinical safety evaluation, human testing could not be performed. As such, MCF-7 cells that were spiked into human blood were applied to the above simulation and used to investigate the influence of erythrocytes, leukocytes, and other interferents in human blood. The results in Table 1 and Figure 3d also demonstrate that MCF-7 cells spiked into human blood could still be captured, although with some efficiency losses.

3.5. *In Vivo* Capture of Circulating Mcf-7 Cells in Rabbits and Wild Type Mice. After confirmation of successful capture with a vein indwelling needle, *in vivo* CTC capture was tested in rabbits and wild type mice. The DAPI stained MCF-7 cells were injected into the left ear veins of the rabbits or the tail veins of the wild type mice. The vein indwelling needles were inserted into the right ear veins of the rabbits or different tail veins of the wild type mice to capture the injected circulating MCF-7 cells (Figure S2a–d). The injected length of the indwelling needle was about 1.7 cm to gain the best capture efficiency. After 3 h of circulation *in vivo*, the needles were withdrawn from the veins, and the cells were eluted and counted. The results in Table 2 and Figure 3d showed that the vein indwelling needles could successfully capture the injected MCF-7 cells circulating in the veins of rabbits and wild type mice.

Table 2. *In Vivo* Capture Efficiencies of the Circulating MCF-7 Cells

	injected cell numbers	mean numbers of the captured cells (n = 3)	capture efficiencies (%)
rabbits	50	6	11.0 ± 4.2
	500	24	4.9 ± 1.4
	1000	34	3.4 ± 1.8
	5000	95	1.9 ± 0.6
wild type mice	50	8	15.0 ± 7.1
	500	26	5.3 ± 0.6
	1000	31	3.1 ± 1.0
	5000	47	0.9 ± 0.1
nude mice	5	2	33.0 ± 23.1
	25	8	32.0 ± 10.8
	50	14	28.0 ± 6.9
	200	34	16.8 ± 4.6
	500	46	9.3 ± 0.3

3.6. *In Vivo* Capture of Circulating MCF-7 Cells in Nude Mice. To further eliminate the effects of the animal's immune system, immunodeficient nude mice were used to test the capture efficiency of CTCs. MCF-7 cells were injected into the tail veins of the nude mice, and the vein indwelling needles were inserted into different tail veins of the nude mice (Figure S2e,f). Because the injected MCF-7 cells would not be cleared by the immune system in nude mice, the capture efficiency was expected to be higher than that in the wild type mice. This expectation was confirmed by the results shown in Table 2. No leukocytes were present in the immunodeficient nude mice (Figure 3d).

3.7. *In Vivo* CTC Capture of Cancer Mouse Model. To investigate the *in vivo* capture of actual CTCs in the blood circulation of a cancer mouse model, the identification of CTC staining with CK8, CD45, and DAPI was applied to the captured cells. To find the first time point of successful CTC capture during the cancer process, the numbers of captured and identified CTCs were counted at 6, 7, 8, 11, 14, 21, and 28 days after subcutaneous inoculation of 4T1 cells. The indwelling time of the needle, namely, the CTC capture time, was 3 h. Results in Table 3 showed that CTCs could be captured by the seventh day after the cancer cell inoculation, when the carcinomas in situ were less than 2 cm and cancer metastasis had not be found. The number of CTCs was increased with the cancer developing process. This indicated to us that the *in vivo* method could capture CTCs at the beginning stages of cancer and thus is promising as an early diagnosis method.

3.8. Comparison with the *in Vitro* Detection Methods. The *in vivo* capture efficiencies seemed far lower than those obtained by *in vitro* methods (generally higher than 80%). The efficiencies were generally calculated by the division of the number of cells captured by the total number spiked into the blood according to eqs 1 and 2. The only difference in the efficiency calculation for the *in vivo* and *in vitro* methods was the denominator. The denominator for the *in vivo* method was the number of cells spiked into the blood throughout the body, but for the the *in vitro* method, it was the number of cells spiked into the blood sampled from the blood circulation. To compare these two methods equivalently, assume there are 500 cells in the blood circulation and detected by these two methods. For the *in vitro* method, assuming the cells were uniformly distributed in the blood circulation and the blood volume throughout the body was 4000–5000 mL for an adult, there would be 1 cell/8–10 mL of blood. When 1 or 7.5 mL of blood was sampled according to reported procedures,[8−10,14] there would be a high probability that the sample would contain no cells, leading to unacceptable false-negative results.

Table 3. *In Vivo* CTC Capture during the Cancer Progress and Comparison with *in Vitro* Capture Methods[a]

	in vivo capture of CTCs								comparison of *in vivo* (column a) and *in vitro* (column b) capture of CTCs											
									1		2		3		4		5		6	
	1	2	3	4	5	6	7	8	a	b	a	b	a	b	a	b	a	b	a	b
6 d	/	/	/	/	/	/	0	/	/	/	/	/	/	/	/	/	/	/	/	/
7 d	/	/	/	/	/	/	0	1	2	0	0	0	1	0	1	0	1	0	0	0
8 d	1	1	/	/	0	3	/	/	/	/	/	/	/	/	/	/	/	/	/	/
11 d	5	6	/	/	/	/	2	1	/	/	/	/	/	/	/	/	/	/	/	/
14 d	/	/	0	0	9	16	43	13	10	0	5	0	5	0	6	0	9	0	10	0
21 d	20	50	17	20	41	35	65	54	34	0	10	0	17	2	21	0	31	0	47	0
28 d	73	82	75	82	*Die*	96	70	82	51	5	39	11	40	8	83	12	59	15	61	0

[a]"/" represents without capture.

DOI: 10.1021/acsami.5b06874
ACS Appl. Mater. Interfaces 2015, 7, 20477−20484

As such, although the efficiency of the *in vitro* method was 100%, the detection remained inaccurate. Namely, CTCs remain undetectable until their numbers are higher than 500 in an average adult blood volume of 4000−5000 mL. In particular, the potential for real time diagnosis of metastatic diseases at earlier stages is hindered by low cell counts in small volumes acquired at fixed time points.[27] However, for the *in vivo* method, the efficiency was relative to the blood volume throughout the body. Accordingly, for the same number of cells (500) injected into the animals, the efficiencies were 5.3 ± 0.6% for wild type mice and 4.9 ± 1.4% for rabbits. Specifically, 26 ± 3 cells and 24 ± 7 cells were captured from mice and rabbits, whose blood volumes were approximately 2 and 200 mL, respectively.

$$\text{the capture efficiency of } \textit{in vivo} \text{ method} = \text{the number of captured cells/the number of cells spiked into the blood throughout the body} \quad (1)$$

$$\text{the capture efficiency of } \textit{in vitro} \text{ method} = \text{the number of captured cells/the number of cells spiked into the blood sampled from the blood circulation} \quad (2)$$

On the basis of the above theoretical analysis, further experimental comparison of the *in vivo* and *in vitro* CTC capture was investigated using the cancer mouse model. Six cancer mouse models were tested during the cancer process. The most frequently used immune-magnetic microbead based *in vitro* method was selected as the comparison method. Results in Table 3 showed that the *in vivo* CTC capture method could capture CTCs at a far more earlier stage of the cancer process and capture far more CTCs at the same stage as the *in vitro* CTC capture method.

3.9. Comparison with Other *in Vivo* Detection Methods. For other *in vivo* methods, an adequate or ambiguous amount of labeled cells were injected into the blood circulation of animals and detected *in vivo*. As such, the capture efficiency or recovery could not be exactly calculated,[15,16,18−21] and the advantages compared with the *in vitro* method and the value of acting as an early diagnosis method of cancer could not be well evaluated. Here, different numbers of labeled cells (5−5000), which were exactly counted beforehand, were injected into the circulation of three types of animals (rabbit, wild type mouse, and nude mouse), and the exact capture efficiencies were tested. The numbers of CTCs were captured at 7, 14, 21, and 28 days after the cancer cell inoculation using *in vivo* and *in vitro* methods during the cancer process. As such, the *in vivo* and *in vitro* methods could be estimated by comparing the capture efficiency.

3.10. Investigation of the Side Effects of the *in Vivo* CTC Capture Method. To investigate the side effect of the coating of the needle with PDMS and adsorbed antibody, unmodified and modified needles were compared. The modified needle did not adsorb more erythrocytes or leukocytes than the unmodified needle. This result may have been observed because the anti-EpCAM antibody on the PDMS played a role in blocking nonspecific cell adsorption. Additionally, as the anti-EpCAM antibody has previously been used as a drug in cancer therapy,[28−30] there should be no unacceptable side effects caused by the surface modification.

4. CONCLUSIONS

In summary, the *in vivo* CTC capture method, which is combined with the process of transfusion with a vein indwelling needle, solved the problem of sampling error and improved the accuracy of the detection method. Experiment results of the *in vivo* CTC capture of the cancer mouse model predicted that the *in vivo* method could be more suitable to be used as an early diagnosis of cancer than the *in vitro* method. This efficient, simple, and less damaging method could be popularized easily, as the use of vein indwelling needles is widespread in clinical practice.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b06874.

Further details of the description of cell staining for circulation and modeled blood circulation (PDF)

Simulation of veins and blood circulation with culture media, perfusion tubes and a constant-flow pump (AVI)

Simulation of veins and blood circulation with human blood, perfusion tubes and a constant-flow pump (AVI)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by 973 Program (2013CB933800) and National Natural Science Foundation of China (21535004, 21227005, 21390411, 21105058).

■ ABBREVIATIONS

CTCs, circulating tumor cells

FSMW, functionalized a structured medical Seldinger guidewire

EpCAM, epithelial cell adhesion molecule

PDMS, polydimethylsiloxane

FITC, fluorescein isothiocyanate

DMEM, Dulbecco's modification of Eagle's medium

DAPI, 4′,6-diamidino-2-phenylindole

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DOI: 10.1021/acsami.5b06874
ACS Appl. Mater. Interfaces 2015, 7, 20477−20484