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High purity microfluidic sorting and in situ inactivation of circulating tumor cells based on multifunctional magnetic composites

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ABSTRACT

Detection and isolation of circulating tumor cells (CTCs) play a pivotal role in the diagnosis and prognosis of cancer, while the high capture efficiency and purity of CTCs are difficult to achieve simultaneously among the various isolation methods. In this work, we designed an inverted microchip integrating silicon nanowires (SiNWs) and multifunctional magnetic nanocomposites (Fe_3O_4@C6/Ce6@silane, Coumarin 6 (C6), Chlorin e6 (Ce6)) for enhanced capture efficiency and purity of CTCs. The Fe_3O_4@C6/Ce6@silane conjugated with antibody can label the CTCs and pull them to the upside SiNWs capture surface by the upward magnetic field with high purity. This inverted structure was also featured with real-time detection and photodynamic therapy (PDT) of CTCs with the confocal laser scanning microscope (CLSM). The results indicate the important role of the composites labels and the magnetic field, which greatly improves the capture purity of the CTCs to 90%. Meanwhile, capture efficiency of CTCs achieve to 90.3% in culture medium and 82% in blood with 2 mL/h flow rate, respectively. Based on the structure of the device and composites, the captured CTCs could be directly inactivated by the in situ photodynamic therapy in the capture process which holds positive impact to block cancer spread.

1. Introduction

The prominent reason for high mortality of cancer-related death is cancer metastasis [1], which is driven by circulating tumor cells (CTCs) that shed from primary tumor mass into the bloodstream and then invade a new organ [2]. CTCs have been thought to hold significant medical insights in cancer diagnosis [3] and treatment [4]. The challenges associated with CTCs detection and analyses begin with the natural scarcity of CTCs, estimated as one to few CTCs among millions of white blood cells (WBCs) and billions of erythrocytes [5]. Presently, CTCs isolation methods with high sensitivity [6], efficiency [7], and reliability are in demand [8]. Several separation techniques [9], such as immunomagnetic [10], filtration [11], and microfluidic devices [12], have been developed based on physical parameter [13]and immunoassays [14]. Nagrath et al. [15,16] focused on the microchannel-based approaches, which were designed and fabricated with various three-dimensional substrates [17] by photoengraving [18] or chemical synthesis [19]. Afterwards, a number of other three-dimensional structures [20] have been designed to increase the separation efficiency [21]. Silicon nanowires [22] (SiNWs) and hierarchical structure [23] developed by Wang et al. [24] show improved the sensitivity of detection due to their high surface area to volume ratio and similar size to biomolecules. Recently, quartz nanowires [25], TiO_2 nanofibers [26], and graphene oxide nanosheets [27] were also used to recognize tumor cells with high capture efficiency.

In spite of the significant progress on nanostructure based CTCs isolation [28], some hindrances related to the rarity and heterogeneity of CTCs still remain and two crucial problems need to be addressed. One is insufficient to compensate for the CTCs capture efficiency and purity, especially for the separation from WBCs [29,30] which may confuse CTCs detection and complicate subsequent analysis [31]. The other is the difficulty of the real-time

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monitoring of CTCs, since the optimal platform relies on a capture layer of maximum cell contact, which, however, is always opaque [32] and set downside [33], utilizing the gravitational force. Note that cancer cell detection and real-time confirmation is desired for diagnosis and treatment.

In search of literature, few studies focused on in situ CTCs therapy during their detection probably because of the consideration of low cost and "point of care". However, for a long term consideration, CTCs detection is not enough and the subsequent inactivation is key important. It can be foreseeable that the meantime capturing and inactivating of CTCs in blood vessels will greatly prevent from the cancer metastasis [34].

Herein we demonstrated a versatile approach upon combination of microchip-based CTCs capture system with multifunctional magnetic assays for diagnosis and therapy, as shown in Fig. 1. It comprised an anti-EpCAM-Fe_3O_4@C6/Ce6@silane (anti-EpCAM, anti-epithelial cell adhesion molecule) and an inverted microchannel with upside SiNWs capture surface. The uniqueness includes several aspects: First, fluorescence from CTCs can be detected directly after capture for the real-time monitoring. Second, high purity of CTCs can be obtained due to the inverted microchannel design and the upward magnetic field, because CTCs are pulled up to the capture surface, while other cells and metabolites are pulled down by gravitational force. Third, by integration of photodynamic therapy (PDT) effect function in magnetic particles, *in vitro* in situ CTCs therapy was realized with confocal laser scanning microscope (CLSM) within microchip.

2. Experimental section

2.1. Synthesis of anti-EpCAM-Fe_3O_4@C6/Ce6@silane

Fe_3O_4 NPs were prepared by co-precipitation with $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$ and ammonium hydroxide solution under N_2 gas protection [35]. The as-prepared Fe_3O_4 NPs were mixed with oleic acid (OA), heated to 80 °C for 30 min under N_2 gas protection. The as-prepared OA–Fe_3O_4 NPs (5 mg/mL, 200 μL), trimethoxy (octadecyl) silane (7.5 mg/mL, 500 μL), Chlorin e6 (Ce6, 1 mg/mL, 200 μL) and Coumarin 6 (C6, 1 mg/mL, 20 μL) were mixed in tetrahydrofuran (THF) under sonication. After 10 min, mixing solution was rapidly injected to 5 mL water (pH ≈ 9, adjusted by addition of ammonium hydroxide and including poly-L-lysine (PLL) for changing surface charge of materials [36], the concentration of pLL is 50 μg/mL) for the hydrolyzing process at 30 °C for 3 h, and the solution was dialyzed for 24 h. In this case, Fe_3O_4@C6/Ce6@silane surface has positive charge, then 20 μl anti-EpCAM antibodies (1 mg/mL in phosphate buffer saline (PBS)) were added into 2 mL Fe_3O_4@C6/Ce6@silane, after reaction at 4 °C, excess antibodies were removed by centrifugation.

2.2. Preparation of the microfluidic device

Silicon nanowires (SiNWs) array were prepared by a wet chemical etching process [23]. Silicon wafers of [100]-oriented P-Si(resistivity of ca.5–10 Ω cm) were successively ultrasonicated in deionized water and ethanol, and then the silicon wafers were placed in Piranha solution (4:1 (v/v) H_2SO_4/H_2O_2) for 1 h. Subsequently, the silicon wafers were immersed into an etching solution containing 4.6 M HF and 0.02 M $AgNO_3$ under room temperature for 1 h, and rinsed with 15% (v/v) nitric acid and deionized water.

After preparing the SiNWs substrates, we employed *N*-hydroxysuccinimide/maleimide chemistry to introduce streptavidin onto the surfaces of the SiNWs substrate [15,23] for conjunction of biotinylated anti-EpCAM. The substrates were treated with 3-mercaptopropyl trimethoxysilane (MPTS, in ethanol, 4% (v/v)) and couple agent *N*-maleimidobutyryl oxysuccinimide ester (GMBS, in

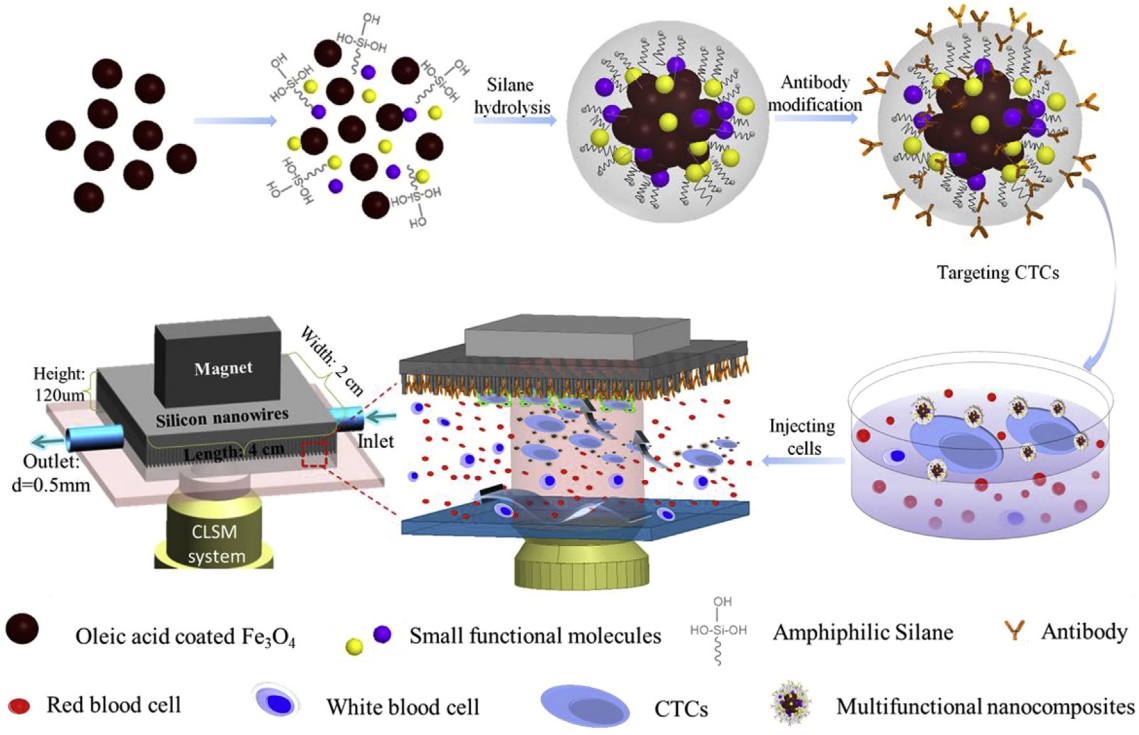

Fig. 1. Schematic of CTCs capture platform, involving the synthesis of the anti-EpCAM-Fe_3O_4@C6/Ce6@silane nanocomposites and microfluidics chip under the confocal laser scanning system. The nanocomposites was prepared by amphiphilic silane capsulation. After labeled with the nanocomposites, CTCs were injected into the novel microfluidic chip with an invert capture trap SiNWs and external magnet. Real-time capturing and in situ PDT treatment were performed with CLSM. The parameters of the chip are described.