Biomaterials 106 (2016) 78–86

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Multifunctional magnetic nanowires: A novel breakthrough for ultrasensitive detection and isolation of rare cancer cells from non-metastatic early breast cancer patients using small volumes of blood

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ARTICLE INFO

Article history:
Received 14 March 2016
Received in revised form
29 July 2016
Accepted 14 August 2016
Available online 16 August 2016

Keywords:
Circulating tumor cells
Magnetic nanowire
Conducting polymer
Colorimetric detection
Breast cancer

ABSTRACT

Circulating tumor cells (CTCs) are recognized as promising biomarkers for diagnosis and indication of the prognosis of several epithelial cancers. However, at present, CTC monitoring is available only for advanced-stage patients rather than for those at an early stage of cancer. This is because of the extraordinary rarity of CTCs and the limited sensitivity of current methods. Herein, we report the development of multifunctional magnetic nanowires for the efficient isolation and detection of CTCs from the blood of patients, especially those with non-metastatic early-stage cancer. The nanowires, which are equipped with a high density of magnetic nanoparticles and five different types of antibodies (Ab mixture_mPpyNWs), offer a significant improvement in cell-isolation efficiency, even from very small amounts of blood (250 μL–1 mL). Notably, CTCs were isolated and identified in 29 out of 29 patients (100%) with non-metastatic early breast cancer, indicating that this procedure allowed detection of CTCs with greater accuracy, sensitivity, and specificity. In addition, we demonstrated *in situ* "naked eye" identification of the captured cancer cells via a simple colorimetric immunoassay. Our results show that antibody-functionalized magnetic nanowires offer great potential for a broad range of practical clinical applications, including early detection, diagnosis, and treatment of cancer.

1. Introduction

The ability to isolate and detect rare cancer cells [i.e., circulating tumor cells (CTCs)] in peripheral blood offers great advantages in developing new approaches to cancer diagnosis and prognostic prediction. As the majority of cancer deaths in patients with solid tumors are strongly associated with metastasis, the potential for CTCs to serve as a non-invasive biomarker has been widely recognized and extensively investigated [1–4]. In recent years, various methods such as immuno-magnetic selection, microfluidic devices, and density gradient- or size-based filtration have been reported for the recovery and detection of CTCs [5–12]. However, despite considerable advances in identifying and enumerating CTCs, many technical barriers remain, which must be overcome for successful clinical application. A major challenge in CTC recovery is their extreme rarity in the bloodstream, estimated to be approximately 1–10 CTCs per 1 mL of whole blood in metastatic cancer patients [13]. Another obstacle is that CTCs exhibit significant phenotypic heterogeneity. There is considerable evidence that epithelial cells in the blood can undergo an epithelia-to-mesenchymal transition (EMT), increasing their invasiveness and metastatic ability, down-regulating the typical epithelial phenotype, and inducing the expression of a mesenchymal signature [14–16]. In addition, because contaminations in cell suspensions of non-specifically bound white blood cells (WBCs) may lead to false positive results, the purity of isolated CTCs is a challenge for subsequent molecular and other downstream analyses. In spite of these limitations, CTCs are still subjects of a variety of basic and clinical studies aimed at translational cancer research. Numerous studies have been conducted on CTC analyses, mostly with metastatic or advanced stage

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http://dx.doi.org/10.1016/j.biomaterials.2016.08.020
0142-9612/

cancer patients rather than those at an early stage of cancer because of the limited sensitivity of existing technologies [17–20]. To increase the utility of CTCs as a diagnostic and prognostic biomarker, the development of an ultrasensitive method to enrich and isolate all CTCs present in the peripheral blood of cancer patients is needed. Technological improvements will broaden the clinical availability and applicability of CTCs to a range of patients at various stages of cancer. Early-stage cancer patients, particularly, would benefit from new methods of cancer detection and diagnosis. Recently, we demonstrated the highly efficient capture of CTCs from whole blood samples of cancer patients, using anti-EpCAM coated polypyrrole (Ppy) platforms [21,22]. In addition, we integrated Ppy nanostructures with polydimethyl-siloxane-based microfluidic devices to develop a high-throughput cell recovery system and demonstrated its potential clinical value via simple manipulation of a series of electrical, chemical, and topographical cues [23]. In this study, we proposed a novel strategy for recovery and detection of CTCs using multifunctional magnetic nanowires (NWs) that can significantly increase sensitivity and specificity in the isolation of CTCs from the blood of patients, even at an early stage of cancer. Ppy NWs, decorated with a mixture of antibodies and doped with a high amount of magnetic nanoparticles (NPs) (Ab mixture_mPpyNWs), are designed to simultaneously allow cell recognition, isolation, and *in situ* colorimetric "naked eye" detection of the captured cells (Fig. 1A). The uniqueness of our approach is in the utility of NWs with a pencil-like morphology, which can highly increase the frequency of contact with CTCs by eliminating steric hindrance with other cellular elements (red and white blood cells and platelets) in the blood, which directly correlates with enhanced CTC recovery (Fig. 1B). In addition, the elongated geometry not only ensures maximum cell-NW attachment by winding NWs around the cells, but also offers multivalent binding sites for targeting ligands. NWs with an average length of 16 μm and diameter of 200 nm are sufficient to accommodate multiple types of antibodies through biotin-streptavidin interactions. Indeed, the combination of epithelial cancer markers with typical mesenchymal markers such as EpCAM, EGFR, TROP-2, vimentin, and N-cadherin, enable the identification of CTC populations with phenotypic variation, which may limit some of the inevitable loss of EpCAM-negative CTCs. From this perspective, the proposed strategy of using Ab mixture_mPpyNWs is reasonable and fully justified, and can be expected to resolve a number of critical issues associated with existing CTC-isolation techniques.

2. Materials and methods

2.1. Chemicals and reagent

Polyvinyl pyrrolidone (MW 29,000) (PVP), iron (II) chloride hexahydrate, 10 nm iron oxide NPs, pyrrole, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxy succinimide (NHS), streptavidin, reduced L-glutathione (GSH), 3,3′,5,5′-tetramethylbenzidine (TMB), dimethyl sulfoxide (DMSO), hydrogen peroxide (H_2O_2), sodium acetate trihydrate, ethyl alcohol (ethanol), paraformaldehyde (PFA), and Triton X-100 were purchased from Sigma-Aldrich (St.Louis, MO, USA).

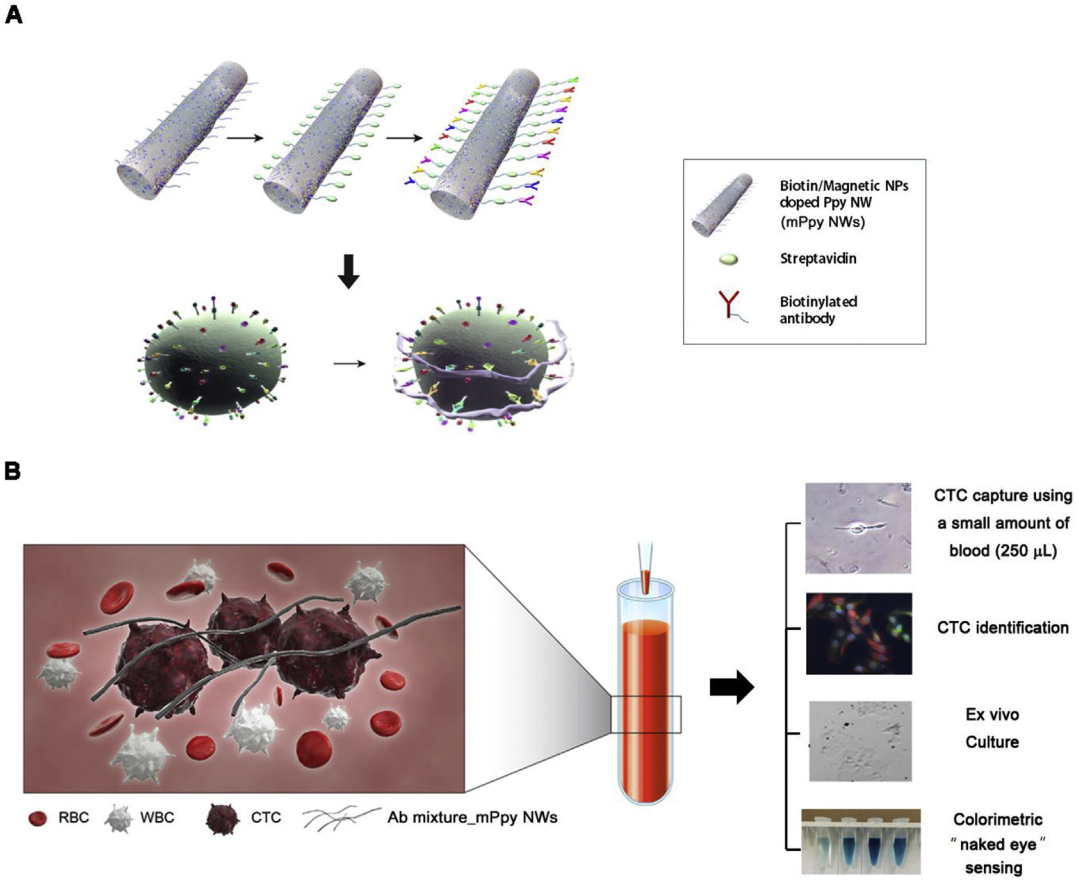

Fig. 1. (A) Antibody mixture-decorated magnetic nanowires (Ab mixture_mPpyNWs) for the isolation of circulating tumor cells (CTCs). Illustration of the labeling of five different types of antibodies on the surface of ss-biotin- and magnetic nanoparticle-doped polypyrrole (Ppy) NWs. (B) Schematic representation illustrating increased multivalent binding between receptors on CTCs and targeting antibodies on the Ab mixture_mPpyNWs.