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An integrated multifunctional platform based on biotin-doped conducting polymer nanowires for cell capture, release, and electrochemical sensing

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ABSTRACT

Here, we propose an integrated multifunctional system constructed by conductive disulfide-biotin-doped polypyrrole nanowires (SS-biotin-Ppy NWs) for capture, release, and *in situ* quantification of circulating tumor cells (CTCs). A well-ordered three-dimensional nanowire structure equipped with a monoclonal antibody offers a significant impact on the cell-capture efficiency, as well as on electrical- or glutathione (GSH)-mediated release of the captured cells. In addition, the electrochemical identification/detection of the captured cancer cells can be directly conducted on the same Ppy NW platform by using horseradish peroxidase (HRP)-labeled and anti-EpCAM-conjugated nanoparticles (HRP/anti-EpCAM Ppy NPs), showing very high sensitivity and specificity. The signal amplification can be clearly attributed to the catalytic response resulting from enzymatic reduction of hydrogen peroxide on Ppy NWs, consequently generating a greatly increased amperometric response with a detection range of 10 to 1×10^4 cells and a detection limit of as low as 10 cells. Overall, the proposed Ppy NWs not only present a promising platform for effective cell capture and release but also permit cytosensing capability for on-site analysis.

1. Introduction

Recently, significant efforts have been devoted for achieving a better understanding of circulating tumor cells (CTCs) in order to unravel the complex mechanisms governing cancer biology [1–4]. As CTCs are very rarely present in the bloodstream, the detection and isolation of viable tumor cells from an individual patient can offer unique opportunities in evaluating the metastasis; predicting cancer progression; and deciding an effective treatment plan after surgery, chemotherapy, or radiotherapy in clinics. Therefore, it is highly desirable to develop simple, sensitive, and reliable methods for efficient capture and release of target CTCs with high recovery and purity rates. Presently, platforms such as microfluidics, microfilters, microchips, conductive nanodots, TiO_2 nanofibers, and silicon nanowire arrays are being developed [5–12]. Such micro/nanotechnology-based approaches enable improved recovery of CTCs with several advantages as compared with the conventional technologies such as centrifugation, flow cytometry, reverse transcription-polymerase chain reaction (RT-PCR), and isolation of epithelial tumor cells by their size (ISET) [13–16]. Particularly, nanoscale topographic features have been reported as an effective way to enhance the detection performance of rare cancer cells.

In our previous research, we demonstrated highly efficient cell capture/release system using a conducting polymer, polypyrrole (Ppy) [17]. By electrochemically polymerizing biotin-doped Ppy on electrodes, we attempted an electrical field-induced capture and subsequent release of the adhered cancer cells. The greatest advantage of Ppy is the reversible polymeric volume change according to the applied electrical field [18,19]. Interestingly, Ppy film expands up to approximately 35% at the oxidation state, ideally suited for entrapping large quantities of biomolecules into the polymeric backbone. Meanwhile, the Ppy backbone shows dramatic shrinkage at the reduction state, thereby releasing the conjugated molecules and attached cells. This intrinsic nature of the conducting polymers has found extensive applications for diverse purposes such as drug delivery and construction of prosthetic devices and tissue engineering scaffolds in the human body [20–25].

In this study, we have proposed an integrated strategy to simultaneously perform cell capture, on-demand release, and *in situ* quantification of the captured cells, all at the same platform.

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This newly introduced platform possesses additional functions by means of (i) fabricating disulfide (SS)-biotin doped Ppy nanowire arrays to enhance the capture efficiency, (ii) exploring the cell release dually via the application of electrical stimulation (ES) or glutathione (GSH) treatment, and (iii) developing a simple and fast electrochemical biosensing strategy for identification/quantification of cancer cells by labeling with horseradish peroxidase (HRP)/antibody-conjugated nanoparticles (HRP/anti-EpCAM Ppy NPs) (Fig. 1). The surface with nano-topographic features allows enhanced cell-capture efficiency by inducing topographical interactions with nanoscale cell components, thereby offering the advantage of very high interaction with cells [26–31]. Our previous studies have illustrated the utility of electric fields in facilitating controlled cell release [17]. In the present study, the release strategy was further extended by encompassing endogenous redox agents, particularly GSH, to collect target cells after capturing them in a nondetrimental manner. Upon treatment with GSH, the adhered cells could be easily detached from the SS-biotin-Ppy NWs as a result of the cleavage of SS linkage in the SS-biotin moiety [32–34]. Dual stimuli-responsive Ppy NWs promise to reveal new perspectives for designing a remotely controlled release system with greater flexibility and versatility. In addition, the Ppy NW structure

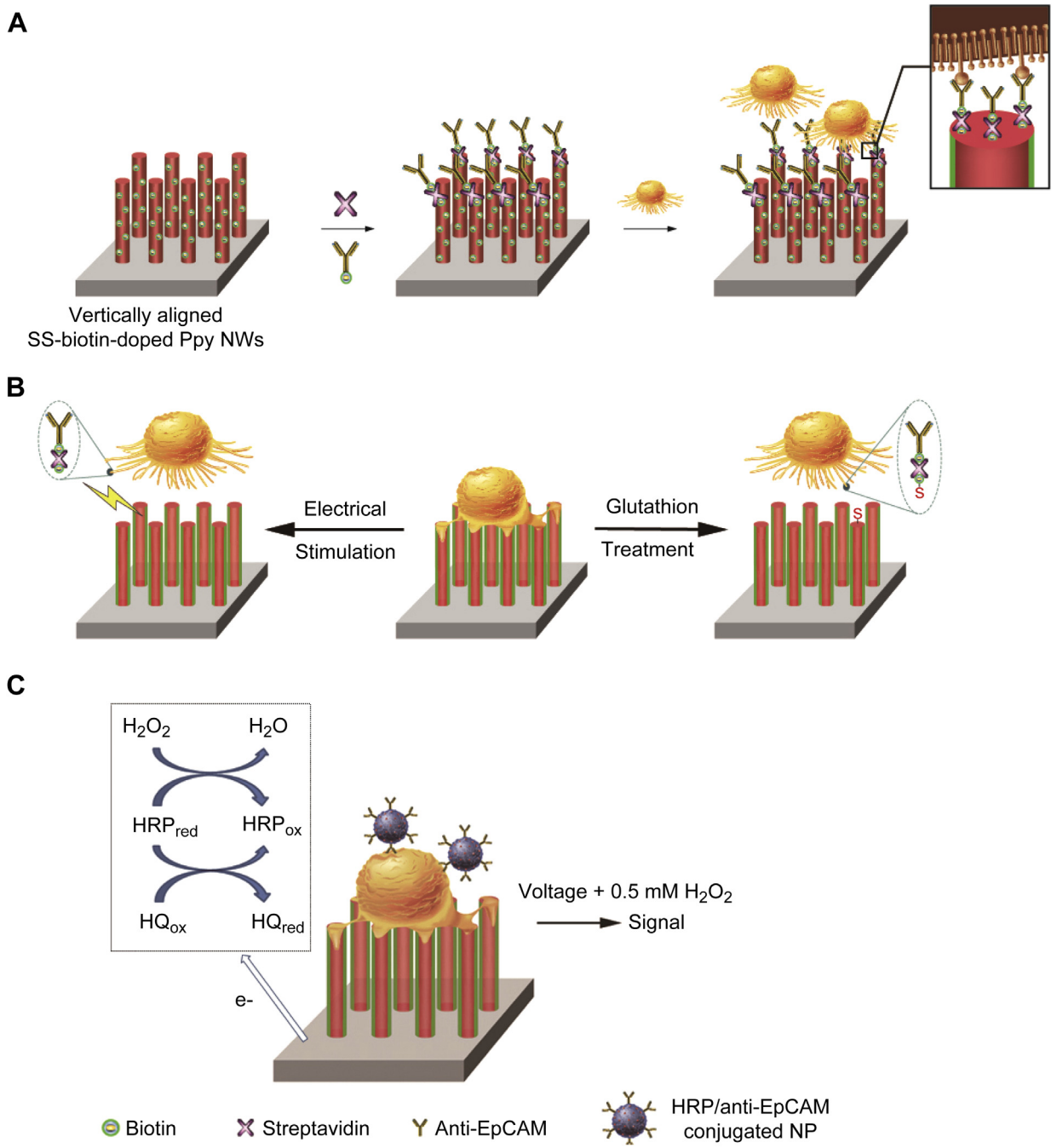

Fig. 1. (A) Fabrication of anti-EpCAM-immobilized SS-biotin-doped polypyrrole nanowires (anti-EpCAM-SS-biotin-Ppy NWs) with 200-nm diameter for capture, release, and *in situ* quantification of cancer cells. (B) The dual electrical simulation (ES)- and glutathione (GSH)-responsive system for the release of the captured cells. (C) On-site electrochemical detection of the captured cancer cells by a nanoparticle-based signal amplification strategy.