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Enhanced fluorescence detection of proteins using ZnO nanowires integrated inside microfluidic chips

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ABSTRACT

Nanostructure-enhanced detection is promising for a number of applications such as early cancer diagnosis, environmental monitoring and mine safety, among which nanostructures integrated microfluidic chips offers unique advantage of ultra-low quantitative analyses. Here, dense ZnO nanowires of varied diameter and length were obtained by changing the content of polyethyleneimine (PEI) and growth time via simple hydrothermal growth in microfluidic channels for protein detection. We showed that this approach was superiorly efficient compared to the conventional hydrothermal method due to the flow-induced replenishment of nutrient and the effect of shear stress. When immobilizing FITC conjugated anti-bovine immunoglobulin G (IgG) on ZnO nanowires, the fluorescence emission was significantly amplified compared to glass substrate and ZnO seed layer. Under the different growth conditions, the most remarkable fluorescence enhancement was observed on the ZnO nanowire substrate grown for 3 h with 5 mM PEI in solution. It is ascribed not only to the increase of the binding surface area of proteins but also the intrinsic fluorescence enhancement of ZnO nanowires as waveguides. We further used the optimized ZnO nanowires to demonstrate multiple detection of cancer biomarkers, achieving a superior limit of detection (LOD) as low as 1 pg/mL in human α-fetoprotein (AFP) assay and 100 fg/mL in carcinoembryonic antigen (CEA) assay with large dynamic range of 6–7 orders, which suggests that ZnO nanowire integrated microfluidic chips are promising for high-throughput fluorescence-based diagnostic assays.

1. Introduction

Over the past decades, ultra-sensitive detection has been widely studied for various applications. In particular, ultra-sensitive detection for cancer diagnosis has attracted increasing attention due to the rise of patient number all over the world. Various strategies have been implemented to monitor cancer biomarkers but early cancer diagnosis is always challenging (Wang and Mountziaris, 2013). It is now a general belief that the analysis of peripheral blood samples of cancer patients, or liquid biopsy, is the way to reach that goal. Although in liquid biopsy the detection of tumor related genetic alterations is perhaps the most promising, the detection of other biomarkers by using enzyme-linked immunosorbent assay (ELISA) (Kai et al., 2012) may also be interesting or complementary to the genitive analysis. The problem is that the popular platform of ELISA is actually costly, time-consuming and limited in detection sensibility. To address these issues, nanomaterials has been used as sensing probes to improve the sensitivity of biodetection, such as quantum dots (Diaz-Diestra et al., 2017; Irshad Ahmad et al., 2016; Wang et al., 2016), noble metal nanoparticles (Choi et al., 2012) Lei et al. (2017) and nanocomposites (Durán-Lobato et al., 2015), showing contribution and versatility as alternatives of tradition fluorophores. Meanwhile, metal nanowires known as “nano antennas” have been demonstrated to show a dramatical amplification of fluorescence (Casadei et al., 2014; Ono et al., 2016). However, the high cost of these materials may impede their practical applications in clinical diagnosis. Nanomaterials have also been utilized as sensing substrate to enhance the fluorescence intensity (Liu et al., 2011; Wang et al., 2015). Recent studies have reported strong fluorescent enhancing capability of non-toxic and inexpensive nanostructured metal oxide (Hu et al., 2013; Li et al.,

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2014; Liu et al., 2014; Sang et al., 2016), holding the great promise for multiplexed biodetection.

Zinc Oxide (ZnO) nanowires have received widely attention due to their desirable optical properties of a wide band gap of 3.37 eV and a large excitation binding energy of 60 meV at room temperature. When used for biomedical detection (Arya et al., 2012; Dorfman et al., 2006a; Rodrigues et al., 2017; Zhao et al., 2013a, 2013b), ZnO nanowires have shown remarkable capability to enhance the fluorescence intensity in various biomedical assays (Dorfman et al., 2006b; Hu et al., 2015; Lupan et al., 2010), allowing high-throughput detection of proteins. Previous work demonstrated that the large surface-to-volume ratio and aspect ratio of ZnO nanowires could greatly enhanced the fluorescence intensity (Ladanov et al., 2013; Sang et al., 2016). Thus, due to the surface dependence of detection performance, high quality of densely packed ZnO nanowires were required. Hydrothermal growth is commonly used to obtain uniform and dense ZnO nanowires (Vayssieres, 2003) and the growth rate depends on the concentration of the ions in the vicinity of growth sites. In a conventional bulk growth, the concentration of ions is determined by both the depletion of crystallization and the replenishment of nutrient. Therefore, to facilitate the synthesis of ZnO nanowires via hydrothermal method, microfluidic devices integrated with deep trenches have been used to supply fresh nutrients continuously (Ladanov et al., 2013). Within the confined space of deep trenches, the number and the surface area of ZnO nanowires were noteworthily increased, which is crucial for the effectiveness of nanowire-based sensors. Nevertheless, it is challenging to fabricate superior ZnO nanowire substrate for multiple fluorescence biodetection on chip since the growth mechanism of ZnO nanowires in a confined spaces was still unclear. Moreover, for the emerging fields of point-of-care testing (POCT) and on-the-spot detection, the idea is portable, robust, and easy to operate and maintain, which requires the low-cost apparatus for routine manipulation (Willmott and Arrowsmith, 2010). ZnO nanostructure integrated microfluidic chips can be used as one of the promising and versatile device for rapid quantitative biomarker detection in POCT.

Here we successfully grew ZnO nanowires in parallel microfluidic channels by hydrothermal method and implemented biosensing of biomarkers. To achieve an optimal growth of ZnO nanowires and investigate the growth mechanism in microfluidic channels, the effect of polyethyleneimine (PEI) and growth time were studied. The fluorescent detection of FITC conjugated anti-bovine IgG (FITC-antiIgG) was performed to evaluate the fluorescence amplification of various ZnO nanowire substrates obtained in different conditions, among which ZnO nanowires grown in the solution with 5 mM PEI for 3 h showed the strongest fluorescence amplification. In addition, the optimal ZnO nanowires were integrated within microfluidic channels to detect human α-fetoprotein (AFP) and carcinoembryonic antigen (CEA) and the limit of detection was found to be as low as 1 pg/mL and 100 fg/mL, respectively.

2. Materials and methods

2.1. Materials

All the chemicals in the experiment were used without further purification. Acetone, isopropyl (IPA), Zinc acetate dehydrate, zinc nitrate hexahydrate, hexamethylenetetramine and ammonium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Polyethyleneimine (PEI) and polyvinyl alcohol (PVA) were purchased from Sigma (China). Polydimethylsiloxane (PDMS, RTV-615) was purchased from Momentive (China). FITC-conjugated anti-bovine IgG and bovine serum albumin (BSA) was purchased from ImmunoReagents, Inc (China). Human AFP antigen, human CEA antigen, FITC-conjugated anti-human AFP and FITC-conjugated anti-human CEA were all purchased from Shanghai Linc-Bio Science Co., Ltd. (China).

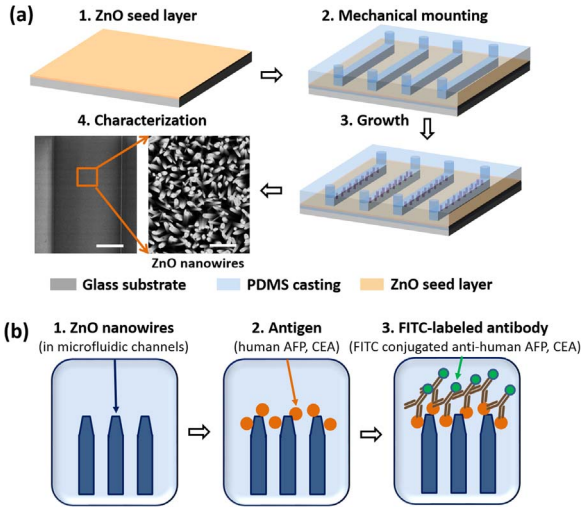

Fig. 1. (a) Schematic of synthesis of ZnO nanowires in microfluidic channels. Scale bar: 200 μm. (b) Schematic diagram of immunoassay in microfluidic channels integrated with ZnO nanowires.

2.2. Preparation of microfluidic device

Microfluidic chips were prepared by standard soft lithographic process. First, a layer of 50 μm thick SU-8 photoresist (MicroChem) was spin-coated onto a silicon substrate. After UV exposure and development, trimethylchlorosilane was evaporated on the mold as release agent. Then, the prepolymer solution of PDMS was prepared at a 10:1 ratio of base polymer to cross-linker and was poured on the SU-8 mold. Before curing, the sample was placed in the vacuum chamber to expel trapped air bubbles. After curing at 80 °C for 4 h, the PDMS replica were peeled off and mounted on the glass substrate with ZnO seed layer by clamping, resulting in the microfluidic channels of 500 μm wide and 50 μm high (Fig. 1(a)).

2.3. ZnO seed layer and hydrothermal growth

A glass substrate (microscope slides, 75 mm × 25 mm, AS ONE, China) was ultrasonically cleaned sequently in acetone, isopropyl (IPA) and Deionized water for 15 min and dried with nitrogen gas. The seed solution was prepared by dissolving PVA (4 wt%) and zinc acetate dihydrate (ZAD, 1 wt%) in DI water and stirred for 1 h at room temperature. To obtain vertical-oriented nanowires, a seed layer was formed by spin coating the seed solution at 3000 rpm for 1 min followed by thermal treatment at 500 °C for 3 h (Lee et al., 2009). After mechanical mounting of PDMS replica and the glass slide, hydrothermal method was used to synthesize ZnO nanowires in microfluidic channels. The growth solution containing $Zn(NO_3)_2 \cdot 6H_2O$ (25 mM), HMTA (12.5 mM), $NH_3 \cdot H_2O$ (0.15 M) and different contents of PEI was introduced to each microfluidic channel by a syringe pump at the flow rate of 10 μL/min at 90 °C for 0.5, 1, and 3 h.

2.4. Fluorescence detection

After synthesis of ZnO nanowires deionized water was perfused into microfluidic channels for 10 min to rinse the ZnO debris. To evaluate the enhancement performance of fluorescence intensity by ZnO nanowires obtained under different conditions, 5 μg/mL FITC-conjugated anti-bovine IgG was introduced into microfluidic channels and incubated for 15 min at the flow rate of 5 μL/min. Fig. 1(b) shows the schematic of immunoassays on ZnO nanowires. After synthesis of the optimal ZnO nanowires, sample solutions in PBS (human AFP, CEA) of different concentation ranging from 100 fg/mL to 10 μg/mL were simultaneously infused into the microfluidic channels at the flow rate