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Versatile synthesis of ZnO nanowires for quantitative optical sensing of molecular biorecognition

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ABSTRACT

A zinc oxide nanowires (ZnO NWs) forest has been grown by a versatile hydrothermal method on solid supports of very different nature, such as flat crystalline silicon, glass fiber and polymer surface. ZnO NWs shown a characteristic photoluminescence (PL) spectrum that has been used for optical transduction of molecular interactions. In this study, ZnO NWs were chemically modified in order to bind a proper bioprobe for selective protein–protein biorecognition. Techniques such as scanning electron microscopy (SEM), water contact angle (WCA), fluorescence microscopy and Fourier transform infrared (FTIR) spectroscopy were used for characterization of nanostructures bioconjugation, demonstrating that ZnO NWs can be easily and efficiently functionalized. Quantitative and label-free sensing of protein–protein interaction was obtained by monitoring PL emission of ZnO NWs under laser irradiation.

1. Introduction

Optical transduction, as alternative to electrochemical and amperometric analytical methods, is an attractive technique for biosensing due to high sensitivity and specificity, real-time monitoring, high throughput, and no sample pretreatment. When a molecular probe (i.e. a protein, an enzyme, a DNA strand, and so on) is conjugated to an optical transducer, an optical biosensor is created, adding natural, high selectivity to the other listed features. Optical biosensors could be integrated in small device and used in applications of social interest such as medical diagnostic, therapeutics, health care, monitoring of environmental pollutants, home and defense security [1–3]. Fluorescence is one of the most used signals in optical transduction, even if the labeling of the probe is often a limiting step in biological sensing [4]. Label free optical biosensors can be realized integrating biomolecular probes on a signaling material which directly transduces the molecular recognition into an optical signal without any external manipulation [5].

Zinc Oxide (ZnO) is one of the most interesting transducer materials for chemical and biological sensing applications: it has a very reactive surface; it is biocompatible and very stable from the chemical point of view; it shows an intense photoluminescence (PL) emission at room temperature under laser irradiation [6]. ZnO is a well-known n-type, direct wide-band-gap II–VI semiconductor with a band gap of 3.37 eV and a large excitonic binding energy of 60 meV, which allows an efficient excitonic emission even at room temperature [7,8]. Moreover, ZnO exhibits the richest family of nanostructures (nanoribbons, tetrapods, nanorods and nanowires) among semiconductor oxides and different methods are available in literature of ZnO nano-objects fabrication, including Vapor–Liquid–Solid growth (VLS), Metal Organic Chemical Vapor Deposition (MOCVD), High Pressure Pulsed Laser Deposition (HP-PLD) [9,10]. However, these technologies always require high temperatures, sometimes the presence of a catalyst, and, in general, complex equipments that make very expensive and energy-consuming the production of ZnO nanowires (NWs).

An alternative approach in ZnO NWs production is the hydrothermal synthesis, an aqueous mediated growth of ZnO nanostructures, which presents several advantages with respect to those aforementioned: it requires not very high temperatures (60–95 °C), simple equipment and low cost reagents. The best asset of hydrothermal synthesis is that different morphologies of nano-objects can be obtained on large surfaces, i.e. tenths of centimeters squares, whereas other nanotechnologies are strongly limited in dimensions of the structured surface. Moreover, the hydrothermal method is not dependent on the nature of the support material: it can be indifferently used on different hard surfaces, such as glasses, metals (gold, aluminum, and so on) thick or thin layers, and other semiconductors (silicon, germanium and other

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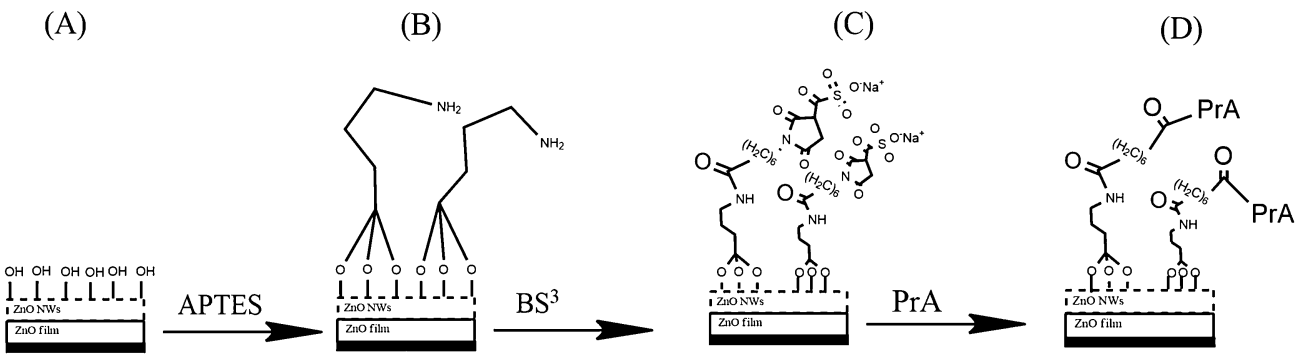

Scheme 1. Schematic representation of ZnO NWs bio-modification: the nanostructured surface of ZnO is highly hydrolyzed (A) and can be silanized by APTES (B). After silanization, the surface exposes ammine groups that bind the cross-linker BS^3 (C). The cross-linker is used to bind the protein A (D).

of electronic industry interest); or on soft, flexible substrates, such as plastics or polymers, of any shape, not necessarily planar, but also curved or even more complicate. Changing the process parameters, it is also possible to modulate the NWs order, density and height [11]. Under laser irradiation, the ZnO NWs show a characteristic photoluminescence (PL) spectrum, which presents a very intense near-band-edge ultraviolet peak at about 380 nm, due to free excitonic emission, and one or two broad bands in the visible-near infrared range related to Zn vacancies, interstitial Zn atoms and lattice defects related to O and Zn, i.e. strongly depending on the preparation conditions. The morphological (such as large surface-to-volume ratio) and physico–chemical (biocompatibility and the PL emission) characteristics of ZnO NWs make this material a good candidate for optical biosensing application [12,13].

In this work, we tested the hydrothermal growth process on different substrates: a flat crystalline silicon wafer, the glassy clad of an optical fiber, and a film of Polyethylene Naphthalate (PEN). ZnO NWs grown on crystalline silicon have been characterized by several techniques such as scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, fluorescence microscopy and water contact angle (WCA). The ZnO NWs surface has been functionalized with a biotinylated-protein A and its interaction with different concentrations of Avidin-Horseradish peroxidase (Avidin-HRP) has been label-free monitored by ZnO NWs PL emissions.

2. Materials and methods

2.1. ZnO NWs hydrothermal synthesis

A uniform ZnO seed layer was deposited on different support materials using a radio frequency (RF) magnetron sputtering equipment from a 99.999% pure ceramic ZnO target. The substrate was placed on the substrate holder and the deposition chamber was pumped down to a base pressure of 3×10^{-6} mbar before introducing the process gases (Ar). A 150 nm ZnO thin film was then deposited at room temperature, with 150 W RF power, 2.5×10^{-2} mbar pressure, 40 sccmAr flux and 30 min deposition time. The solution for hydrothermal process was prepared by dissolving in 200 mL D.I. water an equimolar (0.5 M) solution of alkaline reagent hexamethylenetetramine ($C_6H_{12}N_4$) and the Zn^{2+} salt ($Zn(NO_3)_2$) that act as a precursor. The solution was then heated at 90 °C for 4 h on a P.I.D. controlled hot plate with an immersion thermal sensor and the substrate, with the sputtered ZnO thin film, was immersed upside down. All of the resultant NWs were rinsed with de-ionized water and dried with nitrogen.

2.2. Substrates

The hydrothermal growth method was performed on different substrates with different morphologies to investigate the possibility of using different material like platform for biosensing sensor. In particular we tested a standard optical glass fiber, a plastic film of Polyethylene Naphthalate (PEN) and a silicon wafer. In any case a ZnO seed layer was deposited by sputtering process and the substrate was immersed in the hydrothermal solution.

2.3. ZnO NWs biomodification and biorecognition

Naturally hydrolyzed ZnO NWs (Scheme 1A) grown on silicon were treated with a solution of 5% APTES ((3-aminopropyl)triethoxysilane) (Sigma–Aldrich) in toluene anhydrous for 30 min at room temperature (RT), cured on heater at 100 °C for 10 min. Amino-modified ZnO NWs (Scheme 1B) were then treated by cross-linker BS^3 (Bis[sulfosuccinimidyl] suberate) (Thermo Scientific) 1.7 mM in PBS 1X pH 7.4 at 4 °C for 5 h. The sulfo-NHS-terminated samples (Scheme 1C) were then incubated at 4 °C overnight (ON) with FITC-labeled Protein A (Sigma–Aldrich) 48 μM in PBS 1X pH 7.4 for preliminary bioconjugation evaluation (Scheme 1D) by fluorescence microscopy. ZnO NWs as synthesized, and APTES-modified ZnO NWs were ON incubated with FITC-labeled protein A (48 μM in PBS 1X pH 7.4) as control samples against aspecific adsorption. Protein A modified samples have been reacted with biotin (Sigma–Aldrich) 48 μM in PBS 1X pH 7.4 at RT for 1 h. Avidin-HRP (BioLegend) at 8-4-2-1 μg/mL concentrations (PBS 1X pH 7.4 at RT for 1 h) was drop-deposited onto samples for biorecognition monitoring. The process was carried out in triplicate.

2.4. Scanning electron microscopy

The morphology of ZnO NWs, for each substrate, was investigated by scanning electron microscopy (SEM). SEM images have been collected at 5 kV accelerating voltage and 30 μm wide aperture by a Field Emission Scanning Electron Microscope (Carl Zeiss NTS GmbH 1500 Raith FESEM). Secondary emission and in-lens detectors have been used for imaging.

2.5. Fluorescence microscopy

Fluorescence measurements were made by a Leica Z16 APO fluorescence macroscope equipped with a camera Leica DFC300 and I3 filter (450–490 nm band-pass excitation filter plus a 510 nm dichromatic mirror and a 515 nm suppression filter). Fluorescence intensity values reported in the paper are averaged on three independent determinations.