NANOHYBRID MEDIATED FINELY TUNED NOVEL ZnO/Au NANOSTRUCTURES FOR SELECTIVE BIO-CAPTURE TOWARDS NANODIAGNOSTICS

by

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LIST OF ABBREVIATIONS

AC	Alternating current
AFM	Atomic force microscope
Au	Gold
AuNPs	Gold-nanoparticles
APTES	(3-Aminopropyl)triethoxysilane
BOE	Buffer oxides etch
CO	Carbon monoxide
CO_2	Carbon dioxide
CPE	Constant phase element
CVD	Chemical vapor deposition
DC	Direct current
DEA	Diethanolamine
DIW	Deionized water
DNA	Deoxyribonucleic acid
dsDNA	Double-strain DNA
EDX	Energy-dispersive X-ray
EtOH	Ethanol
FESEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
FWHM	Full-width at half-maximum
GNPs	Gold nanoparticles
HAP1	Hemolysis-associated protein-1
HMT	Hexamethylenetetramine
IDE	Interdigitated electrode

IEP	Isoelectric point
IPA	Isopropyl alcohol
IS	Impedance spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
I-V	Current-Voltage
MEA	Monoethanolamine
MBE	Molecular beam epitaxy
MOCVD	Metal-organic chemical vapor deposition
NWs	Nanowires
NRs	Nanorods
NaOH	Sodium hydroxide
NaCl	Sodium chloride
ncDNA	Non-complementary DNA
NRs	Nanorods
NFs	Nanoflowers
NWs	Nanowires
O_2	Oxygen gas
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pDNA	Probe DNA
PL	Photoluminescence
PVD	Physical vapour deposition
Rct	Charge transfer resistance
RF	Radio frequency
RMS	Root-mean-square

RT	Room temperature
SAED	Selected area diffraction
SiO_2	Silicon dioxide
SPR	Surface plasmon resonance
smDNA	Single-mismatched DNA
ssDNA	Single-strain DNA
tDNA	Target DNA
tmDNA	Triple-mismatched DNA
T	Transmittance
TEM	Transmission electron microscope
T-RFLP	Terminal-restriction fragment length polymorphism
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
VLS	Vapour-liquid-solid
VS	Vapour solid
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
ZnO	Zinc Oxide

LIST OF SYMBOLS

a.u.	Arbitrary Units
α	Absorption coefficient
Å	Angstrom
λ	Absorption band edge
Al	Aluminum
Ar	Argon
C	Carbon
σ	Conductivity
°C	Degree Celsius
E_g	Energy band gap
F	Farad
Hz	Hertz
Au	Gold
Z	Impedance
Fe	Iron
µm	micrometer
mg	milligrams
mm	milimeter
nm	nanometer
N	Nitrogen
Ω	Ohm
$\alpha h\nu$	Optical band gap
ε_∞	Optical dielectric constant
O	Oxide

P	Phosphorus
hv	Photon energy
n	Refractive index
Ag	Silver
Zn	Zinc

Pembentukan ZnO/Au Nanostruktur Novel Melalui Pengabungan Nanohibrid Bertujuan Untuk Penderian Biologi Terpilih Bagi Applikasi Nanodiagnostik

ABSTRAK

Struktur nanoskala bergabung dengan logam unik dijangka akan meningkat hasil bahan novel yang akan membentuk kaedah baru untuk diagnosis dan terapeutik. Antara pelbagai jenis logam/ semikonduktor hibrid nanostruktur yang telah dibangunkan, nanostruktur ZnO dikaji dengan lebih intensif kerana sifat nanomorfologinya yang unik, biokompatibiliti, kestabilan kimia, sensitiviti, ketidak toksidan dan sifat-sifat pemangkinnya yang tinggi. Ciri-ciri biokompatibiliti ZnO menjadikan bahan ini sebagai pilihan yang lebih sesuai untuk kefungsian permukaan konduktor dan penggabungan kompaun kimia dan biologi di pH yang ekstrem. Nanohibrid yang terdiri daripada ZnO dan nanokluster logam novel telah menarik perhatian sejak beberapa tahun lalu disebabkan potensinya yang mempunyai sifat pemangkin tinggi, nisbah luas permukaan kepada isipadu yang tinggi dan beberapa fungsi yang mampu mendahului sifat asal bahan ZnO. Objektif kajian ini adalah untuk mensintesis pelbagai jenis nanostruktur ZnO yang telah mengalami pendopan Au menggunakan pengabungan kaedah penyalutan sol gel dengan cara putaran serta kaedah hidroterma pada suhu rendah untuk mengenali profil biomolekul (DNA) yang menerajui ke arah pengesanan patogenik leptospira dan cholera. Satu lapisan nipis benih ZnO telah disalutkan atas interdigitated elektrod emas untuk penyediaan tapak penukleusan bagi pertumbuhan nanostruktur ZnO. Nanorod ZnO telah ditumbuh menggunakan cara pertumbuhan hidroterma suhu rendah. Berterusan dengan itu, nanorod ZnO telah dipercikkan dengan Au untuk pembentukan nanostruktur ZnO/Au. Ciri-ciri morfologi permukaan, struktur, optik dan sifat elektrik nanostruktur ZnO/Au yang disintesis dikaji dengan menggunakan FESEM, TEM, AFM, XRD, PL, XPS, IS dan meter sumber. Penyelidikan ini berjaya menunjukkan penggabungan kaedah sol gel dan pertumbuhan hidroterma untuk mensintesiskan nanostruktur yang berbeza. Selain itu, ZnO yang direka berasaskan peranti nano juga telah mengalami pendopan dengan nanopartikel Au untuk aplikasi penderiaan biologi. Penyelidikan ini, mengkaji kesan Au ke atas sifat optik filem nipis nanostruktur ZnO. Penggabungan Au dengan filem nipis ZnO membawa kepada perubahan ke atas pemukaan morfologi serta sifat optik dan elektrik. Ia telah diperhatikan bahawa nanopartikel Au cenderung untuk tumbuh bersama-sama dengan salutan nipis ZnO dan kepadatan nanopertikel Au meningkat menyebabkan pembentukan lapisan yang berterusan selari dengan peningkatan ketebalan pemercikan meningkat dari 10 nm hingga 50 nm. Menurut analisi struktur, optic dan elektrik, penggabungan Au telah meningkatkan prestasi salutan filem nipis nanostruktur ZnO. Kesan pemercikan nanopartikel Au atas kekonduksian peranti nanorod ZnO juga dikaji. Ia telah diperhatikan bahawa pemercikan nanopartikel Au yang mempunyai ketebalan yang berbeza sangat mempengaruhi pemalar dielektrik nanorod ZnO serta kekonduksian peranti. Dalam usaha untuk menyelidik sifat penderiaan biologi, struktur nanowayar berbunga baru telah difabrikasi dan diuji dengan menggunakan impedans spektroskopi bagi membezakan spesis leptospira patogenik dan tidak patogenik. Pemilihan yang spesifik bagi mengenali prob molekular atas nanopartikel Au telah menjadi bukti kepada interaksi dengan DNA dari leptospirosis pathogenik melalui penghibridan dan analisi tidak sepadan. Had pengesanan yang 100 fM telah ditentukan menggunakan impedans spektroskopi. Selain daripada itu, kestabilan, keboleh ubahan dan pemulihan semula penderiaan peranti ini telah dikaji dengan teliti. Akhirnya, nanostruktur baru difabrikasi daripada hibrid ZnO/Au melalui proses akueus hidroterma yang menggunakan pendopan nanopartikel Au atas kekisi ZnO yang menyerupai struktur ulat. Di samping itu,

kebolehan untuk menfabrikasi struktur hibrid ZnO/Au baru untuk penderiaan impedans telah terbukti membezakan serovar cholera. Ia telah diperhatikan bahawa peranti ini adalah lebih sensitif berbanding dengan pendopan nanopartikel Au dengan nanorod ZnO dalam literasi yang didokumenkan untuk mengesan DNA dalam suhu yang optimum. Peranti yang difabrikasi itu telah memaparkan peningkatan dalam rintangan sempadan butiran dari 0.17 hingga 1.13MΩ apabila konsentrasi DNA sasaran ditingkatkan daripada 1 μM ke 10 fM. Penderiaan biologi berdasarkan struktur Au-decorated hibrid nano-worm memaparkan kelinearan yang cemerlang dalam pelbagai konsenstrasi DNA sasaran. Selain itu, kestabilan yang lebih tinggi, keboleh ubahan dan pemulihan semula atas pemukaan peranti telah dipaparkan. Disamping itu, struktur hibrid ZnO/Au mempunyai kelebihan dari segi kestabilan struktur dimana kokosongan zink and oksigen diisi oleh nanopartikel Au sekaligus meningkatkan keupayan penderian biologi peranti berbanding dengan nanowire berbunga. Kesimpulannya, kajian ini telah berjaya menunjukkan proses untuk mensintesis, menfabrikasi, menyifat dan mengesahkan pendopan Au ke atas nanostruktur ZnO bagi penderiaan biologi.

Nanohybrid Mediated Finely Tuned Novel ZnO/Au Nanostructures for Selective Bio-Capture towards Nanodiagnostics

ABSTRACT

Nanoscale structures combined with noble metals are expected to yield novel materials that will create new avenues for diagnosis and therapeutics. Among various types of nanostructured metal/semiconductor hybrid that have been developed, nanostructured Zinc oxide (ZnO) has been intensively studied because of its unique nano-morphological, functional bio-compatible, chemical stability, sensitivity, non-toxicity, and high catalytic properties. The biocompatibility characteristics of ZnO make this material a convenient choice for conducting surface functionalization and interfacing with chemical and biological compounds at pH extremes. Nanohybrids that comprise ZnO and noble metal nanoclusters have attracted tremendous interest in recent years owing to their potential for improved catalytic activity, excellent surface area-to-volume ratio and several functionalities that are superior to those of pure ZnO nanomaterials. The objective of this research is to synthesize different type of Au doped ZnO nanostructures using simple sol-gel spin coating and low-temperature hydrothermal method to tract the profile of bio-molecule (DNA) that lead to pathogenic leptospirosis and cholerae detection. Firstly, a thin seed layer of ZnO prepared by sol-gel method was deposited on the gold interdigitated electrode to provide the nucleation sites for growth of ZnO nanostructures. Next, ZnO NRs were grown using simple low temperature hydrothermal growth method. Consequently, ZnO NRs were sputtered with Au to form ZnO/Au nanostructures. Therefore, the fabricated ZnO/Au nanostructure was examined through various characterization for surface morphology (FESEM, TEM, AFM), structural (XRD, XPS), optical (PL, UV-VIS) and electrical properties (EIS, IV) investigation. Thus, the research has successfully demonstrated the application of novel two-step method, the combination of sol-gel and hydrothermal process to synthesize different nanostructures. Secondly, we have studied the effect of Au on the localized surface plasmonic of ZnO thin film. Incorporation through sputtering of Au into a ZnO thin film resulted in changes in the surface morphology as well as the optical and electrical behaviour. It was observed that the AuNPs tends to grow along with the ZnO thin film and the density of the AuNPs increases forming a continuous layer as the Au thickness increased from 10 nm to 50 nm. Based on the structural, optical and electrical analyses, incorporation of Au substantially improves the ZnO thin film. Furthermore, the effect of sputtered AuNPs on the conduction mechanism of ZnO Nanorods was also studied. It was observed that different sputtered thickness of AuNPs greatly affects the dielectric constant of ZnO Nanorods as well the conductivity of the device. Thirdly, we have selected novel spotted nanoflower structure among the different structure of nanowire fabricated to investigate the biosensing properties for impedance sensing to distinguish pathogenic and non-pathogenic leptospira species. Selective capture of molecular probes onto the seeded AuNPs was evidence for the specific interaction with DNA from pathogenic leptospirosis-causing strains via hybridization and mis-match analyses. The attained detection limit was 100 fM as determined via impedance spectroscopy. Furthermore, stability, reproducibility and regeneration of this sensing surface were demonstrated. Finally, we created a new worm like nanostructure with ZnO/Au hybrid through aqueous hydrothermal method, by doping Au-nanoparticle (AuNP) on the growing ZnO lattice. Further, the ability to create Au-decorated hybrid nano-worm structure for impedance sensing was proved to distinguish serovars of cholera. It was observed that the sensor was more sensitive over the literature documented AuNP incorporated doped ZnO nanorods

in detecting DNA at ambient temperature (10 fM). The fabricated sensor displayed the increment in grain boundary resistance from 0.17 to 1.13 MΩ when the target DNA concentration was increased from 1 µM to 10 fM. The biosensor based on Au-decorated hybrid nano-worm structure exhibited excellent linearity over a wide range of target DNA concentrations. Further, higher stability, reproducibility and regeneration on this sensing surface were demonstrated. The worm like nanostructure with ZnO/Au hybrid has superior sensitivity and selectivity compared to novel spotted nanoflower due to the improvement over structural defects such as Zinc- and/or Oxygen-vacancies. Such improvement facilitates the chemisorption of organic molecules towards the substrate, which is beneficial for the high loading of DNA during immobilization and hybridization processes. In addition, the AuNPs nano-radii in combination with the increased surface area due to random curving, significantly enhances the detection efficiency due to increased immobilization rates and enhanced hybridization efficiency. In conclusion, this research successfully demonstrated the process to synthesize, fabricate, characterize and validation of Au doped ZnO nanostructures based biosensor.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Trends have been changed with the transition between microstructure and nanostructure, involves nanoscale creations. These creations include devices, chips, sensors, ovonic and semiconductors. In the past, semiconductor were given special attention due to its high-performance with improved conductivity variance towards making sensitive devices. The appealing characters of semiconductor include withstand with increasing temperature along with increment in conductivity, which cannot be achieved by metals. Semiconductor is made from elemental or compound materials, capable of conducting electricity with the value falls between conductor and insulator. Moreover, the unique electrical and optoelectronic properties of semiconducting metal-oxide nanostructure serve as an excellent scaffold for various application such as bio-sensing (Cheng et al., 2014; Jiang et al., 2014; Solanki et al., 2011). Zinc oxide (ZnO) which derived from Metal oxide group II–VI series in the periodic table has recently become dominant due to distinct optoelectronic properties which draw researcher attention for various application such as solar cell, light emitting diode, ultraviolet (UV) photodetector, chemical and gas sensor, biosensor, analytical devices and etc. (Chang et al., 2010; Guo et al., 2008; He et al., 2012; Jin et al., 2014; Tzeng et al., 2013; Vijayalakshmi et al., 2013; Zhang et al., 2014).

Development of ZnO nanostructure has broad range of advantages due to its unique physiochemical properties such as high electron mobility and suitability for simple fabrication of various nanostructures, which potentially results in lower cost ZnO based devices. During the past decade, many researcher has work to develop various type of ZnO nanostructures in order to optimize the ZnO nanostructure fabrication process and yield improve properties of ZnO nanostructures (Chen et al., 2006; Kashif et al., 2012a; Tak et al., 2014; Wang et al., 2005, 2006). ZnO nanostructures can be synthesized through various method such as chemical vapour deposition (CVD), radio frequency sputtering (RF), pulse laser deposition (PLD), molecular beam epitaxy (MBE), vapour-solid (VS), vapour-liquid-solid mechanism (VLS), spray pyrolysis, sol-gel, hydrothermal, inkjet printing and etc. (Ismail and Abdullah, 2013; Lai et al., 2010; Lee and Kim, 2013; Singh and Chakrabarti, 2013; Tarwal et al., 2013; Tzeng et al., 2013; Zhang et al., 2013; Znaidi et al., 2012). However, the researchers are still remain in challenge to produce ZnO nanostructure with desirable physiochemical properties.

The unique characteristics of ZnO nanostructure such as excellent surface-area-to-volume ratio, high electron mobility and suitability for simple fabrication attributes to ZnO has become a rising hope and a motivation for development of highly sensitive, stable and selective analytical devices. In general, there are two major techniques to produce Zinc oxide nanostructures, namely top-down and bottom-up. The bottom-up fabrication approaches has been preferred as it possible to produce less defects, highly crystalline and control shape and size of ZnO nanostructure compared to top-down methods. There are various methods available under bottom-up approaches to produce ZnO nanostructures in desirable properties, such as chemical vapour deposition (CVD), aqueous hydrothermal growth, sol-gel, spray pyrolysis, molecular beam epitaxy (MBE), vapour-solid (VS), vapour-liquid-solid mechanism (VLS) methods. (Kashif et al., 2012b; Lee and Kim, 2013; Suh et al., 2010; Zhang et al., 2012). ZnO nanostructures has been intensively studied because of its unique nano-morphology, functional bio-compatibility, chemical stability, sensitivity, non-toxicity, and high catalytic properties. The biocompatibility characteristics exhibited by ZnO is highly desired for surface functionalization and interfacing with chemical and biological which provide great prospective for highly stable analytical device developments (Foo et al., 2015). Furthermore, ZnO nanostructures possessed high isoelectric point (IEP) among the various metal-oxides semiconductor (8.7-10.3 IEP). On the other hand, DNA bio-molecule has lower IEP of (5.0), which allow the direct immobilization and hybridization of DNA bio-molecule onto the surface of ZnO nanostructure through electrostatic interaction (Kumar et al., 2006; Pradhan et al., 2010). Hence, the immobilization and hybridization can be easily monitored by various transduction method such as electrochemical impedance spectroscopy measurement.

Nanohybrids that comprises ZnO and noble metal nanoclusters have attracted tremendous interest in recent years owing to their potential for improved catalytic activity, excellent surface-to-volume ratio and several functionalities that are superior to those of pure ZnO nanomaterials (Geng et al., 2012; Lee et al., 2011). Moreover, the addition of metal nanoparticle on metal oxide forming of hybrid nanostructures may facilitates the improvement in band-edge emission and reducing the defect emission (Lee and El-sayed, 2006; Lim et al., 2011; Pawinrat et al., 2009). In this conjunction, gold nanoparticles (AuNPs) have high electron affinity, and the Schottky barrier produced between AuNPs and other metals is high (Pawinrat et al., 2009). The unique characteristics of gold (Au) such as surface chemical functionalization, biocompatibility, anti-oxidative characteristics, high conductivity and the ability to tailor gold to uniform and different nano-sizes has drawn researcher attention in recent years (Guirgis et al., 2012; Lim et al., 2011; Ryu et al., 2010; Upadhyayula, 2012). Therefore, the agglutination of AuNPs into a ZnO nanostructure enables thiolated biomolecules to bind easily, directly and selectively. These compatibilities permit the selective binding of a nucleic acid-based probe to the agglutinated AuNP for the specific bio-recognition.

To demonstrate performances of these nanostructures, detection of disease causing agents profound to be a right way and proves their applications. Among different diseases, leptospirosis is an epidemic zoonosis bacterial disease caused by spirochetes (specifically, leptospira species), which infect humans and other species of vertebrates. Pathogenic leptospira bacteria is the prime causative agent of severe pulmonary haemorrhage, acute respiratory failure, myocarditis, meningitis and renal failure in humans (Branger et al., 2005; Fearnley et al., 2008). Furthermore, given the existence of more than three hundred leptospira serovars, distinguishing between pathogenic and non-pathogenic strains is mandatory. To address these issues, a specific gene that is expressed only in pathogenic leptospira: Hemolysis-associated protein-1 (Hap1) can be selected as a probe design and interactive analyses can be performed by hybridization and mis-matching (Branger et al., 2001; Lee et al., 2000).

1.2 Problem statements

In general, there are two major techniques established in nanofabrication of metal oxide nanostructures: ‘‘top-down’’ and ‘‘bottom-up’’. Top-down approach is not a promising method because it has spatial resolution of lithography limitation and not cost-effective. Whereas bottom-up approach own its superiority compared to top-down approach in-terms of nanostructure synthesis, which is capable of producing various nanostructures with simple and low cost fabrication (Foo et al., 2014; Nuraje et al., 2009). ZnO nanostructures prepared by bottom-up approach are catalytically synthesized by chemical vapour deposition (CVD) and vapour liquid solid (VLS) methods, where structures are assembled from their atomic level (Foo et al., 2014b, 2013). Hence, ZnO nanostructure from bottom-up fabrication approaches always has been preferred as it possesses unique physical, optical and electrical properties, which is highly suitable for down-stream applications. Unfortunately, ZnO nanostructures synthesized by bottom- up approach has its own limitations such as catalyst dependence and difficulties in constructing reliable ohmic contact (Mohapatra et al., 2008).

Constructing reliable ohmic contact in bottom-up synthesized ZnO nanostructures that can be overcome by introducing interdigitated electrode (IDE) on the substrate. The IDE consists of twin electrodes which are arranged in a comb-like structure to form gaps between the two electrodes and a metal contact at the end of each electrodes. Futhermore, the fringing electrical field lines of an interdigitated electrodes will set randomly around the electrodes for planar IDE sensor. Hence, ZnO nanostructure coating on the electrode surface would allowed the confinement of the fringing electric field concentrated around the coated area within the sensing layer. In addition, an insulating layer (SiO2) were introduced using wet oxidation on a cleaned silicon wafer to avoid the electron leakage. The IDE serve as transducer and metal contact point in which ZnO nanostructured will be synthesized.

Nanostructured ZnO can be synthesized using chemical and gas phase route via number of methods such as spray pyrolysis, sol-gel, hydrothermal, inkjet printing, chemical vapour depositon (CVD), radio frequency sputtering (RF), pulse laser deposition (PLD), molecular beam epitaxy (MBE), vapor solid (VS), vapor liquid solid (VLS) method. The VS and VLS require high temperature for the growth of nanostructures. On the other hand, RF requires high cost instrument and specialized laboratory setup. Moreover, most of the gas phase synthesis process acquired catalyst which increases the impurities of end product. On the contrary, the chemical route through sol-gel method and hydrothermal is cost effective and can synthesize ZnO with controlled nanostructures, morphology and dopants.

For the novel metal doped ZnO sensing applications, researchers usually use Au or Ag as a dopant source along with ZnO nanorods. For the introduction of dopant or catalyst, a special equipment is required. For the synthesis of Au-sputtered ZnO nanorods, the predominant methods are chemical vapor deposition (CVD) and molecular beam epitaxy (MBE) (Cheng et al., 2010; Heo et al., 2002; Ryu et al., 2010; Sun et al., 2012). However, both CVD and MBE methods involve high temperature growth and expensive instrumentation which are not available in ordinary laboratory settings. Moreover, the Au doping onto the synthesized ZnO require RF sputtering which also needs expensive laboratory set-up. Based on above mentioned issues, this research proposed the alternative ways in order to minimize the cost and time. In order to resolve the above addressed issues, the current work was carried out and successfully demonstrated the alternative ways to resolve the current issues.

At present, leptospirosis cases in Malaysia have increased leaps and bounds causing it to be one of the major infectious zoonotic diseases in this nation (Lim, 2011). Leptospirosis is caused by the strain of leptospira bacteria, an epidemic causal agent that initially infects blood and cerebrospinal fluid, and consequently affects kidneys. Symptoms occur during leptospirosis is similar to other diseases and also in several cases, there will be no symptoms at all (Bharti et al., 2003). These conditions led the necessity to generate the detection systems/strategies and currently, assays/methods developed for the detection of leptospirosis are limited. Many symptoms, the most clinical features of leptospirosis include fever, headache, myalgia (particularly in calf muscle), jaundice, general malaise in addition to other symptoms/signs (Bharti et al., 2003; Ooteman et al., 2006). These symptoms are easily confused with other common diseases in the tropics,