

ONE-DIMENSIONAL CONDUCTING POLYMER NANOSTRUCTURES FOR CHEMICAL AND BIOLOGICAL SENSOR

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ABSTRACT

One-Dimensional Conducting Polymer Nanostructures for Chemical and Biological Sensor Applications by Nicha Chartuprayoon Doctor of Philosophy, Graduate Program in Chemical and Environmental Engineering University of California, Riverside, December 2012 Dr. Nosang V. Myung, Chairperson Despite of their short history, conducting polymers such as polypyrrole (PPy) have emerged as a novel building block for label-free chemiresistive /FET chemical/biological sensors owing to a great environmental stability, active functional monomers for direct covalent immobilization of bioreceptors, remarkable optical, magnetic, and electrical properties like a semiconductor as well as mechanical property and ease of fabrication possessed by polymers. Tunable electrical conductivity can also be realized by several orders of magnitude via the process called 'doping' where anions or dopants is chemically or electrochemically incorporated to oxidized CP backbones to attain the charge neutrality.

Confining conducting polymers to one-dimensional (1-D) nanoscale has ultimately increased the surface area to volume ratio and facilitated the electron transport through the bulk of 1-D nanomaterials. Therefore, a small perturbation by adsorbed charged chemical/biological molecules on their surfaces significantly affected the charge distribution within the bulk of 1-D nanomaterials, enhancing the sensitivity and detection limits. While this nano-electronic chemical/biological sensor showed a potential for advanced technology in detection and monitoring, device fabrication and assembly for 1-D conducting polymer nanostructures still appeared challenging to be scalable and reproducible in a cost-effective manner. Herein, this dissertation focused on fabrication and assembly of 1-D conducting polymer nanostructures based chemiresistive /FET chemical/biological sensors in a cost-effective manner. First, lithographically patterned nanowire electrodeposition (LPNE) was used to batch-scale fabricate single PPy nanoribbon with controlled dimensions and defined location on various substrates for NH₃ detection. Various bioreceptors were also surface functionalized on LPNE grown PPy nanoribbon to investigate sensing performance in terms of sensitivity, selectivity, dynamic range, and detection limits towards the specific virus and the target protein. Polyclonal antibodies (pAbs) that recognized cucumber mosaic virus (CMV) were anchored on PPy nanoribbons for the detection of CMV. Single chain fragment variables (scFvs) specific for mycobacterium tuberculosis antigen 85 complex protein (Ag85) were functionalized on PPy nanoribbons to accommodate the electrostatic screening effect caused by dissolved salt concentration in buffer solution and diluted human serum. On the other hand, template directed electrodeposition was employed to synthesize PPy nanowires using various dopants and solvents in order to evaluate the structure dependent sensing performances for detection of Ag85B protein.

INTRODUCTION

Rapid, inexpensive, and reliable biosensors for massive parallel sequences analysis of biological activities have been considerably researched as a promising analytical tool to numerous applications involving bioterrorism, agriculture, pharmaceutical research, genomic and proteomic studies, and point-of-care (POC) diagnostics. A gamut of biosensor configurations including optical, piezoelectric, electrical and electrochemical biosensors prepared by planar thin films of metals, metal oxides, conducting polymers and other carbon materials as conventional transducers were developed and have been extensively investigated by many research groups.

However, over the past decades, the advent of nanotechnology has prompted the integration of one-dimensional (1-D) nanostructures (NS) as transducers, which offered ample benefits. Miniaturized lab-on-chips (LoCs) using high density arrays of 1-D NS based nanodevices could be realized by various complementary innovative top-down fabrication and bottom-up synthesis along with assembly techniques. The ultra-high surface area to volume ratio, their comparable sizes to biomolecules, especially singlewalled carbon nanotubes (SWNTs) where all atoms were on the surface, subtle structural properties, and electrical transport within the bulk of 1-D NS improved sensitivity and limit of detection possibly down to a single molecule detection. The response time was also enhanced by a virtue of the two-dimensional diffusion. A great research effort on 1-D NS based bio-detection has been given particularly to optical, electrochemical, and electrical biosensors. In optical biosensors based 1-D NS, segmented metallic nanowires exhibited distinctive optical reflectivity to their adjacent segments were used as barcodes to enable a variety of recognition patterns. This alleviated the limitation of encoding or identifying large numbers of parallel biological analyses owing to inadequate number of fluorophores that displayed different fluorescences. Strano and coworkers also pioneered the use of carbon nanotubes as robust labels for near-infrared (IR) optical sensors and solution based in-vivo biological imaging, since they could endure long-term excitation without photo bleaching and sustain chemical staining. This sensor configuration, despite several advantages, may not be practical to cost-effective portable analytical devices caused by expensive optical instrumentations that hampered the full exploitation of this technology in the field operation. Interfacing the biological activities with 1-D NS based nano-electronics therefore had a great potential to achieve the demand for portable biosensors in terms of low cost associated with sensor fabrication, sensor operation as well as ease of sensor miniaturization.

Electrical transduction of 1-D NS towards biological activities such as electrochemical reactions of biomolecules, antibody-antigen interactions or catalyzed enzymatic reaction was accomplished by several sensor designs and configurations that eliminated the use of chemiphores and fluorophores. 1-D NS functioned as a working electrode in electrochemical sensors illustrated exceptional sensing performance towards detection of biomolecules due to their inherited high surface area and a fast electron transport. The sensor response time was remarkably enhanced because of improved mass transfer caused by non-planar radial diffusion as compared to planar diffusion of macroscale microelectrodes. The limit of detection of 1-D NS based electrochemical sensors such as CNTs was achieved down to 160 zmol for proteins and 1.3 zmol for DNA, respectively. Another promising approach for direct

and label-free electrical detection was chemiresistive/chemFET biosensors, which solely relied on the modulation of the electrical conductivity/resistivity of 1-D NS between microelectrodes. Since the pioneer of 1-D based FET biosensors by Cui et al. in a decade, this sensor configuration has been increasingly explored as an excellent promise to inexpensive, miniaturized and reliable sensing devices for field operation. Herein, this review will highlight the recent application of 1-D NS based label-free chemiresistive/chemFET biosensors and the mechanism associated with the biomolecular detection. In addition, the sensitivity, selectivity, and reliability of chemiresistive/chemFET biosensors will be reviewed based on current material synthesis and fabrication approaches, the surface functionalization schemes with selection of bioreceptors, and the sensor operation. Finally, the outlook to chemiresistive/chemFET biosensors will be recommended for subsequent advancement in this field.

WAFER-SCALE FABRICATION OF SINGLE POLYPYRROLE NANORIBBON BASED AMMONIA SENSOR

One-dimensional nanostructures, such as nanowires, nanotubes, and nanobelts, are attractive platforms for sensors as their high surface-to-volume ratio has the potential to enhance responses to surface processes, and diminutive sizes endow high density systems characteristic of complex olfactory organs. Among the myriad of actively studied nanomaterials, conducting polymers, such as polyaniline (PANI) and polypyrrole (PPy), offer an intriguing combination of properties, with electrical behavior tunable from insulator to near metallic and polymeric mechanical characteristics providing flexibility and low density. In addition, their room temperature operation, diverse monomer chemistry, and ease of functionalization have prompted the use of conducting polymers in chemical and biological sensors with various transduction mechanisms including conductometric, electrochemical, and field effect transistors (FET).

Various synthesis techniques, from physical to chemical and electrochemical deposition, utilizing both soft and hard templates, have been successfully reported, along with less traditional approaches such as electrospinning, electron-beam (Ebeam) lithography, and dip-pen lithography to fabricate conducting polymer nanowires with precisely controlled dimensions. The major challenge for most of these fabrication techniques is accurate positioning of the polymeric constructs for reproducible integration with existing micro-/macro fabrication. While some methods have addressed these issues, they are typically associated with costly and time-consuming serial patterning techniques, such as atomic force microscopy (AFM), focused ion beam (FIB), and e-beam lithography. Moreover, practical and fundamental constraints of each approach impose limits to the lengths and aspect ratio that have been demonstrated with each process. In this work, we employ Lithographically Patterned Nanowire Electrodeposition (LPNE) for the first time to fabricate ultra-long (> 1cm) complex shaped PPy nanoribbons. LPNE overcomes the drawbacks encountered with other synthesis techniques by combining attributes of top-down “photolithography” and bottom-up “electrodeposition” approaches to fabricate nanowires to pre-determined locations with precisely controlled widths and thickness. The incredible lengths and precise placement of these nanoribbons facilitate alignment of numerous lithographically patterned electrode

along the length of PPy nanoribbons anchoring the wires to the substrate and delineating multiple devices from a single wire, permitting four-point probe measurements for reduction of contact resistance. Furthermore, LPNE is a cost-effective process with a wafer-scale batch nature and reproducibility that is amenable to manufacturing. PPy nanoribbons with patterned electrodes fabricated using LPNE demonstrated significant potential as chemical gas sensors based on chemiresistive responses to ammonia vapor. Finally, the sensitivity toward ammonia was controlled by tuning the conductivity of PPy nanoribbons.

VIRAL PLANT PATHOGENS DETECTION USING SINGLE POLYPYRROLE (PPy) NANORIBBON IMMUNOSENSOR

Cucumber mosaic virus (CMV), an economically important virus belonging to the genus *Cucomovirus* (family *Bromoviridae*), is commonly found throughout the world and is transmitted by different aphid species to a wide range of vegetable crops including cucumbers, tomatoes, grapes, peanut, tobacco, etc. Similar to other plant viruses, no chemical substances have been reported to significantly prevent and eliminate CMV infection in plants. Current CMV mitigation strategies mainly involve eradication of infected sources so that they will not serve as inocula for other infected agricultural crops in the field. Thus, early detection is of the utmost importance and key to the successful management of CMV.

Many serological and molecular detection techniques have been developed and exploited to identify and quantify viral plant pathogens ranging from enzyme-linked immunosorbent assays (ELISA), including direct double antibody sandwich ELISA (DASELISA) and triple antibody sandwich ELISA (TAS-ELISA), electrochemical immunoassay (ECIA) to real-time PCR. Although these techniques can effectively detect viral plant pathogens, they are bound to laboratory settings, requiring well-trained individuals, costly instruments, and time-consuming processes. Development of rapid, accurate, reliable, and miniaturized field deployable sensors for minimally trained personnel has prompted research on one dimensional (1-D) semiconducting nanostructures based chemiresistors or chemical field effect transistors (ChemFET). Owing to their ultra-high surface area to volume ratio, enhancing surface adsorptive capacity, and tunable electrical properties, these label free, ultra-sensitive, and selective electronic biological/chemical sensors show a great promise towards fulfilling this need. When a molecule binds on the surface of a nano structured transducer (e.g., PPy nanoribbon), such a minute perturbation leads to depletion/accumulation of charge carriers within the bulk of the nanostructure which results in significant alterations of their electrical properties. This feature greatly improves the sensitivity as well as the low detection limit. One-dimensional nanostructures including single-walled carbon nanotubes (SWNTs), metal oxide nanowires (NWs), and silicon NWs configured as chemiresistors and ChemFETs have been developed for the detection of various biomolecules such as viruses, DNA, proteins, bacteria and cells. While these works demonstrated greater potential for advancing the sensing technology, one of the major challenges is the development of scalable, reproducible and cost-effective methods to fabricate and assemble high density devices. Despite their short history compared to inorganic materials, conducting polymers such as polypyrrole (PPy), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT) have emerged as excellent transducers for label-free electronic

chemical/biosensors. Besides the aforementioned advantages of 1-D nanostructures, 1-D conducting polymer nanostructures can be chemically or electrochemically synthesized under mild conditions, which allow entrapment of biorecognition molecules during electropolymerization, bypassing post-surface immobilization treatment. Their diverse monomer chemistries and derivatives also promote post-surface covalent immobilization using well-studied bioconjugation chemistry. Herein, we demonstrate the detection of CMV using anti-CMV polyclonal antibodies functionalized PPy nanoribbon-based chemiresistive immunosensors. The lithographically patterned nanowire electrodeposition (LPNE) technique previously developed by our group was employed to massively batch-fabricate PPy nanoribbons at pre-determined locations with integration of patterned microelectrodes. 60 N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC)/N-hydroxysuccinimide (NHS) chemistry was used to surface functionalize anti-CMV onto the PPy nanoribbons. The sensing performance of anti-CMV functionalized PPy nanoribbon-based immunosensors was optimized by adjusting dimensions, electrical conductivity, and the ionic strength of pH buffer.

CONCLUSION

Recently, the development of field deployable, label-free, ultra-sensitive sensory devices that enable reliable and direct electrical detection has been intensively researched to overcome drawbacks associated with traditional optical and electrochemical methods. Advancement of nanotechnology and biotechnology has prompted utilization of 1-D nanostructures as a novel sensing platform, owing to their ultra-high surface area to volume ratio, size dependent electrical properties, and possibility of a device miniaturization.

Conducting polymers (CP) are π -conjugated system which has single and double bonds alternating along the polymer chain. They emerged as another class of materials for chemiresistive/chemFET biosensors because they exhibit remarkable optical, magnetical, and electrical properties like a semiconductor while possessing mechanical properties and ease of fabrication as polymers. Tunable electrical conductivity can be realized by several orders of magnitude (i.e., 10^{-10} to 10^3 S/cm) via the process called 'doping' where anion is chemically or electrochemically incorporated to oxidized CP backbone to attain the charge neutrality. Amongst CPs, polyheterocyclines such as polypyrrole (PPY), polyaniline (PANI), poly(3,4-ethylenedioxythiophene) (PEDOT) and their derivatives are used as transducers due to great environmental stability, high conductivity, and functional monomers for direct immobilization of the bioreceptors by various surface immobilization routes such as physical adsorption, entrapment, crosslinking, and covalent bonding.

Thus, CP nanowires/tubes configured as chemiresistors and FETs have been developed as analytical tools for detection and monitoring of various chemical and biological molecules. While this nano-electronic biosensor illustrates strong potential for advanced technology in detection and monitoring, limitation in device fabrication and assembly for high density sensor arrays appears challenging to be scalable and reproducible in a cost-effective manner.