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Bioaffinity Sensing Using Biologically Functionalized Conducting-Polymer Nanowire

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Recent advances in electronic detection based on nanowires (NWs) and nanotubes (NTs) has revolutionized our ability to provide label-free and real-time, yet sensitive and selective detection of a wide range of chemical and biological species using the NW or NT as the gate of a planar field effect transistor (FET).[1] Unlike two-dimensional FETs, one-dimensional nanowires avoid the reduction in conductance changes caused by lateral current shunting to the point that even single-molecule detection is possible. The selectivity of the nanosensors can be further enhanced by modification with specific bioreceptors such as antibodies. Examples of silicon nanowire (SiNWs) and carbon nanotubes (CNT) functionalized with biotin and human autoantigen (U1A), respectively, for label-free, sensitive, and real-time detection have been reported.[1,2] The suggested mechanism for the resulting high sensitivity, potentially to single molecule, is the extremely sensitive modulation of the electrical conductance/resistance of the NWs and NTs brought about by the changes in the electrostatic charges from surface adsorption of various molecules, leading to the depletion or accumulation of carriers in the "bulk" of the nanometer diameter structure.

While these reports demonstrated the power of nanoengineered materials as biosensors, the fabrication methods employed are seriously limited. The techniques of manipulating individual carbon nanotube onto prepatterned electrodes by an atomic force microscope,[3] random dispersion of suspended carbon nanotubes onto prepatterned electrodes,[4,5] and lithographically patterning catalyst (as CNT nucleation sites) on electrodes,[6,7] while adequate for demonstrating the operational characteristics of individual devices, have low throughput and limited controllability and hence are unattractive for scaling up to high-density sensor arrays. More importantly, surface modifications, typically required to incorporate bioreceptors, have to be performed post-synthesis and post-assembly. Attempts to improve fabrication controllability using either electric field alignment[8,9] or fluidic alignment followed by e-beam lithography have been reported.[1,10] However, no report to date has demonstrated the ability to assemble these nanomaterials into a functional sensor circuit and to individually address each nanostructured sensing element with the desired bioreceptor, a requirement necessary for the successful fabrication of nanosensor arrays.

Conducting polymers such as polypyrrole (Ppy) because of their electronic conductivity, environmental stability, easy and controlled processing by electrochemical polymerization, and biocompatibility have emerged as promising materials in the development of planar electrochemical biosensors.[11,12] Biomolecules can be incorporated into the conducting polymer in a single step during polymer synthesis rather than the multiple steps needed for synthesis of surface-modified silicon nanowires and carbon nanotubes. Recently, Hernandez et al. reported a template method for synthesis of biologically functionalized Ppy nanowires.[13] Although elegant, application of template-fabricated biologically functionalized nanowire is limited by some of the same problems as those for SiNWs and CNT. Because the nanowires synthesized in the template have to be separated and collected by dissolving the template in a solution, the nanowires are either floating or remain attached to the substrate, forming a vertical array. For use in biosensors these nanowires will need a time-consuming and arduous method for adhering to patterned electrodes. Additionally, the harsh conditions, 25% nitric acid followed by 3 M sodium hydroxide, required to dissolve the alumina template might not be suitable for many biological molecules of interest. We report herein for the first time a simple, biomolecular friendly, single-step protocol for the fabrication of a polypyrrole nanowire biosensor of controlled dimension and composition, large aspect ratio, and most of all, site-specific positioning and its application to label-free bioaffinity sensing.

We recently demonstrated the feasibility of fabricating single and multiple individually addressable polypyrrole and polyaniline nanowires of controlled dimension (100 nm wide and up to 13 μm long) and location by electrodeposition within a channel between two electrodes on the surface of a silicon wafer and their application as pH sensors.[14] A similar electrode structure with a channel 100 or 200 nm wide by 3 μm long was employed for the entrapment of the model protein, avidin, during electrochemical polymerization of polypyrrole in a single step. The initial investigations on biomolecular functionalization of Ppy nanowires by entrapment in a single-step during electropolymerization were performed using avidin-conjugated ZnSe/CdSe quantum dots (Aqd). This was done to facilitate microscopic characterization and establishment of the location of the biomolecule in the nanowire.

Scanning electron micrograph images of the Aqd-functionalized Ppy nanowire demonstrated that the nanowire was continuous, well defined, dendrite free, spanning the entire length of the channel, and making a good contact with both electrodes. Energy-dispersive X-ray (EDX) analysis of the nanowires confirmed the presence of Cd within the nanowire, an indication of the presence of quantum dot and thereby streptavidin within the Ppy nanowire (Figure 1).

The integrity and good ohmic contact with the two electrodes of the biomolecule-modified Ppy nanowire was further confirmed by the linear dependence of current as a function of the applied potential (see Supporting Information Figure S2).

To demonstrate the utility of functionalized nanowires as sensors, biotin conjugated to a 20-mer DNA oligo (biotin−DNA) was applied. Figure 2 shows that the resistance of the 200 nm wide avidin-functionalized nanowires increased rapidly to a constant value upon addition of 1 nM of the biotin−DNA conjugate, and the resistance change increased with increasing concentrations up to 100 nM. Increasing the concentration further to 1 μM resulted in only a 4% increase over 100 nM, indicating saturation of recog-

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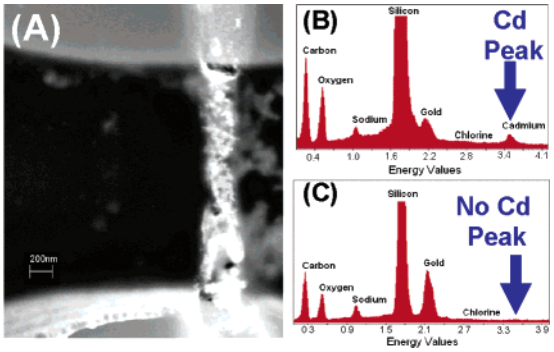

Figure 1. (A) SEM image of an Aqd-embedded polypyrrole nanowire (200 nm wide). The EDX analysis of polypyrrole nanowire with embedded Aqd (B) and without embedded Aqd (C).

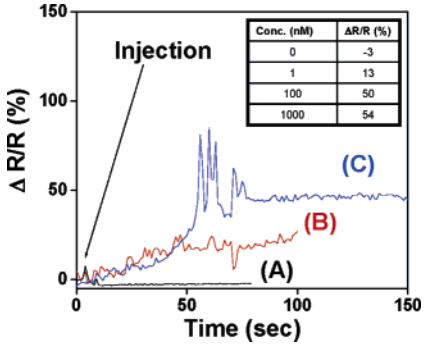

Figure 2. Electrical responses of an unmodified nanowire (A) to 100 nM biotin−DNA (single stranded) and avidin-embedded polypyrrole (200 nm) nanowires to 1 nM (B) and 100 nM (C) biotin−DNA. The responses were recorded on two separate polypyrrole−avidin nanowires. Polypyrrole nanowire containing entrapped avidin were grown using 25 nM pyrrole in 10 mM NaCl and of avidin.

nition sites. In contrast, addition of buffer and/or DNA oligo without conjugated biotin to the functionalized nanowires did not result in any observable changes in resistance. These observations together with the absence of any response to the addition of biotin−DNA to an unmodified Ppy nanowire confirmed that the changes in resistance were due to the binding of biotin−DNA with avidin in the functionalized nanowires. A similar response of a 100 nm Ppy−Aqd nanowire upon exposure to similar concentrations of biotin−DNA further validated the sensing strategy. While the % changes were very similar to those of the 100 nm Ppy−Aqd nanowire as expected, the absolute resistance values in 200 nm nanowires were lower due to the higher cross-sectional area of such nanowires.

For the detection of larger target analytes that cannot diffuse into the Ppy pores and a fast response of small molecules, the entrapped biomolecule should be on the surface of the nanowire. To determine the location of the Aqd in the Ppy, AFM PhaseImaging was performed on a 50 nm thick (the depth of the channel used for nanowire formation) Aqd−Ppy film electrochemically deposited on a gold electrode. AFM PhaseImaging is an extension of tapping mode which allows detecting variation in composition and hardness. As shown in Figure 3, Aqd−Ppy film shows a much higher contrast compared to Ppy film, demonstrating the composite nature of Aqd−Ppy film and presence of Aqd on the film surface.

In conclusion, a facile yet powerful method for fabrication of biologically functionalized nanowires of controlled dimension and high aspect ratio in confined channels and its application to bioaffinity sensing were demonstrated. The one-step incorporation of functional biological molecules into the conducting-polymer nanowire during its synthesis and built-in electrical contacts is the major advantage of the new fabrication method over the reported silicon nanowire and carbon nanotube biosensors that require post-

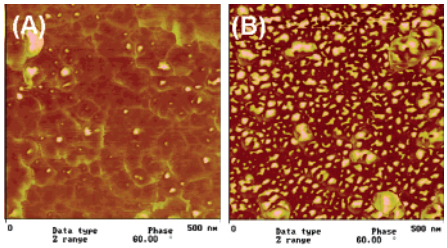

Figure 3. AFM phase images for a 50 nm thick film on an area for (A) polypyrrole and (B) polypyrrole with entrapped Aqd.

synthesis modification and positioning. Combined with the already demonstrated ability to make individually addressable nanowires that are a few micrometers apart sequentially, one at a time, this will enable fabrication of high-density biosensor nanoarrays. While the concept has been demonstrated for biological modification of Ppy nanowires, other monomers such as aniline and thiophene that can be electropolymerized from an aqueous environment benign to biomolecules can also be employed. The nanomolar sensitivity of the present biosensor, while lower than that for SiNW and CNT, was obtained with nanowires that were not optimized with respect to the conductivity. A sensitivity of potentially single-molecule detection is possible by adjusting the nanowire's conductivity to a value closer to the lower end of a semiconductor. Wrighton's group has already demonstrated that the conductivities of Ppy or polyaniline can be modulated by simply controlling the oxidation state of these polymers.[11,12,15] Additionally, the diversity of monomers, dopants, and electropolymerization conditions and modes adds many dimensionalities to the reported technique in terms of the ability to design tailor-made nanowire biosensors. These are currently being investigated.

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Supporting Information Available: Experimental procedure for fabrication of biologically functionalized Ppy nanowires, chronopotentiogram during nanowire growth, and *I*−*V* plot. This material is available free of charge via the Internet at http://pubs.acs.org.

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