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Wafer-scale fabrication of patterned carbon nanofiber nanoelectrode arrays: A route for development of multiplexed, ultrasensitive disposable biosensors

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ABSTRACT

One of the major limitations in the development of ultrasensitive electrochemical biosensors based on one-dimensional nanostructures is the difficulty involved with reliably fabricating nanoelectrode arrays (NEAs). In this work, we describe a simple, robust and scalable wafer-scale fabrication method to produce multiplexed biosensors. Each sensor chip consists of nine individually addressable arrays that uses electron beam patterned vertically aligned carbon nanofibers (VACNFs) as the sensing element. To ensure nanoelectrode behavior with higher sensitivity, VACNFs were precisely grown on 100 nm Ni dots with 1 μm spacing on each micro pad. Pretreatments by the combination of soaking in 1.0 M HNO_3 and electrochemical etching in 1.0 M NaOH dramatically improved the electrode performance, indicated by the decrease of redox peak separation in cyclic voltammogram (ΔE_p) to ~100 mV and an approximately 200% increase in steady-state currents. The electrochemical detection of the hybridization of DNA targets from *E. coli* O157:H7 onto oligonucleotide probes were successfully demonstrated. The 9 arrays within the chip were divided into three groups with triplicate sensors for positive control, negative control and specific hybridization. The proposed method has the potential to be scaled up to $N \times N$ arrays with N up to 10, which is ideal for detecting a myriad of organisms. In addition, such sensors can be used as a generic platform for many electroanalysis applications.

1. Introduction

With the recent increase in pathogen outbreaks in water, food and other media, new methods and technologies for detection and quantification are needed. These devices and systems will need to be fast, reliable, ultrasensitive, portable, and automated. For more than several decades, detection heavily relied on an indicator organism approach to assess the microbiological quality of drinking water. But an increased understanding of the diversity of waterborne pathogens has concluded that the use of bacterial indicators may not be as universally protective as was once thought (National Research Council, 2004). Newer methods involving immunofluorescence techniques and nucleic acid analysis provide valuable opportunities for rapid and more specific analytical methods. Particularly, electrochemical (EC) biosensors are attractive for detecting a wide range of species, including proteins, nucleic acids, small molecules and viruses because of their relative simplicity, portability, low cost and low power requirement. EC biosensors consist of two primary components: a recognition layer containing a biomolecule and an electrochemical signal transducer. They make use of electrochemical reactions or the surface property changes upon target binding. Advances in microfabrication technology have provided electrode configurations such as microelectrode arrays (MEAs) (Dill et al., 2004), and interdigitated arrays (IDA) (Niwa and Tabei, 1994) but their performance can be further enhanced by miniaturizing to nanoscale. Recent progress in nanofabrication technologies like electron beam lithography and nanoimprinting enable fabrication of one-dimensional nanostructure electrodes like carbon nanofibers (Li et al., 2004a,b, 2005; Guillorn et al., 2002), carbon nanotube bundles (He and Dai, 2004; Yun et al., 2006), nanoscale IDA (Gerwen et al., 1998), silicon nanowires (Patolsky et al., 2006) and diamond nanowires (Yang et al., 2008), which are capable of high spatial and temporal resolutions, possibly yielding sufficient sensitivity to single molecule detection. Among various types of one-dimensional nanoscale electrodes, vertically aligned carbon nanofibers (VACNFs) have received

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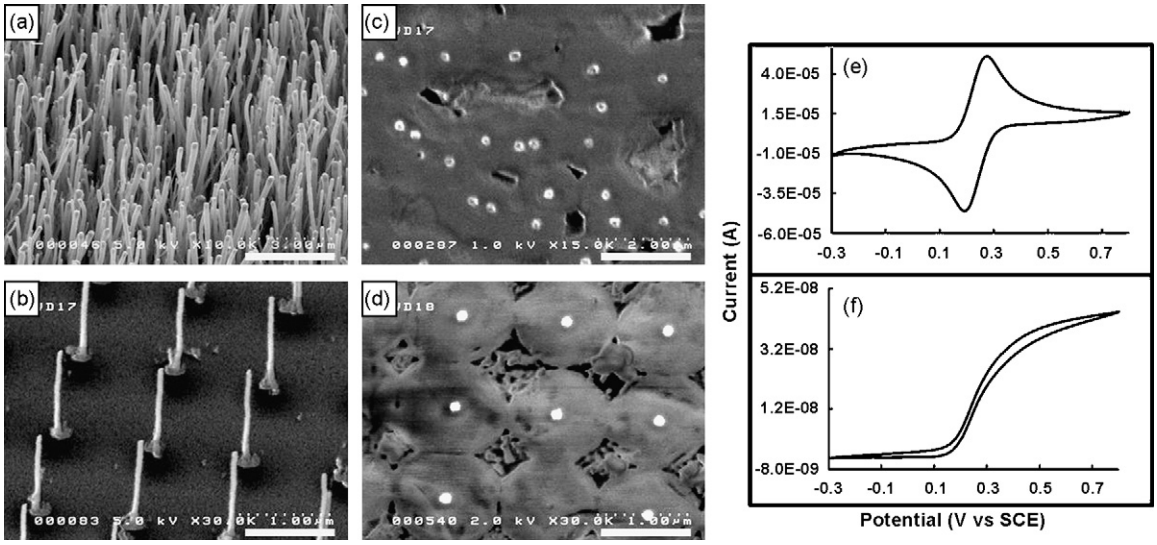

Fig. 1. SEM images of (a) as-grown forest-like VACNFs, (b) as-grown patterned VACNF arrays on 100 nm diameter Ni spots using e-beam lithography, (c) the surface of a polished VACNFs embedded in SiO_2 matrix, and (d) the surface of a polished patterned VACNF array embedded in SiO_2 matrix. (a and b) are 30° perspective views while (c and d) are top views. The scale bars in (a)–(d) are 3,2,1 and 1 μm, respectively. (e) and (f) are the cyclic voltammetry curves for a high-density array chip with forest-like VACNFs and a low-density array chip with patterned VACNFs from one of the micro pads. Curves were taken in 4.3 mM $K_4Fe(CN)_6$ in a 1.0 M KCl at 20 mV/s scan rate.

tremendous attention because of their attractive properties such as high electrical and thermal conductivities, superior mechanical strength, a wide electrochemical potential window, flexible surface chemistry and biocompatibility (Li and Meyyappan, 2004; Melechko et al., 2005). Compared to other carbon materials such as glassy carbon, carbon black, carbon microfibers, and pyrolytic graphite, the open-ended VACNF arrays present well-defined edge-plane structure suitable for selective covalent functionalization of primary amine-terminated oligonucleotide probes.

Initial work on VACNF based EC sensors from our group (Li et al., 2003a,b; Koehne et al., 2003) and others (Baker et al., 2006; Guillorn et al., 2002) primarily used forest-like CNFs or MWCNTs. In these studies, the detection sensitivity and electrochemical properties were initially improved by controlling the nanofiber density (Li et al., 2004a,b, 2005). If more CNFs were exposed with spacing being smaller than $\sim 6R_{ave}$, where R_{ave} is the average electrode radius, then the cyclic voltammogram (CV) from those high-density arrays would behave similar to a macroelectrode with anodic and cathodic peak currents and high charging/discharging (background) currents. In contrast, a low-density array with individual nanoelectrodes separated by more than $\sim 6R_{ave}$ ensures that there is no overlap of the diffusion layers from neighboring electrodes and each CNF truly behaves as a single nanoelectrode showing a sigmoidal CV curve with a steady-state current dominated by radial diffusion. Such low-density arrays are best suited for ultra-sensitive detection because of improved signal-to-noise ratio (S/N), reduced ohmic drop, and thus the capability to reach lower detection limits. The most common fabrication approach is a bottom-up scheme where VACNFs are grown with plasma enhanced chemical vapor deposition (PECVD) as high-density arrays from a catalyst film (Meyyappan et al., 2003) or as low-density arrays from precisely positioned individual catalyst dots (Moser et al., 2003; Li et al., 2003a,b; Teo et al., 2003). The as-grown VACNFs are then embedded in a SiO_2 matrix that provides electrical isolation and mechanical support. For example, Fig. 1a and b shows the as-grown VACNFs from a Ni film and an array of Ni dots, respectively, and Fig. 1c and d shows the corresponding VACNF tips after embedded in silicon oxide followed by re-exposing the CNF tips by planarization. The oxide was grown by thermal chemical vapor deposition (CVD) of tetraethlyorthosilicate (TEOS). The open ends of the CNFs protruding above the matrix surface behave like nanoelectrodes. As shown before, the open ends are dominated by edge planes, which serve as highly active carbon electrodes with fast electron transport for electroactive species. Fig. 1e and f shows CVs from a high-density NEA and a low-density NEA in consistency with theory.

Previous reports have demonstrated patterned VACNF arrays as potential electrochemical probes, where the chip was mostly fabricated from small pieces of substrates (Guillorn et al., 2002). They underline the importance of revealing only the tips to the redox solution but not the VACNF sidewalls and the usually long metal interconnects (several millimeters), which is critical in minimizing the background "leakage" currents. Typically, a passivation layer is deposited on the fibers and interconnects followed by dry plasma reactive ion etch (RIE) to re-expose the tips. The widely used passivation layers include silicon dioxide, electrochemically inert polymers such as photoresist, epoxy, SU-8 or a combination of polymer and oxide. Here, we used a chemical mechanical polishing (CMP) process instead of RIE. The CMP process demonstrates significant advantages, particularly in preserving structural integrity around the tips. In this study, for the first time, we report a 3 × 3-array biosensor using nanopatterned VACNF array for *E. coli* O157:H7 detection. High S/N ratio and on-chip positive and negative controls were achieved with such arrays. We also confirm the feasibility of a simple and reliable wafer-scale fabrication process that utilizes standard semiconductor processes and can be easily scaled up to $N \times N$ arrays (with N up to 10), critical for the development of highly multiplexing disposable biosensor chips.

2. Materials and methods

2.1. Fabrication of VACNFs nanoelectrode arrays

The wafer-scale fabrication of VACNF NEAs includes six major steps: (1) metal deposition for micro pads, contact pads and electrical interconnects; (2) nanopatterning of Ni catalyst dots; (3) directional growth of CNF; (4) silicon dioxide deposition for electrical isolation and mechanical support; (5) chemical mechanical polishing (CMP) to expose CNF tips, and (6) a wet etch with 7:1 HF to expose contact pads. All processes are done on a 4-in. silicon (1 0 0) wafer coated with 500 nm thick thermal oxide (Silicon Quest International, Inc., Santa Clara, CA). The sequence for this fabrication process and corresponding scanning electron microscope