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Vertically aligned carbon nanofiber nanoelectrode arrays: electrochemical etching and electrode reusability

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Abstract

Vertically aligned carbon nanofibers in the form of nanoelectrode arrays were grown on nine individual electrodes, arranged in a 3×3 array geometry, in a 2.5 cm^2 chip. Electrochemical etching of the carbon nanofibers was employed for electrode activation and enhancing the electrode kinetics. Here, we report the effects of electrochemical etching on the fiber height and electrochemical properties. Electrode regeneration by amide hydrolysis and electrochemical etching is also investigated for electrode reusability.

Index terms

Carbon nanofibers; electrochemical etching; Atomic Force Microscopy; cyclic voltammetry; Differential pulse voltammetry

1 Introduction

Recent advances in nanomaterial synthesis, manipulation and nanofabrication technologies involving hybrid bottom-up/top-down processes have enabled a substantial growth in the development of electrochemical (EC) sensors for biological and chemical sensing.¹⁻³ Integration of variety of 1-D nanomaterials such as carbon nanotubes (CNTs), carbon nanofibers (CNFs),^{4, 5} silicon and other nanowires,^{8, 9} with microelectrode arrays¹⁰ or interdigitated arrays^{11,12} and associated micro/nanofluidics can provide interesting lab-on-a-chip devices for point-of-care-testing applications. Carbon nanostructures such as CNTs and CNFs have been explored extensively for sensor applications due to their unique advantages such as enhanced electrical properties, higher chemical and mechanical stability, rapid electrode kinetics, easy surface functionalization, modification and probe attachment for specific biotargets. The general requirements for the development of an ideal electrode for biosensors include high sensitivity, low detection limits, low power consumption, assay multiplexing, reproducibility and fast response time (high electron transfer kinetics).

Recently, nanoelectrode arrays (NEAs) constructed using vertically aligned CNFs (VACNFs) grown by plasma enhanced chemical vapor deposition (PECVD) have gained much attention for biosensor applications due to their unique properties such as superior

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electrical and thermal conductivities, mechanical robustness, higher signal to noise ratio, a wide electrochemical window, ease in surface modification and biocompatibility.¹³ The detection limit and temporal resolution of electrochemical measurements improve with reduced size of the sensing electrodes.¹⁴⁻¹⁶ Properly spaced and electrically isolated VACNFs, either regularly patterned or randomly grown on the substrate, have been studied for ultrahigh sensitivity and lower detection limit in biosensing applications.^{17,18} The integration of the nanoelectrode array with microelectronics and associated microfluidics can lead to a fully miniaturized system in the near future.¹⁹ The non-linear diffusion of redox species to the electrode surface results in the sigmoidal steady state cyclic voltammetry (CV) response²⁰ with high electrode kinetics, which makes encapsulated VACNF NEAs attractive for rapid and ultrasensitive biosensing applications. They offer unique advantages such as high current density (compared to micro and macro electrodes), higher sensitivity, lower detection limit, low background current, high signal-to-noise ratio and faster response.²¹ Various biosensing applications such as DNA hybridization analysis,¹⁷ nucleic acid detection,¹⁸ electrochemical sensing of neurotransmitters,¹⁹ glucose detection,²² neural electrical reading,²³ label-free detection of ricin A chain and cardiac troponin,^{24,25} and electrochemical protease biosensor²⁶ have been reported using VACNF NEAs.

In spite of the enormous work done over the past decade, the use of VACNF NEA in lab-on-a-chip applications faces challenges in meeting simultaneously all the general properties of an ideal electrode listed above. In the context of improving the biosensor reliability, the effects of extreme environment and process conditions on the dimensional characteristics and quality of CNFs were studied earlier.²⁷⁻²⁹ More efforts for improving the stability of the essential characteristics of the sensor such as chemical, electrical, mechanical, and reproducibility are needed before system integration with microfluidics and launching them into the market. Electrode preconditioning is done to activate and enhance the electrode kinetics by performing electrochemical etching (ECE) in NaOH aqueous solutions. In this study, we investigate the effect of ECE on the resulting height and electrochemical properties of the VACNFs. Electrode regeneration has also been investigated by amide hydrolysis using ECE.

2 Experimental work

A. Chemicals and Reagents

For electrochemical etching, 1.0 mM solution was prepared by dissolving appropriate NaOH pellets (Sigma Aldrich, St Louis, MO) in high purity deionized water (18.2 MΩcm) from super-Q Millipore system. For protein (C-reactive protein, CRP) binding experiments, CRP (2.1 mg protein per ml (lowry)), anti-human C-reactive protein (anti-CRP, 54.7 mg protein per ml (Biuret)) antibody (produced in goat), linker reagents that include 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%) and N-hydroxysuccinimide sodium salt (sulfo-NHS, 98% (HPLC)) were purchased from Sigma Aldrich (Saint Louis MO). CRP and anti-CRP stock solution were stored at 4 °C and -20°C respectively as instructed by the manufacturer. Phosphate buffered saline (PBS, 10 mM, pH 7.4) was prepared for protein dilution by dissolving PBS sachet (Sigma Aldrich, St. Louis, MO) in deionized water and

filtering using a 0.22 μm membrane filter before every use. Potassium hexacyanoferrate (III) was used as electrolyte for all electrochemical evaluation. All other reagents used in the study were of analytic grade.

B. Fabrication of e-beam patterned VACNF NEA Chips

A detailed discussion on wafer scale fabrication of VACNF NEA chips has been reported previously.^{17-19,21} A brief account relevant to the present study is given below. Figure 1 shows various fabrication steps including the following six key processes: (1) defining of micro pads (electrodes), contact pads and interconnections on silicon wafer using optical lithography, (2) metal deposition by e-beam evaporation process, (3) e-beam nanopatterning of Ni catalyst particles, (4) DC-biased PECVD of VACNFs, (5) oxide passivation of freestanding individual nanofibers and (6) electrode surface planarization and exposure of nanofiber tips by reactive ion etching (RIE) and chemical mechanical polishing (CMP).

A 4-inch silicon (100) wafer with ~ 500 nm oxide layer was lithographically patterned to contain 30 device chips. Each chip contains nine identical micro pads (3×3 format, $200 \mu\text{m}$ by $200 \mu\text{m}$, Fig. 1A), nine contact pads (1 mm by 1 mm) and nine individual channels for interconnection between micro pads (electrodes, Fig. 1B) and contact pads for external electrical contact. A $200 \mu\text{m}$ thick chromium (Cr) under layer was deposited on the wafer by e-beam evaporation. The 100 nm nickel catalyst dots were patterned using e-beam lithography (2 nA , $1950 \mu\text{C}/\text{cm}^2$ at 100 keV) where each dot was placed at a distance of $1 \mu\text{m}$ from its neighbor. These dots were created by depositing 10 nm Cr adhesion layer followed by 30 nm Ni catalyst layer. Subsequently the wafer was inspected by optical microscope (see Fig. 1C).

VACNFs were grown from these patterned Ni catalyst particles with previously reported growth protocols¹⁹ using DC-biased PECVD reactor (Aixtron, Cambridge, UK). The growth recipe includes acetylene (C_2H_2 , 125 sccm) feedstock for carbon source diluted with ammonia (NH_3 , 444 sccm) at 6.3 mbar , plasma power of 180 W and 700°C . A 15 min . deposition yields ~ 39000 freestanding VACNFs (shown in Fig. 1D) on each patterned electrode where each fiber has a tip diameter of $\sim 80 \text{ nm}$, base diameter of $\sim 100 \text{ nm}$ and an average height of $\sim 1.5 \mu\text{m}$ with Ni catalyst particle on the tip. To provide mechanical support to the freestanding fibers and electrical isolation of the underlying Cr layer, $3 \mu\text{m}$ SiO_2 was deposited using CVD with tetraethylorthosilicate (TEOS) vapor precursor from a liquid source. Figure 1E depicts the oxide deposition on electrodes containing the nanofibers. The electrode surface planarization and exposure of the fiber tips were achieved by CMP in two steps, using $0.5 \mu\text{m}$ alumina ($\text{pH } 4$) for stock removal (Fig. 1F, CNFs with Ni particles on the tips) and $0.1 \mu\text{m}$ alumina ($\text{pH } 4$) for final polish as shown in Fig. 1G. Figure 1H is an atomic force microscope (AFM) micrograph of the electrode 2-D cross section further validating the successful exposure of the CNFs tips after the CMP steps. Contact pads for electric connection were exposed by wet chemical etching of the SiO_2 layer using HF etchant. The wafer was finally diced into 30 equal size (2.5 cm by 2.5 cm) chips.

A final polishing step was carried out prior to the use of the electrode, using non-crystallizing colloidal silica suspension ($0.02 \mu\text{m}$, Buehler, Lake Bluff, IL) on polishing pad (MicroCloth, 73 mm dia., Buehler, Lake Bluff, IL) for 5-7 minutes, followed by rinsing with

isopropyl alcohol (IPA), deionized water and sonication in deionized water for 15 minutes. This step removes the residual debris off the CNF tips, which could be from the final CMP procedure. Following sonication, the chips were soaked in nitric acid for 30-50 minutes to ensure the complete removal of any Ni catalyst particles and further clean the nanofiber surface. The chip was then rinsed with de-ionized water and stored in petri dish for future use.

C. Electrochemistry and Microscopy

Electrochemical etching and characterization measurements were carried out using a standard three-electrode electrochemical cell connected to a H-CHI660D Instrument, Electrochemical Workstation/ Analyzer (CHI Instruments, Inc, Austin, TX). The instrument was interfaced to a personal computer and controlled by associated data processing/ analyzing software, chi660d. The electrochemical cell set up includes a high quality platinum (Pt) wire as counter electrode, saturated calomel electrode (SCE, Princeton Applied Research, Oakridge, TN) as reference electrode and individual VACNF NEAs as working electrode connected in a custom designed Teflon liquid cell. The cell defines an O-ring of 3 mm diameter and connects 9 gold spring-loaded pins (IDI, Kansas City, KS) for electrical contacts to the 9 working electrodes through respective contact pads.

Electrochemical etching of an electrode was performed at a potential of 1.5 V in 1M NaOH aqueous solution from 0.0 to 22.5 s with a 2.5 s increment. AFM imaging and electrochemical evaluation of the electrode were performed after every 2.5 s of ECE. Chips used in this study were from the same wafer that ensured uniformity in the physical properties, chemical composition, and electrochemical or electrical characteristics of the VACNFs.

A specialized carbon nanotube (CNT) modified AFM-tip (Performance Line, $k = 2 \text{ N/m}$, $f = 60 \text{ kHz}$, Carbon Design Innovations Inc., Burlingame, CA) was used to map the electrode surface topography for measuring the height of CNFs protruding from the SiO_2 matrix. All images were collected in tapping mode^{27,28} using a Multimode AFM (Digital Instruments, Santa Barbara, CA) at 1 Hz scan rate and 512×512 pixels frame. 2-D cross-sectional images were collected to obtain the height of CNFs protruding above the SiO_2 matrix. Electrodes were imaged in two different regions and the heights of more than 220 CNFs were measured to calculate the averages and standard deviations. After every etching operation and before collecting AFM images, the chip was sonicated in water for at least 5 minutes at a frequency of 30 kHz (to remove the debris) and dried at room temperature for at least 10 minutes.

Scanning electron microscopy (SEM) images were recorded using a field emission SEM (S-4000, Hitachi, Pleasanton, CA) to further verify the VACNF presence above the silicon oxide surface after every etching operation. Energy Dispersive Spectroscopy (EDS) was also performed on the electrode to examine the composition of the VACNFs. Electrochemical measurements were performed after each timed etching to investigate the effect of electrochemical etching on electrochemical properties of the VACNFs. A 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 1 M KCl electrolyte solution was used to evaluate the electrode characteristics using cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

D. Electrode Recovery using Electrochemical Etching

The reusability of the sensor was evaluated by electrochemically removing the target-probe molecules. Complete antigen-antibody binding experiments were performed on a chip. Five electrodes in a chip were studied for the electrode regeneration process. Figure S1 depicts the surface functionalization and probe binding steps; Fig. S1 (A) and (B) show a schematic of the VACNF NEA and the fiber tip exposed from the SiO₂ matrix respectively. Electrode activation and the generation of carboxylic functional groups on the fibers tips were generated by NaOH treatment (similar to electrochemical etching for 5-7 s only, see Fig. S1 (C)). The electrochemical readout for bare electrodes was collected before the probe molecule binding. To achieve a stable terminal activation of carboxylic groups, a reaction of coupling reagents (linker) solution containing 0.4 M EDC and 0.1 M sulfo-NHS in PBS (10 mM, pH 7.4 and 1:1 volume) was carried out with the VACNFs for 30 minutes. The functionalized electrodes were then rinsed gently with PBS twice for 5 minutes each to remove loosely attached linker molecules off the electrode surface. Immediately after the linker binding, a 50 μ L of anti-CRP (5 μ M) solution in PBS (10 mM, pH 7.4) was spotted on these electrodes and incubated at room temperature for 90 minutes. The anti-CRP probe immobilization was achieved through covalent bonding of the probes with carboxylic functional groups through the reaction between the primary amine groups in anti-CRP and –COOH groups at the VACNF tips, yielding stable amide bonds between anti-CRP and VACNFs, shown in Fig. S1(D). Stringent rinsing was performed for 15 minutes in three steps for removing non-specific by-products and loosely bonded anti-CRP molecules off the electrode surface: twice with PBS (2 $\times$ 5 minute) and once with de-ionized water (5 minutes) while shaking the sensor at room temperature. The electrodes were then allowed to dry at room temperature before the electrochemical characterization from the probe (linker/anti-CRP) binding. Then the electrodes were rinsed gently with de-ionized water and incubated with specific concentration of CRP (2 μ M) on anti-CRP immobilized electrode surface for 1 hour. Electrochemical data were collected on each electrode after CRP binding. Later these CRP modified electrodes were treated with 1 M NaOH in two steps: 1) the reaction between NaOH and amide bonds (formed between amine terminated CRP-antibody and –COOH) for couple of minutes and 2) applying a potential of 1.5 V for one sec only. After the NaOH treatment the electrodes were rinsed with deionized water and allowed to dry.

3 Results and Discussion

A. AFM Scanning and Measurements

In order to estimate the fiber etching rate, the height of individual fibers (above the SiO₂ layer) was measured for different durations (0 to 22.5 s) of electrochemical etch using the AFM images. Figure 2 shows 2-D and 3-D AFM images of $\sim 144 \mu\text{m}^2$ cross section of the electrode after performing ECE for different durations. Figure 2A shows the 2-D image after performing ECE for 5 s where the VACNFs are clearly seen as raised features above the oxide surface (shaded background). The presence of the fibers above the oxide surface is further confirmed by the vertical features in the corresponding 3-D image of Fig. 2B. The variations in the initial height of VACNFs (as shown in Fig. 2B) can be attributed to the non-uniform growth of CNFs from Ni catalyst particles under the set growth protocols. The 2-D and 3-D images in Fig. 2C and D after 12.5 s of ECE show a reduction fiber height

compared to the one shown in Fig. 2A and B. A further decrease in fiber height and density above the oxide surface can be seen after 15 s of ECE (see Fig. 2E & F). The topograph shows recessed (holes) into the oxide surface for 20 s of ECE as shown Fig. 2G. The recessed CNFs into the oxide surface can be seen clearly as sharp trenches in Fig. 2H. The decrease in the fiber height is due to the electrochemical etching of carbon atoms from the tips.^{14, 30} The decrease in the density of fibers above the surface with the increase in electrochemical etch duration, before all the CNFs are recessed, is due to the fact that the fibers with much smaller initial height recess faster into the oxide surface than the taller fibers. Besides the etching of CNFs, excessive electrochemical etch can result in the etching away of the oxide surface, for instance, bigger grooves or cavities are seen in Fig. 2F and H.

The discussion above indicates qualitatively that the height of the CNFs decreases with the increase in electrochemical etch duration. However for quantitative analysis, the height of CNFs is measured for estimating the etch rate. Figure 3A depicts a graphical 2-D cross sectional height profile image of a fiber obtained by drawing a line across the fiber. The CNF height as well as diameter can be measured from Fig. 3A. The height of the fiber is obtained by measuring the vertical distance between the CNF tip and the SiO₂ matrix at the CNF base (blue markers) and the CNF diameter can be obtained by measuring the full width at half (FWH) of the CNF (red markers) as shown in Fig. 3A. To further illustrate the effect of electrochemical etching on fiber height, three line sections (blue, red and green markers) were drawn on Fig. 2A, C, E, G and the corresponding graphical 2-D cross sectional height profile images are shown in Fig. S2 (B, D, F & H) (in supporting information). The decrease in fiber height as shown in the line graphs of Fig. S2 is consistent with the discussion above.

The fiber height above the oxide surface and the tip diameter are important parameters for device sensitivity and detection limit.¹⁴⁻¹⁶ Figure 3B shows a plot between the average height (nm) of the fibers vs. electrochemical etching duration where error bars represent the standard deviation in the height. A linear decrease in CNF height (etching of carbon) is seen with respect to etch time. The CNF height is measured above as well as below the oxide surface. The slope of the plot in Fig. 3B determines the etch rate, which is ~ 1.7 nm/s. VACNF preconditioning is done commonly in NaOH aqueous solution through ECE in order to activate the tips for higher sensitivity and to generate the -COOH functional groups at the tips through oxidation, which eventually enhance the probe molecule binding with CNFs. However, the results here reveal that electrochemical etching of CNFs in NaOH proceed at a rate of ~ 1.7 nm/s. An excessive conditioning could potentially render the VACNF electrodes unsuitable for sensing applications. Thus an accurate estimation of electrochemical etching rate of VACNFs in NaOH becomes necessary for biosensing applications.

B. SEM imaging and EDS Data Analysis

Figure 4 shows SEM images of the electrode after various etch times. The bright round dots in Fig. 4A represent the VACNFs embedded in shaded SiO₂ background in the electrode after 5s of ECE. The distance between a pair of CNFs is ~ 1 μ m which is consistent with the AFM results. The etching of VACNFs occurs with the increase in etching duration. Figures 4B and C are SEM images of the electrode after 12.5 and 15 s of etching where the

formation of holes at the CNF locations indicates the etching of fibers. Some of the CNFs can be seen recessed into the oxide surface and the density of recessed CNFs increases with the etch duration. All the CNFs are shown recessed completely into the oxide surface for 20 s of etching, which is also seen in the corresponding AFM images (Fig. 2G, H).

Figure 5 shows the SEM images of an un-etched (Fig. 5A) and an etched electrode (17.5 s of ECE, Fig. 5C) and Figure 5B and D represent the EDS spectrum for the same electrodes respectively, showing the peaks for elements (oxygen (O), silicon (Si), chromium (Cr) and carbon (C)). The height of the element peaks represents the % by weight of specific materials in the total composition. The C peak is smaller than the peaks of the other elements, which is consistent with the fact that VACNF growth is assisted by discretely and sparsely patterned Ni nano-particles over the micro pad. Similarly, Si and O peaks are higher due to the SiO₂ layer and silicon substrate. The C peak in Fig. 5D has a smaller amplitude than in Fig. 5B, which reflects the etching of carbon atoms. The EDS spectra for specific regions (CNF sites and SiO₂ surface) in Fig. 5A and C can be analyzed for the presence of C using INCA software application tools. Figure S3 (in supporting information) shows distinct data points (spectrums) studied and quantitative values (% by weight) for the different elements. Six data points in Fig. S3 (A) and fourteen data points in Fig. S3 (B) were explored for C using EDS. Quantitative values of constituent elements for all the data points in Fig S3 (A) and (B) are returned by the INCA and given in Table 1 (Fig. S3 (C)) and Table 2 (Fig. S3 (D)) respectively. The weight % of C is the lowest (~13-15% for un-etched and ~5-8% for etched CNFs) including all other elements (Si, O and Cr) of all the data points under study, which is true due to the reason discussed above. The % by weight of C is also lower for an etched electrode than for the un-etched electrode. The % of all other components (Si, O and Cr) shows almost the same values for all the data points in Fig. S3 (A) and (B) (see the min-to-max range for Si, O & Cr), which further confirms that ECE of CNFs removes C atoms. In Table 2, no value for C is recorded for some regions, which is anticipated for complete etching of CNFs. Etching of SiO₂ and the underlying Cr layer was also observed for excessive electrochemical etching (as seen in Fig. S4). The complete removal of C and Cr would affect the electrochemical properties of the electrodes as discussed in the next section.

C. Effect of Electrochemical Etching on Electrochemical Properties of the VACNF NEA

The CV curves were recorded in a potential window from -400 mV to 800 mV vs. SCE at 20 mV/sec and DPV curves were obtained with 5 mV step potential, 25 mV modulation amplitude, 0.8 s modulation time, 1.2 s interval time and voltage scan from -0.3 to 0.7 V. Figure 6 presents CV and DPV curves from an electrode recorded at 2.5 s intervals for ECE performed on the electrode for 0 to 22 s. From 0 to 15 s (during when CNFs exist above the oxide surface), the CV and DPV curves acquire typical sigmoidal shape, increase in steady state and exhibit a reduction in the potential gap between the two steady states (forward and reverse scan), which in turn indicates an improvement in electrode kinetics. This significant improvement in electrode kinetics can be credited to the progressive cleaning (removal of debris off the CNFs surface) and activation of the fibers, and removal of carbon atoms from the CNF tips (edge planes) or from the side walls (basal planes) creating defects (open edges), which can act as charge transferring sites²⁷. The current amplitude, however,

decreases significantly for etching time greater than 15 s which is due to excessive etching of carbon material.^{17,18} Moreover, recessing of VACNFs much deeper into the oxide layer (holes) creates hollow cylinders on top of the CNF tips, and accumulation of redox reaction products in these holes can block the electrolyte species to reach the CNFs for charge exchange, thus resulting in a decrease in current.

Similar behavior can be seen for the DPV peak current as shown by the baseline corrected DPV scans in Fig. 6B. The DPV current improves with etch duration from 0 to 15 s and then starts decreasing afterwards. Excessive electrochemical etching can etch away not only carbon but also SiO₂ as well as the underlying Cr layer, therefore resulting in no steady state CV current or DPV peak current (see CV and DPV curves for 20 s of ECE in Fig. 6A and B). The improvement in electrode kinetics is an important factor for higher sensitivity and lower detection limit unless the carbon atoms are removed substantially.^{17,18,32}

D. Electrode Regeneration Evaluation

Sensor regeneration was evaluated by electrochemically removing the probe (antibody) molecules from the electrodes. Five of the nine electrodes in the chip were modified with anti-CRP probes and CRP antigens and treated with NaOH (post antigen bindings) as described earlier. Figure 7 shows selective CV and DPV curves for bare, linker\anti-CRP and linker\anti-CRP\CRP modified and regenerated electrodes. The CV steady state current amplitude decreases from its value for bare (curve I) to subsequent surface modified electrodes: linker\anti-CRP (curve II) and linker\anti-CRP\CRP antigen (curve III). The subsequent decrease in current amplitudes is due to the coverage of effective electrode area first by antibody (anti-CRP) insulating interface and later by the binding of target CRP molecules with anti-CRP probes, which further screens the fibers blocking the charge transfer between fibers and (Fe[CN₆]^{3-/4-}). However, the current (curve IV, regenerated) regains to its bare electrode value after the NaOH treatment of antigen-anti-body modified VACNFs NEAs electrode.

Similar trends are seen for the DPV current peaks shown in the baseline subtracted DPV scans in Fig. 7B. Figure 7C shows the average DPV peak current values for bare, linker\anti-CRP and linker\anti-CRP\CRP modified and regenerated electrodes. The current decreases with surface coverage (linker\anti-CRP and linker\anti-CR\CRP) however the current value is regained after the NaOH treatment. Figure 7D shows the percentage regeneration results obtained from five electrodes and the average is ~ 100 %.

These results imply that the NaOH treatment removes the antibody-antigen molecules selectively from the electrodes and regenerates the original electrochemical or electrical properties. Antigen-antibody complex dissociation and electrode regeneration using NaOH in ethanol has been reported by Bryan *et al.*³² Regeneration of electrodes can be explained with amid hydrolysis reaction and ECE in two steps: 1) amide bonds holding the antibody with CNFs (covalent bonding between –COOH and amine associated with anti-body molecules) are dissociated by NaOH to evolve ammonia gas and sodium salt of carboxylic acid during the couple of minutes reaction of NaOH according to the reaction³³ shown below

and 2) the sodium salt is etched during the 1 s electrochemical etching. The etch time for removing the antibodies is kept low in order to avoid the etching away of carbon atoms as discussed earlier. Electrode regeneration can provide advantages in multiple use and assay multiplexing biosensor applications.

E. Electrode Repeatability Studies

Each individual electrode of the 3×3 array shows high repeatability both before and after amide hydrolysis and electrochemical etching steps. The electrochemical etching of electrodes in NaOH was performed just before attaching the probe molecules as well as after the amide hydrolysis step i.e., post target molecule binding.

The NaOH treatment of the electrodes creates functional groups such as carboxylic groups ($-COOH$) as well as hydroxyl groups ($-OH$) at the fiber tips. The $-COOH$ groups help in covalent bonding of amine terminated probe molecules to the fiber tip; the $-OH$ groups, however, contribute to the electrode current response because of hydrogen reduction. Since the $-OH$ groups are not stable, they could lead to an unstable initial CV response curve^{18,21}. It is important to obtain a stable electrochemical response before using these electrodes for specific probe binding and target molecule detection experiments. The contribution to the response curve due to the $-OH$ groups ceases completely on repeating the CV scans several times after which overlapping (reproducibility) of the response curves starts to appear.

In order to evaluate the repeatability of the electrode response, CVs were taken before immobilizing the probe molecules (antibodies) as well as after performing the amide hydrolysis and electrochemical etching post target molecule bindings. Figure 8 shows the CVs for an electrode recorded before and after the hydrolysis and electrochemical etching steps. Figure 8 (A) and (C) show the current vs. potential plots whereas Fig. 8 (B) and (D) give the corresponding current vs. time representations. It is clear from these figures that after the initial instability, the CV curves start overlapping approximately after the seventh scan of the CV curve. The repetition of CVs is further evident from the time plots after ~ 600 seconds.

4. Conclusions

Electrochemical etching of vertically aligned carbon nanofibers was performed in NaOH aqueous solution for various etch times and the effects on the resulting CNF height and electrochemical properties were studied. The reusability of electrodes using ECE was also investigated. The height of the CNFs reduces at a rate of ~ 1.7 nm/s and the electrode kinetics improves with etch time. Electrode activation (fast electron transfer kinetics) enhances the electrode sensitivity and lowers the detection limit. However, longer duration etching can substantially remove the VACNF below the passivating oxide matrix, which in turn can render the electrodes unsuitable for sensor application. Electrochemical etching in 1 M NaOH at 1.5 V for a couple of seconds can regenerate the VACNFs electrodes for multiple use. The current challenges include chip-to-chip variation arising from inactive Ni

catalysts particles failing to grow CNFs on some locations resulting in VACNF density variations and potential excessive etching that can remove CNF tips and even the underlying chromium layer. Future growth and processing optimization studies are expected to address these issues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Fabrication steps for the VACNF nanoelectrode array; A) Scanning Electron Microscopy (SEM) image of a 3×3 array device, B) individual electrode, C) patterned Ni catalyst particles, D) patterned growth of CNFs (30° view), E) conformal deposition of SiO₂ on VACNFs, F) exposure of CNFs, G) CNFs embedded in SiO₂ and H) AFM image of 2-D cross-section of electrode.

Figure 2. AFM 2-D (A,C,E,G) and 3-D (B,D,F,H) images of the electrodes scanned after electrochemical etching of different durations in an aqueous solution 1 M NaOH at 1.5 V; (A and B) 5.0 s, (C and D) 12.5 s, (E and F) 15.0s and (G and H) 20.0s.

Figure 3.

VACNF height measurement. A) 2-D graph (topographical profile) obtained by drawing a line cross section across CNF on 2-D AFM image and B) Average height of VACNFs after different durations (in seconds) of ECE performed in an aqueous solution of 1 M NaOH at 1.5 V.

Figure 4. SEM 2-D images (top view) of the electrodes scanned after performing different durations of ECE in an aqueous solution of 1 M NaOH at 1.5 V; A) 5.0s, B) 12.5 s, C) 15.0s and D) 20.0s.

Figure 5. VACNF NEA electrode EDS Spectrum for un-etched and electrochemically etched for 17.5 seconds in an aqueous solution of 1 M NaOH at 1.5 V. (A and C) SEM images of un-etched and etched and (B and D) EDS spectrum for un-etched and etched VACNF NEA electrode; peaks of constituent components represent the %w (normalized) values of the elements present in the 2-D cross-section of electrode surface under study.

Figure 6.

CV and DPV curves for VACNF electrode recorded in electrolyte solution containing 5 mM $\text{Fe}[\text{CN}_6]^{3-/4-}$ in 1 M KCL after performing different durations of ECE in an aqueous solution of 1 M NaOH at 1.5 V. (A) CVs recorded at scanning speed of 20 mV/sec and (B) DPV. Base line fitted and subtracted curves (fitted between 0 to 400 mV from the original DPV curves).

Figure 7.

Selected CV and DPV curves for bare and subsequently modified VACNF NEAs recorded in electrolyte solution containing 5 mM $\text{Fe}[\text{CN}_6]^{3-/4-}$ in 1 M KCL. (A) CV; bare (red), linker\anti-CRP (blue), linker\anti-CRP\CRP (green) and regenerated (maroon) and (B) DPV; bare (red), linker\anti-CRP (blue), linker\anti-CRP\CRP (green) and regenerated (maroon), (C) average DPV peak current for bare, linker\anti-CRP, linker\anti-CRP\CRP and regenerated electrodes (5 electrodes) with respective SDV error and (D) % regeneration of five electrodes. The electrodes were regenerated by performing ECE for 1 s in 1 M NaOH at 1.5 V post CRP binding on each of the five anti-CRP immobilized electrodes.

Figure 8. Electrode repeatability study. A) current vs. potential (20 CV scans), B) current vs. time before the hydrolysis and electrochemical etching and C) current vs. potential (15 CV scans), and D) current vs. time after the hydrolysis and electrochemical etching.