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Ultrasensitive Electrocatalytic DNA Detection at Two- and Three-Dimensional Nanoelectrodes

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Nanostructured materials constitute new platforms for biomolecular sensing that may provide increased sensitivity and amenability to miniaturization. However, the use of nanoarchitectures for electrochemical biomolecular detection represents an undeveloped area. Several types of arrayed nanostructures composed of electroactive materials are available[1−3] and show great but unrealized promise for ultrasensitive biosensing. Gold nanoelectrode ensembles (NEEs) are a particularly attractive substrate for biosensing, given the low detection thresholds previously achieved with nonbiological analytes.[2] Here, we report a nanoscale approach to DNA biosensing that uses oligonucleotide-functionalized Au NEEs. The marriage of these novel electrodes with an electrocatalytic nucleic acid detection assay[4] provides a very sensitive nanoscale DNA detection system.

A nanoelectrode platform for DNA detection was generated that features Au nanowires templated by a polycarbonate membrane. Figure 1A shows scanning electron micrographs of the structures made using a modified version of an electroless plating method previously described.[2] These two-dimensional (2D) nanoelectrodes are approximately 10 nm in diameter and have an average separation of 200 nm. Using oxygen plasma etching to remove a thin layer of polycarbonate,[5] the same materials are used to prepare three-dimensional (3D) NEEs featuring exposed Au nanowires. Plasma etching resulted in consistent exposure of ∼200 nm of the gold nanowires (Figure 1B). Sealing of the polycarbonate membrane around the NEEs was achieved by heat treatment and was a crucial step that significantly reduced the double-layer charging currents.[2]

The NEEs were tested as a biosensing platform using an electrocatalytic DNA detection method developed in our laboratory.[4] This label-free system reports on the binding of a target DNA sequence to an immobilized probe oligonucleotide using a catalytic reaction between two transition-metal ions, $Ru(NH_3)_6^{3+}$ and $Fe(CN)_6^{3-}$.[4] The Ru(III) electron acceptor is reduced at the electrode surface and then reoxidized by excess Fe(III), making the electrochemical process catalytic. The increased concentration of anionic phosphates at the electrode surface that accompanies DNA hybridization increases the local concentration of $Ru(NH_3)_6^{3+}$,[6] and therefore produces large changes in the electrocatalytic signal. This approach works with sequences of varied composition[4] and is thus widely applicable to any target gene of interest. In these studies exploring the utility of NEEs for DNA detection, oligonucleotide sequences that correspond to a portion of the 23S rRNA gene from *Helicobacter pylori* (a pathogen implicated in gastric ulcers and cancer)[7] were used. A single-stranded DNA probe was thiolated[8] and covalently attached to NEEs through a gold-thiol bond (see Supporting Information for DNA sequences used in these experiments). The DNA-modified surfaces were then analyzed using Ru(III)/Fe(III) electrocatalysis before and after the hybridization of target sequences. Conventional macroelectrodes were also modified, analyzed, and compared to the NEEs.

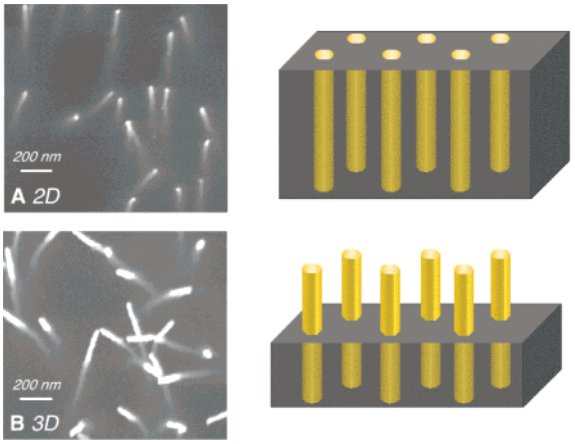

Figure 1. Scanning electron micrographs and schematic illustrations of 2D (A) and 3D (B) NEEs. See Supporting Information for details concerning the preparation, assembly, and imaging of NEEs.

As shown in Figure 2, the electrocatalysis signals obtained using NEEs exhibit large increases upon the hybridization of a target DNA sequence.[9] While very small currents are measured at probe-modified NEEs relative to macroelectrodes, the signals observed upon DNA hybridization at NEEs approach or surpass those obtained with Au macroelectrodes (Table 1 and Figure 2).[10] To quantitate the effect of DNA hybridization on the signals obtained with different electrodes, ΔQ values (reflecting integrated electrocatalysis currents before and after introduction of the target oligonucleotide)[11] were compared for 2D NEEs, 3D NEEs, and macroelectrodes. Average ΔQ values of 730, 420, and 80% were obtained (Table 1), respectively. Thus, the electrocatalytic signals measured at NEEs were more strongly modulated by DNA hybridization relative to those observed at macroelectrodes. Control experiments where noncomplementary sequences were tested did not generate significant increases in the electrochemical responses measured with any of the electrodes,[12] indicating that the signal changes observed reflect the formation of a specific complex between target and probe.

The success of the DNA hybridization experiments conducted using NEEs clearly indicates that these nanostructures are useful substrates for biosensing. While both types of NEEs exhibited positive signal changes when a target DNA strand was present, several differences in the behavior of these electrodes were observed, indicating that the 3D nanostructures were more suitable for practical applications. The electrocatalysis currents measured at 3D NEEs were larger than at 2D NEEs, consistent with the increased active surface area produced by etching of the polycar-

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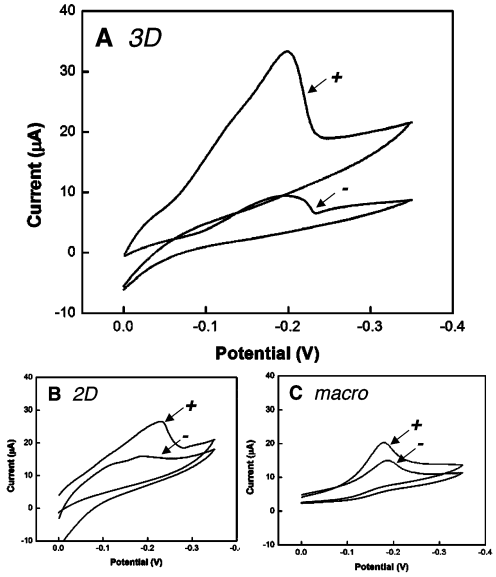

Figure 2. Representative cyclic voltammograms for Ru(III)/Fe(III) electrocatalysis at (A) 3D NEEs, (B) 2D NEEs, and (C) Au macroelectrodes in the absence (−) and presence of (+) a DNA oligonucleotide complementary to the immobilized probe. Data shown was obtained at a scan rate of 100 mV/s. See Supporting Information for experimental conditions and reagent concentrations.

Table 1. Comparison of Electrochemical Signals Obtained at Macro and Nanoelectrodes

electrode	ΔQ %[a]	Q (μC)[b]	geometric area (cm^2)[c]
Au macro	80 ± 10	6.6 ± 0.6	0.02
2D NEE	730 ± 80	8.5 ± 0.5	0.07
3D NEE	420 ± 80	13 ± 4	0.07

[a] See ref 11. [b]Integrated charge after hybridization. [c]Geometric area of the NEEs is defined by the exposed area of the nanowire ensemble.

bonate substrate.[13] Moreover, the etching process used to generate the exposed nanowires increased the yield of functional electrodes obtained from a single membrane (85% for 3D and 45% for 2D NEEs) and produced electrodes with smaller capacitive currents and more stable background signals during DNA hybridization experiments. The results we obtained with the 3D NEEs are significant not only because of the DNA detection capabilities of this platform but also because this is the first report of their use as electrodes.

The 3D NEEs were used to establish the sensitivity of the electrocatalytic DNA detection assay performed on the nanoscale architecture (Figure 3). When a probe-modified 3D NEE electrode was titrated with a target DNA strand, solutions containing picomolar concentrations of the analyte produced detectable changes in the electrochemical signal. Indeed, a sample containing 5 attomoles of target DNA increased the electrocatalysis signal by >200% (see Supporting Information for quantitation). This analysis was performed on an electrode with an exposed geometric area of 0.07 cm^2, indicating that zeptomole detection limits could easily be achieved with a modest decrease in the size of the aperture used in the electrochemical analysis. Previous studies that used the Ru(III)/Fe(III) electrocatalysis assay to detect the same DNA sequences using macroscopic gold electrodes achieved femtomole sensitivity.[4] An attomole-level detection limit compares favorably with recently reported electrochemical methods for the direct detection of oligonucleotides.[14] The achievement of this detection limit with nanoscale electrodes generated by a simple and lithography-free

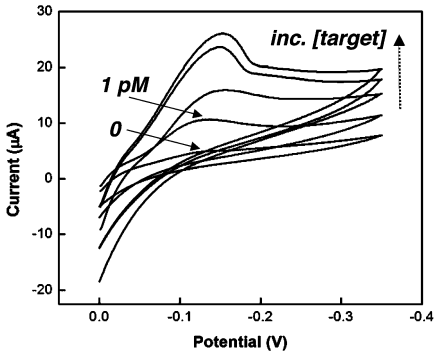

Figure 3. Evaluation of DNA detection limit at a 3D NEE using cyclic voltammetry. Experimental conditions are the same as in Figure 2. Data shown correspond to 0, 1 pM, 1 nM, 1 μM, and 20 μM target DNA.

method is a significant advance that will facilitate the development of miniaturized devices for biomolecular sensing.

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Supporting Information Available: Experimental protocols, electrode assembly and preparation, oligonucleotide synthesis and purification, SEM imaging parameters, and additional experimental data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) See Supporting Information for hybridization conditions and sequences.
(10) Currents measured at 3D NEEs were typically ~2-fold higher than those measured at 2D NEEs. The surface area of exposed Au on 3D NEEs (A = 0.025 cm^2) should be increased by a factor of ~850 over 2D NEEs (A = 2.9 × 10^{-5} cm^2), on the basis of areas approximated from SEM images. However, depletion of the diffusion layer between adjacent 3D nanostructures has previously been observed to decrease the participation of exposed sidewalls in electrochemical processes.[3] This effect may be responsible for the relatively small signal increases that are observed at 3D relative to 2D NEEs in our assay.
(11) Signal changes were calculated as follows. ΔQ (%) = {(($Q_{final} - Q_{initial}$)/$Q_{initial}$)100}, where Q_{final} and $Q_{initial}$ are integrated cathodic charges after and before DNA hybridization, respectively.
(12) ΔQ values observed in control experiments where probe-modified NEEs were incubated with noncomplementary DNA were typically <50%, a small difference relative to the 400−700% changes in Q observed with complementary DNA targets. See Supporting Information for electrochemical data corresponding to these controls.
(13) Currents observed at NEEs are larger than what would be predicted on the basis of the active surface areas of these structures.[10] However, strong electric fields are predicted to exist around the NEEs, which may facilitate the reduction of $Ru(NH)_6^{3+}$ through favorable electrostatic interactions (Smith, C. P.; White, H. S. *Anal. Chem*. **1993**, *65*, 3343.)
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