Electrically Addressable Biomolecular Functionalization of Conductive Nanocrystalline Diamond Thin Films

Wensha Yang,[†] Sarah E. Baker,[†] James E. Butler,[‡] Chang-soo Lee,[†] John N. Russell, Jr.,[‡] Lu Shang,[†] Bin Sun,[†] and Robert J. Hamers*[,†]

Department of Chemistry, University of Wisconsin—Madison, 1101 University Avenue, Madison, Wisconsin 53706, and Gas/Surface Dynamics Section, Chemistry Division, Naval Research Laboratory, Washington, D.C. 20375

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Recent studies have shown that when diamond surfaces are covalently bonded to biomolecules such as DNA or antibodies, the resulting biologically modified surfaces exhibit extraordinary chemical stability and excellent specificity in biomolecular recognition studies.[1−4] These properties make diamond a nearly ideal material for integration of biological systems with microelectronics. Because most biosensing applications involve fabrication of arrays in which many small surface regions are modified with distinct biomolecular recognition elements, there is great interest in methods that are able to "address" specific surface regions for functionalization. We previously showed that diamond surfaces could be functionalized via a photochemical reaction of olefins.[2,5] However, more recent studies on electrodes modified with carbon nanotubes and nanofibers have demonstrated the use of electrical signals to control the functionalization chemistry, thereby leading to electrically addressable fabrication of biomolecular arrays.[6] Here we show that it is also possible to use a surface electrochemical reaction to achieve electrically addressable biomolecular functionalization of diamond thin films. Our results provide a pathway for fabricating arrays of distinct biomolecular recognition elements on diamond surfaces, without the use of microfluidics or spotting methods.

To make diamond arrays, we started with a Si wafer that was coated with a 300 nm insulating silicon nitride layer. Standard photolithographic patterning procedures were used to deposit Ti/Mo contacts, followed by seeding the wafer with nanocrystalline diamond powder and then lifting off the photoresist. Conductive nanocrystalline diamond thin films (∼0.5 μm) were then preferentially grown on the Ti/Mo contacts in a 2.45 GHz microwave plasma reactor (Astex Model PDS-17) at the Naval Research Laboratory using purified hydrogen (900 sccm), methane (3 sccm), and B_2H_6 (6 sccm).[7] Figure 1a shows a scanning electron microscope image of a nanocrystalline diamond thin film that was grown on Ti/Mo contacts. High-resolution images show a highly facetted surface structure with grain sizes ranging from 20 to 100 nm. Formation of a thin, continuous, pinhole-free film is important to protect the underlying metal electrodes and to achieve good electrical response in thin film sensing devices.[8,9]

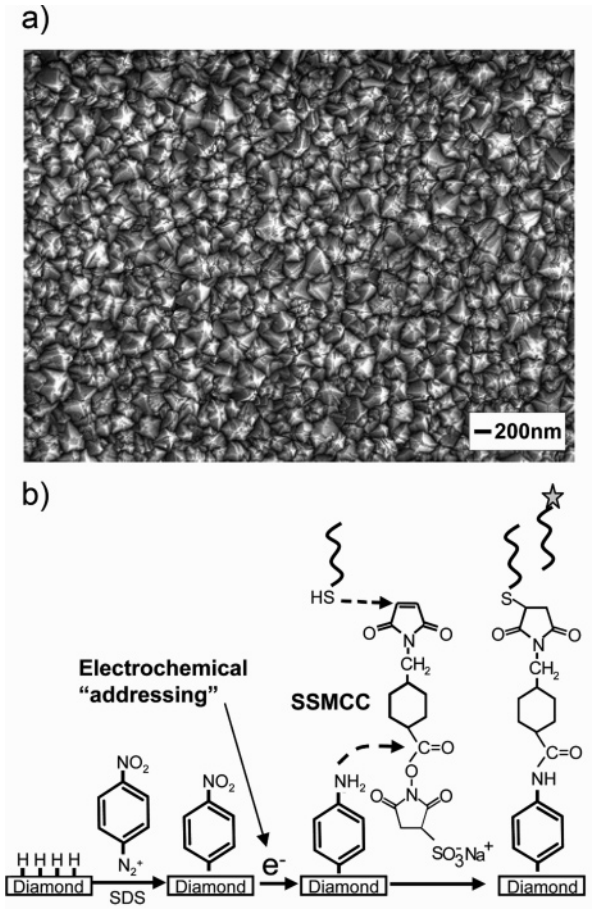

Figure 1. (a) Scanning electron microscope image of a diamond thin film deposited on Ti/Mo contacts. (b) Schematic illustration of addressable biomolecular functionalization of diamond-coated electrodes.

The electrically addressable functionalization starts with hydrogen-terminated diamond thin films,[2,5] followed by functionalization of the diamond with nitro groups using 40 mM 4-nitrobenzenediazonium tetrafluoroborate in 1% sodium dodecyl sulfate (SDS) solution; this produces surface terminated with aryl nitro groups, as depicted in Figure 1b. The key to electrically addressable biomolecular functionalization lies in the ability to selectively reduce nitro groups on specific electrodes to primary amines, followed by

* To whom correspondence should be addressed. E-mail: rjhamers@wisc.edu.
[†] University of Wisconsin—Madison.
[‡] Naval Research Laborator.

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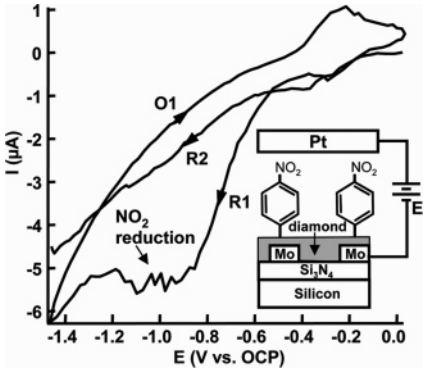

Figure 2. Cyclic voltammograms of diamond-coated Ti/Mo contacts after modification with nitrophenyl groups. The reduction of the nitro groups to amine groups appear as a negative-going peak on the first cycle of reduction sweep (R1). Voltages are given with respect to the open-circuit potential, using a platinum counter-electrode.

reaction with bifunctional linkers that will selectively react with the amines and covalently link them to DNA or other biomolecules of interest.

To achieve selective functionalization of an individual diamond electrode, we make use of cyclic voltammetry to selectively reduce nitro ($-NO_2$) groups to primary amine ($-NH_3$) groups.[10] Figure 2 shows cyclic voltammograms of diamond grown on Ti/Mo contacts starting at a potential of 0 V (vs open circuit potential), in a solution of 0.1 M KCl in a 90% H_2O/10% ethanol. As the potential becomes more negative during the first reduction sweep (labeled R1), the nitro groups are reduced to amino groups at potentials between -0.8 and -1.2 V, leading to a clear negative-going (reduction) peak. Neither the subsequent oxidation sweep (labeled O1) nor a subsequent reduction sweep (labeled R2) show any peaks characteristics of reduction. These results show that the reduction of the $-NO_2$ groups to $-NH_3$ groups complete during the first cycle of the reduction sweep. The importance of using conductive diamond is indicated by the fact that no reduction peaks were observed in a similar experiment on intrinsic diamond grown on Ti/Mo contacts; this result indicates that effective electron transfer through the bulk diamond is necessary to reduce the molecules that are attached to the diamond surface. Integrating the peak in Figure 2 and correction for the sample area, capacitive background, and the fact that 6 electrons are required for reduction of a single nitro group to an amine ($-NO_2 + 6H^+ + 6\,e^- \rightarrow -NH_2 + 2H_2O$) yields a density of approximately 5×10^{14} molecules/cm^2 that are reduced within the electrode area. This is, within experimental error, identical to the 7×10^{14} cm^{-2} amine groups produced by photochemical modification[2] and the value of 4.6×10^{14} molecules/cm^2 reported for self-assembled monolayers on gold,[11] suggesting that the molecular layers are densely packed.

DNA was covalently linked to the surface by first immersing the entire chip in a 1 mM solution of the heterobifunctional cross-linker sulfo-succinimidyl 4-(*N*-maleimidomethyl)cyclohexan-1-carboxylate (SSMCC, Pierce) in 0.1 M triethanolamine buffer (Aldrich), pH 7.0, for 20 min to expose reactive maleimide groups. After thiol-modified DNA (250 μM in 0.1 M triethanolamine buffer, pH 7.5) was applied to the entire surface, the sample was kept in a humid chamber for 4 h and then copiously rinsed in hybridization buffer.[2,3,12]

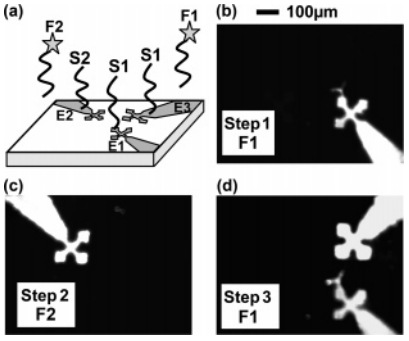

Figure 3. Sequential images demonstrating fabrication of diamond arrays. (a) Schematic illustration of diamond array modified with sequence S1 and S2. (b) Fluorescence image after electrode E1 was modified with S1 and the entire chip exposed to the complementary fluorescently-labeled sequence F1. (c) Fluorescence image after sample in Figure 3b was denatured, electrode E2 was functionalized with DNA sequence S2, and the entire chip was exposed to the complementary fluorescently-labeled sequence F2. (d) Fluorescence image after sample in 3c was denatured, electrode E3 was functionalized with DNA sequence S1, and the entire chip was exposed to fluorescently labeled sequence F1.

The significance of using an electrochemical reaction to control the functionalization is that it provides a way to modify different diamond-coated electrodes with distinct biomolecule of interest without using microfluidics or spotting methods.[6] Figure 3a demonstrates the process. In this experiment, a silicon nitride chip with three independent diamond-coated electrodes was first modified with nitrophenyl groups by immersing the entire chip into 4-nitrobenzenediazonium tetrafluoroborate solution. The chip was then immersed into 0.1 M KCl in a 90% H_2O/10% ethanol solution, and the potential applied to one of the electrodes (electrode 1) was swept from 0 V down to -1.5 V to reduce the nitro groups to amino groups. A platinum wire was used as a counter-electrode. In step 1 a thiol-modified DNA oligonucleotide with a sequence S1[13] was linked to amine groups via its thiol groups using SSMCC as described above. To test the specificity of the DNA-modified diamond electrode, we expose the entire chip to a solution containing fluorescently labeled DNA oligonucleotide F1,[13] which has a sequence complementary to that of S1. Figure 3b shows the fluorescence image obtained after step one; the high fluorescence intensity on electrode 1 shows that F1 exhibits a strong preference for binding to electrode E1, which was functionalized with the complementary sequence S1.

After denaturing the surface with urea solution (8.3 M), the electrochemical potential was applied to the electrode E2, followed by the modification with thiol-modified DNA (S2)[13] (step 2). The entire chip was then immersed in the fluorescently labeled complementary DNA, F2, which is complementary to S2.[13] The fluorescence image in Figure

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(13) S1: 5′ Thiol TTT TTT TTT TTT TTT GC TTA TCG AGC TTT CG 3′. F1: 5′ FAM CGA AAG CTC GAT AAG C 3′. S2: 5′ Thiol TTT TTT TTT TTT TTT GC TTA AGG AGC AAT CG 3′. F2: 5′ FAM CGA TTG CTC CTT AAG C 3′.

3c shows that the electrode E1(functionalized with sequence S1 in step 1) no longer exhibits significant fluorescence and that bright emission is now observed from electrode E2. The strong decrease in fluorescence signal from electrode E1 shows that the DNA hybridization is reversible, while the intense emission from electrode E2 (modified with S2) shows that F2 binds selectively to it. Quantitative measurements show that, compared with the denatured sample, exposure to S2 increases the fluorescence intensity on electrode E1 by 350 (arbitrary units) and electrode E2 by 1750. Thus, we conclude that F2 shows a strong (>5-fold) preference for binding to the complementary sequence S2 on the surface, but with some detectable nonspecific binding to the electrode (E1) functionalized with sequence S1.

Finally, electrode 3 was similarly electrochemically addressed and functionalized with DNA sequence S1.[13] Figure 3d shows that, after exposure to F1, high fluorescence intensity is observed on electrodes 1 and 3, while little fluorescence is observed from electrode 2. This result demonstrates that the sequential use of electrochemical addressing can be used to create biomolecular arrays capable of distinguishing perfectly matched and mismatched DNA sequences.

Our previous studies showed that DNA-modified diamond surfaces are extraordinarily stable to repeated hybridization-denaturation cycles[2] and at elevated temperatures,[4] making diamond a very attractive substrate for a variety of biosensing applications. While in conventional biochips the spatial distribution of biomolecular recognition elements is controlled by spotting, microfluidics, or ink-jet printing technologies,[14] our present results demonstrate the ability to fabricate biomolecular arrays on diamond surfaces without the use of microfluidics, by using an electrochemical step to control the surface functionalization. This ability provides a simple pathway for fabrication of high-density, highly stable biomolecular arrays for applications such as electrical biosensing[8,15] and surface-based biochemical processes.[16]

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