

Preparation and Electrochemical Characterization of DNA-modified Nanocrystalline Diamond Films

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ABSTRACT

Nanocrystalline diamond thin films of sub-micron thickness have been covalently modified with DNA oligonucleotides. Quantitative studies of hybridization of surface-bound oligonucleotides with fluorescently tagged complementary and non-complementary oligonucleotides were performed. The results show no detectable non-specific adsorption, with extremely good selectivity between matched and mismatched sequences. Impedance spectroscopy measurements were made of DNA-modified boron-doped nanocrystalline diamond films. The results show that exposure to non-complementary sequences induce only small changes in impedance, while complementary DNA sequences produce a pronounced decrease in impedance. The combination of high stability, selectivity, and the ability to directly detect DNA hybridization via electrical means suggest that diamond may be an ideal substrate for continuously-monitoring biological sensors.

INTRODUCTION

Recent advances in biotechnology and molecular electronics have fueled an interest in fabrication of well-defined, highly stable interfaces between biomolecules and solid substrates.[1-4] The desire to link biomolecules to substrates compatible with current electronic technologies is leading to increased interest in substrates such as gold,[5] silicon,[3,4,6,7] and diamond.[8] Diamond is especially attractive since, in addition to having good electrical[9] and chemical properties,[10] it is also biocompatible, and can be deposited as a robust thin film on silicon and other substrates at moderate temperatures that are compatible with microelectronics processing.[11-13]

However, to achieve very thin, continuous, pinhole-free diamond films, the nucleation density during growth must be extremely high, producing diamond films with crystallites of nanometer dimensions. Recent advances in seeding processes and other techniques have made this readily obtainable on a variety of substrates.

EXPERIMENT

Experiments were performed using diamond thin films grown via two very different procedures. Nanocrystalline diamond (NCD) thin films were grown in a 2.45 GHz microwave plasma chamber under hydrogen-rich conditions, using purified hydrogen (900 sccm) and methane (99.999%, 3 sccm) at a total pressure of 15 torr.[14] In some cases, these films were intentionally boron-doped to produce highly-conductive, p-type films. “Ultrananocrystalline” diamond (UNCD) films, were grown in a 2.45 GHz microwave plasma reactor under hydrogen-deficient conditions, using 1% CH₄ and 99% Ar at 150 torr pressure at 800°C.[15] Nitrogen gas (up to 20%) was added to make samples where are highly n-type conductive.[16] The samples were cleaned in acid baths to remove metals, amorphous carbon, and silicates. The diamond thin films were then heated to approximately 800°C in a 13.56 MHz inductively coupled hydrogen plasma (15 Torr H₂) for 20 minutes to terminate the surface with hydrogen.[17]

Chemical functionalization of the diamond surface was accomplished by placing a H-terminated diamond sample in a Teflon reaction vessel and dropping 5 μL of trifluoroacetamide-protected 10-aminodec-1-ene (“TFAAD”) directly onto the surface. The chamber was covered with a quartz window, and the sample was then exposed to ultraviolet light from a low-pressure mercury vapor quartz grid lamp ($\lambda_{\max} = 254 \text{ nm}$ 0.35 mW/cm²) for approximately 12 hrs, while purging with dry nitrogen to produce a monolayer film.[4] After deprotecting TFA from amine group, the sample was first modified by a cross-linker molecule (SSMCC), and then 5' thiol-modified DNA.

Electrochemical impedance measurements were performed using a small microfluidics-based electrochemical cell with a Ag/AgCl reference electrode and a platinum counter electrode, using a Solartron 1260/1287 electrochemical impedance spectroscopy system. The diamond film was pressed against the bottom of the cell, leaving a 0.13 cm² sample area exposed. All measurements were obtained at room temperature in SSPE buffer solution (300mM NaCl, 20 mM sodium phosphate, 2 mM EDTA, and 6.9 mM sodium dodecyl sulfate). The diamond thin films were modified with single stranded DNA before the impedance spectroscopy measurement, and impedance spectra of the DNA-modified interface were recorded at the beginning of the experiment, after exposure to a mismatched sequence (4-base mismatch out of 16 total), and after exposure to a perfectly complementary sequence.

RESULTS AND DISCUSSION

The photochemical attachment and subsequent chemistry leads to DNA molecules that are covalently linked to the diamond surface as depicted in Fig. 1A. Two different DNA sequences were attached to the surface in different locations. The sequences attached to the surface were 5'HS-C₆H₁₂-T₁₅- GC TTA TCG AGC TTT CG3' (**S1**) and 5'HS -C₆H₁₂-T₁₅-GC TTA AGG AGC AAT CG3' (**S2**). The oligonucleotides used for hybridization

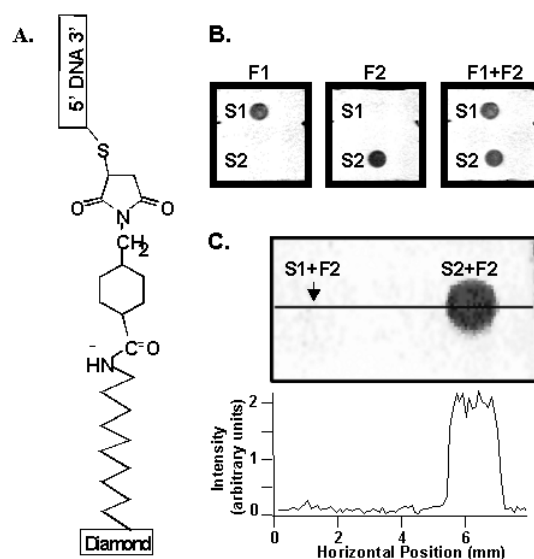


Figure 1: DNA modification and hybridization experiments on UNCD diamond film. Fig. 1A: Schematic illustration of covalent linkage of DNA to diamond thin film Fig. 1B: Fluorescence images showing fluorescence after a UNCD sample modified with two different sequences of DNA (sequences S1 and S2) was then exposed to fluorescently-labeled complementary and non-complementary sequences (F1, F2). The top figure panel shows a single sample that was successively hybridized with sequence F1, imaged, denatured, hybridized with sequence F2, imaged, denatured, and then hybridized with a mixture of F1 and F2. Fig. 1C: Fluorescence image and variation in intensity along the indicated line after a diamond surface modified with sequences S1 and S2 was hybridized with sequence F2.

were modified with fluorescein on the 5'-end using 6-FAM phosphoramidite. The two sequences used were 5'-FAM-CG AAA GCT CGA TAA GC-3' (F1, 16 bases complementary to S1 and a four-base mismatch to S2) and 5'-FAM-CG ATT GCT CCT TAA GC-3' (F2, 16 bases complementary to S2 and with a four-base mismatch to S1). Fig. 1B shows a fluorescence image (black = high intensity) of a diamond surface that was covalently modified at one location with sequence S1 and at another location with S2. After exposure to F1 (Fig. 1B, left panel) a clear single spot appears at the location of S1, indicating that the applied oligonucleotide (F1) efficiently hybridized to its complement (S1). However, there is no detectable hybridization between F1 and the four-base mismatch (S2). After this image, the sample was denatured in 8.3 M urea, rinsed in deionized water, and a second experiment was performed in which sequence F2 was applied to the surface for 20 minutes. The resulting fluorescence image (Fig. 1B, middle panel) shows fluorescence intensity only at the second spot. Fig.1C presents these data in a more quantitative way by showing the intensity variations along a specified line. Finally, the sample was denatured again and a mixture of F1 and F2 was applied to the surface. The fluorescence image (Fig. 1B, right) shows hybridization at both locations.

To quantitatively evaluate the selectivity of hybridization, the average and standard deviation of the fluorescence intensity were measured in specific regions of the sample. The average fluorescence intensity from an area of 0.42mm^2 (encompassing $\sim 50\%$ of the area of the hybridized spots) was 2436 ($\sigma=144$) for the perfect match (F2+S2) and 525 ($\sigma=38$) for the four-base mismatch (F2+S1). The background in the non-functionalized region also was 525 ($\sigma=38$). Thus, the signal from the mismatched sequence is indistinguishable from the background, while the perfect match yields a signal-to-noise ratio (intensity due to hybridization divided by the pooled standard deviation) of 12.9 from this 0.42mm^2 area. Similar measurements on a NCD sample produced a signal level of 862 ($\sigma=66$) for a perfect match, and 284 ($\sigma=38$) for the background, yielding a signal-to-noise of 7.6. The small difference between NCD and UNCD may be related to the different film thickness and needs to be explored more systematically before any clear conclusion could be drawn. For both UNCD and NCD diamond, our experiments show that DNA-modified diamond thin films exhibit a strong preference for binding to complementary vs. non-complementary sequences.

Perhaps the most remarkable feature of thin-film diamond as a material for biological modification is its extreme stability. We have found that DNA-modified diamond surfaces can be hybridized and denatured more than 30 times with no detectable decrease in sensitivity or selectivity,[18] while other substrates (such as glass and gold) that are widely used in biochip technologies showed significant degradation.[18]

In addition to its extremely high stability, diamond is also of interest because of its semiconducting properties. Fig.2 shows impedance spectroscopy of a p-type (boron-doped) nanocrystalline diamond thin film that was grown on a silicon substrate. It was

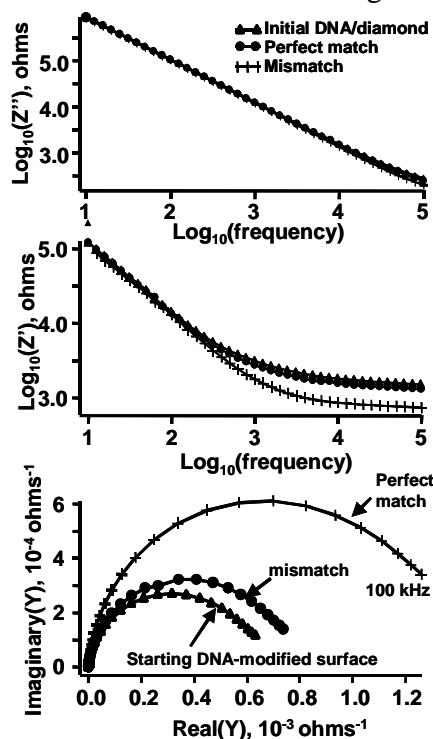


Figure 2 Impedance spectra of a p-type, nanocrystalline diamond thin film that was modified with DNA and then exposed to and mismatched (non-complementary) and matched (complementary) sequences of DNA in solution.

modified with single-stranded DNA using the same chemistry described above, and the impedance across the interface was measured using an electrochemical impedance analysis system. The complex impedance data can be presented in a number of different ways. The top and middle plots, respectively, show logarithmic plots of the real and imaginary parts of the complex impedance as a function of frequency.

From these graphs, it can be seen that the real and imaginary parts of the impedance vary smoothly with frequency. While exposure to a mismatched sequence produces almost no change in the real or imaginary parts of the impedance, when the sample is exposed to a perfectly complementary sequence (a "perfect match"), there is a decrease in the real and imaginary parts of the complex impedance. On the logarithmic scale it is difficult to see the changes induced by biological binding on top of the much larger changes as a function of frequency. For biosensing applications, one goal is to find robust electronic "signatures" of biological binding. We have found that plotting the complex admittance Y ($Y=1/Z$) more clearly brings out those changes induced by hybridization, especially when plotted as a Cole–Cole plot, which consists of the locus of admittance values measured at different frequencies (here, 10 Hz to 100 kHz) plotted in the complex plane. As shown in the bottom panel of Figure 2, when presented in this manner the biochemically-induced changes are much more apparent. In the plots shown here, a DNA-modified diamond sample was exposed to a non-complementary sequence and then to a complementary sequence. The non-complementary sequence shows only a small increase in admittance, while the complementary sequence causes a much more pronounced increase in admittance. Because the hybridization on diamond is very reversible, it is possible to conduct these experiments multiple times, verifying that the changes are indeed induced by the DNA hybridization. Further confirmation was obtained by measuring the fluorescence after hybridization and denaturation.

The above spectra show that DNA hybridization can produce a recognizable "signature" that can be used for direct electronic detection of DNA hybridization via impedance spectroscopy. Results have also been obtained on surfaces of UNCD diamond on a Mo substrate, silicon, and gold.[19] The results presented here on NCD diamond agree with those measured in previous studies which indicate that DNA hybridization increases the conductivity through the molecule, due to the π -stacked array formed in the duplex.[20-22] However, while most previous studies that have added a redox-active reagent to detect changes in DC conductivity of the DNA film, our results show that it is possible to directly observe DNA hybridization via the changes induced in the AC frequency response without the need for any additional reagents, thereby providing a completely label-free method of detecting DNA hybridization.[19]

CONCLUSIONS

Nanocrystalline diamond thin films were modified with DNA, and the resulting sample shows very good specificity and stability in DNA hybridization and denaturation. By monitoring the changes in AC impedance across the DNA-modified surface, it is possible to directly detect DNA hybridization entirely via the changes in electronic response at the interface. The above work demonstrates that thin film nanocrystalline diamond can be used as a highly-stable substrate for selective biomodification and biosensing; by combining this with the direct electronic sensing, it should be possible to

fabricate completely electronic sensing systems suitable for use in applications where continuous monitoring and/or harsh environmental conditions may be encountered.

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