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Short communication

Direct attachment of DNA to semiconducting surfaces for biosensor applications

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ABSTRACT

In this work we propose a novel method of immobilizing nucleic acids for field effect or high electron mobility transistor-based biosensors. The naturally occurring 5′ terminal phosphate group on nucleic acids was used to coordinate with semiconductor and metal oxide surfaces. We demonstrate that DNA can be directly immobilized onto ZrO_2, AlGaN, GaN, and HfO_2 while retaining its ability to hybridize to target sequences with high specificity. By directly immobilizing the probe molecule to the sensor surface, as opposed to conventional crosslinking strategies, the number of steps in device fabrication is reduced. Furthermore, hybridization to target strands occurs closer to the sensor surface, which has the potential to increase device sensitivity by reducing the impact of the Debye screening length.

1. Introduction

Nucleic acid hybridization can be detected using electrical (Ganguly et al., 2009), mechanical (Su et al., 2003), acoustic (Hur et al., 2005), or optical (Piliarik et al., 2009) methods. For detection to occur, oligonucleotide probes must be immobilized onto the detection system/substrate. Silane-based chemistries are commonly used for linkages to oxides (Ganguly et al., 2009), while thiols are used for noble metal linkages (Hur et al., 2005). There have been a limited number of reports of phosphate/phosphonate-based binding and coordination to certain elements such as Ti, Zr, and Hf (Gawalt et al., 1999; Jespersen et al., 2007; Lane et al., 2008). A limited number of studies have shown phosphate/phosphonate–metal oxide interactions for nucleic acid immobilization (Acton et al., 2008). Our group has previously reported that the 5′ terminal phosphate group on DNA can be used to directly immobilize DNA on HfO_2, and that once immobilized it can be hybridized with complementary DNA (Fahrenkopf et al., 2009). Here, we demonstrate the feasibility of using this approach on other materials commonly used in electrical-based biosensors such as field effect transistor (FET) or high electron mobility transistor (HEMT) based sensors. This strategy reduces the number of steps needed to immobilize nucleic acids, and brings the hybridized strands closer to the sensor surface. This is substantiated by Stern et al., who have shown that Debye screening length has a major impact on the sensitivity of FET-based biosensors (Stern et al., 2007). The materials we present here include SiO_2 (a ubiquitous material in device fabrication), TiO_2 (a material often used for biomedical implants), ZrO_2 (a dielectric material that is similar to TiO_2 and HfO_2), AlGaN, and GaN (materials used in light emitting diodes and high electron mobility transistors).

2. Materials and methods

Tris–EDTA (TE) buffer was obtained from National Diagnostics (Atlanta, GA). Sulfuric acid, hydrogen peroxide (30%), and 20× SSC buffer were obtained from Sigma–Aldrich (St. Louis, MO). PicoGreen fluorescent dye (Invitrogen, Carlsbad, CA) was diluted into TE buffer 1:400 (v/v) prior to use. Bovine serum albumin (BSA) (New England Biolabs, Ipswich, MA) was diluted to 0.1 μg/μL in TE prior to use. Single stranded DNA oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) having 22 base-pairs. Single stranded sequences were obtained either with ("probe-5P") or without ("probe") the 5' terminal phosphate group, along with the complementary strand to the probes ("target"). Upon receipt, all oligonucleotides were reconstituted to 500 μM with filter sterilized, autoclaved diH_2O and stored at −20 °C.

Prior to patterning, surfaces were cleaned by immersion in piranha solution (30% H_2O_2:98% H_2SO_4, 1:2 (v/v)) for 5 min followed by a triple rinse with dH_2O before being blown dry with nitrogen gas. Single-stranded probe DNA was patterned onto sur-

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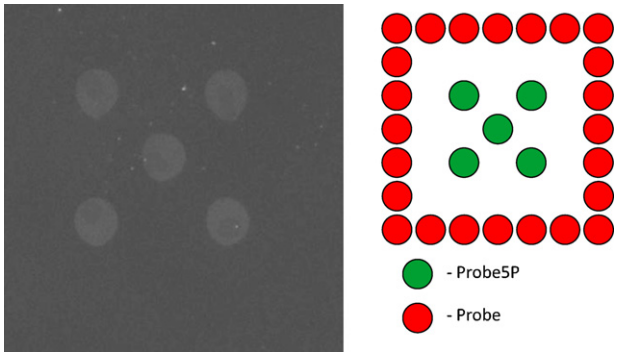

Fig. 1. (Left) Fluorescence micrograph of a GaN surface that was printed with 5 μM single-stranded probe (sequence: 5′ GAC CRA TCC TGT CAC CTC TGA C) DNA, hybridized with 10 μM target (sequence: 5′ GTC AGA GGT GAC AGG ATY GGT C) DNA and stained with PicoGreen dye. Other surface materials used in this study showed similar results. (Right) Schematic of the printing pattern used to determine the location of phosphorylated vs. non-phosphorylated single-stranded target DNA.

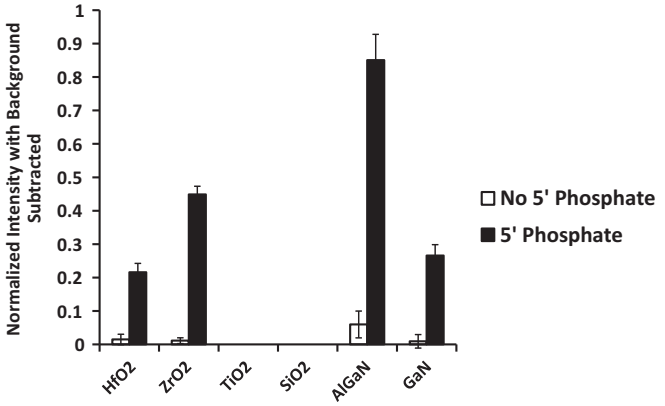

Fig. 2. Comparison of target DNA hybridization to phosphorylated (black bars) and non-phosphorylated (white bars) DNA that was printed onto various materials. HfO_2 films were prepared by ALD as previously described (Clark et al., 2008). ZrO_2 films were prepared by reactive e-beam evaporation with oxygen flow using a SPECS (Berlin, Germany) EBE-1 mini evaporator, followed by a 600 °C, 60 s rapid thermal anneal. TiO_2 films were prepared using a Varian (Palo Alto, CA) e-beam evaporator, followed by a 450 °C, 3 h anneal in air. Commercially available 100 mm silicon wafers with a native oxide were used as the substrate for SiO_2 experiments without additional processing. GaN films were grown using a Veeco (Somerset, NJ) D180 MOCVD reactor, on to a sapphire substrate. Approximately 20% aluminum gallium nitride (20% AlGaN) films, on the order of 2 μm thick, were grown in a similar manner. Single-stranded probe DNA was printed, hybridized to target, then stained with PicoGreen dye. These data represent the average fluorescence intensity of five spots normalized to background. For both TiO_2 and SiO_2 immobilization could not be evaluated.

faces using a BioForce Nano eNabler (Xu et al., 2009). Following printing, the samples were allowed to incubate for 12 h at room temperature. Surfaces were then triple rinsed with TE before being blocked in a solution of BSA for 20 min similar to (Xu et al., 2009). Samples were then triple rinsed with TE buffer and immersed in a hybridization solution of 5× SSC buffer and 100 nM "target" DNA. The hybridization solution was incubated at 70 °C for 5 min and allowed to cool to room temperature before being triple rinsed with TE. Hybridized samples were labeled with PicoGreen fluorescent dye per the manufacturer's instructions. After 20 min the samples were rinsed with TE, covered with a glass coverslip, and imaged using a Nikon Eclipse 80i microscope with 490/520 nm excitation/emission. Fluorescence intensity analysis was performed using ImageJ (Rashband, 1997–2008). For each sample, the average intensity of five spots was taken, along with a background area next to each spot. The fluorescence signal for each spot was determined by normalization to the background intensity: Signal = [Spot Intensity/Background Intensity] − 1.

3. Results and discussion

Both phosphorylated and non-phosphorlyated probes were printed onto surfaces, enabling evaluation of ssDNA immobilization and hybridization to its complement. Fig. 1 shows the printing pattern used for comparing "probe" with "probe-5P", along with a typical fluorescence image. Likewise, Fig. 2 shows the results of the image intensity analysis comparing "probe" and "probe-5P" printed and hybridized with "target" on the surfaces studied. Little, if any, signal was recorded where the non-phosphorylated DNA was printed, while the phosphorylated DNA spots yielded a comparatively high fluorescence signal. This strongly suggests that the DNA with the 5′ terminal phosphate group was successfully immobilized and available for hybridization, whereas DNA without a terminal phosphate was not immobilized, or was not available for hybridization. For both SiO_2 and TiO_2 there was significant difficulty in creating a pattern due to surface hydrophilicity, which caused significant spot spreading, and prevented our ability to assess immobilization. AlGaN surfaces showed affinity for DNA adsorption while still exhibiting phosphate dependency. We suggest that the incorporation of Al is the root of this increase, which is supported by others who have reported multiple immobilization strategies of DNA onto Al (Pellerite et al., 2003). Although we were unable to demonstrate phosphate dependent immobilization onto Ti, it is theoretically possible since Ti, Zr, and Hf fall into the same group of elements in the periodic table (group IV). It is also likely that any material containing Al, as well as other group III-nitride surfaces would also have this affinity for phosphate.

4. Conclusions

We have shown that DNA can be directly immobilized onto ZrO_2, AlGaN, GaN, and HfO_2 in a phosphate dependent manner. Immobilization was not successful for SiO_2 or TiO_2 surfaces, although alternative deposition methods could yield successful DNA immobilization on these materials. The primary application of this immobilization technique is for electrical-based nucleic acid detection systems, such as field-effect transistors (FETs) and high electron mobility transistors (HEMTs) that utilize these materials for active device components such as gate oxides/dielectrics. In each case, nucleic acid probes can be directly immobilized onto the substrates tested without complex surface chemistry, cross-linking, or probe modifications.

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