Botulinum toxin detection using AlGaN/GaN high electron mobility transistors


1Department of Materials Science and Engineering, University of Florida, Gainesville, Florida 32611, USA
2Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA
3Constellation Technology Corp., Largo, Florida 33777, USA
4SVT Associates, Eden Prairie, Minnesota 55344, USA

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Antibody-functionalized, Au-gated AlGaN/GaN high electron mobility transistors (HEMTs) were used to detect botulinum toxin. The antibody was anchored to the gate area through immobilized thioglycolic acid. The AlGaN/GaN HEMT drain-source current showed a rapid response of less than 5 s when the target toxin in a buffer was added to the antibody-immobilized surface. We could detect a range of concentrations from 1 to 10 ng/ml. These results clearly demonstrate the promise of field-deployable electronic biological sensors based on AlGaN/GaN HEMTs for botulinum toxin detection. © 2008 American Institute of Physics. [DOI: 10.1063/1.3056612]

Biological weapons are particularly attractive tools for terror because biological agents are available and easy to manufacture, small amounts are required to cause large-scale effects, and attacks can easily overwhelm existing medical resources. Reliable detection of biological agents in the field and in real time has proved to be challenging. Clostridium botulinum neurotoxins are among the more deadly toxins and are listed as a NIAID-category A agent for bioterrorism.

The lethal dose in unvaccinated humans is estimated at 1 ng/kg. Conventional methods of detection involve the use of high performance liquid chromatography (HPLC), mass spectrometry, and colorimetric enzyme-linked immunosorbent assay (ELISAs); but these are impractical because such tests can only be carried out at centralized locations, and are too slow to be of practical value in the field. Another test for botulinum toxin detection is the “mouse assay,” which relies on the death of mice as an indicator of toxin presence. Clearly, such methods are slow and impractical in the field.

AlGaN/GaN high electron mobility transistors (HEMTs) have shown promise for biosensing applications, since they include a high electron sheet carrier concentration channel induced by piezoelectric and spontaneous polarization of the strained AlGaN layer. There are positive counter charges at the HEMT surface layer induced by the electrons located at the AlGaN/GaN interface. Any slight changes in the ambient can affect the surface charge of the HEMT, thus changing the electron concentration in the channel at the AlGaN/GaN interface.

In this Letter, we report the use of antibody-functionalized Au-gated AlGaN/GaN HEMTs for detecting botulinum toxin. The botulinum toxin was specifically recognized through botulinum antibody, anchored to the gate area. We investigated a range of concentrations from 0.1 to 100 ng/ml.

The HEMT structures consisted of a 3 μm thick undoped GaN buffer, 30 Å thick Al0.3Ga0.7N spacer, and 220 Å thick Si-doped Al0.3Ga0.7N cap layer. The epilayers were grown by a molecular beam epitaxy system (MBE) on sapphire substrates. Mesa isolation was performed with an inductively coupled plasma (ICP) etching with Cl2/Ar based discharges at −90 V dc self-bias, ICP power of 300 W at 2 MHz, and a process pressure of 5 mTorr. Ohmic contacts of 10×50 μm2 separated with gaps of 5 μm consisted of e-beam deposited Ti/Al/Pt/Au patterned by lift-off and annealed at 850 °C, with 45 s under flowing N2. Polymethyl methacrylate (PMMA) of 400 nm thick and 4% was used to encapsulate the source/drain regions, with only the gate region open to allow the liquid solutions to cross the surface. The source-drain current-voltage characteristics were measured at 25 °C using an Agilent 4156C parameter analyzer with the gate region exposed.

Figure 1 shows a schematic device cross sectional view with the immobilized thioglycolic acid, followed by botulinum antibody coating. The Au surface was functionalized with the specific bifunctional molecule thioglycolic acid. We

FIG. 1. (Color online) Schematic of AlGaN/GaN HEMT sensor. The Au-coated gate area was functionalized with botulinum antibody/antigen on thioglycolic acid.
anchored a self-assembled monolayer of thioglycolic acid HSCH$_2$COOH, an organic compound and containing both a thiol/mercaptan and a carboxylic acid functional group, on the Au surface in the gate area through strong interaction between gold and the thiol-group of the thioglycolic acid. The devices were first placed in the oxygen plasma chamber and then submerged in 1 mM aqueous solution of thioglycolic acid at 4 °C. This resulted in binding of the thioglycolic acid to the Au surface in the gate area with the COOH groups available for further chemical linking of other functional groups. X-ray photoelectron spectroscopy and electrical measurements confirming a high surface coverage and Au–S bonding formation on the GaN surface have been previously published.\textsuperscript{28} The device was incubated in a phosphate buffered saline (PBS) solution of 200 μg/ml botulinum polyclonal rabbit antibody for 18 h before real time measurement of botulinum toxin subtype A acquired from Metabiologics Inc.

After incubation with a PBS buffered solution containing botulinum antibody at a concentration of 200 μg/ml, the device surface was thoroughly rinsed off with PBS and dried by a nitrogen blower. The source and drain current from the HEMT were measured before and after the sensor was exposed to 100 ng/ml botulinum toxin at a constant drain bias voltage of 500 mV, as shown in Fig. 2 (top). Any slight changes in the ambient of the HEMT affect the surface charges on the AlGaN/GaN. These changes in the surface charge are transduced into a change in the concentration of the 2-dimensional electron gas (2DEG) in the AlGaN/GaN HEMTs, leading to the decrease in the conductance for the device after exposure to botulinum toxin.

Figure 2 (bottom) shows a real time botulinum toxin detection in PBS buffer solution using the source and drain current change with a constant bias of 500 mV. No current change can be seen with the addition of buffer solution around 100 s, showing the specificity and stability of the device. In clear contrast, the current change showed a rapid response in less than 5 s when the target 1 ng/ml botulinum toxin was added to the surface. The abrupt current change due to the exposure of botulinum toxin in a buffer solution was stabilized after the botulinum toxin thoroughly diffused into the buffer solution. Different concentrations (from 0.1 to 100 ng/ml) of the exposed target botulinum toxin in a buffer solution were detected. The sensor saturates above 10 ng/ml of the toxin. The experiment at each concentration was repeated four times to calculate the standard deviation of source-drain current response. The limit of detection of this device was below 1 ng/ml of botulinum toxin in PBS buffer solution. The source-drain current change was nonlinearly proportional to botulinum toxin concentration, as shown in Fig. 3. Figure 4 shows a real time test of botulinum toxin at different toxin concentrations with intervening washes to break antibody-antigen bonds. This result demonstrates the real-time capabilities and recyclability of the chip.

In summary, we have shown that through a chemical modification method the Au-gated region of an AlGaN/GaN HEMT structure can be functionalized for the detection of botulinum toxin with a limit of detection less than 1 ng/ml. This electronic detection of biomolecules is a significant step towards a field-deployed sensor chip, which can be integrated with a commercial available wireless transmitter to realize a real-time, fast response, and high sensitivity botulinum toxin detector.

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![FIG. 2. (Color online) (Top) I-V characteristics of AlGaN/GaN HEMT sensor before and after exposure to 100 ng/ml botulinum toxin. (Bottom) Drain current of an AlGaN/GaN HEMT vs time for botulinum toxin from 0.1 to 100 ng/ml.](image1)

![FIG. 3. Change of drain current vs different concentrations from 0.1 to 100 ng/ml of botulinum toxin.](image2)

![FIG. 4. The real-time test from a used sensor which was washed with PBS in pH 5 to refresh the sensor.](image3)