Real Time Bacterial Water Sensors

(OTT ID 1225)

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Shortfalls of Current Water Testing

Problems/Unmet Needs:

- The *E. coli* 0157:H7 strain of bacteria causes symptoms such as bloody diarrhea, hemorrhagic colitis, and kidney failure
- It is highly virulent and is infectious with a very low dose such that 10 to 100 CFU can lead to infection
- Current methods demonstrate low sensitivity, less specificity, and are time-consuming

Technological Solution:

- The inventors have utilized self-assembly of thermally-reduced monolayer graphene oxide (TRMGO) nanosheets on photolithographically patterned gold electrodes for highly sensitive detection of *E. coli* 0157:H7
- The TRMGO FET device shows great electronic stability and high sensitivity to *E. coli* cells with a concentration as low as 10 CFU per milliliter
- This immunosensor has high sensitivity with a short response time as well as high reproducibility
- Fabrication can easily be scaled up with good reproducibility and high electrical stability
- The TRMGO FET device is an attractive replacement for these methods due to its rapid response, high sensitivity, and ability for real-time monitoring

Road to Commercialization: Market, Intellectual Property, and Partnering

Market

- The EPA notes that around 3.5 million Americans become ill annually from bacteria and other toxins released from sewage spills.
- The water market represents one of the largest business sectors with global revenues around $550 billion (2010 Innovaro Technology Foresight)
- The global environmental sensor and monitoring market was valued at $11 billion in 2010 and was predicted to reach $15.6 billion in 2016 (BCC Research)

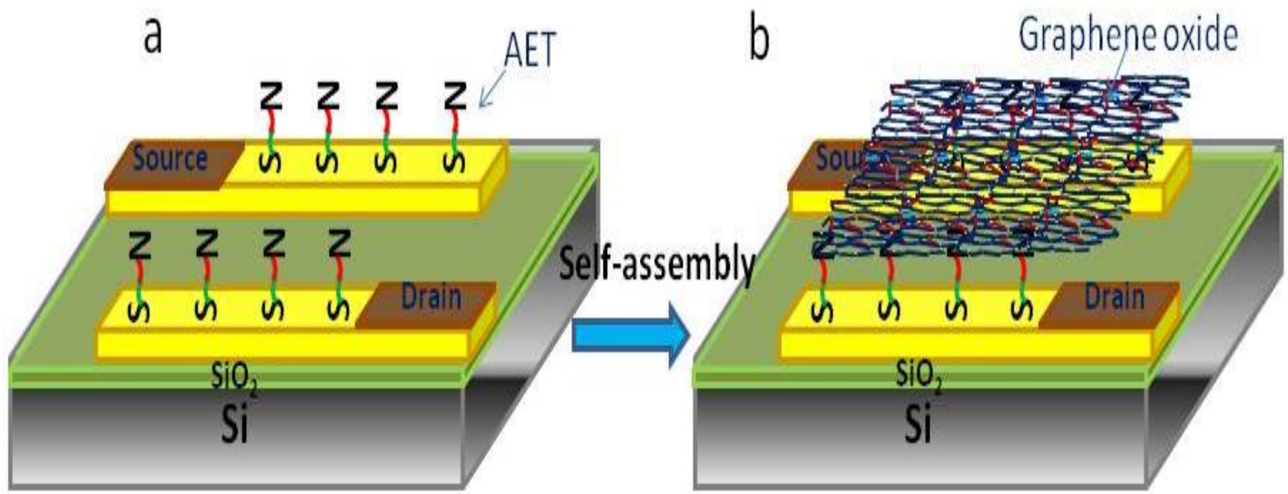
Intellectual Property

- **8,268,405** Controlled Decoration of Carbon Nanotubes with Aerosol Nanoparticles
- **8,240,190** Ambient Temperature Gas Sensor
- **US-2012-0214172-A1** Graphene-Based Field-Effect Transistor Biosensors

Partnering

- The UWM Research Foundation has licensed the above patents to NanoAffix Science, LLC (http://www.nanoaffix.com/) and together the parties are working find additional partners to develop the technology into a final product. This technology is available for developmental research support and licensing

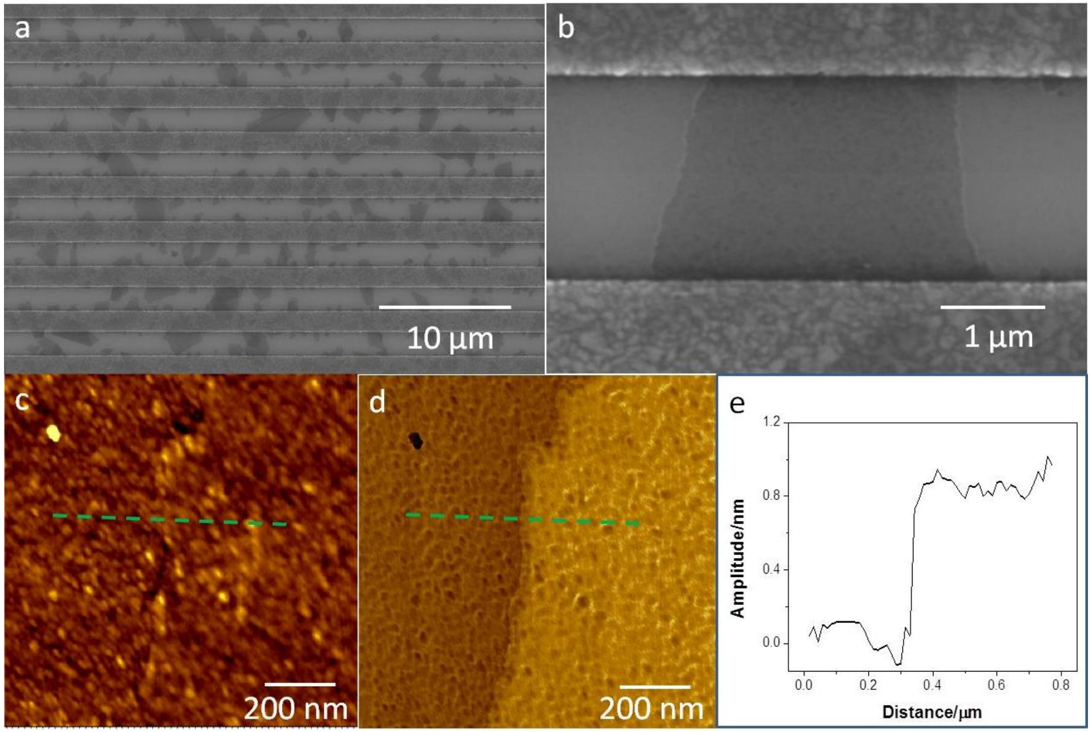
Device Fabrication



Schematic illustration of (a) a layer of AET coating on the electrode surface, and (b) self-assembly of GO sheets on the AET-modified electrodes.

- TRMGO FETs were fabricated by self assembly of GO sheets on the AET-modified AU interdigitated electrodes
- The electrodes were fabricated using a photolithography process on a highly doped Si wafer with a top layer or SiO_2
- A monolayer of AET was assembled on the electrodes and immersed in a GO dispersion
- Gold nanoparticles were deposited on the TRMGO

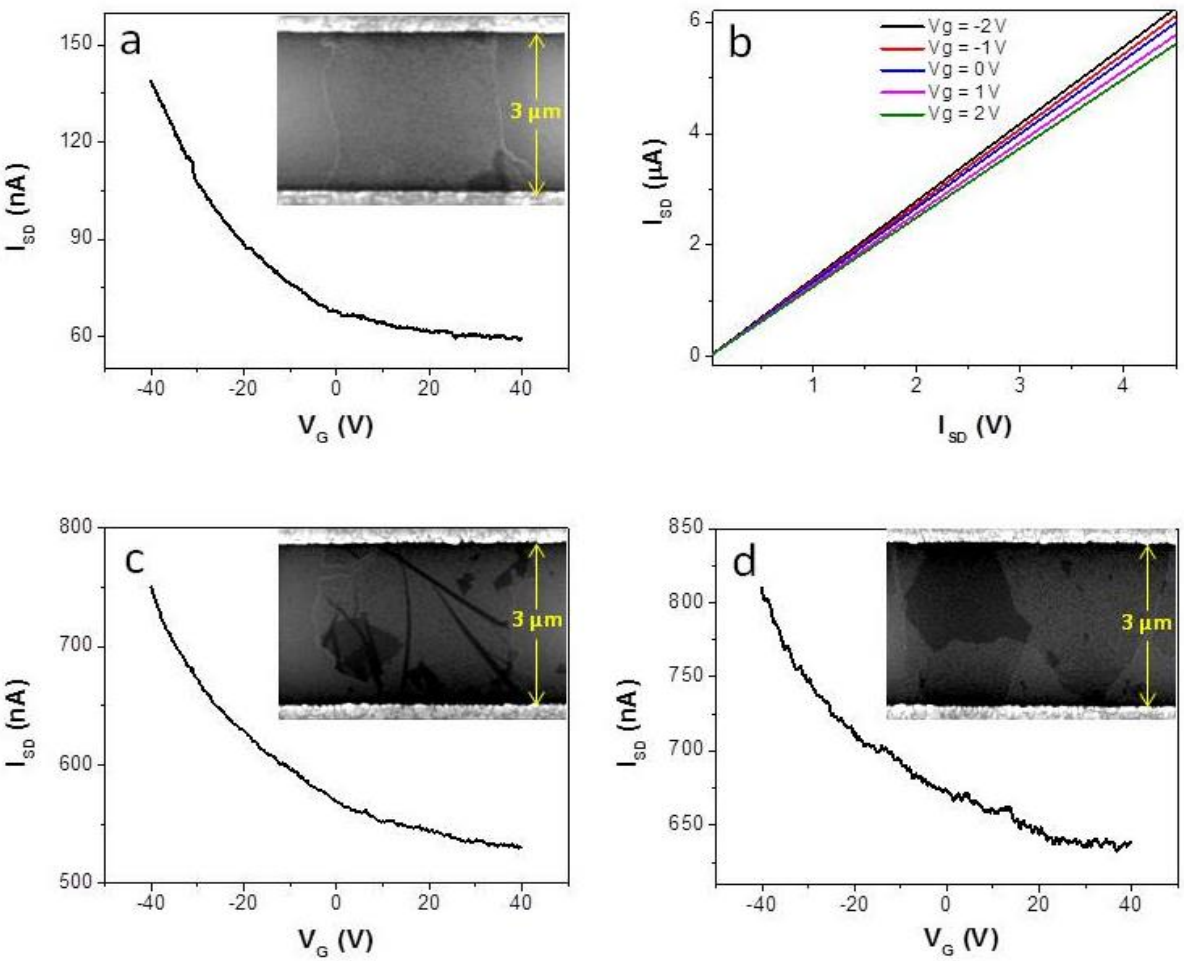
GO Layers are Morphologically Stable



- Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were used to image the self-assembled TRMGO sheets on the electrodes
- The TRMGO sheets were uniformly distributed on the modified electrodes at 5 sheets per 10 μm^2

(a) and (b) SEM images of TRMGO sheets across the electrode gaps. AFM data (tapping mode) of TRMGO on the silicon wafer: (c) height and (d) phase images of the same zone at a cross-sectional area. The dashed line indicates a scanning trace of the TRMGO. (e) Height profile of TRMGO obtained by scanning from bare silicon wafer to TRMGO.

Electrical Properties



(a) The FET I-V curve of TRMGO on SiO_2/Si substrate (I_{SD} = 100 mV). Inset shows an SEM image of a monolayer GO sheet bridging the electrode gap.

(b) I_{SD}–V_{SD} output characteristics of the TRMGO FET device at different bottom-gate V_G from -2 to 2 V with an interval of 1 V.

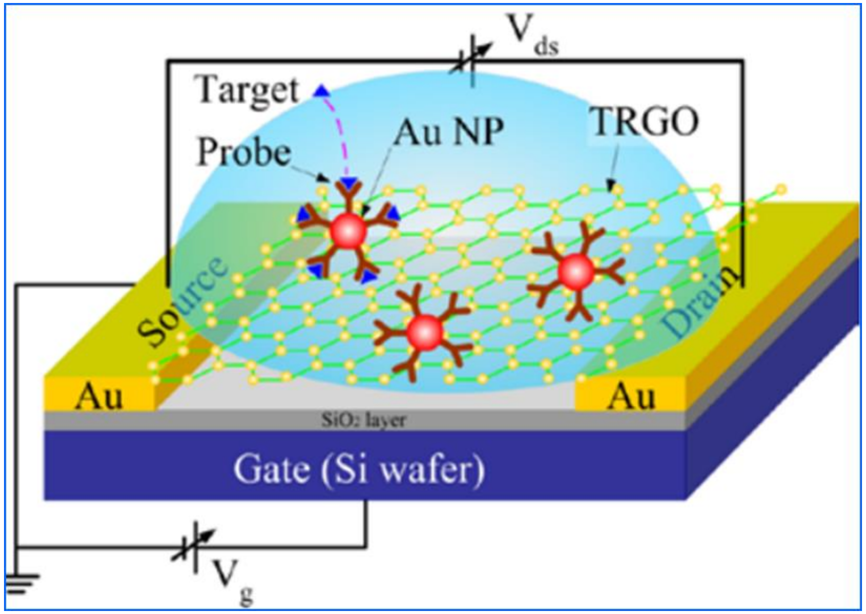
(c) The FET I-V curve of the crumpled GO FET device. Inset shows an SEM image of a crumpled GO across the electrode gap.

(d) The FET I-V curve of the multilayer GO FET device. Inset shows an SEM image of multilayer GO sheets across the electrode gap.

Electrical Properties Summary

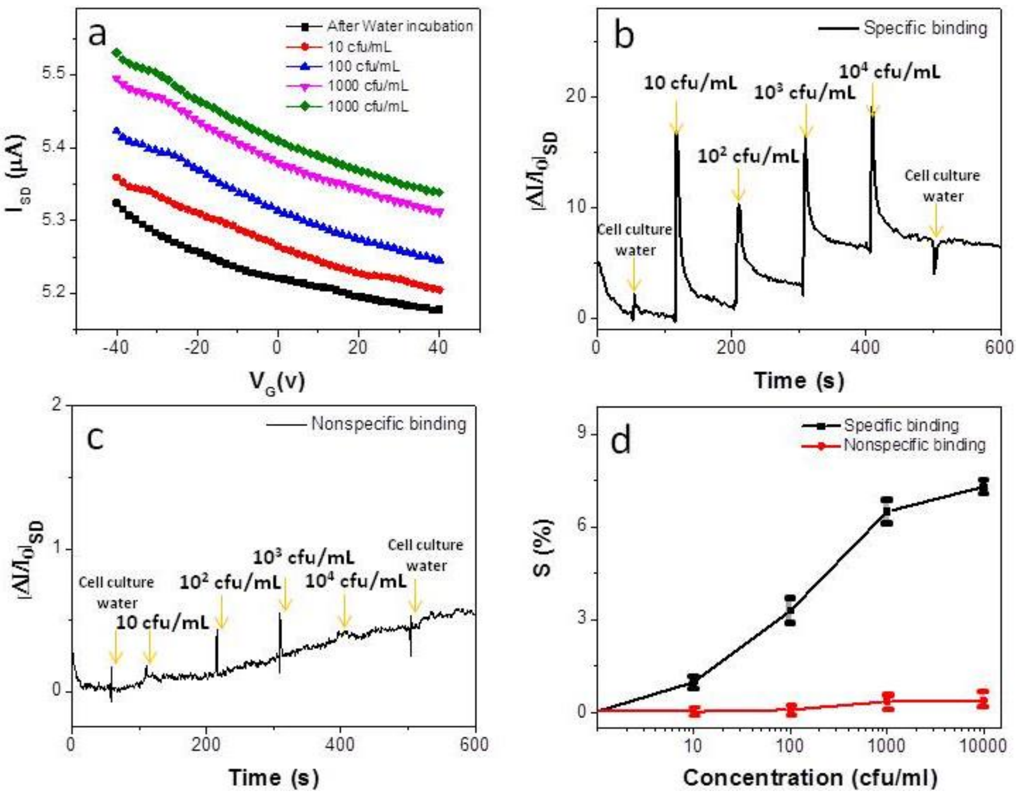
- The decrease in conductivity with the increasing voltage indicates that the TRMGO sheets are p-type semiconducting materials
- The device shows good switching performance with an on/off current ratio of 2.35
- More than 100 devices have been repeated showing similar properties of conductivity (~140 kΩ) and current on/off ration (~2.3)
- The drain-source current decreased with increasing gate voltage (slide 6b) indicating that the device response is sensitive to the gate voltage
- Devices displayed Ohmic-contact behavior, indicating that the sensing mechanism is dominated by electrostatic gating

E. coli O157 Detection



- The device is incubated with anti-*E. coli* 0157 antibodies followed by a blocking buffer to avoid non-specific interactions
- The device was exposed to various concentrations of *E. coli* cells in cell culture grade water
- Concentrations tested were 10, 10^2, 10^3, and 10^4 cfu per mL

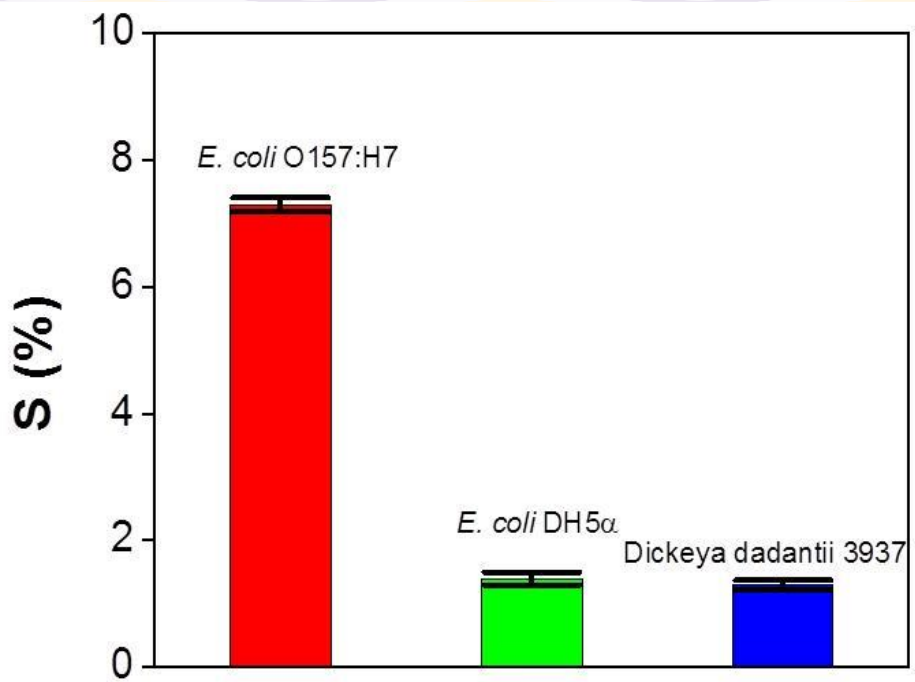
The Sensor Effectively Detects *E. coli*



(a) Typical gate voltage dependence (V_{SD} = 0.1 V) of I_{SD} upon the introduction of *E. coli* cells of different concentrations.
(b) Dynamic response of the devices exposed to different concentrations of *E. coli* cells for specific binding in the TRMGO FET device.
(c) Non-specific binding in the TRMGO FET device (without anti-*E. coli* antibody probes).
(d) The calibration curve of the TRMGO FET device (sensitivity $S = \Delta I/I_0$ vs. concentration).

- Conductance of the devices continued to increase with increasing concentrations of *E. coli* cells
- Injection of *E. coli* in a device without antibody probe showed no effect (c)
- Sensitivity increased linearly with increasing concentrations of *E. coli*

Sensors are Specific to *E. coli* Cells



- Comparison of the sensor sensitivity in response to *E. coli* O157:H7 (10^4 cfu/mL), *E. coli* DH5α (10^4 cfu/mL), and Dickeya dadantii 3937 (10^4 cfu/mL). Error bars were obtained through multiple measurements.

- The sensor sensitivity was 1.4% for *E. coli* DH5a, 1.3% for plant pathogen *D. dadantii*, and 7.3% for *E. coli* O157:H7

Summary

- The inventors have demonstrated ultra-sensitive detection of *E. coli* cells using TRMGO FET devices
- Fabrication of the devices can be easily scaled up with good reproducibility and high electrical stability
- Monolayer GO sheets were selectively deposited on the electrodes by the self-assembly method with a density of 5 sheets per 10 μm^2
- The sensor platform demonstrated real-time, label-free, step-wise, target-specific, highly sensitive electrical detection of *E. coli* cells at concentrations as low as 10 cfu per mL using immobilized anti-*E. coli* antibodies
- This sensor platform offers a promising route for large-scale, high-performance chemical sensor and biosensor applications

Next Steps

Further Testing and Applications

- Fabricate a fluidic cell for continuous water sensing
- Demonstration of sensor incorporation into existing water equipment for real-time measurements
- Also testing a related platform for heavy metal ion detection (mercury, lead, cadmium, and arsenic)

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