Biosensors and Bioelectronics 32 (2012) 69–75

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Rapid, sensitive and label-free detection of Shiga-toxin producing *Escherichia coli* O157 using carbon nanotube biosensors

Sowmya Subramanian, Konrad H. Aschenbach, Jennifer P. Evangelista, Mohamed Badaoui Najjar, Wenxia Song, Romel D. Gomez *

University of Maryland, College Park, MD 20742, United States

ARTICLE INFO

Article history:
Received 30 September 2011
Received in revised form
15 November 2011
Accepted 18 November 2011
Available online 2 December 2011

Keywords:
Biosensor
Deoxyribonucleic acid (DNA)
E. coli
Field-effect transistor (FET)
Carbon nanotube (CNT)

ABSTRACT

An electronic platform to detect very small amounts of genomic DNA from bacteria without the need for PCR amplification and molecular labeling is described. The system uses carbon nanotube field-effect transistor (FET) arrays whose electrical properties are affected by minute electrical charges localized on their active regions. Two pathogenic strains of *E. coli* are used to evaluate the detection properties of the transistor arrays. Described herein are the results for detection of synthetic oligomers, unpurified and highly purified genomic DNA at various concentrations and their comparison against non-specific binding. In particular, the capture of genomic DNA of *E. coli* O157:H7 by a specific oligonucleotide probe coated onto the transistor array results in a significant shift in the threshold (gate-source) voltage (V_{th}). By contrast the signal under the same procedure using a different strain, *E. coli* O45 that is non-complementary to the probe remained nearly constant. This work highlights the detection sensitivity and efficacy of this biosensor without stringent requirement for DNA sample preparation.

1. Introduction

Food borne illnesses are a growing cause of concern in the USA and worldwide. The yearly incidences of food-borne illnesses are estimated to be about 81 million cases just in the USA, of which 91% of the total outbreaks are associated with bacteria (Beran et al., 1991; Potter et al., 1997). *E. coli* O157:H7 is considered to be one the most dangerous food borne pathogens (Griffin and Tauxe, 1991; Buchanan and Doly, 1997). This strain produces large quantities of a potent toxin that can cause hemorrhagic colitis or hemolytic uremic syndrome which may lead to death (Quadri and Kayali, 1998; Jaeger and Acheson, 2000; Ivnitski et al., 1999). In North America, *E. coli* O157:H7 has been implicated as the major cause of hemolytic uremic syndrome (85–95%). A similar strain the *E. coli* O104 has caused an outbreak in Europe in June 2011 that has left at least 22 dead, thousands sickened and nearly $600 M per week in lost agricultural revenue (Associated Press, 2011).

Early detection of this pathogen in food production from farm to table (Ivnitski et al., 1999) is key in minimizing its spread and arresting potential outbreaks. The traditional methods for the detection of bacteria involve a series of steps: pre-enrichment, selective enrichment, biochemical screening and serological confirmation (Helrich, 1990; Kaspar and Tartera, 1990; Tietjen and Fung, 1995; Hobson et al., 1996). The steps are labor-intensive, time-consuming tests and require specialized expensive equipment and trained personnel. For example recent detection methods use polymerase chain reaction (PCR) that is very sensitive but require purified DNA samples, hours of processing and considerable bio-molecular expertise (Meng et al., 1996; Sperveslage et al., 1996) for confirmed identification to be made. Such a process could take 48 h to about 5 days (Karch et al., 1999) to complete. Additionally, transportation of the specimen to reference state labs causes additional delays, which further extends the confirmation time from a few days to a few weeks in some cases. Therefore, there is a clear and present need for rapid, simple and sensitive identification techniques that could be deployed in the field with minimal sophisticated instrumentation. In this paper, a method for electronic label-free sensing of *E. coli* specific pathogenic DNA is reported that addresses this need. A proof of concept is presented which shows specific detection of minute amounts of *E. coli* O157:H7 DNA extracted from highly purified samples and also crude *E. coli* O157:H7 DNA extracted by the boiling method. Detection is on the order of picomoles quantities within several hours and is compared against non-specific binding.

2. Materials and methods

2.1. Design of the carbon nanotube field-effect transistor

The central component of the system is a large array of carbon nanotube field-effect transistors (CNTFETs) (Pandana et al., 2008).

* Corresponding author at: University of Maryland, 2313 A.V. Williams Building, Department of Electrical and Computer Engineering, College Park, MD 20742, United States. Tel.: +1 301 405 7755.
E-mail address: rdgomez@umd.edu (R.D. Gomez).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.11.040

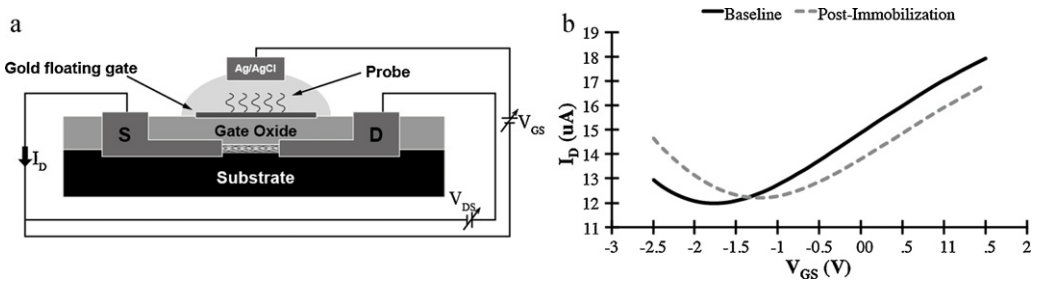

Fig. 1. (a) Principle of operation. When target DNA is captured at the gold floating gate, the curve representing the drain-source current (I_D) versus gate voltage (V_{GS}) shifts by an amount, ΔV_{th}, commensurate with the amount of extra charges that have been captured. (b) Typical $I_{DS} - V_{GS}$ to show baseline (solid) and post-immobilization (dashed). Post-immobilization refers to the measurement after the probe has attached to the gold using thiol–gold chemistry. Au–Cr films are patterned to form S and D electrodes.

Briefly, each transistor is comprised of carbon nanotubes grown on 500 μm thick silicon oxide substrate and contacted by two electrodes labeled source (S) and drain (D). A schematic diagram is shown in Fig. 1a. The nanotubes are covered by a thin layer of high quality oxide material, with thickness between 30 and 60 nm which electrically insulates and prevents liquids from making direct electrical contact with the carbon nanotubes. A segment of the oxide directly on top the carbon nanotubes is designated as the active area, and coated with gold. It becomes the repository of single stranded probe and target DNA charges that will ultimately be detected. The sensing strategy relies on the fact that the DNA is intrinsically charged from its phosphate backbone. A voltage applied between the drain and source, V_{DS}, provides the bias to push the electrons across the carbon nanotubes creating a drain current, I_D. The voltage, V_{GS}, applied between the Ag–AgCl electrode immersed in the liquid buffer and the source, provides a gate potential to increase (or decrease) the current of the carbon nanotube transistors by modulating the number of mobile charges on the carbon nanotubes. For small V_{DS} (~100 mV), the current I_D versus V_{GS} has the generic form shown in Fig. 1b. The typical leakage current between gate and source is on the order of a few pico amperes. The value of the voltage V_{GS} at minimum current is called V_{th} or threshold voltage for conduction. When additional charges accumulate at the oxide surface, e.g. due to capture of target DNA, the electric field from these charges shifts the threshold voltage. Under the conditions in this study, the voltage shift has a simple relation with the bound surface charges given by,

$$\Delta V_{th} = \frac{\Delta \sigma_{DNA}}{C_D} \quad (1)$$

where $\Delta\sigma_{DNA}$ is the added surface charge per unit area on the transistor and C_D is the effective capacitance per unit area arising from the double layer the screening effect of the mobile ions around the bound charges. C_D is given by

$$C_D = \frac{\varepsilon_{buffer}}{\lambda_{Debye}} \quad (2)$$

where λ_{Debye} is the Debye length and ε_{buffer} is approximately $80\varepsilon_0$, the permittivity for water. The phenomenon described by Eq. (1) is exploited for the detection of complementary DNA–DNA binding by tethering an oligonucleotide probe whose sequence is complementary to a target DNA or RNA. The singly ionized phosphate backbone of the nucleic acid strands gives rise to charges. Thus, in the process of Watson–Crick base pairing the target DNA captured by the probe is concommitant to an increase the surface charge density which results in the voltage shift given in Eq. (1).

Fig. 2a shows the sensor chip and the platform. The chip is mounted on a removable carrier with appropriate wire-bonding. A reservoir for liquid buffer containment is made from cut polypropylene tube and mounted on the chip. It can accommodate up to 100 μl. A Ag–AgCl electrode is kept in contact with the buffer from the top of the liquid reservoir during measurement. The Ag–AgCl electrode is attached to a plastic lid which facilitates the alignment of the electrode inside the reservoir. The measurement is automated with the aid of two multiplexers that allow each transistor to be scanned. The optical micrograph shown in Fig. 2b identifies the placement of the transistors and the electrical connections that bring the signal from the active areas in the interior to the contact pads (not shown) at the edges of the die. Fig. 2c is a scanning electron micrograph of the active areas of the devices. The dark contrast region are the metal pattern, the intermediate contrast (green) region are the semiconductor with oxide passivation layer and the bright region (gold) are the gold coated gate active areas upon which the gold oligomers are immobilized. Six complete transistors are shown in this figure, which are connected by a single source electrode running through the center. The six horizontal lines that terminate into rectangular patterns are the individual drain electrodes. The dashed line indicates the overlap with the Au gate active areas. The separation between the source and drain contacts for each transistor is 15 μm. The carbon nanotubes underneath are in the form of a mesh. The CNTs are the conduction channels that function very similar to the depletion layer of a conventional MOSFET. Based upon the drain current we estimate approximately 10 carbon nanotubes channels contribute that to the conductance. A systems level block diagram is shown in Fig. 2d. With the source and gate voltages common to all transistors, the multiplexer unit selects each transistor one after another through the drain electrodes. For every transistor, the I_D is measured as the V_{GS} is swept from $-V_o$ to $+V_o$ (V_o typically 1.5 V).

Other groups, have used similar techniques, to demonstrate detection of DNA binding as applied to 30-mer ssDNA and 21-mer ssDNA target oligomers, respectively (Star et al., 2007; Maehashi et al., 2004). The present device differs from the earlier implementations by having oxide encapsulation of the CNTs sensors, incorporation of gold-coated active areas and gating through the oxide. By encapsulating the CNTs with oxide, they become isolated from the chemical environment, which reduces spurious signals from nonspecific binding of molecules to the CNT walls and thus minimizing false positives. Furthermore by using Au as the gate surface, a standard thiol chemistry protocol for probe immobilization can be used. A new 45-mer probe specific to *E. coli* O157:H7 was developed in this work along with the protocols for immobilization, hybridization, dehybridization and blanking that are essential for the proper operation and accurate analysis of results.

2.2. DNA immobilization

The thiol modified DNA probes were synthesized by Integrated DNA Technologies. The probes were HPLC purified and lyophilized. They were resuspended in Top Gate Buffer (TGB) (1 mM TE pH 8.0, diluted to 0.1× via Ultra Pure Water). The probes were DTT treated to cleave the disulphide bond and create a free sulphide needed to covalently attach to the Gold Attachment Surface (GAS) of the