Sensors and Actuators B 257 (2018) 96–104

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Research paper

Enumeration of circulating tumor cells and investigation of cellular responses using aptamer-immobilized AlGaN/GaN high electron mobility transistor sensor array

Anil Kumar Pulikkathodi[a], Indu Sarangadharan[a], Chen-Pin Hsu[a], Yi-Hong Chen[b], Lien-Yu Hung[b], Geng-Yen Lee[d], Jen-Inn Chyi[d], Gwo-Bin Lee[a,b,c,*], Yu-Lin Wang[a,b,*]

[a] Institute of NanoEngineering and MicroSystems, National Tsing Hua University, Hsinchu 300, Taiwan, ROC
[b] Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu 300, Taiwan, ROC
[c] Institute of Biomedical Engineering, National Tsing Hua University,Hsinchu 300, Taiwan, ROC
[d] Department of Electrical Engineering, National Central University, Jhongli City, Taoyuan County 320, Taiwan, ROC

ARTICLE INFO

Article history:
Received 28 July 2017
Received in revised form 15 October 2017
Accepted 21 October 2017
Available online 28 October 2017

Keywords:
Whole cell sensor
AlGaN/GaN HEMT
Biosensor array
Colorectal cancer
Circulating tumor cells

ABSTRACT

This study reports the fabrication and characterization of electrical double layer gated AlGaN/GaN High electron mobility (HEMT) biosensor array to capture, detect and count circulating tumor cells (CTCs) of colorectal cancer (CRC). GaN HEMT chips were assembled into a sensor array on a thermo-curable polymer substrate in a simple and robust packaging process. The array had multiple aptamer immobilized sensing sites and was highly sensitive and selective with up to single cell resolution. The present method can detect cells in their native electrolyte composition, in a very short time with extremely low cost compared to the conventional circulating tumor cell assays. The effect of cellular binding in different test environments was further studied and reported. The electrical response of the sensor was discovered to be relevant to the transmembrane potential of the cell. This whole cell sensor array has the potential to be used as a point-of-care diagnostic tool in varied applications such as detection of rare cells, pathogens and studying the dynamics of cellular interactions.

1. Introduction

Since the 1960s, the field of biosensors has rapidly evolved to detect and monitor various biological analytes using technologies with diverse principles, such as detection based on optical, electrochemical, electronic and piezoelectric sensors [1–4]. The most desirable specifications for biosensors include high sensitivity and dynamic range, low sample volumes, low cost and high throughput. Multiple biosensing sites can be used to increase the throughput and achieve the specifications [5,6]. Thus, a biosensor chip with sufficiently high density sensor array can perform simultaneous analyte detection and improve the overall performance by large. Among its contemporaries, FET-based biosensors have great significance, mainly because it can directly translate the interaction of biological samples to readable, electrical signals without any labeling [7,8]. Combined with the increased device scalability due to advanced semiconductor fabrication techniques, FET based biosensors are an excellent choice for implementing biosensor array. The AlGaN/GaN high electron mobility transistor (HEMT) is ideal for biosensing applications owing to the high mechanical, chemical and thermal resistance, superior electronic properties and ease of mass production [9,10]. However, the use of FET-based biosensors is limited to low ion concentration environments due to the severe charge screening effect observed in physiological fluids [11]. This requires extensive sample pre-treatment methods which may adversely affect the sample integrity.

Recently, we developed an electric-double-layer (EDL) gated FET biosensor using AlGaN/GaN HEMTs that can directly detect analytes in high salt concentrations such as 1X PBS and human sera, without sample pre-processing [12]. By taking advantage of the high transconductance gain from the EDL-FET structure, receptor-ligand binding can be detected with very high sensitivity. The sensor chips fabricated using conventional semiconductor microfabrication processes have been packaged using a simple thermo-curable polymer-based technology [13]. This method is highly flexible in terms of design and fabrication, allowing us to integrate high density sensor array on a single chip, as required for the assay. The combination of the highly sensitive detection technique and the

* Corresponding authors at: Institute of NanoEngineering and MicroSystems, National Tsing Hua University, Hsinchu 300, Taiwan, ROC.
E-mail addresses: gwobin@pme.nthu.edu.tw (G.-B. Lee), ylwang@mx.nthu.edu.tw (Y.-L. Wang).

https://doi.org/10.1016/j.snb.2017.10.127
0925-4005/

stable sensor array chip can be used to detect rare cell types that are difficult to detect using current laboratory techniques, for early disease detection and monitoring disease prognosis.

Colorectal cancer (CRC) is the world's fourth most deadly cancer which kills almost 700,000 people every year [14]. CRC is in a rise now in western countries (∼10% of cancer related deaths) owing to modern dietary habits and lifestyle [15]. Circulating tumor cells (CTCs) are migrating cells that have clinical importance as a cancer biomarker for early detection and metastases [16–18]. It is increasingly difficult to detect the presence of CTCs, often called 'liquid biopsy', due to its extreme rare occurrence in blood, about 1–10 cells per mL. The progression of cancer and related mortality worldwide, despite the significant advancements in science and technology, warrants an effective strategy to detect, monitor and thus control the onset and spread of cancer. Among current technologies, CellSearch, which is approved by the FDA, is the most popular and considered as the industrial standard [19]. It performs positive selection technique using immunomagnetic separation and enumeration by immunofluorescence. However, the lack of receptor specificity due to inefficient anti-EpCAM antibody targeting leads to inconsistent results. This has restricted its clinical usage. Flow cytometric techniques also use immunomagnetic enrichment and immunocytochemical analysis [20]. The large sample size and dependence of sensitivity on sample volume are some of its drawbacks. CTC detection and enumeration has been implemented without the use of targeting cell surface receptor by studying their morphology [21,22]. Although this technique overcomes the limitations set by the receptor, it requires the interpretation of anatomic pathologists, due to the varying sizes of CTCs and contamination caused by apoptopic cells, introducing the variability factor of operator bias. The above-mentioned techniques are all benchtop and optical based detection systems that require very long turnaround time. Several CTC microdevices are being developed that can offer higher sensitivity, specificity and much less process times [22,23]. The sinusoidal microchip offers desirable characteristics such as elimination of the need for fluorescence staining as it uses a conductivity sensor for enumeration [23]. However, it relies on monoclonal antibodies for CTC capture which are often diverse due to varied host sources. It also uses a two electrode system to carry out conductivity based enumeration which has a low signal to noise ratio. Moreover, the smaller sized CTCs and CTC debris may go undetected by the conductivity sensor and larger sample size takes longer time for enumeration.

In the present research, we have developed an aptamer-based biosensor array using AlGaN/GaN HEMTs to capture and count the number of CTCs in a small sample volume. The high sensitivity of this detection technique offers up to single cell resolution in high ionic strength solutions, requiring only a short duration (5 ∼ 10 min) of test. The current method eliminates the need for sample dilutions, thus minimizing sampling errors, and provides specific detection using CTC specific aptamer, with high throughput via the sensor array, which is perfectly suitable to be used as a point-of-care diagnostic device with minimal user protocols and extremely low cost. The electrical response of CTC in different buffer solutions is also discussed. The present biosensor array can not only be used to detect and count CTCs, but also can be used to study the dynamics of cell binding and interactions.

2. Materials and methods

2.1. AlGaN/GaN HEMT fabrication

GaN epi-wafer consists of 1 mm silicon substrate with 3 μm GaN buffer layer, 260 Å AlGaN and GaN cap layer. Transistor active area is defined by mesa isolation using Inductively Coupled Plasma (ICP) etching by BCl_3/Cl_2 gas mixture. Ohmic contacts consisting Ti/Al/Ni/Au (200/800/400/1000 Å) are deposited using electron beam evaporator, followed by rapid thermal annealing at 850 °C in N_2 environment. Metal interconnects consisting Ti/Au (200/2000 Å) are then deposited using evaporator. The HEMT device is fabricated on a die area of 1 mm^2. The top view of the device is shown in Fig. 1(a) with the in-plane gate electrode. The device is passivated using photoresist, and using photolithography openings of $10 \times 60\ \mu m^2$ are made on the transistor channel and the gate electrode, which are separated by a fixed distance of 65 μm as shown in Fig. 1(a). The gate electrode opening is designed so considering the size of cells to be captured which are typically 5–20 μm in size.

2.2. Biosensor array fabrication

The sensor array fabrication is a robust and flexible process that allows us to have any number of HEMT sensors depending on the substrate dimension of the sensor array chip. Two step GaN HEMT biosensor packaging technology has been described in our previous works [13]. Briefly, HEMT devices are embedded in epoxy resin and thermally cured at temperatures of 125 and 165 °C for 1.5 and 1 h, respectively. The transistor chips can be arrayed with sufficient density in user defined pattern. Post curing, metal probing pads are deposited using electron beam evaporator, for biasing the HEMT device and measuring the drain current response. A simple microfluidic channel with an inlet and outlet is placed on top of the chip such that sensors come in contact with the test solution flowing in. The top view of the biosensor array chip is shown in Fig. 1(b) with an 8-sensor array. Depending on the final size of the substrate and the application specifications, the array density, periodicity and positions can be varied and optimized.

2.3. Cell line and reagents used

The cell model used in this study was HCT-8 cell line (colorectal cancer). The HCT-8 cells were cultured in RPMI-1640 medium (Invitrogen Co., USA) prior to use. Cells were then suspended in media that contained a physiological salt concentration such as cell culture medium, phosphate buffered saline (PBS) and Hanks balanced salt solution (HBSS) with and without calcium and magnesium ions. After cells were captured and tested, sensors can be regenerated by washing the bound cells away with tris-EDTA (ethylenediaminetetraacetic acid) buffer or near neutral pH protein elution buffer.

2.4. CTC specific aptamer immobilization

Aptamer specific to HCT-8 cells have been screened by Lee et al. [24] with the sequence, TACAGCACCACAGACCATGGTTGT-GTTTTTTTTTGTGTGGCTTCGTATGTTGTTGCGTGTTTGTCTTCCTGCC (5′ to 3′). The entire array chip is passivated with photoresist, and the openings on the gate electrode is used for aptamer immobilization. The sensor chip was cleaned prior to aptamer functionalization using UV ozone cleaning in DI (deionized) water for 10 min followed by diluted acid and DI water wash. Thiol modification was carried out on one end of the aptamer sequence and it was immobilized on the gold gate electrode of the HEMT chip for 24 h, allowing the formation of a self-assembled monolayer. The aptamer-immobilized sensor is shown as in Fig. 1(c).

2.5. Sensor measurements

Once the aptamer is immobilized on the gate electrode area of the sensor, the cells introduced into in the flow channel can