Lab on a Chip

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: A. K. Pulikkathodi, I. Sarangadharan, Y. Chen, G. Lee, J. Chyi, G. Lee and Y. Wang, *Lab Chip*, 2018, DOI: 10.1039/C7LC01305A.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/loc

View Article Online
DOI: 10.1039/C7LC01305A

Dynamic Monitoring of Transmembrane Potential Changes: Study of Ion Channels using Electrical Double Layer gated FET Biosensor

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

[a] Institute of NanoEngineering and MicroSystems, National Tsing Hua University, Hsinchu, Taiwan 300, R.O.C.

[b] Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan 300, R.O.C.

[c] Institute of Biomedical Engineering, National Tsing Hua University, Hsinchu, Taiwan 300, R.O.C.

[d] Department of Electrical engineering, National Central University, Jhongli City, Taoyuan County 320, Taiwan, R.O.C.

***Correspondence to ylwang@mx.nthu.edu.tw**

***Co-correspondence to gwobin@pme.nthu.edu.tw**

Abstract

In this research, we have designed, fabricated and characterized electrical double layer (EDL) gated AlGaN/GaN High electron mobility transistor (HEMT) biosensor array to study the transmembrane potential changes of cells. The sensor array platform is designed to detect and count circulating tumor cells (CTCs) of colorectal cancer (CRC) and investigate cellular bioelectric signals. Using EDL FET biosensor platform, cellular responses can be studied in physiological salt concentrations, thereby eliminating complex automation. Upon investigation, we discovered that our sensor response follows the transmembrane potential changes of the captured cell. Our whole cell sensor platform

View Article Online
DOI: 10.1039/C7LC01305A

can be used to monitor the dynamic change in membrane potential of cells. The effect of continuously changing electrolyte ion concentrations and ion channel blocking using cadmium are investigated. This methodology has the potential to be used as an electrophysiological probe for studying ion channel gating and interaction of biomolecules in cells. The sensor can also be a point-of-care diagnostic tool for rapid screening of diseases.

Keywords: EDL FET biosensor; transmembrane potential; ion channel; circulating tumor cells

Introduction

Biosensing technologies developed for live cell sensing, cellular diagnostics, cell counting, and analysis have provided means to identify and classify cell based biomarkers for diseases and study the fundamental biology of cell. Currently optical based sensing methodologies are used for cell based diagnostics and investigation of cell signaling and communication (1-3). Cancer cells released into blood known as circulating tumor cells (CTCs) are detected and analyzed using immunofluorescence, which is the only FDA approved CTC detection technique used in clinical applications (4). But the technology suffers from poor sensitivity, complexity and lack of receptor specificity. Flow cytometry is one of the oldest optical technologies developed and is used widely in downstream applications for live cell imaging and several other cellular manipulations (5). Surface Plasmon Resonance (SPR) and Resonant Waveguide Grating (RWG) are other optical based techniques used in laboratory to perform whole cell sensing and in cell biology related studies (6, 7). However, the above-mentioned techniques are all primarily bench-

View Article Online
DOI: 10.1039/C7LC01305A

top instruments that are bulky and extremely costly, and require trained laboratory staffs to perform the experiments.

Currently vital bioelectric signals of cells like transmembrane potential or simple membrane potential (V_m) is monitored using electrophysiological techniques like patch clamp and molecular potentiometric probes (8-10). The patch clamp technique is invasive and often suffers from unstable cell-device interface. Potentiometric probes are non-invasive, but suffer from problems commonly associated with fluorescent probes such as photo-bleaching and cross reaction with other biomolecules leading to variations. With the advancements in nanotechnology, newer platforms to study cellular biology have been developed (11). However, a whole cell sensing platform that can non-invasively detect cellular functions such as ion channel gating, in a single cell, is yet to be realized.

Compact and simplistic diagnostic platforms can be developed with the help of miniaturization, such as in microfluidics-based cellular diagnostics (12). In this genre, semiconductor based electronic sensors are particularly interesting, as they are highly sensitive, simple and compact, have very low time and sample volume requirements and are extremely cost effective. Field effect transistor (FET) based biosensors have been reported to possess high sensitivity, less turnaround times, easy signal read out capabilities and offers label free cell detection (13, 14). However, the pronounced charge screening effect in high ionic strength solutions such as physiological solutions, and drift

View Article Online
DOI: 10.1039/C7LC01305A

in electrical characteristics due to harsh test environments are some of the disadvantages of FET based biosensors that have hindered their clinical applications (15).

Previously, we developed a whole new sensing methodology using AlGaN/GaN high electron mobility transistor (HEMT) based biosensors, where electrical double layer (EDL) formed in the test solution is used to modulate the channel conductivity (16). Biological detection was also achieved in high ionic strength media in electrolyte gated organic FETs (17, 18). In the current research, we have employed the sensing platform of EDL FET biosensor to detect and enumerate CTCs in physiological salt environment without the need for any sample pre-treatments, and to study and investigate the bioelectric signals of cells. The sensor offers very high sensitivity due to the unique ion gating mechanism, with single cell resolution. A simple and robust biosensor array is fabricated to increase the throughput of enumeration of rare cell types such as CTCs of colorectal cancer (CRC) and to provide multiple recognition sites on a single sensor chip. This technology can be used along with microfluidic CTC enrichment platform to perform quick and easy liquid biopsy tests. In this study, we have also systematically investigated the cellular response in different external media and confirmed that our EDL FET biosensor can be used to monitor the changes in transmembrane potential of the cell. Based on this discovery, we have investigated the dynamic sensor response to continuously changing extracellular ion concentrations and ion channel blocking effect as

View Article Online
DOI: 10.1039/C7LC01305A

well. This sensor methodology can be used in commercial diagnostics such as point of care diagnostics and in various biomedical applications related to fundamental cell biology.

Materials and Methods

AlGaN/GaN HEMT fabrication

The GaN epi was grown on a 1mm silicon substrate with 3 µm GaN buffer layer, 260 Å AlGaN and 10 Å GaN cap layer using Metal-organic Chemical Vapor Deposition (MOCVD) system. Inductively Coupled Plasma (ICP) etch was performed by using BCl_3/Cl_2 gas mixture to form the transistor active area. Electron beam (E-Beam) evaporation method was used to deposit ohmic contacts consisting of Ti/Al/Ni/Au (200/800/400/1000 Å), which was followed by rapid thermal annealing (RTA) at 850 °C in N_2 environment. Finally, E-Beam is used to deposit metal interconnects consisting of Ti/Au (200/2000 Å).

Biosensor array fabrication

In our previous works (19), we have shown a two-step GaN HEMT biosensor packaging technology. Briefly, HEMT devices are embedded in epoxy resin and thermally cured in steps of 125 and 165 °C, for 1 and 1.5 hours, respectively. Probing pads are deposited using e-beam evaporator and the entire array is passivated using photoresist. The sensing

View Article Online
DOI: 10.1039/C7LC01305A

regions are opened using photolithography so that sample solution can come in contact. The schematic of AlGaN/GaN HEMT is shown in Figure 1 (a). The gate electrode and transistor channels are opened using photolithography with an area of 10x60 μm^2 separated by a gap distance of 65 μm^2. This is defined as the sensing region of the sensor. The packaged biosensor array chip image is shown in Figure 1 (b). The density and periodicity of sensor array can be optimized according to the requirements of the biosensing applications.

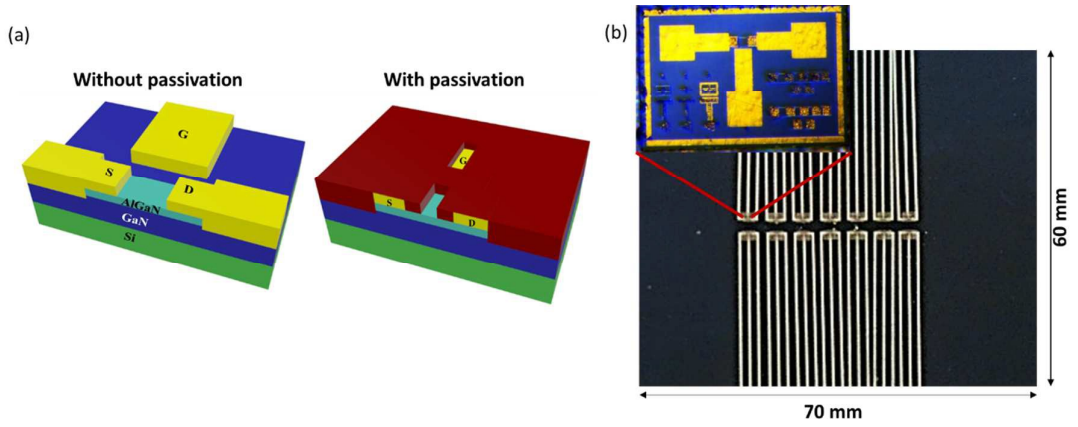

Figure 1. Structure and design of EDL FET sensor and biosensor array. (a) Schematic diagram of AlGaN/GaN HEMT with and without passivation. (b) Top view image of AlGaN/GaN HEMT biosensor array.

Cell line and reagents used

The cell model used in this study is HCT-8 CRC cell line. Cells were cultured in RPMI-1640 medium (Invitrogen Co., USA). While performing electrical measurements, cells were suspended in culture medium, 1X-phosphate buffered saline (PBS) or balanced salt solutions such as Hanks Balanced Salt Solution (HBSS) with and without calcium and

View Article Online
DOI: 10.1039/C7LC01305A

magnesium. The composition of each test medium is denoted in Table 1. After cells were captured and tested, sensors can be regenerated by washing the bound cells away with Tris-EDTA buffer or near neutral pH protein elution buffer.

Contents	RPMI 1640 (Cell culture medium)	HBSS with Ca^{2+} & Mg^{2+} ions	HBSS without Ca^{2+} & Mg^{2+} ions	1X PBS
Calcium Chloride ($CaCl_2$) (anhyd.)	1.05	1.25	-	-
Magnesium Sulfate ($MgSO_4$) (anhyd.)	0.41	0.81	-	-
Magnesium Chloride (anhydrous)	0.30	-	-	-
Sodium Bicarbonate ($NaHCO_3$)	29.02	4.16	4.16	-
Sodium Chloride (NaCl)	120.61	136.89	136.89	137
Potassium Chloride (KCl)	4.16	5.36	5.36	2.7
Sodium Phosphate monobasic (NaH_2PO_4-H_2O)	0.45	-	-	-
Sodium Phosphate dibasic (Na_2HPO_4) anhydrous	0.5	0.34	0.34	8.0
Potassium Phosphate (KH_2PO_4) anhydrous	-	0.44	0.44	1.53
Glucose	17.5	5.55	5.55	-

Table 1. Composition of test media (culture medium, PBS and HBSS) in which CTCs are suspended. Concentrations expressed in mM.

View Article Online
DOI: 10.1039/C7LC01305A

CTC specific aptamer immobilization

An aptamer specific to HCT-8 cells was screened by Lien et.al (20). The sequence is 5'-TACAGCACCACAGACCATGGTTGTGTTTTTTTTGTGTGGCTTCGTATGTTGTTGCGTGTTTGTCTTCCTGCC - 3'. The aptamer was reported to exhibit high specificity and high affinity towards HCT-8 cells. The aptamer was thiolated at one end to bind to the gold gate electrode. TCEP (tris(2-carboxyethyl)phosphine) was used to reduce the disulfide bonds and created free thiols to link the aptamer to the gold surface, during an incubation period of 24 hours at room temperature. After the incubation period, the unbound aptamer was washed away from the device surface using 1X PBS.

Sensor measurements

The source drain characteristics are measured and recorded by Agilent B1530/B1500A semiconductor parameter analyzer. The transistor is operated under constant source drain bias of 2.5 V and pulsed gate voltage of 2 V is applied as the gate bias to the gold gate electrode. Initially drain bias is turned on and gate bias is held at 0 V for 2 μs and then held at 2 V for 50 μs, after which gate and drain biases are turned off. The change in transistor drain current before and after applying the gate bias, is defined as current gain of the sensor. Measurement parameters are shown in detail in supplementary information.

View Article Online
DOI: 10.1039/C7LC01305A

Results and Discussion

The sensing platform utilizing electrical double layer (EDL) gated FET biosensor is able to detect and count the number of CTCs present in the test solution with up to single cell resolution, and also be used to dynamically monitor the changes in the transmembrane potential of the captured cell. The sensing structure and working principle of EDL FET have been studied in our previous work (16). The sensing region of the HEMT biosensor is defined as the gate electrode open area (10x60 μm^2) and the transistor channel open area (10x60 μm^2), separated by a fixed and controlled distance of 65 μm. When high ionic strength solutions such as physiological fluids are dropped on the sensing region, electrical double layer is formed on the gate electrode and transistor channel interfaces. Further application of pulsed gate bias creates a potential drop across the test solution and the transistor dielectric, which modulates the channel conductivity and hence the transistor drain current. Therefore, the applied gate voltage is the sum total of potential drop across the solution and the transistor dielectric, as described in Equation 1,

$$V_g = \Delta V_s + \Delta V_d \qquad \text{Equation 1}$$

The EDL created at the gate electrode and transistor dielectric interfaces sets up a solution capacitance C_s which modulates the dielectric capacitance C_d. If the solution and

View Article Online
DOI: 10.1039/C7LC01305A

dielectric capacitances are connected in series, from equation 1, the potential drop across transistor dielectric V_d can be described by Equation 2,

$$\Delta V_d = \frac{\frac{1}{j\omega C_d}}{\frac{1}{j\omega C_d}+\frac{1}{j\omega C_s}} \times V_g = \frac{C_s}{C_d+C_s} \times V_g \qquad \text{Equation 2}$$

Thus any change in solution capacitance influences the potential drop across transistor dielectric, which modulates the channel conductivity. In the case of GaN HEMT, the highly dense electron gas trapped in the interface of AlGaN and GaN layers forms the channel. When V_d changes as a result of changes in C_s, it readily influences the 2 dimensional electron gas channel of HEMT as it is highly sensitive, thereby changing the transistor drain current I_d. A detailed schematic showing the potential drops and capacitances across the sensor structure is provided in the supplementary information.

When the EDL FET is used as a biosensor, the test solution is normally a biological solution with physiological salt concentration, containing biological analytes. In this study, the test solutions used are cell culture media, standard reaction buffers like 1X PBS or balanced buffers like HBSS. In these test solutions, the salt concentration is very high and roughly the same as physiological ionic strength. According to equation 2, when ionic strength increases, solution capacitance increases, which would lead to an increase in potential drop across dielectric and hence an increase in transistor drain current. This means that our sensor is gated by the means of effective capacitance of the test solution.

View Article Online
DOI: 10.1039/C7LC01305A

This solution capacitance is modulated not only by the ionic strength, but also by surface functionalization of the gate electrode and the subsequent capture of biological analytes at the gate electrode. In other words, when aptamer is immobilized on the gate electrode open area, it changes the local charge distribution within the EDL, which directly influences the solution capacitance C_s. Similarly, when cells are captured by the immobilized aptamer, the solution capacitance changes, corresponding which the transistor drain current changes. Hence the transistor drain current can follow the capture of cells on the gate electrode area and the magnitude of change in drain current is proportional to the number of cells captured. Throughout this study, we have used current gain as the sensor signal instead of absolute drain current. Current gain is defined as the difference of transistor drain current before and after applying gate voltage V_g. Current gain or simply gain is more stable sensor index as absolute drain current of the transistor may be prone to variations.

View Article Online
DOI: 10.1039/C7LC01305A

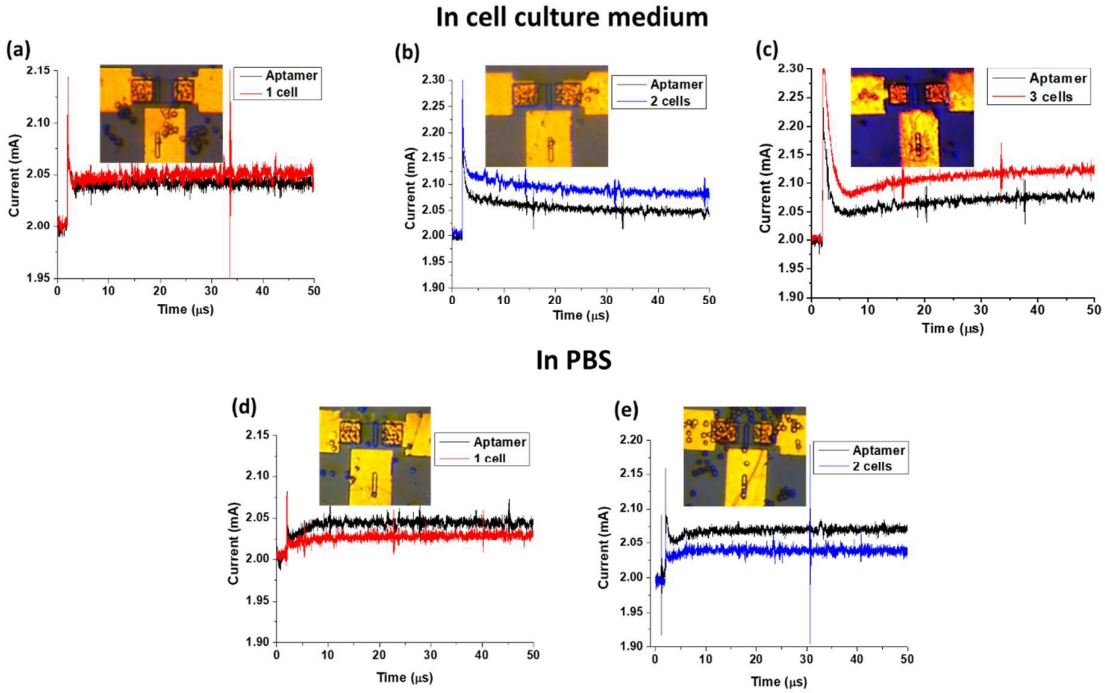

Figure 2. Detection and enumeration of CTCs in culture medium and 1X PBS. (a) through (c) drain current characteristics of EDL FET biosensor when 1 cell, 2 cells and 3 cells are captured on the device (in inset), respectively, in cell culture medium. (d), (e) drain current characteristics of EDL FET biosensor when 1 cell, 2 cells are captured on the device (in inset), respectively, in 1X PBS.

Figure 2 depicts the capture, detection and enumeration of CTCs in culture medium and 1X PBS. The baseline of the sensor is measured after the immobilization of the aptamer, in the same test solution in which CTCs are suspended. Figure 2 (a) through (c) shows the electrical characteristics of detection of CTC in culture medium. After measuring aptamer baseline, CTCs suspended in culture medium is introduced into the flow channel and allowed to incubate on the sensor surface for 10 mins. Gentle perturbation using pipetting is provided so that the frequency of collision between the cells and their aptamer increases. After incubation, the unbound cells are gently washed away and optical microscope is used to monitor the capture of CTCs on the sensor surface and the

View Article Online
DOI: 10.1039/C7LC01305A

corresponding electrical readings of the EDL FET sensor are recorded. Figure 2 (a) through (c) shows the change in current gain for 1 cell, 2 cells and 3 cells captured on the sensor, in culture medium. The change in sensor signal, that is the current gain, is proportional to the number of cells captured on the sensor. Similarly, when cells are suspended in 1X PBS, the change in sensor signal follows the number of cells captured on the sensor, as seen in Figure 2 (d) and (e). Although the change in current gain, which is regarded as the sensor signal is indicative of the number of cells captured, both in culture medium and 1X PBS, the direction of change, that is change in current gain with respect to the aptamer baseline, is different in different test media, That is, in culture medium (Figures 2 (a) through (c)) the current gain increases from the aptamer baseline and in 1X PBS (Figure 2 (d) and (e)) the current gain decreases with respect to the aptamer baseline. This phenomenon of relative change in current gain was found to be interesting and indicated that the EDL FET sensor platform can not only be used for detection and enumeration of rare cell types such as CTCs, but also be used in the study of cellular response as a function of external stimuli.

View Article Online
DOI: 10.1039/C7LC01305A

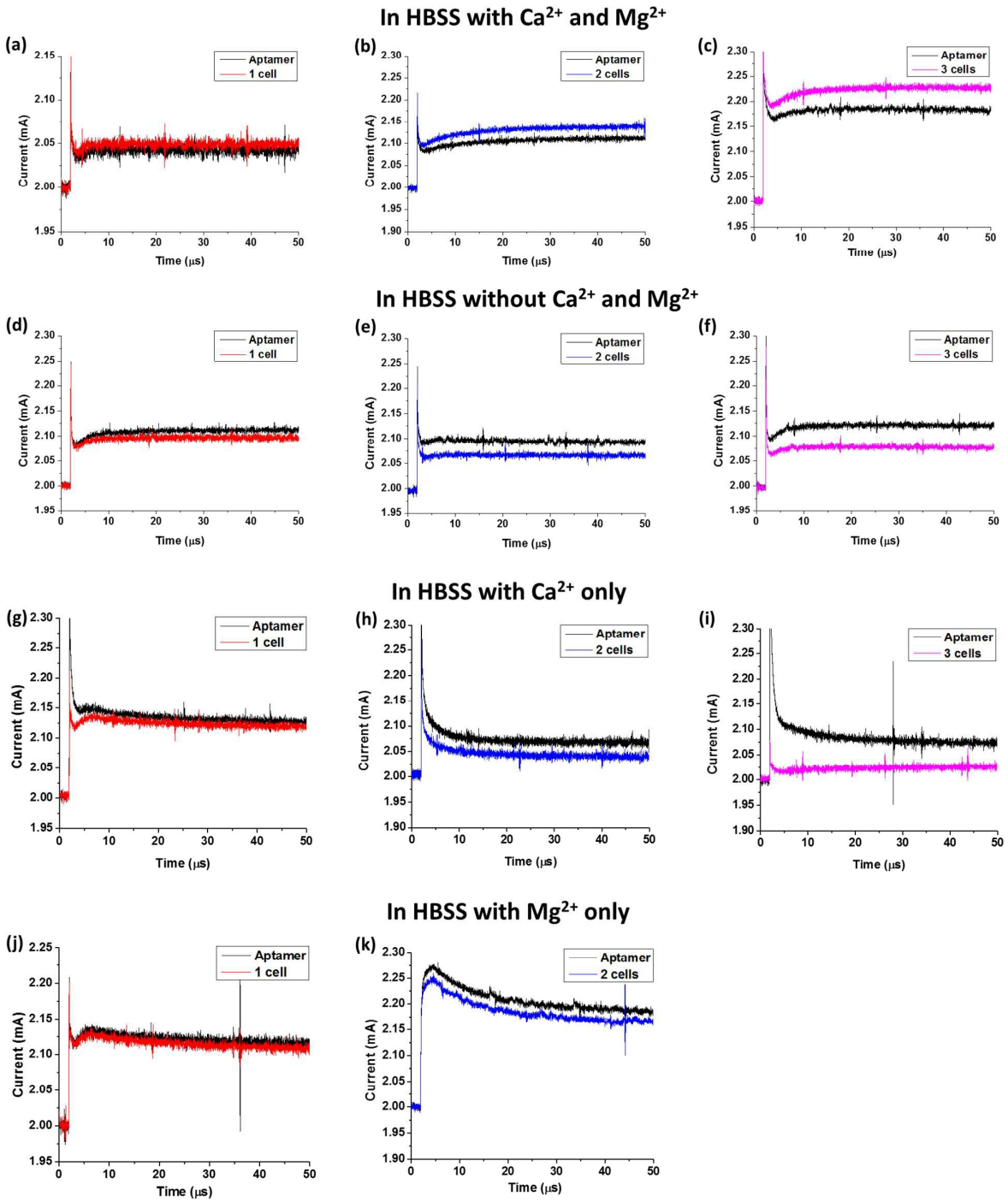

Figure 3. Sensor characteristics in different test solution in the presence and absence of divalent cations. Drain current characteristics of EDL FET biosensor (a) through (c) in HBSS with Ca^{2+} and Mg^{2+}. (d) through (f) in HBSS without Ca^{2+} and Mg^{2+}. (g) through (i) in HBSS with only Ca^{2+}. (j) and (k) in HBSS with only Mg^{2+}.

View Article Online
DOI: 10.1039/C7LC01305A

A quick comparison of the contents of culture medium and 1X PBS, indicated the presence and absence of divalent cations such as Ca^{2+} and Mg^{2+}, respectively. Since 1X PBS is not a balanced buffer, osmotic pressure needs to be balanced in order to prevent the cells from dying of osmotic shock. Hence we performed experiments in HBSS and intentionally provided external stimuli to CTCs, in the form of the presence and absence of Ca^{2+} and Mg^{2+} ions. When extracellular Ca^{2+} and Mg^{2+} ions, denoted as $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ where present in HBSS, the sensor response was similar to that in culture medium, i.e., the current gain increased with respect to aptamer baseline. This is depicted in Figure 3 (a) through (c). On the other hand, when $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ was absent from HBSS, the capture of CTCs caused a decrease in current gain with respect to the aptamer baseline, as seen in Figures 3 (d) through (f). This behavior of sensor is similar to that recorded in 1X PBS test environment. From the results in Figures 3 (a) through (f), it can be concluded that divalent cations do provide stimuli to CTCs leading to a cellular response that results in change in sensor signal. To find out the effect contributed by each of the divalent cation, tests were performed with CTCs suspended in only $[Ca^{2+}]_o$ containing HBSS solution and only $[Mg^{2+}]_o$ containing HBSS solution. Consequently, in both cases, depicted in Figures 3 (g) through (k), the current gain decreases with respect to the aptamer baseline, indicating a response similar to that in 1X PBS. This sensor behavior prompted us to investigate previous literature that suggests what type of

View Article Online
DOI: 10.1039/C7LC01305A

response is exhibited by cells when they are subjected to external stimuli such as modulating the concentration of divalent cations. Cancer cells have been known to possess increased permeability to divalent cations and leaky channels by which their transmembrane potential is easily modulated (21). Previous studies have demonstrated that the absence of divalent cations such as Ca^{2+} and Mg^{2+}, alter the transmembrane potential from their resting potential, towards more depolarized potential (21-25). Similarly, when either of the divalent cations are absent, i.e., $[Ca^{2+}]_o$ or $[Mg^{2+}]_o$, the transmembrane potential attains more depolarized potential values. This is due to the imbalance in the electrochemical and ionic gradient caused by the extracellular and intracellular distribution of ions, which results in a shift in transmembrane potential (21). When the transmembrane potential of the cell changes, the impedance is altered, like an RC element, which influences the local charge distribution in the vicinity of EDL (like a biorecognition event), resulting in a change in C_s and hence the current gain.

View Article Online
DOI: 10.1039/C7LC01305A

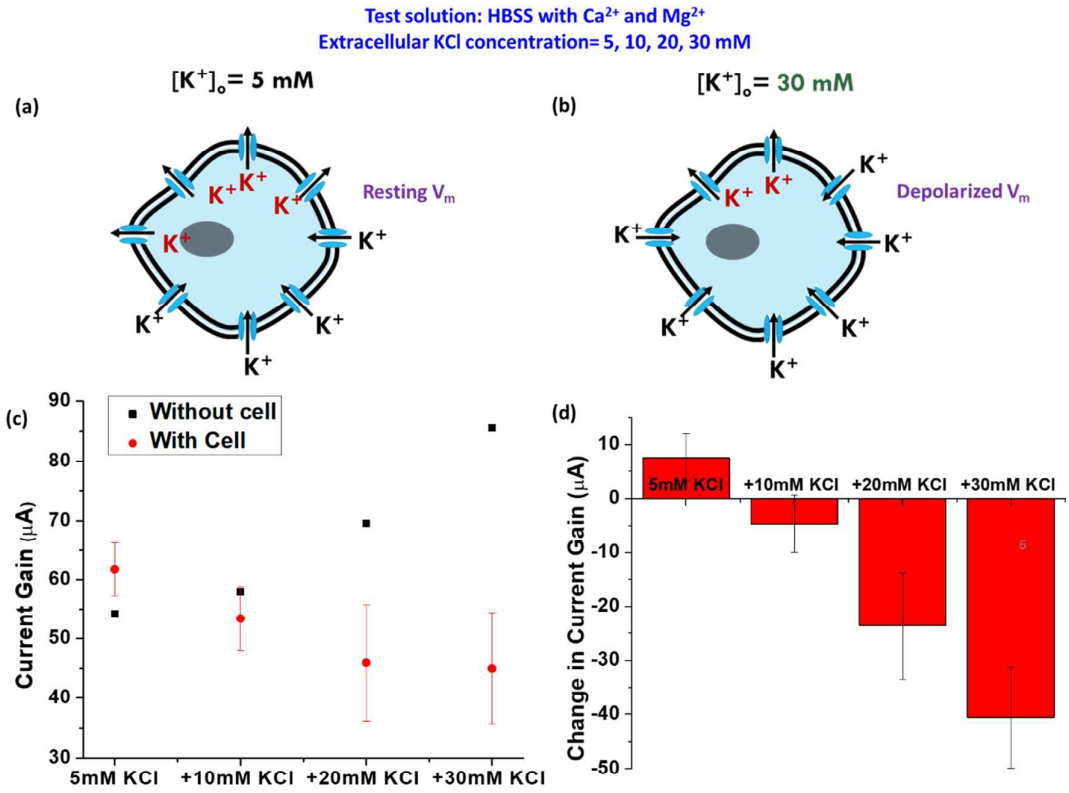

Figure 4. Sensor response during KCl induced step depolarizations. (a) and (b) schematically represent the cell responses with Ca^{2+} and Mg^{2+} buffer and higher concentration (30mM) of KCl, respectively. (c) Current gain vs. external KCl concentration of the sensor, with and without cell. (d) Sensor response (change in current gain with respect to aptamer baseline) to external KCl concentration.

The sensor response to changes in transmembrane potential of the cell can be confirmed by providing known depolarizing stimuli to the cells and recording the sensor signal. Figure 4 depicts the sensor characteristics when captured CTCs are subjected to step depolarizations by increasing the extracellular KCl concentrations. This method of depolarizing the membrane potential by changing KCl concentrations has been used to calibrate the existing membrane potential measurement technique which utilizes potentiometric fluorescent probes (26, 27). Figures 4 (a) and (b) schematically depict the

View Article Online
DOI: 10.1039/C7LC01305A

cellular responses when step depolarizations are introduced. As observed from Figure 4 (c), when KCl concentration in HBSS is increased from 5 mM to 30 mM, the current gain of the sensor increases. This is due to the significant increase in total ion concentration of the test solution, which increases the Cs and hence the current gain. Incidentally, when CTCs are allowed to be captured by the aptamer, and extracellular KCl concentration is increased, the current gain decreases with respect to their corresponding aptamer baseline, as shown in Figure 4 (c). This decrease in current gain is synonymous with results in Figures 2 (d) and (e), Figures 3 (d) through (k), where the membrane potential attains more depolarized values. In the case of increasing extracellular KCl concentrations, depolarization is induced in cells because $[K^+]_i$ concentration increases due to increased influx of K^+ from outside the cell membrane, which leads to the inside of cell being less negative and hence, depolarized. Figure 4 (d) depicts the sensor signal, defined as the change in current gain with respect to the aptamer baseline, as a function of extracellular KCl concentrations, or step depolarizations. Thus, our EDL FET sensor can be used to monitor the bioelectric signals of cells, in their native conditions. This method of monitoring transmembrane potential of cells has several advantages over the conventional methods. Our method is non-invasive, unlike microelectrode or patch clamp method. The currently popular method uses potentiometric molecular probes to measure the membrane potential of a group of cells. Fluorescent probes are prone to photo-bleaching issues and

View Article Online
DOI: 10.1039/C7LC01305A

cellular uptake of probes. Our EDL FET biosensor platform can provide the means to monitor membrane potential changes of a single cell, without the need for extensive calibration procedures. Several studies have reported the key role of transmembrane potential in indication of disease progression in non-excitable cells (28), cell proliferation and differentiation (29) and cell-cell communication (30). A simplistic and sensitive platform like EDL FET biosensor for membrane potential monitoring can be useful in cancer therapeutics, regenerative medicine and early disease diagnosis.

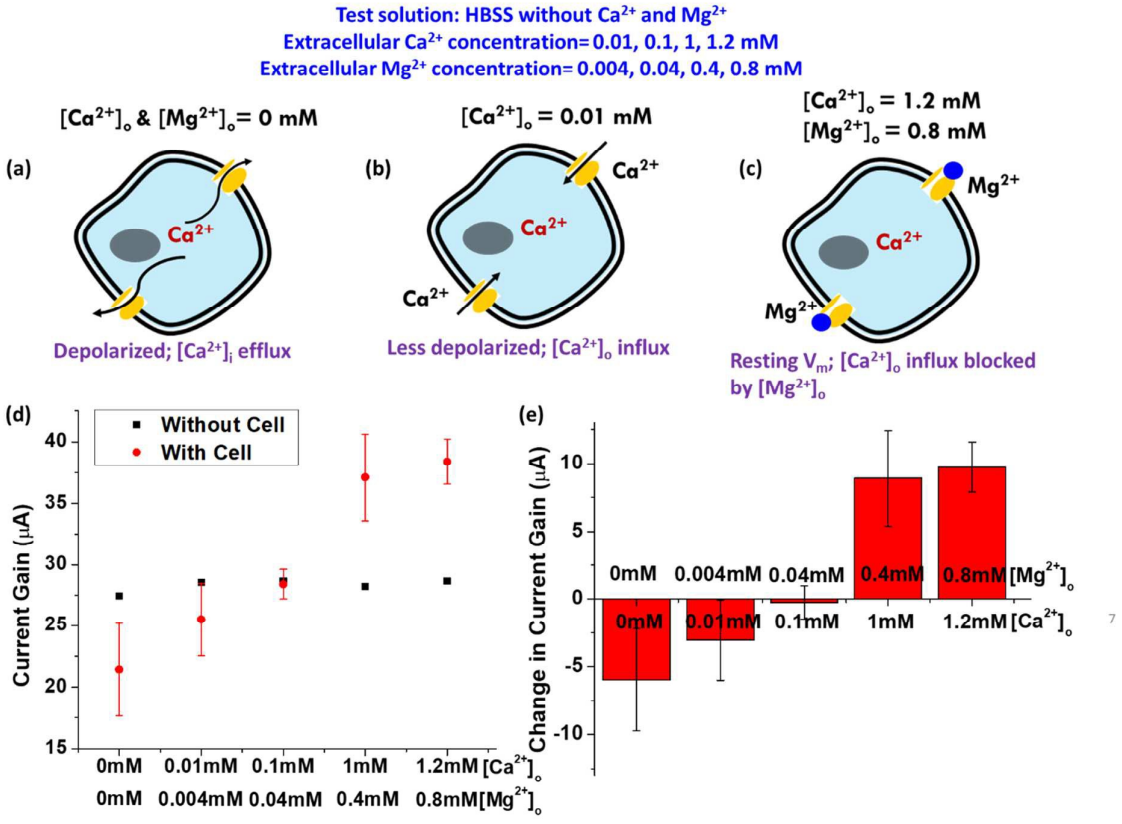

Figure 5. Dynamic sensor response to continuously changing divalent cation concentrations. (a) through (c) schematically represent the cell responses without Ca^{2+} and Mg^{2+}, low concentration (10μM) Ca^{2+} and physiological concentrations of Ca^{2+} (1.2mM) and Mg^{2+} (0.8mM), respectively. (d) Current gain vs. external Ca^{2+} and Mg^{2+}

View Article Online
DOI: 10.1039/C7LC01305A

concentration of the sensor, with and without cell. (e) Sensor response (change in current gain with respect to aptamer baseline) to external Ca^{2+} and Mg^{2+} concentration.

As described in Figure 3, the presence and absence of divalent cations changes the membrane potential and sensor signal accordingly. In Figure 4, we have verified that EDL FET biosensor does indeed follow the changes in transmembrane potential, by causing step depolarizations using well known method of extracellular stimulus. It was interesting to investigate the effects of varying concentrations of divalent cations or in other words, dynamically monitor the sensor response with continuous change of external stimulus. We designed an experiment to steadily increase $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ from extremely low concentrations ($[Ca^{2+}]_o$=0 mM; $[Mg^{2+}]_o$=0 mM) to the physiological concentrations ($[Ca^{2+}]_o$=1.2 mM; $[Mg^{2+}]_o$=0.8 mM) and record the sensor response dynamically. Figure 5 describes the sensor response to continuously changing divalent cation concentrations. Figures 5 (a) through (c) schematically depict the cellular responses to changing divalent cation concentrations. As observed from Figure 5 (d), the aptamer baseline does not change significantly when the divalent cations' concentrations are increased. This is because the change in overall ionic concentration of the test solution is negligible and hence the current gain of the sensor remains steady. When CTCs suspended in HBSS without Ca^{2+} or Mg^{2+}, are allowed to be captured on the sensor, the current gain decreases with respect to aptamer baseline (shown in Figure 5 (d)), as previously seen in Figure 2. The $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ concentrations are increased in steps

View Article Online
DOI: 10.1039/C7LC01305A

and after 5 minutes sensor signal is recorded. As seen in Figure 5 (d), the current gain keeps increasing with respect to the signal at zero concentration of Ca^{2+} and Mg^{2+}. Figure 5 (e) shows the change in current gain with respect to aptamer baseline, as a function of changing concentrations of divalent cations. The current gain decreases in zero concentration of divalent cations, indicating depolarized membrane potentials and keeps increasing with further addition of divalent cations, which represent repolarization of cell. This dynamic sensor response to continuously changing ion concentrations indicates that EDL FET biosensor can be used to monitor the effect of ions or biomolecules on the transmembrane potential or cell membrane capacitance. Investigating these capacitive changes in cell as a response to ion or biomolecular interaction can have profound implications in biomedical engineering.

View Article Online
DOI: 10.1039/C7LC01305A

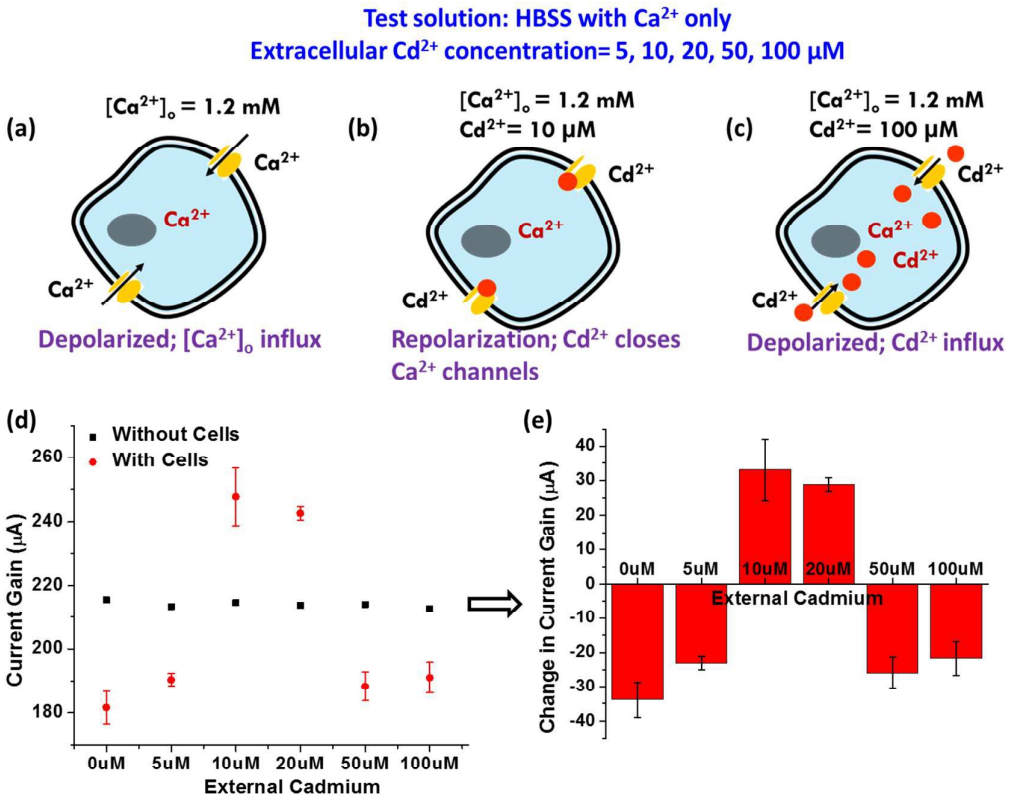

Figure 6. Cadmium block of calcium channels. (a) through (c) schematically represent the cell responses with only Ca^{2+} buffer, low concentration (10µM) Cd^{2+} and higher concentration (100µM) Cd^{2+}, respectively. (d) Current gain vs. external cadmium concentration of the sensor, with and without cell. (e) Sensor response (change in current gain with respect to aptamer baseline) to external cadmium.

One of the methods to study the effects of ion interaction with cell is to use a well-known channel blocker to close ion channels present on the cell membrane, which would result in a shift in transmembrane potential of cell. Cadmium ions are well-known calcium channel blockers (31). Divalent cations can behave as calcium antagonizer, which is the reason why $[Mg^{2+}]_o$ can close calcium channels and maintain physiological $[Ca^{2+}]_i$ concentrations (25). The outer radius of Ca^{2+} and Cd^{2+} ions are similar and hence Cd^{2+} ions can also block calcium channels present on the cell membrane (32). Cd^{2+} is known to

View Article Online
DOI: 10.1039/C7LC01305A

be a permeant channel blocker sensitive to the changes in membrane potential (31). This means that Cd^{2+} can not only close the calcium channels but also enter into the intracellular space and compete with $[Ca^{2+}]_i$ in the cytoplasm (32). Since Cd^{2+} block of calcium channels are known to be more effective in depolarized membrane potentials, we first suspended CTCs in HBSS containing only Ca^{2+} and allowed CTCs to be captured on the sensor and sensor signal was recorded. Figures 6 (a) through (c) schematically depict the cellular responses to changing external cadmium concentrations. Figure 6 (d) shows the decreased current gain with respect to aptamer baseline, when CTCs are suspended in Ca^{2+} only solution. Cd^{2+} concentrations in the extracellular medium was increased in micromolar concentrations, from 5 μM to 100 μM and sensor signal was recorded. Figure 6 (e) shows the dynamic sensor response as a function of continuously changing concentrations of extracellular Cd^{2+}. Current gain increased with respect to zero concentrations of Cd^{2+} until 20 μM Cd^{2+}. This sensor response agrees with the physiological phenomenon of channel blocking by Cd^{2+}, as low micromolar concentrations of extracellular Cd^{2+} have been previously known to block calcium channels (33). Thus, the membrane potential which was depolarized due to open calcium channels (decreasing current gain values in Figure 6), steadily progresses towards more negative membrane potentials or in other words achieves repolarization due to the closing of calcium channels (increasing current gain values in Figure 6). When the Cd^{2+}

View Article Online
DOI: 10.1039/C7LC01305A

concentration is further increased from 20 μM to 100 μM, the current gain decreases. This phenomenon can be attributed to the property of Cd^{2+} being a permeant channel blocker and higher concentrations of Cd^{2+} have been previously reported to increase intracellular concentration of Cd^{2+} (32, 34). This is also the pathway of Cd^{2+} induced cytotoxicity. The permeant Cd^{2+} ions increase basal $[Ca^{2+}]_i$ which further depolarizes the cell, and hence the current gain of the sensor decreases, as shown in Figure 6. Test results depicted in Figure 6 demonstrate that EDL FET biosensor can offer a highly sensitive platform to study the bioelectric signature of cell, focusing on ion channel investigating and cellular response to drugs. The study of ion channels is a constantly evolving field of cell biology and our EDL FET sensor may be used to study the functioning of ion channels. Since we have demonstrated that we can capture close biomolecular interactions, EDL FET biosensor may find applications in drug discovery and development as well.

Conclusion

We have designed, fabricated and characterized an EDL FET biosensor for the detection and enumeration of CTCs and the investigation of bioelectric signals of captured cells. In this research, we have used EDL FET biosensor platform to monitor transmembrane potential changes of the cell and apply the methodology to study the functioning of ion channels. High electric field applied at the gate electrode creates EDL in the test solution

View Article Online
DOI: 10.1039/C7LC01305A

which modulates the transistor drain current. Since our FET biosensor is EDL gated, it offers very high sensitivity, with single cell resolution, in physiological salt concentrations without any sample pre-treatments. The sensor signal changes as the number of CTCs captured on the gate electrode area changes. This change in sensor response is different in different external test media. The investigation of cellular response and hence sensor response in different test environments, elucidated that sensor response changes as the transmembrane potential of cell changes. This has been confirmed by causing step depolarizations using well known method and recording the corresponding sensor response. The electrical response of sensor is dynamically monitored by continuously changing divalent cation concentrations and the effect of cadmium as a calcium channel blocker is investigated as well. Our cell based EDL FET biosensor has bright prospects in cell based diagnostic platforms, the study of fundamental cell biology and drug discovery and development.

Acknowledgements

This work was partially supported by research grants from Ministry of Science and Technology (MOST 104-2221-E-007-142-MY3, MOST 106-2221-E-007-001), National Health Research Institutes (NHRI-EX104-10428EI) and National Tsing Hua University (104N2047E1). We thank the technical support from National Nano Device Laboratories

View Article Online
DOI: 10.1039/C7LC01305A

(NDL) in Hsinchu and the Centre for Nanotechnology, Material Science and Microsystems (CNMM) at National Tsing Hua University.

References

1. S. Varma, A. Fendyur, A. Box and J. Voldman, Analytical Chemistry, 2017, 89, 4663-4670.

2. D. W. Domaille, E. L. Que and C. J. Chang, Nat Chem Biol, 2008, 4, 168-175.

3. K. Kikuchi, H. Takakusa and T. Nagano, TrAC Trends in Analytical Chemistry, 2004, 23, 407-415.

4. B. Hong and Y. Zu, Theranostics, 2013, 3, 377-394.

5. N. S. Barteneva, E. Fasler-Kan and I. A. Vorobjev, Journal of Histochemistry and Cytochemistry, 2012, 60, 723-733.

6. P. N. Abadian, C. P. Kelley and E. D. Goluch, Analytical Chemistry, 2014, 86, 2799-2812.

7. Y. Fang, A. M. Ferrie, N. H. Fontaine, J. Mauro and J. Balakrishnan, Biophysical Journal, 2006, 91, 1925-1940.

8. C.-C. Chen, C. Cang, S. Fenske, E. Butz, Y.-K. Chao, M. Biel, D. Ren, C. Wahl-Schott and C. Grimm, Nat. Protocols, 2017, 12, 1639-1658.

View Article Online
DOI: 10.1039/C7LC01305A

9. P. J. White, B. Biskup, J. T. M. Elzenga, U. Homann, G. Thiel, F. Wissing and F. J. M. Maathuis, Journal of Experimental Botany, 1999, 50, 1037-1054.

10. E. Fluhler, V. G. Burnham and L. M. Loew, Biochemistry, 1985, 24, 5749-5755.

11. Peter B. Kruskal, Z. Jiang, T. Gao and Charles M. Lieber, Neuron, 86, 21-24.

12. S. Tsuda, in Encyclopedia of Nanotechnology, ed. B. Bhushan, Springer Netherlands, Dordrecht, 2014, DOI: 10.1007/978-94-007-6178-0_136-2, pp. 1-6.

13. Y. Zhang, J. Clausmeyer, B. Babakinejad, A. López Córdoba, T. Ali, A. Shevchuk, Y. Takahashi, P. Novak, C. Edwards, M. Lab, S. Gopal, C. Chiappini, U. Anand, L. Magnani, R. C. Coombes, J. Gorelik, T. Matsue, W. Schuhmann, D. Klenerman, E. V. Sviderskaya and Y. Korchev, ACS Nano, 2016, 10, 3214-3221.

14. T. Sakata and Y. Matsuse, Genes to Cells, 2017, 22, 203-209.

15. M. Kaisti, Biosensors and Bioelectronics, 2017, 98, 437-448.

16. C.-H. Chu, I. Sarangadharan, A. Regmi, Y.-W. Chen, C.-P. Hsu, W.-H. Chang, G.-Y. Lee, J.-I. Chyi, C.-C. Chen, S.-C. Shiesh, G.-B. Lee and Y.-L. Wang, Scientific Reports, 2017, 7, 5256.

17. G. Palazzo, D. De Tullio, M. Magliulo, A. Mallardi, F. Intranuovo, M. Y. Mulla, P. Favia, I. Vikholm-Lundin and L. Torsi, Advanced Materials, 2015, 27, 911-916.

View Article Online
DOI: 10.1039/C7LC01305A

18. J. Rivnay, R. M. Owens and G. G. Malliaras, Chemistry of Materials, 2014, 26, 679-685.

19. C.-P. Hsu, P.-C. Chen, A. K. Pulikkathodi, Y.-H. Hsiao, C.-C. Chen and Y.-L. Wang, ECS Journal of Solid State Science and Technology, 2017, 6, Q63-Q67.

20. L.-Y. Hung, C.-H. Wang, Y.-J. Che, C.-Y. Fu, H.-Y. Chang, K. Wang and G.-B. Lee, 2015, 5, 10326.

21. C. D. Cone, Annals of the New York Academy of Sciences, 1974, 238, 420-435.

22. A. Zhang, T. P. O. Cheng and B. M. Altura, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1992, 1134, 25-29.

23. F. C. Mooren, M. Moreno Geada, J. Singh, R. Stoll, W. Beil and W. Domschke, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1997, 1358, 279-288.

24. M. T. González-Martínez, Journal of Biological Chemistry, 2003, 278, 36304-36310.

25. T. Fazekas, B. J. Scherlag, M. Vos, H. J. J. Wellens and R. Lazzara, Clinical Cardiology, 1993, 16, 768-774.

View Article Online
DOI: 10.1039/C7LC01305A

26. X.-B. He, S.-H. Yi, Y.-H. Rhee, H. Kim, Y.-M. Han, S.-H. Lee, H. Lee, C.-H. Park, Y.-S. Lee, E. Richardson, B.-W. Kim and S.-H. Lee, STEM CELLS, 2011, 29, 1861-1873.

27. E. S. Levitan, R. Gealy, J. S. Trimmer and K. Takimoto, Journal of Biological Chemistry, 1995, 270, 6036-6041.

28. M. Yang and W. J. Brackenbury, Frontiers in Physiology, 2013, 4, 185.

29. S. Sundelacruz, M. Levin and D. L. Kaplan, Stem Cell Reviews and Reports, 2009, 5, 231-246.

30. J. Cervera, S. Meseguer and S. Mafe, 2016, 6, 35201.

31. The Journal of General Physiology, 1991, 98, 751-770.

32. M. Li, T. Kondo, Q.-L. Zhao, F.-J. Li, K. Tanabe, Y. Arai, Z.-C. Zhou and M. Kasuya, Journal of Biological Chemistry, 2000, 275, 39702-39709.

33. D. Swandulla and C. M. Armstrong, Proceedings of the National Academy of Sciences of the United States of America, 1989, 86, 1736-1740.

34. C. Marchetti, ISRN Toxicology, 2013, 2013, 184360.