A Comprehensive Model for Whole Cell Sensing and Transmembrane Potential Measurement Using FET Biosensors

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In this research, the development of a highly sensitive FET based biosensor technology for whole cell sensing and cellular transmembrane potential measurement is discussed and a quantitative model is proposed to elucidate the sensor mechanism. Electrical double layer (EDL) gated FET biosensor platform offers high sensitivity target analyte detection, even in high ionic strength solutions, thereby eliminating the need for complex sample pre-processing methods. Using this sensor platform, we have developed a whole cell sensing technology that can detect and count cells, and monitor cellular bioelectric signals such as transmembrane potential changes. A quantitative model is developed to explain the sensor mechanism and theoretical prediction shows good agreement with experimental results. The changes in the cellular transmembrane potential upon extracellular stimulus is modeled based on the EDL FET sensor response. Our investigation reveals that EDL FET biosensor platform can be used to study the bioelectric signals of cells, dynamically which is highly relevant to cell biology in applications of drug development, ion channel studies and disease model establishment. This sensor technology can also be used for point of care diagnostics, in the diagnosis and prognosis of diseases such as cancer.

Since the discovery of cells, the basic building blocks of life, they have remained as crucial links to unraveling the pathophysiology of disease and understanding the complex, fundamental processes that sustain life. Several technologies are employed for the identification and the study of fundamental cell biology. Optical technologies are commonly used for most types of cell based experiments. Flow cytometry is the traditional choice where cells are flown in through a channel and optical microscopy is used to image, analyze and characterize cells.12 Often fluorescent probes are used to image specific molecules within the cell to either identify the type of cell or track particular cellular functions.13,14 Newer technologies such as surface plasmon resonance (SPR) and resonant waveguide grating (RWG) have been well established for cellular investigation.15,16 However, these technologies require sophisticated instrument and trained laboratory staff to perform the experiments, which significantly increases the associated cost and inconvenience. Also, long turnaround times, photobleaching of fluorescent probes and poor signal to noise ratio are some of the issues that need to be addressed to improve the efficiency and reliability of the optical technologies.

Recently, several micro/nano technology based cellular diagnostic and analytical platforms have been developed to overcome the limitations of traditional optical based technologies.1–11 Among them, electronic micro/nano sensors are particularly attractive owing to their high sensitivity response, miniaturized design, cost-effectiveness and speedy response. Field effect transistors (FETs) are surface affinity type sensors that can amplify the biological interactions into electrical signals, due to their high transconductance gain.12 FET based biosensors have been used for a variety of bio applications.12–15 However, in high ionic strength solutions with physiological salt concentration (such as 1X PBS), the Debye screening effect hinders the sensor from detecting the bio-recognition process.16–18 Previously, FET sensors were used in bio applications by diluting the test medium to increase the Debye length and facilitate detection of target analytes. However, the structure and functions of biomolecules are specifically dependent on their interaction with solvent molecules which is governed by the surrounding ionic strength of the medium.

To overcome this limitation, we have developed a unique sensing methodology for FET based biosensors to directly detect target analytes in high ionic strength solution, without any sample pretreatments, to facilitate biomolecule recognition in their native state.19 During sensor operation, electrical double layer (EDL) is created in the high ionic strength solution which modulates the transistor channel conductivity. In this research, we have systematically investigated the mechanism of whole cell sensing using the EDL FET biosensor and developed quantitative model of the enhanced sensitivity in physiological salt environment. In this study, circulating tumor cells (CTCs) of colorectal cancer (CRC) are used as the model cell line. CTCs are important diagnostic and prognostic biomarkers of metastatic cancer.20,21 We demonstrate that the sensitivity of CTCs detection using EDL FET biosensor is dependent on the external electric field applied, which modulates the channel conductivity. The predicted model describes a critical field required for enhanced sensitivity in high ionic strength medium. Detailed investigation of CTCs capture and detection reveals that the high sensitivity is extended at least 10 times more than the Debye length in 1X PBS. Moreover, EDL FET biosensor is shown to be responsive to the transmembrane potential changes of even single cell and the sensor model is used to describe how the capacitive changes across the cell membrane and the test solution affects the overall sensor response. Through this study we evaluate the micro environment of the sensor-biomolecule interface which can be applied to the study of fundamental cell biology and cell based diagnostic technologies.

Materials and Methods

**AlGa**N/GaN HEMT fabrication.—Figure 1a demonstrates the cross-sectional view of our AlGa**N/GaN HEMT structure constituting 1 mm thick silicon substrate on which the GaN buffer layer is grown with 260 Å AlGaN and 10 Å GaN cap layer using Metal-organic Chemical Vapor Deposition (MOCVD). Transistor active area was defined by performing Inductively Coupled Plasma (ICP) etch using BC**3/C**2 gas mixture. ohmic contacts comprising Ti/Al/Ni/Au

Received January 2, 2018. Published February 7, 2018. This paper is part of the JSS Focus Issue on Semiconductor-Based Sensors for Application to Vapors, Chemicals, Biological Species, and Medical Diagnosis.
Figure 1. (a) Schematic diagram of AlGaN/GaN HEMT whole cell sensor (b) Top view image of HEMT sensor with CTCs captured at the sensing region (c) Schematic illustration of GaN HEMT biosensor array.

(200/800/400/1000 Å) were deposited using Electron beam (E-Beam) evaporation, followed by rapid thermal annealing (RTA) at 850 °C in N₂ environment. The final step is to deposit metal interconnects comprising Ti/Au (200/2000 Å) using E-Beam.

Biosensor array fabrication.— A simple two-step HEMT biosensor packaging methodology was discussed in detail, in one of our previous works. To explain in brief, 1 mm² GaN HEMT devices are embedded in epoxy resin and cured thermally at high temperatures of 125 and 165 °C, for 1 and 1.5 hours, respectively. These devices are arranged in parallel to form an array of sensors in the chip. The entire chip is passivated and using photolithography openings are made for metal interconnects. Using E-Beam evaporator, metal interconnects are deposited. After metal lift-off, the chip is passivated again and using photolithography, the sensing regions are opened with an area of 10 × 60 μm² separated by a gap distance of 65 μm² which is defined as the sensing region of our sensor. These are depicted in Figures 1a, 1b showing the schematic and real images of AlGaN/GaN HEMT, respectively. The schematic representation of our packaged biosensor chip is as shown in Figure 1c.

Cell line and reagents used.— HCT-8 CRC cells that were cultured in RPMI-1640 medium (Invitrogen Co., USA) were used for this study. Cells were suspended in 1X-Phosphate Buffered Saline (PBS) or 1X-Hanks Balanced Salt Solution (HBSS) with calcium and magnesium with proper concentrations, while performing electrical measurements. After the test, near neutral pH protein elution buffer or Tris-EDTA buffer could be used to regenerate the sensors in the array by washing away the captured cells.

CTCs specific aptamer immobilization.— Previously, HCT-8 cell-specific aptamer (72 base pairs) have been screened by Hung et al with a sequence as follows: 5'- TACAGCACCACG-ACCATGTTTGGTTTTTTTGTGCTCCTGATGTTGGCGTGTGTGGTCTTCTGCC - 3'. In order to bind to the gold gate electrode, the aptamer was thiolated at one end. Then, the aptamer was immobilized to the gold surface by reducing the disulfide bonds and creating free thiols using TCEP (tris(2-carboxyethyl)phosphine), during an incubation period of 24 hours at room temperature. When the incubation period was complete, the excessive aptamer was washed away in 1X PBS.

Sensor measurements.—Agilent B1530/B1500A semiconductor parameter analyzer is used to measure and record the source drain characteristics. Unless otherwise mentioned, the transistor is operated at constant source-drain bias of 2.5 V with a pulsed gate bias of 2 V. The gate pulse is generated by turning on the drain bias and holding the gate bias at 0 V for 2 μs, and then maintaining at 2 V for 50 μs, after which gate and drain biases are turned off. Current gain of our sensor can be defined as the change in transistor drain current before and after applying the gate bias.

Results and Discussion

Figure 1a depicts the schematic of our HEMT structure. Using photolithography, openings of 10 × 60 μm² are made on the gate electrode and channel regions, respectively, as shown in Figure 1b. The gate electrode and channel openings are separated by a short gap of 65 μm. When the test solution is dropped on the sensor surface, it connects the openings on the gate electrode and channel, by forming a sensing region that simulates a two-plate capacitor: the openings on the gate electrode and channel acting like two metal plates (by ignoring the voltage drop in the highly conductive channel) and the test solution acting as the dielectric in between. Figure 1c shows the schematic illustration of the packaged sensor array capturing cells present in the flow channel. The sensor array fabrication method is described in detail in our previous studies. Sensor array can significantly improve the throughput of measurement by providing multiple target binding sites on a single chip. The CTCs specific aptamer was selectively immobilized on the gate electrode opening and CTCs introduced into the flow channel were allowed to be captured by the CTCs-specific
aptamer. The cell containing test solution was incubated in the flow channel for 5~10 minutes, by occasionally gently stirring with pipette to increase the cell to aptamer collision frequency. Following this, the unbound cells were gently washed away. The sensors with bound cells were then measured electrically and compared with respect to the aptamer reading as baseline, to determine the number of cells captured, electrically. Cell capture was also recorded through optical microscopy for confirmation.

The mechanism of detection and the enhanced sensitivity in high ionic strength is investigated in here. In our sensing structure, gate bias is applied to the gate electrode which separated from the transistor channel. This applied potential forms electrical double layer (EDL) in the test solution which modulates the channel conductivity. The purpose of the separated gate electrode is not merely to function as a reference electrode but to modulate the potential drop across the transistor dielectric. In fact, our sensor is a high field gated EDL FET biosensor. The difference between traditional FET sensor and our unique sensing structure which leads to increased sensitivity in high ionic strength is detailed in this section. Firstly, the electric field strength applied to the transistor is varied by applying different gate bias $V_g$ and altering the gap between the gate electrode and transistor channel. Figure 2 shows the change in sensor signal for different electric field strength. The current gain of the sensor is denoted as the signal and not the absolute drain current. Current gain or $\Delta I_D$ is the change in drain current before and after applying gate voltage. This is a more stable index compared to the absolute drain current. Figure 2a shows the structure of the sensor adopted to perform the varying gap experiment. The in-plane gate electrode on the sensor surface is not used to apply gate bias. Instead, a top gate electrode with a $10 \times 60 \ \mu m^2$ opening is positioned vertically above the transistor opening, to form a vertical, parallel plate capacitor. The distance between the top gate electrode is controlled and varied using PDMS spacers of different thickness such that the gaps between the gate electrode and the channel are 30, 330, 930, 1530, 2430, 3480, 4980 $\mu m$. Figure 2b shows the change in current with increasing gap and increasing $V_g$. When $V_g$ is increased the current gain increases, as the potential across the transistor dielectric is increased. Similarly, when the gap between gate electrode and channel is increased, current gain decreases. In other words, when the electric field strength increases, current gain increases and vice versa. However, the change in current gain saturates after a certain gap and does not vary considerably when gap is further increased. More intuitively, as seen in Figure 2c, we can say that the slope or sensitivity does not change significantly after a saturation point. Therefore, we can define two regions of device operation: linear and saturation regions or high field and low field operation. In linear/high field operation, the gap between gate electrode and channel is sufficiently small such that the applied field can modulate the channel conductivity. In other words, the change in current gain is linearly dependent on the gap. But in saturation/low field operation, the applied field no longer modulates the channel conductivity or it assumes a low and constant value which is not modulated by further changing the gap. A traditional FET sensor operates in the saturation/low field region, because the reference electrode is either grounded or maintained at very large (arbitrarily large), uncontrolled distance away from the transistor channel. Thus, the potential gradient generated across the test solution, between the reference electrode and the transistor channel cannot influence the conductivity. The gating mechanism is solely dependent on the potential difference caused by receptor-ligand binding at the transistor gate region, which is hindered in high ionic strength solution. On the contrary, in our sensing structure, we employ the linear/high field operation, where the gap between gate electrode and channel is very small such that it generates a large electric field strength or potential gradient across the test solution. This structure may have similarity to that of electrophoresis, which

Figure 2. (a) Varying electric field applied to top gate electrode by varying the gap between gate electrode and channel (b) Gain versus gap between gate electrode and channel (c) Gain versus applied $V_g$. 

aptamer. The cell containing test solution was incubated in the flow channel for 5~10 minutes, by occasionally gently stirring with pipette to increase the cell to aptamer collision frequency. Following this, the unbound cells were gently washed away. The sensors with bound cells were then measured electrically and compared with respect to the aptamer reading as baseline, to determine the number of cells captured, electrically. Cell capture was also recorded through optical microscopy for confirmation.

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uses electric field gradient to drive biomolecules between two metal electrodes. In electrophoresis, the applied electric field strength is at least 1 V/cm. In our typical sensing structure, gate bias is applied at 2 V over a gap of 65 μm which generates an electric field strength much greater than the minimum field applied in electrophoresis. If 1 V/cm is considered as the critical field strength, our sensor operates in the linear region, where the applied high field can modulate the channel conductivity. From Figures 2b and 2c, we can see that the linear region extends till 2500 μm after which the current gain gets saturated. Therefore, the critical field strength in this device structure occurs around the gap of 2500 μm.

To confirm that sensitivity is indeed enhanced by the high field modulation, we performed CTCs detection under 3 different field strengths, by varying the gap between the gate electrode and channel. The gaps were: 65, 1000 and 3000 μm. As seen from Figures 3a and 3b, when aptamer is immobilized directly on the gate electrode and far away from the gate electrode opening, the sensitivity of CTCs detection decreases. Since the saturation point is around 2500 μm, it is not surprising that even though CTCs are captured at the gate electrode opening by the aptamer, there is no significant change in sensor signal at 3000 μm, where the electric field is less than the critical field. In fact, traditional FET biosensors work with field strength lesser than the critical field and hence, they face poor sensitivity in high ionic strength solution and charge screening effect. Figure 3 also demonstrates that our sensor platform can be used to count the number of cells captured on the sensing region. When biosensor array is used, enumeration of rare cell types such as CTCs can be performed with high throughput, in a rapid and simple manner without extensive and complicated automation.

We have established that high field modulation of the EDL FET biosensor does enhance the sensitivity in highionic strength. But the exact physical phenomenon that allows detection of biomolecules and complex membrane bound structures like cells, beyond the Debye length in high salt concentration is unclear. In our sensor, the aptamer immobilized on the gate electrode captures the CTCs and the current gain of the sensor changes. One can imagine that this may be due to the strong electrostatic interaction of the aptamer and cell membrane bound ligand, which not only pulls the cells very close toward the sensor surface, but also creates charge re-distribution near the EDL region. If so, the proximity of captured cells to the sensing region must be sufficient to cause charge re-distribution in the local region. We performed experiments to verify the proximity effect. Figure 4 shows the test results where CTCs were captured by the aptamer which was immobilized directly on the gate electrode and far away from the gate electrode opening. The typical aptamer immobilization process is detailed in the methods section and is followed for experiment shown in Figures 4a and 4b. However, to increase the distance at which CTCs are captured, from the sensor surface, as shown in Figure 4c, we added a poly-dT sequence with 20 bases to the 3′ end (previously thiol modified) of the CTCs aptamer. Thiol modification was carried out at the free end of the poly-dT sequence and before immobilization on the gate electrode, the sensitivity is significantly reduced, as shown in Figures 4c and 4d. The results in Figures 4b...
and 4d demonstrate that the captured cell need to be at very close proximity to the local region of the EDL, to exert sufficiently strong electrostatic interaction in the EDL neighborhood, for optimal sensitivity. They also depict that our EDL FET biosensor platform can detect biomolecules in physiological salt concentration, for at least one order higher than the Debye length. Moreover, when CTCs are allowed to stay afloat on the gate electrode opening without aptamer to capture them (as illustrated in Figure 4e, the sensor signal does not change appreciably, as seen in Figure 4f. This further explains that when aptamer is present at the sensor surface, it binds with the specific ligand on the cell membrane, while pulling the cells closer to vicinity of the EDL on the gate electrode. Without aptamer, cells may still occupy the gate electrode opening (as is seen from optical images) by staying afloat in the region, but do not contribute to appreciable sensor response. This is because the applied electric field can circumvent the cells that are afloat in the region and drop at the transistor dielectric on the other end. In other words, the cells that are afloat on the gate electrode without the strong interaction of aptamer binding, do not cause sufficiently strong electrostatic interaction in the local region of EDL, to yield sensor response. This phenomenon is useful in whole blood diagnostics using EDL FET biosensor, where the blood cells that do not possess tight intercellular junctions, do not yield significant sensor signal and thus, do not cause non-specific signaling. The results in Figure 4 are quite interesting for biomedical applications, because they imply that only cells captured via strong electrostatic binding (or strong molecular recognition forces) can provide sufficient sensor response. This indicates that our EDL FET biosensor can differentiate between target cells and non-specific cells and this may prove beneficial for the detection of rare cells such as CTCs or pathogens directly from whole blood. The combined effects of high field modulation and proximity (leading to strong electrostatic interaction), confirmed with results in Figure 4, demonstrate the mechanism of biomolecular detection beyond Debye length in physiological salt concentration, using our EDL FET biosensor platform.

In here, a quantitative model for cell sensing and signal transduction is proposed. When we apply pulsed bias $V_g$ to the gate electrode, the voltage drops across the test solution and the transistor dielectric. Therefore, the total voltage drop in the sensor can be envisioned as in Equation 1:

$$\Delta V = \Delta V_s + \Delta V_d$$  

[1]

Where $V_s$ and $V_d$ are the potential drops across the test solution and the transistor dielectric. The change in $V_d$ eventually modulates the transistor drain current. When $V_d$ is higher, the drain current will be lower and vice versa. From the traditional FET model equation for linear transistor operation, the transconductance gain $g_m$ is denoted as follows.

$$g_m = \frac{\Delta I_D}{\Delta V_g} = \frac{W}{L} \mu C_d V_d$$  

[2]

Where $I_D$ and $V_D$ are the drain current and voltage, respectively. $W$ and $L$ are the width and length of the active device area, respectively, $\mu$ is the carrier mobility and $C_d$ is the transistor dielectric capacitance. Figure 5a depicts the ionic charge distribution in the EDL FET sensor, in 1X PBS, without cell capture. When equation 2 is applied to our sensor structure, $\Delta V_d$ is the effective change in applied voltage that modulates the transistor drain current. From equation 1, we can see that the applied gate bias $V_g$ influences $V_s$ and $V_d$, which eventually modulates channel conductivity. Thus, it is reasonable to assume that at any fixed condition of test solution and applied gate bias, the drain current is dependent on the potential drop across the transistor dielectric, $\Delta V_d$. Therefore, we can rewrite equation 2 as follows.

$$g_m = \frac{\Delta I_D}{\Delta V_g} = \frac{W}{L} \mu C_d V_d$$  

[3]

At constant $V_D$ and $V_g$ varied from 0 to 2 V. Since $\Delta V_d$ governs our sensor response, we can analyze it in terms of the capacitance change in the sensing structure. When $V_g$ is applied to the gate electrode, EDL is formed on the gate electrode-solution and transistor channel-solution interfaces, which sets up a solution capacitance $C_s$. If the transistor dielectric has a fixed capacitance $C_d$, we can model the potential drop across the dielectric for applied $V_g$ in terms of the
capacitances across the solution and transistor dielectric. If the capacitances are in series connection, $\Delta V_d$ can be denoted according to equation 1, as follows.

$$\Delta V_d = \frac{1}{C_s + C_d} \times V_g$$

When cell is captured on the gate electrode, as shown in Figure 5b, it contributes to a series addition of another capacitance $C_{cell}$, as the phospholipid bilayer structure of the cell membrane is often modeled as a capacitor. Thus, equation 4 can be modified to represent the influence of the captured cell on $\Delta V_d$ as follows.

$$\Delta V_d = \frac{1}{C_s + C_{cell} + C_d} \times V_g$$

Thus, by combining equations 3 and 5, we can see that the current gain of the sensor is dependent on $C_s$ and $C_{cell}$. When the ion concentration of the test solution changes considerably, $C_s$ also changes. This is observed in Figures 5c and 5d. When KCl is increased from 5 mM to...
30 mM, the current gain increases. This means that $C_2$ is a function of the changing ion concentration, in this case $K^+$. So we can denote $C_2$ as a function of the ion concentration as follows.

$$C_S = C_2 e^{α[K^+]}$$

[6]

Where $C_2C_3$ is the initial solution capacitance at physiological conditions and $α$ is a constant of non-linearity. Figure 5c shows the fitted results using equation 6, which describes the change in $C_2$ as a function of extracellular $K^+$ concentration.

When CTCs are captured by the aptamer, the current gain increases compared to the aptamer baseline at extracellular $K^+$ concentration denoted as $[K^+]_0 = 5$ mM. This condition simulates the physiological condition of cell suspended media. The electrical measurement results are depicted in Figure 5d. In this state, the cell is in its resting transmembrane potential $V_m$. However, when $[K^+]_0$ is increased further by steps of 10, 20 and 30 mM, the cell is depolarized or $V_m$ becomes less negative. Extracellular KCl induced depolarization is a widely used method to calibrate membrane potential measurement techniques. When $V_m$ is less negative or cell is depolarized, the capacitance of cell membrane also changes, as the electric field gradient across the membrane changes. As seen in Figure 5d, the changing $K^+$ concentration influences $C_{cell}$, which is primarily the capacitance across the cell membrane, and current gain decreases with respect to the aptamer baseline at $[K^+]_0 = 10, 20$ and 30 mM. This enables us to use the EDL FET biosensor platform to monitor the dynamic changes in $V_m$. If we follow the curve in Figure 5d, we can see that at a particular $[K^+]_0$, the current gain should be equal to the aptamer baseline at the initial condition. We can define this test condition as $[K^+]_{critical}$. In other words $[K^+]_{critical}$ is the ionic concentration at which the difference of current gain $C_{cell}$ and current gain $C_{aptamer}$ is zero. The value of $[K^+]_{critical}$ is important during dynamic monitoring of changes in $C_{cell}$. Also, from the results in Figures 3 and 4, the parameter $C_{cell}$ is also influenced by the number of cell captured on the sensor surface. Thus, we can model the parameter $C_{cell}$ as follows.

$$C_S = n^C_{cell}e^{-β[K^+]-[K^+]_{critical}}$$

[7]

Where $n$ is the number of cells captured on the sensor and $C_{cell}$ is the cell capacitance parameter at initial physiological conditions. Figure 5d shows the fitted results of change in $C_{cell}$ using equation 7. Thus equations 6 and 7 which are used to model the influence of varying extracellular ion concentrations on the $V_m$ of captured cell and subsequent sensor response, can quantify the capacitances in the sensor structure and using equation 5 in 3, we can quantitatively model the change in current gain of the sensor (signal) as a function of $C_{cell}$ and $C_{cell}$, which is dependent on the number of cells captured and also on the effect of extracellular ion concentrations on the transmembrane capacitance of cell. The change in current gain when CTCs are captured at the gate electrode and extracellular $K^+$ concentration is varied in steps, is demonstrated in Figure 5e. Thus, we can use the EDL FET sensor platform to monitor the changes in $V_m$ dynamically. Our robust sensor technology offers non-invasive and label free means to monitor the cellular bioelectric signature, unlike the traditional methods such as patch-clamp technique and potentiometric probes. This sensor model can elucidate the two applications of our EDL FET biosensor, such as patch-clamp technique and potentiometric probes. This sensor technology offers non-invasive and label free means to monitor the changes in physiological salt environment. Furthermore, a quantitative model is proposed to incorporate the cell sensing mechanism in high ion strength and the mechanism of sensor response as extracellular stimuli changes the transmembrane potential of cells.

Acknowledgments

This work was partially supported by research grants from Ministry of Science and Technology (MOST 104–2221–E–007–142–MY3), National Health Research Institutes (NHRI-EX104-10428EI) and National Tsing Hua University (104–2047E–007–142–MY3). We thank the technical support from National Nano Device Laboratories (NDL) in Hsinchu and the Centre for Nanotechnology, Material Science and Microsystems (CNMM) at National Tsing Hua University.

Summary

In this research, we have developed a quantitative whole cell sensor model for the illustration of enhanced sensitivity in CTCs detection in high ionic strength solution and the mechanism of cellular interaction with the sensor surface via transmembrane potential changes. The enhanced sensitivity of EDL FET biosensor is shown to be dependent on the applied electric field strength. When the sensor is operated in linear/high field region, the sensitivity is significantly increased and on the contrary, in saturation/low field region of operation, the sensitivity is very low. The results depict the advantages of our EDL FET sensing structure as opposed to the traditional FET biosensors. The importance of critical field strength is also emphasized. The physical phenomenon that governs the sensor response during aptamer-cell binding is demonstrated via electrical results. High field modulation and close proximity of captured cell (to influence the local region of EDL) are found to be underlying principles of detection beyond Debye length in physiological salt environment. Furthermore, a quantitative model is proposed to incorporate the cell sensing mechanism in high ion strength and the mechanism of sensor response as extracellular stimuli changes the transmembrane potential of cells.

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