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Application and Development of Biomimetic Solid-State Nanopore in Biosensing Technique

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Abstract. Biomimetic solid-state nanopore is a nano-level technology, which can be effectively used for many detection work, including DNA sequencing. The design of biomimetic solid-state nanopore is inspired by biological ion channels. Biomimetic solid-state nanopore displays many advantages. It can increase the processing characteristics while it can also ensure the performance of similar biological ion channels. It also exhibits controllable surface chemical properties, making biomimetic solid nanopores have more application space. When compared with traditional biosensors, biomimetic solid nanopores have many advantages such as low price, high sensitivity and high specificity. At the same time, biomimetic solid-state nanopores have full help for medicine, analytical chemistry, materials science, single molecule biophysics, single molecule in vitro diagnostics, and genetics. Therefore, in this work, the working mechanisms of several biosensors are introduced. In addition, the applications of nanopores in biosensing are outlined. Besides, the circuit design schemes for improving the efficiency of nanopore has been discussed. In conclusion, the help of nanopores at the single-molecule level is undoubtedly huge, and it could help mankind to understand the nanoscale world.

Keywords: Nanopore; Biosensor; Bionic; DNA Sequencing.

1. Introduction

Nanopores play an important role in living organisms [1-3]. In any organism, the essence of life is an exchange or a transformation to obtain the needed substance. When everything involves conversion, the channel is a necessary thing. In the biological cell, the cell membrane is embedded with many different kinds of biological ion channels. These channels are composed of membrane proteins, which can intelligently regulate the transport of matter. It plays a very important role in maintaining cell ion homeostasis, signal transduction, and energy conversion in various life processes. With the help of these channels, the entire body system can reach a state of equilibrium.

At present, there are two kinds of mainstream nanopores which are biological nanopores and biomimetic solid-state nanopores. The biological nanopores are extremely dependent on the environment and have harsh use conditions, so they are not very versatile. As for the biomimetic solid-state nanopores, they are composed of highly controllable materials with mechanical and chemical stability. Their subtle modification can make effective changes to any conditions. Therefore, this nanopores are more effective. This kind of biomimetic solid-state nanopore is divided into two mainstreams including organic synthesized nanopore and inorganic nanopores. The organic synthesized nanopores include polyethylene terephthalate (PET), polyimide (PI), polycarbonate (PC). These nanopores can be obtained in different shapes by track etching techniques, including cone, hourglass, bullet, and cigar. The other category is inorganic nanopores, such as silicon nitride, alumina film, glass and graphene.

The important application of these nanopores lies in nanopore biosensors. In this work, the working mechanisms of different types of nanopore biosensors are introduced. The application of nanopore biosensors has been discussed including in-situ detection of cells and DNA sequencing. In addition, the design ideas for further improving the performance of nanopore biosensors have been summarized.

2. Working Mechanisms of Biosensors

The detection performance of biosensors is significantly influenced by the design of the measurement circuit. The difference in this measurement mechanism is particularly important for nanopore biosensors.

The most basic principle of all biosensors is to generate and visualize the current [4], which is generally carried out in a dual-stage system. As it is shsown in Fig.1(a), two electrolytic modules fix the solid nanopore film in the middle. The ions in the electrolyte are driven and observed when an electric field is applied. There are generally two types of output in this mode. The first one is the resistance pulse method, as it is shown in Fig.1(b). When a constant voltage is applied, under the action of the electric field, the movement of ions will produce a constant current. If different objects are blocked, the substances block this limited channel. The volume of the analyte will directly affect the content of the ions passing through, thus affecting the final current size. This would result in fluctuations in the results, which include the increase of resistance, the decreases of current. When the analyte leaves, the current will return to its previous normal value. Thus, by analyzing the time of flow, the peak value of the current, and the frequency, various information about the object can be collected. In this wawy, the quantitative and qualitative detection can be achieved [5]. The second approach is the steady-state ion-current method, as it illustrated in Fig.1(c). A probe is fixed with a specific functionalization on the surface of the nanopore to effectively limit the entry of analytes. By limiting the effective pore size, the surface charge affects the change of current. Detection of analytes is obtained by observing the voltage and current of the added and unadded analytes.

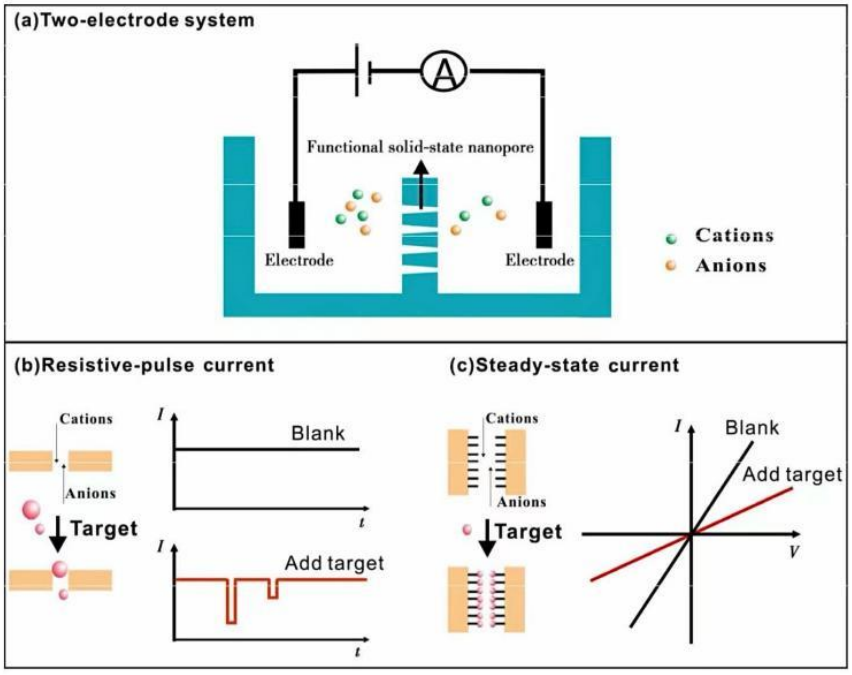

Figure 1. Basic principle of all biosensors: (a) a two-stage system, (b) a visualization of the resistance pulse method, (c) a visualization of a steady-state ion current [4].

3. Application of Nanopore in In-Situ Detection of Cells

Nanopores can be used to achieve in-situ detection of cells. When there is a detection delay and contamination, the detection often cannot obtain the most original state of the cell material. The false signals and results will often be produced. Therefore, the cell in situ detection nanopore is generated, which can monitor the behavior of heterogeneous cells in real time at the single-cell level. Its detection realms include metabolism, signals, and ion movement. The miniature sensor can be directly embedded into the cytoplasm, ensuring the stability through glass or quartz material. This detection method displays very small damage. This nanopore was effectively proposed in 2016, and

Nascimento [1] reported a nanotransplant liquid tube device functionalized using glucose oxidase. It is reported that changes in intracellular pH can be detected in real time using resistance pulse technology, which can then be converted into intracellular glucose levels. This nanosensor was used to quantify single-cell intracellular glucose levels in human fibroblasts and metastatic breast cancer cell lines. The results showed that cancer cells exhibited significantly elevated blood glucose levels compared to non-malignant cells. The cells remained viable before and after the measurement, demonstrating the feasibility of this platform and making the cell-in-situ detection nanopores a diagnostic tool to distinguish whether cancer cells are malignant or not. The redox reaction can also provide rich pathological information. The nicotinamide adenine dinucleotide (NADH) molecule is a key marker in the energy production chain in mitochondria. An increase in NADH levels signals the emergence of a metabolic imbalance. A new type of asymmetric nanopore electrical grade (ANE) was invented by Long Yi et al., which has the advantages of easy modification and size control [2]. ANE can use catechol-modified nanopore electrodes to measure the concentration of NADH in a single cell. It can convert the concentration index into a clear electronic signal, and realize the detection of NADH solution at a concentration as low as 1 ppm. This new type of asymmetric electrode can also be controlled to a diameter of less than 30 nm, so that it can be precisely embedded in the cell for real-time monitoring of intracellular reactions.

However, this type of nanopore sensor embedded in cells also has its drawbacks. That is, it will cause a certain degree of damage to the cell, and there are certain requirements for the detection environment. The complex environment in the cell will have a great impact on the weak pulse signal. This affects the accuracy of the observations. Therefore, a strategy based on functionalized solid-state nanochannels reported by Xia Fan et al. perfectly solves this problem [3]. In-situ detection of cells can be done without the need to embed in cells. This method is used to detect the hydrogen peroxide released by the cells.

4. Application of Nanopore in DNA Sequencing

Nanopore sequencing represents a theoretical platform for DNA sequencing that is fast and inexpensive. Compared to other sequencing technologies, nanopore sequencing increases accuracy by several degrees. A nanopore is a type of tiny pores fixed at both ends of a biofilm, through which an ionic solution moves under the action of an electric field. When a DC voltage is applied between the chambers, it generates a current corresponding to the dissolved ions transmitted through the channels. The local electric field can also attract charged target molecules, such as DNA, through the pores, resulting in an instantaneous change in the measured ionic current. This is also the most important quality of nanopores for DNA sequencing [6]. The key to nanopore technology for single-molecule sequencing is that long DNA molecules are arranged in a single sequence through the nanopores under the action of an external electric field, generating a unique ionic current and recognizing each of the four base types in real time.

Nanopore DNA sequencing is time-consuming. Each base has a characteristic resistance, which ultimately determines the precise base sequence. However, a significant amount of computational work is required to decipher potential sequences from the digital current measurement flow that is distorted by time and noise. Therefore, researchers have discovered effective ways to improve the efficiency of nanopores through extensive work exploration.

4.1. The Direct Injection Readout Circuit (DI)

Fig. 2 shows a basic design of direct injection readout circuit [7]. In the DI circuit, the common gate PMOS device MDI, is used to bias and sense the current of the sensing unit, as it is illustrated in Fig. 2 [6]. The common gate MDI can be controlled by a bias during the integration process. The DI circuit has a simple structure without active power consumption, which makes it suitable for high-density array applications. Therefore, this method is not suitable for reading low-noise array data.

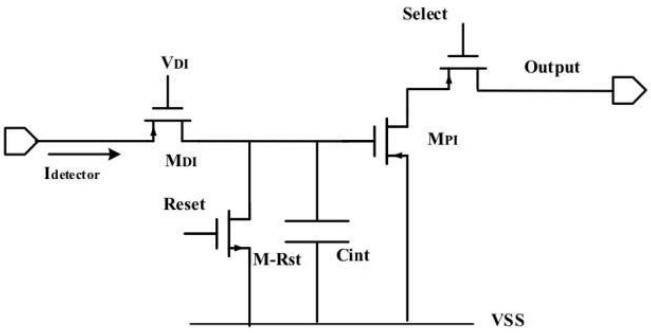

Figure 2. A basic design of direct injection readout circuit [7].

4.2. Switch Current Integration (SCI)

In SCI circuits, the integral capacitance is placed outside the array and shared by the circuit units in the same column, as it is shown in Fig. 3 [8]. The Idetector and row select switch MR-SEL, are the core parts of this detection method. The structure performs the current switching and integration, utilizing shared buffer technology to achieve the gain level of SCI. The cascaded current mirrors M1-M4 act as current buffers before selecting the switch, providing current mode gain determined by the size ratio of M2 and M4.

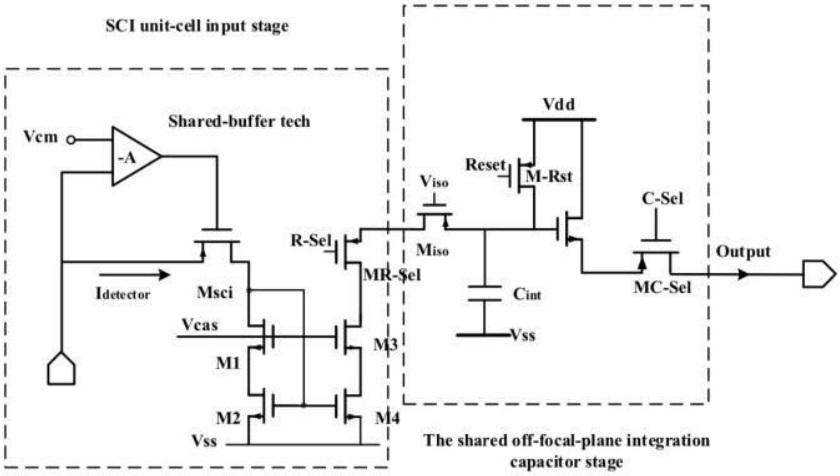

Figure 3. A basic design of switch current integration [8].

4.3. Capacitive Transimpedance Amplifier (CTIA)

It has a better signal-to-noise ratio. Because the signal needs to be integrated to obtain a sufficiently large output swing, it usually has a low bandwidth, making it ideal for nanopore sequencing (which is typically in kHz). In the integrator which is shown in Fig. 4, the integrator capacitor is placed across the op amp as a feedback loop. The reset switch Mn2 controls the switching of the integrating capacitor, and the common-mode voltage Vcm is used as the amplifier input reference voltage. The bias Vcm of the detection unit is also controlled by the imaginary short characteristic of the amplifier. The CTIA can obtain good detection bias control. Due to the effect of the Miller effect on the integrated capacitance, it is possible to make its capacitance very small, resulting in low noise and high sensitivity performance [9].

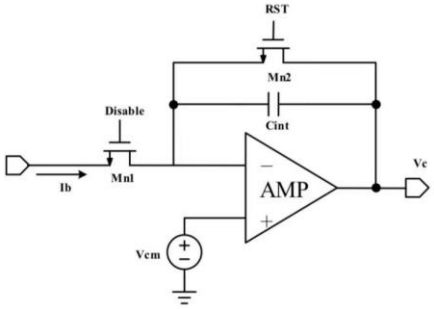

Figure 4. A basic design of capacitive transimpedance amplifier [9].

4.4. Analysis

Comparing the three different circuits, in the circuit DI, the circuit requires a stable, low-noise DC-biased VDI. However, the threshold voltage non-uniformity and high KTC noise are still problems with the DI readout circuit. But DI is the simplest circuit, reducing unnecessary complexity. In the second circuit, the integration time of the SCI readout structure is limited by the processing time of one line, which may result in a decrease in detection sensitivity. The reduced sensitivity can be compensated by increasing the current gain of the current mirror. However, the SCI circuit also has a disadvantage that SCI has a large array structure, which means it cannot meet the high-throughput design requirements of the nanopore DNA detection array. Finally, CTIA circuits require an operational amplifier to charge and discharge capacitors. However, the trade-off effect of the reset clock is coupled to the detection unit node, resulting in a decrease in stability. In the CTIA, it is also necessary to increase the area and power consumption of the reverse gain stage.

5. Conclusion

In conclusion, this work focuses on nanopore biosensors and their applications. The working principles of three types of biosensors are introduced. The application of nanopore biosensors is discussed. Effective circuit design schemes to improve the efficiency of nanopore detection are also discussed in detailed. Improving circuit design to enhance the detection efficiency of nanopore biosensors is a promising research direction in the future. The application of nanopore biosensors in the biomedical field will be even greater in the future.

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