Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2016, Article ID 5284786, 13 pages
http://dx.doi.org/10.1155/2016/5284786

Review Article

Solid-State Nanopore-Based DNA Sequencing Technology

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Received 28 March 2016; Accepted 5 May 2016

Academic Editor: William Yu

The solid-state nanopore-based DNA sequencing technology is becoming more and more attractive for its brand new future in gene detection field. The challenges that need to be addressed are diverse: the effective methods to detect base-specific signatures, the control of the nanopore's size and surface properties, and the modulation of translocation velocity and behavior of the DNA molecules. Among these challenges, the realization of the high-quality nanopores with the help of modern micro/nanofabrication technologies is a crucial one. In this paper, typical technologies applied in the field of solid-state nanopore-based DNA sequencing have been reviewed.

1. Introduction

Since the chain-termination method, which was proposed by Sanger et al. in 1977 [1], firstly provided a feasible way to detect nucleic acid sequences, the DNA sequencing technologies have been closely concerned and the development rate has equaled and now even outpaces Moore's Law [2, 3]. These DNA sequencing technologies provide people with an opportunity to get genomic information to prevent, diagnose, and cure human diseases and would lead medical research and medical care to a new era [4, 5]. However, the sequencing cost was huge (~$10 million) by traditional Sanger sequencing technology because of the enormous amount (~3 billion base-pairs) of DNA found in human genomes, which made DNA sequencing uneconomical to be a part of routine medical procedure (https://www.genome.gov/pfv.cfm?pageID=12513210). In 2008, the cyclic-array sequencing technology (including wash-and-scan, PCR, and termination process) [6–8] led the first leap in DNA sequencing field. The second-generation DNA sequencers (e.g., 454 Genome Sequencers [8], Illumina [9], HelioScope, and SOLiD [7]) have gradually replaced the first-generation ones and reduced more than 3-fold of the cost in sequencing one megabase (https://www.genome.gov/pfv.cfm?pageID=27541954). More details about the second-generation sequencing technologies can be found in previous reviews [10–13].

To further reduce the cost, operation time, and equipment size of DNA sequencing process and meanwhile continually increase the contiguous read length, throughput, and accuracy, researchers have proposed different approaches for DNA sequencing, leading to the emergence of the third-generation sequencing technologies, such as the real-time sequencing by synthesis technology [14] and direct image technology [15, 16]. Nanopore-based DNA sequencing technology has become one of the most attractive and promising third-generation sequencing technologies because of its outstanding characteristics of label-free, amplification-free, great read length, and high throughput, which offer possibilities of high-quality gene sequencing applications, such as *de novo* sequencing, high-resolution analysis of chromosomal structure variation, and long-range haplotype mapping [17].

The nanopore-based DNA sequencing was first proposed by Church et al. in 1995 (awarded in 1998) [18]. One year later, Kasianowicz's group [19] demonstrated the detection of the electronic behavior of ssDNA passing through an α-hemolysin (2.6 nm in diameter) nanopore, which marked the beginning of the nanopore-based DNA sequencing field. Since then, various biological nanopores were used to sequence DNA and RNA molecules [20, 21], for example, the octameric protein channel of *Mycobacterium smegmatis* porin A (MspA) [22]. The biological nanopores have many advantages [23, 24], such as the dimension reproducibility,

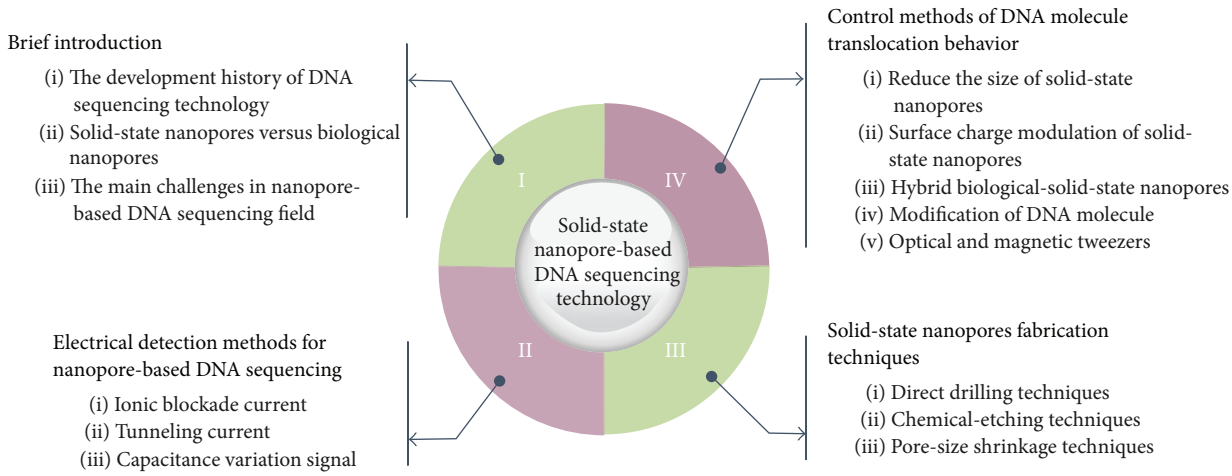

Figure 1: The schematic presentation to overview the contents of this paper.

the compatibility of genetic or chemical modification process, and relatively slower DNA translocation velocity through the nanopores. However, there are also some undeniable drawbacks [23, 24]: (a) the rigor environmental demands (e.g., temperature, electrolyte concentration, and PH) of nanopores to keep their biological activities; (b) the fragility of the lipid bilayer which makes the biological nanopores break down easily; (c) the incompatibility with the standard semiconductor fabrication process.

With the rapid development of advanced fabrication technologies, solid-state nanopores have become an inexpensive and superior alternative to biological nanopores due to the following superiorities [25–28]: (a) the better robustness and durability; (b) the superior mechanical, chemical, and thermal characteristics; (c) the easier-handled shape and size fabrication process with nanometer precision; (d) the compatibility with semiconductor technology allowing the integration with other nanodevices.

Although biological and solid-state nanopore-based DNA sequencing technologies have plenty of superiorities, substantial long fragment of DNA molecules have yet been sequenced with high accuracy [29, 30]. Several bottlenecks hold the further development of nanopore-based sequencing technologies. For instance, high translocation velocity of DNA molecule passing through the nanopore results in low temporal resolution of single-base sequencing; large channel length leads to a poor spatial resolution in separating adjacent bases (might be solved by nanopores fabricated on two-dimensional materials [31]). All of these challenges demand high-quality nanopores with sufficiently small feature size, good pore shape, and proper materials. In the following sections, we firstly give a brief review of the electrical detection methods of nanopore-based DNA sequencing. Then we introduce the fabrication techniques of the solid-state nanopores, including direct fabrication techniques, chemical-etching techniques and pore-size shrinkage techniques. Among these techniques, the chemical wet etching method presents unique advantages in its intrinsic pyramidal shape. Finally, the modulation methods of DNA molecule's behavior inside the nanopores are summarized. Figure 1 shows a schematic presentation that can overview the contents of this paper. Through the evolution of the solid-state nanopore fabrication technologies, it can be seen that the nanopores fabricated with the combination of advanced nanomanufacturing techniques and pore-size reduction/modification techniques will be the critical fabrication platform of low cost single-molecule DNA sequencing systems.

2. Electrical Detection Methods for Nanopore-Based DNA Sequencing

Since DNA bases (adenine, thymine, cytosine, and guanine) are different from each other in atomic scale, it is essential to collect base-specific information at atomic level to correspond to the DNA sequence with the measured signals. According to the different types of the signals, the detection methods can be roughly classified into two categories: the electrical detection methods and the optical readout methods [32]. The former is more promising due to its potential to reduce the cost and scale of the sequencers. In this section, several nanopore-based electrical methods to detect the sequence of DNA molecule, as well as the major challenges of these methods, have been reviewed.

2.1. Detection Method Based on Ionic Blockade Current. Measuring the ionic blockade current is the most common and original method to detect the sequence of DNA molecule [1, 17, 33, 34]. Figure 2(a) schematically illustrates the basic principle of the detection method based on ionic blockade current. A membrane with a solid-state nanopore separates the container into two chambers which are filled with electrolyte solution. A constant bias voltage applied astride the membrane will induce a steady-state ionic current through the pore. By adding DNA molecules into the negatively biased chamber, the electrophoresis force will drive the

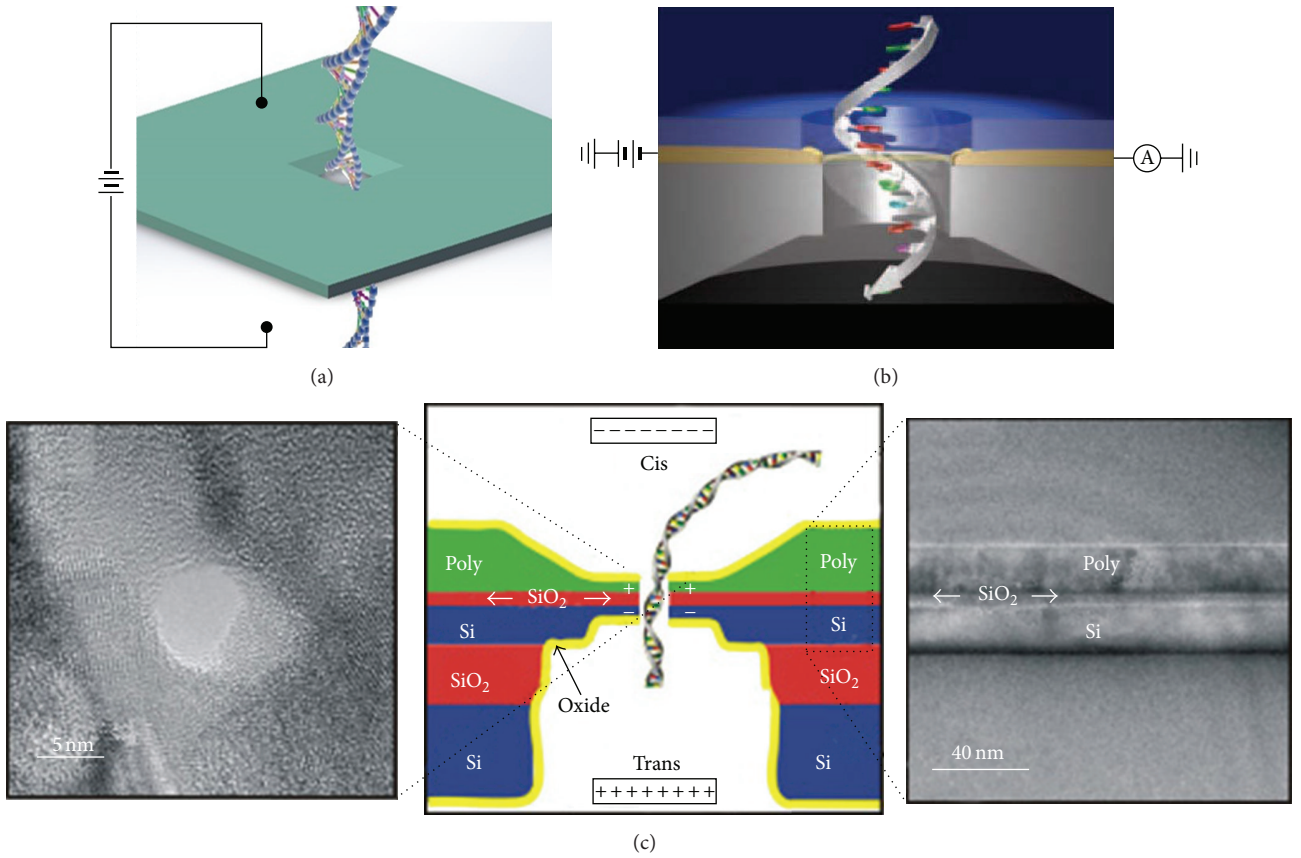

Figure 2: The electrical detection methods to sequence DNA molecule based on solid-state nanopores. (a) Basic principle of DNA sequencing via ionic blockade current signal [18]. (b) Schematic of tunnel-current detection method via a pair of nanoelectrodes fabricated at the edge of the nanopore [42]. Copyright © 2010, Nature Publishing Group. (c) Middle: schematic of MOS capacitive synthetic nanopore to detect DNA sequence. Right: A TEM image of the cross-section of the capacitor membrane structure. Left: A TEM micrograph of ~8 nm diameter pore drilled through the capacitor [48, 49]. Copyright © 2006, IOP Publishing.

self-charged DNA molecule passing through the nanopore and different bases theoretically cause base-specific ionic blockade current, providing corresponding information of DNA molecule sequence.

The size of an ideal nanopore should be small enough to unfold the DNA molecule from its coiled state, which allows DNA to pass through the nanopore in a linear configuration. The effective length of the nanopores should be short enough to distinguish each single base of DNA molecule. However, most of the existing manmade nanopores cannot meet such requirements. Even an "infinitely short" channel (e.g., conical nanopore) might not achieve enough spatial resolution, due to the extension of the electrical "read" region in the channel caused by the high electric field region [29]. Thus, the ionic blockade current detection method sets high requirements for solid-state nanopores morphology and fabrication technologies. This method also face another problem: the current signal might not entirely indicate the actual DNA translocation events, because significant reduction of ionic current can also occurs when the DNA is occluded at the pore mouth due to the strong hydrophobic DNA-pore interaction [35].

According to the above-mentioned challenges, the DNA sequencing technology simply based on ionic blockade current signal seems to lack the spatial and temporal resolution to obtain the DNA structural information at single-base level. To improve the situation, Akeson et al. proposed that the detection accuracy can be enhanced with the assistant information of the DNA bases duration time [36]. Several other assistant methods have been put forward, such as hybridization-assisted [37] and exonuclease-assisted nanopore sequencing [17]. The latter can identify nucleoside $5'$-monophosphate molecules with accuracies averaging 99.8%. These assistant methods require the modification of nanopores with biomolecules (e.g., oligonucleotides and enzyme), which means a higher requirement of biocompatibility of solid-state nanopores. More details can be found in the reviews by Branton et al. [29] and Zwolak and Di Ventra [38].

2.2. Detection Method Based on Tunneling Current. The tunneling-current-based detection method relies on the measurement of tunneling-current signals, which is generated when DNA bases pass through a pair of voltage biased tips in

a very close distance, as displayed in Figure 2(b). Tunneling-current signals are theoretically base-specific due to the different chemical and electronic structures of four different DNA bases. Instead of monitoring the ionic blockade current which occurs due to the occupancy of bases in the entire nanopore channel, the tunneling current is slightly influenced by adjacent bases. It is because the signal is controlled by base-electrode coupling and the energy of the molecular states, which provides an opportunity to achieve single-base sequencing resolution [38]. This method might be the least expensive and fastest route for DNA sequencing. The tunneling current can be measured by both scanning tunneling microscopes (STM) [15, 39] and embedded electrodes incorporated with current readout instruments. Xu et al. detected the electronic properties of different DNA bases by an ultrahigh vacuum (UHV) STM at room temperature and obtained base-specific electronic signatures (e.g., molecular energy level and tunnel current). However, the distribution of the signatures was broad and overlapped each other [40]. He et al. promoted this method by introducing a guanine-functionalized STM probe, and the result showed unique decay of tunneling current of the Watson-Crick base-pair [41].

With the development of advanced nanofabrication technologies, the embedded electrodes sequencing method has drawn growing attention, because of its potential to realize portable and integrable sequencers. Various of electrodes materials, such as Au [42], Pt [43], and carbon [44], are adopted to detect the electronic properties of individual nucleotides and DNA strands. Furthermore, graphene is considered to be one of the most promising materials for transelectrode membrane [28, 45], because of its in-plant electronic conduction sensitivity to the immediate surface environment and transmembrane solution potential, as well as its atomic thinness structure [46].

The experimental data support the feasibility of tunneling-current-based detection method; however, it might not be adopted into actual application until the following challenges are overcome [29, 38]. Firstly, the DNA molecule should be specifically located and oriented when it is passing through the electrode pairs. Tunneling current is very sensitive to the electrode spacing and nucleotides' orientations (perpendicular or parallel to the electrode surface) and exponentially affected by the distance between the bases and electrodes, which will lead to orders of magnitude fluctuations in the value of current [39]. Secondly, the translocation rate of DNA molecule should be slow enough (<0.1 ms/base) to meet the requirement of high-bandwidth current readout instruments and to minimize the inevitable noise. Thirdly, the fabrication of tunneling-current-based sequencing device remains a challenge. More economical and efficient methods to manufacture nanopores with precisely aligned tunneling electrodes are still needed. Fourthly, the prediction of the tunneling-current behavior caused by different bases of unknown DNA strand is difficult, because of the variation of voltage bias, ions condition, and DNA state fluctuation [47]. In general, more theoretical and experimental research is in urge need, involving the fundamental factors that influence tunneling-current and controlling methods to dominate the DNA molecules spatial manners.

2.3. Detection Method Based on Capacitance Variation. Utilizing metal-oxide-semiconductor (MOS) capacitive synthetic nanopores to detect and sequence the DNA structure is another potential alternative electrical method for DNA sequencing [48, 49]. As illustrated in Figure 2(c), when a DNA molecule translocates through the capacitive nanopore, the capacitor will be polarized because of the unique electrostatic charge distribution of different DNA bases, thus triggering base-specific voltage signatures to identify the sequence of DNA molecules.

Heng et al. integrated a nanopore into a MOS-capacitor (associated with heavily doped silicon electrode) and attempted to measure the voltage signal corresponding to the translocation of a double-strand DNA (dsDNA) [48]. However, due to the narrow bandwidth of the voltage amplifier, RC time constant, and large diameter of the nanopore, no base-specific signal was obtained [48]. Besides proof-of-principle experiments, plenty of theoretical research using molecular dynamics (MD) simulation software have been conducted. Gracheva's group proposed a numerical multiscale approach to simulate the electric signal detected by a MOS capacitive nanopore [49], and the voltage signal obtained from the simulation supported the possibility of such kind of device with single nucleotide resolution [49, 50]. But the conformational disorder of DNA molecules was not taken into account in those simulation. Later, Sigalov's group demonstrated a back-and-forth motion of DNA in a 1 nm nanopore. A sequence-specific hysteresis can be detected through the electrostatic potential at the electrode of MOS capacitive nanopore [51].

However, simulation results cannot totally represent the actual situation, and there are several challenges obstructing this method for practical application. First, the bases should be well oriented to the electrodes, because the electrical signature is sensitive to the dipole moment which associates with each unique base. Second, because of the fact that the DNA molecule is negatively charged and most of the charge is accounted for by the phosphate backbone [52], the desired signal would be easily masked by the larger backbone signal. Third, the large fluctuations of ions at the entrance of the pore may contribute to the voltage fluctuation. Furthermore, the size of nanopore should be controlled at about 1 nm to enhance the voltage signature by orienting the DNA bases along the pore axis [50].

3. Solid-State Nanopores Fabrication Techniques Used in DNA Sequencing

To achieve high-quality sequencing performance, solid-state nanopores should have the following characteristics. First, the size of the nanopores should be comparable to the DNA molecule diameter (~1 nm for ssDNA and ~2 nm for dsDNA) and thus can enhance the change in ionic current and avoid space-folding when DNA pass through the pore. Second, the effective length of the nanopore should be

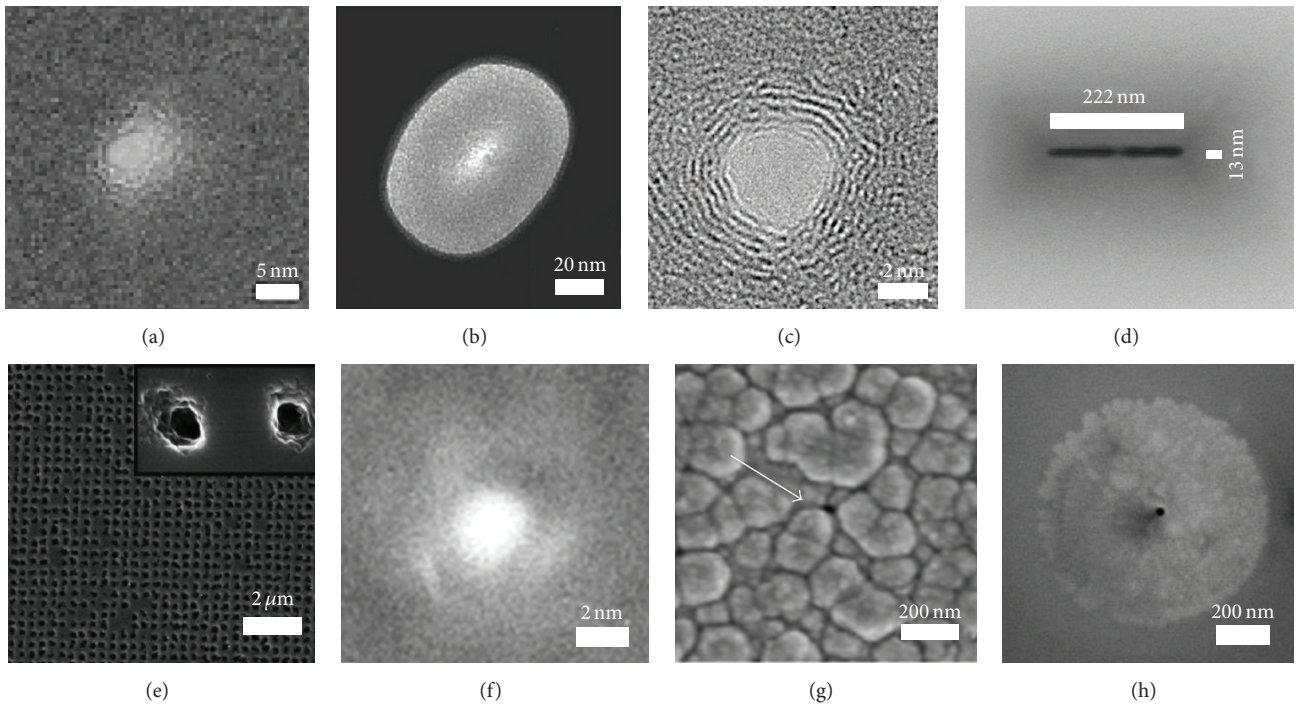

Figure 3: TEM and SEM images of various nanopores fabricated by typical and advanced technologies. (a) A 3.7 nm nanopore formed on free-standing SiN membrane by helium ion microscope (HIM) direct drilling technique [64]. Copyright © 2011, IOP Publishing. (b) A 1.8 nm SiN_x nanopore obtained by Ar-ion beam sculpting technique [65]. Copyright © 2001, Nature Publishing Group. (c) A graphene nanopore formed by FEB sculpting technique whose diameter was about 3.5 nm [31]. Copyright © 2008, AIP Publishing LLC. (d) A silicon nanoslit with feature size down to 13 nm fabricated by KOH wet etching process [73]. (e) Nanopore arrays manufactured by MaPE technique combined with nanoimprint lithography technology. Inset figure shows representative zoomed-in image of the nanopore arrays [76]. Copyright © 2012, American Chemical Society. (f) A 2 nm Al_2O_3 nanopore formed by ALD process [78]. Copyright © 2004, American Chemical Society. (g) ~18 nm nanopore created by electrodeposition process [79]. Copyright © 2010, IOP Publishing. (h) A FIB-fabricated nanopore shrunk from 1 μm to 25–30 nm by local oxide deposition method [81]. Copyright © 2006, John Wiley and Sons.

theoretically no more than the distance between two adjacent bases (~0.5 nm for ssDNA) to realize sing-base sequencing precision. Furthermore, the fabrication method should be effective, controllable, and economical. For decades, a large amount of effort has been centered on seeking proper methods of nanopore fabrication. In this section, several typical and advanced fabrication methods have been reviewed; more details can be found in previous reviews [53–56].

3.1. Direct Drilling Techniques. Focused ion beam (FIB) and focused electron beam (FEB) drilling techniques are the most common methods to fabricate nanopores directly. Utilizing these techniques, nanopores with various shapes and sizes have been obtained in different materials, such as Si_3N_4 [57, 58], SiO_2 [59], graphene [60], magnesium [61], SiC, and polymer membranes [56]. In general, because of the limited beam diameter, short penetration range, and interaction, the feature size of the nanopores fabricated by traditional FIB method is usually above 10 nm, even with the help of feedback system [62]. However, sub-5 nm nanopores have been realized by adopting various assistant approaches, such as the adoption of a dedicated FIB nanowriter equipment developed by Gierak's group [63] and a helium ion microscope (HIM) whose ion source beam diameter could reach atomic scale demonstrated by Yang's group [25, 64], as shown in Figure 3(a). Compared with the FIB drilling technique, the FEB drilling can achieve higher resolution and smaller pore size, making it a predominant fabrication method of one-step formed nanopore.

It should be noticed that by changing the energy of the ion/electron beam (low energy for ion beam [65] and high energy for electron beam [57]), a prefabricated nanopore could be fine-tuned with single-nanometer precision. This fabrication process is known as "sculpting." The boundaries between the "drilling" and "sculpting" process are ambiguous, and most of the nanopores fabricated by focused ion/electron beam technology usually experience both processes to achieve smaller scales and more precise control. Figure 3(b) displays a 1.8 nm SiN_x nanopore fabricated by Ar-ion beam sculpting process [65].

As ultrathin membranes (e.g., graphene whose thickness is 0.335 nm per layer [66]) gain their popularity due to their potential to realize single-base sequencing resolution [66], the FEB techniques are considered to be the most convenient way to fabricate ultrathin nanopores by one step. After the first FEB-drilled graphene nanopore (~3.5 nm in diameter, as shown in Figure 3(c)), which is demonstrated by Fischbein and Drndić in 2008 [31], ultrathin nanopores with various

materials such as hexagonal boron nitride (BN) [67] and MoS_2 [68] have also been realized by the same method. Additionally, both kinds of nanopores presented irregular circular structure. These nanopores showed better responses to DNA translocation events. However, undesired defects and damage existing in membrane which is introduced by the fabrication process may results in lower signal-to-noise ratio of the nanopores [67, 69, 70], and further optimization is still needed.

3.2. Chemical-Etching Techniques. Different from FIB/FEB drilling and sculpting techniques, chemical-etching techniques can achieve massive and cost-effective production of solid-state nanopores and nanopore arrays. Nanopores with various materials can be fabricated, such as polyimide nanopores [71], ethylene terephthalate nanopores (could be realized by ion-track etching technique) [72], semiconducting nanopores (could be realized by wet anisotropic etching technique) [73, 74], and metal nanopores (could be realized by electrochemical anodizing technique) [75]. In general, it seems that the major roadblock in the development of chemical-etching technologies is the relatively low control precision of the nanopore size.

The fabrication of nanopores based on traditional anisotropic silicon wet etching had been proposed by Liu's group in Tsinghua University [73], and nanoslit with feature size down to 13 nm was successfully obtained (Figure 3(d)) using KOH wet etching process with a color-indicator-based feedback system [73]. Thanks to the anisotropic characteristic of the wet etching process, the inner shape of the nanopores is pyramidal, which could theoretically enhance the sequencing resolution for its sharp edge at atom level. From the results which appeared in the SEM picture, it can be found that the size and the geometric shape of the nanopore can be easily improved by several nanometers with the optimized mask and the precise control of the etching process. In fact, the sizes of these nanopores massively produced with the wet etching process can be further reduced by pore-size shrinkage techniques described in the next section.

Metal-assisted plasma etching (MaPE) technique, which was first proposed by James et al. [76], is another way to achieve asymmetry nanopores. Under the assistance of gold nanoparticles (Au NPs), conical etch profiles will be formed in Si, which is caused by the enhanced silicon etching rate surrounding and beneath the Au NPs. Individual nanopores and nanopore arrays were realized using this technique. The smallest diameter of the pore reached ~20 nm, as shown in Figure 3(e). More details about metal-assisted chemical-etching technologies can be found in Huang's review [77].

3.3. Pore-Size Shrinkage Techniques. Material deposition is considered to be the most direct and convenient way to reduce the size of prefabricated nanopores. This idea triggered the appearance of various deposition-induced nanopore shrinkage technologies. Atomic layer deposition (ALD) is one of the pore-size reduction technologies that can achieve single Angstrom controlling precision. Using ALD technology, an Al_2O_3 nanopore with diameter down to 2 nm (Figure 3(f)) was obtained by Chen et al. [78]. Evaporation and electrodeposition are common choice for fabricating metal nanopores. The latter can achieve real-time control by monitoring the ionic conductance of the pore. As shown in Figure 3(g), a Si_3N_4 nanopore coated with Pt (~18 nm in diameter) was successfully fabricated by this method [79].

Instead of changing the entire membrane character due to the deposited material, ion/electron beam induced deposition (I/EBID) techniques offer an alternative approach of local-area deposition. With the ion/electron-mediated decomposition of a precursor, the certain material can be deposited at local area of a nanopore. The final diameter of the pores could be controlled at subnanometer resolution, because of the monolayer-by-monolayer process [80]. The prefabricated pores with sizes of 500–1000 nm were shrunk down to 25–30 nm by IBID of SiO_2 (Figure 3(h)) [81] and from 120 nm to 5 nm by EBID of carbonaceous compound [82], respectively. However, the above-mentioned techniques share a common problem in shrinking cylindrical nanopore: the effective length will increase during the deposition process. Therefore, the deposition-induced shrinkage techniques may be more suitable for conical or pyramidal nanopores.

Our group proposed that the dry oxidation process could be another effective and economical approach to contract Si nanopores. As SiO_2 has chemically reactive agents, it is suitable for further functionalization with biomolecules which directly interact with the translocate polymers. Under high temperature (~900°C) and sufficient O_2 supply, the SiO_2 forms upon the Si nanopore surface and diffuses to the edge area that has lower surface free energy [83–85]. Experimental results showed that nanopores whose diameters range from 150 to 60 nm could be contracted to sub-10 nm with single-nanometer precision. Moreover, the pyramidal nanopore will transform into an "hour-glass" structure after the oxidation shrinkage process, leading to an unincreased effective length of the pore.

4. Control Methods of DNA Translocation Behavior through a Solid-State Nanopore

As mentioned above, high velocity of DNA passing through a nanopore (27 bases/μs [86]) is one of the most serious obstacles to the further development and actual application of solid-state nanopore-based DNA sequencing technology. The reasonable value of the DNA translocation speed is considered to be 0.01~1 ms per base [87]. In order to take control of the DNA molecule translation process, various theories and approaches have been put forward in recent decades, involving the physical control of solution viscosity, voltage bias, and temperature [87, 88]. However, the changes of these factors may lead to kinds of problems: the sequencing process might not be conducted under optimal conditions and the readout signals at nanoampere level would be further weakened, and these methods might not reduce the variations in the DNA translocation dynamics, which were caused by nonspecific DNA-pore interactions [29, 89]. Therefore, in this section some more effective methods to control DNA translocation behaviors have been reviewed, such as the modification of nanopores with physical, biological, and

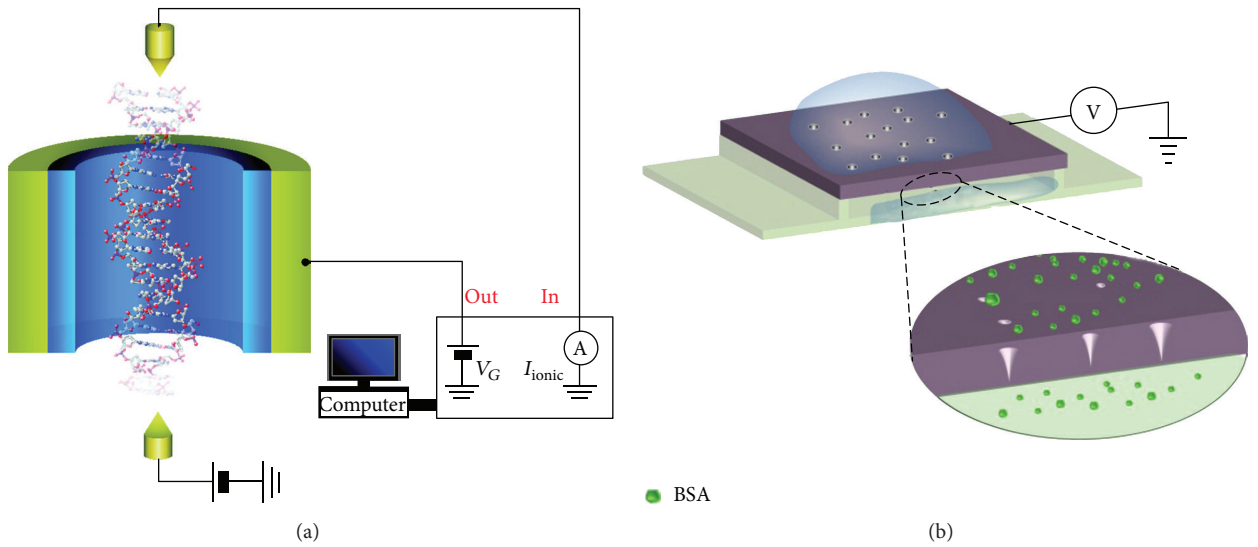

FIGURE 4: Surface charge condition modulation via gate voltage control. (a) Schematic of gate-controlled DNA translocation via the ionic feedback current signal [95]. Copyright © 2011, American Chemical Society. (b) Schematic illustration of fluorescently labeled BSA protein transported across the nanoporous membrane controlled by different gate voltage biases [76]. Copyright © 2012, American Chemical Society.

chemical method, the decoration of DNA molecule, and the tweezers techniques.

4.1. Reduction of Solid-State Nanopore Size. The research carried out by Wanunu's group, showed an order of magnitude decrease in DNA translocation velocity when a SiN nanopore's diameter decreased from 5 nm to 2.7 nm [90]. By decreasing the feature size of the nanopores, the interaction between the pore and the DNA molecule is enhanced. This interaction is caused by series of thermally activated jumps over small energy barriers (~$12k_BT$) and influenced by factors such as the size of the nanopores, temperature, and DNA length [90]. Similar phenomenon was found by Keyser's group, a small change in diameter of a nanopore will affect the translocation time due to the hydrodynamic coupling between molecules and nanopores [91]. These experimental results indicate that it is a key issue to fabricate nanopores with well-controlled diameter. However, the DNA translocation behavior in a smaller pore is more complex and will introduce more collision, which might result in lower signal-to-noise ratio, compared with larger nanopores.

4.2. Surface Charge Condition Modulation of Solid-States Nanopores. As DNA molecules are self-charged in electrolyte solutions, the surface charge density conditions of a nanopore will affect the DNA translocation performance in the pore. Both positively charged (e.g., Al_2O_3 [26]) and negatively charged (e.g., SiO_2 [92]) nanopores can be used to modulate the DNA translocation process [93].

Venkatesan's group put forward a three-step pore formation method by changing the dose of electron beam in a TEM system to fabricate and optimize Al_2O_3 nanopores [26]. By this method, amorphous alumina ($Al:O \approx 2:3$) transformed to heterophase crystalline structure ($Al:O \approx 1:0.6$), leading to an irregular surface charge distribution in the nanopore (positively charged), which enhance the interaction between the DNA molecule and nanopore. The subsequent experiment showed a 10-fold decrease in average translocation velocities (≈1.4 bases/μs), compared with the Si_3N_4 and SiO_2 nanopores under similar feature sizes and experimental conditions. It should also be noticed that the Al_2O_3 nanopores have a better performance in signal-to-noise ratio and DNA capture rate [86, 94].

Instead of obtaining a nanopore with fixed surface charge condition, He et al. proposed a theory to modulate wall-surface charge density ($\sigma_w{}^*$) of the nanopore (SiO_2) by controlling the gate-bias voltage (V_G) [95]. The schematic of this gate-controlled modulation system with ion-current feedback method is shown in Figure 4(a). In the capture stage, positive gate-bias ($V_G > 0$) is demanded to decrease the effective wall charge density $\sigma_w{}^*$, thus weakening $\sigma_w{}^*$-induced advection flow to enhance the DNA capture rate. In the translocate state, the negatively biased gate ($V_G < 0$) will create another electrical double layer (EDL), which will introduce an electroosmotic force opposite to the DNA translocation direction. This strong interaction leads to a dramatic decrease in DNA translocation speed at a rate of about 55 μm/s per 1 mV/nm [95]. However, the wall-surface charge density is sensitive to the gate voltage (V_G), especially at a low salt concentration. Additionally, the demanded electric field (E_p), which is generated by the gate voltage, reaches the electric field breakdown limit of the SiO_2 layer and needs further optimization [95].

The rectification property [96] found in asymmetry nanochannel, especially in conical nanopores [97–99], provides an effective way to modulate DNA molecule velocity through the nanopore. The modulation mechanism is also based on the manipulation of surface charge condition by a

varying gate voltage applied across the nanopore. Utilizing this characteristic, Karnik et al. successfully enhanced and stopped the protein in their transistor-reservoir-transistor circuit [100]. A few years later, James et al. achieved active control of the bovine serum albumin (BSA) transport performance by modulating the voltage applied across the semiconducting conical nanoporous membranes (Figure 4(b)) [76]. Compared with cylindrical nanopores under the same feature sizes, conical structures have lower resistance and higher sensitivity to the variation of gate voltages, resulting in a stronger ionic current and better manipulation on DNA molecule behaviors. Therefore, the asymmetry nanopores seem to be a better platform for DNA sequencing.

4.3. Hybrid Biological-Solid-State Nanopores. As mentioned in Section 1, biological nanopores can achieve better control of DNA translocation time and performance [23, 24]. Therefore, hybrid biological-solid-state nanopores might theoretically provide a new platform with both enhanced robustness and unique biological qualities [101–103]. In 2010, Hall's group demonstrated hybrid pores by inserting α-hemolysin (α-HL) nanopores into SiN nanopores (2.4 nm–3.6 nm in diameter), as shown in Figure 5(a) [101]. The hybrid pores showed better robustness and the bionanopore in the structure remained nondenatured and functional, signifying a superior control ability of DNA translocation velocity [101]. However, the size of the solid-state nanopores should be precisely controlled to ensure the smooth and effective assembly process with the α-HL protein nanopores.

Different from inserting a bionanopore into a solid-state one, DNA origami can also be used to modify the solid-state nanopores [104, 105]. Hernández-Ainsa et al. put forward two kinds of decoration modes to enhance the control of DNA translocation velocity: one was "physical" mode (top figure in Figure 5(b)), which was based on a self-tuning nanopore formed in the DNA origami structure attached to the entrance of a solid-state nanopore; another was "chemical" mode which relied on the strong base-pair interaction introduced by the complementary DNA origami hung on the edge of the solid-state nanopore (the bottom figure in Figure 5(b)). These enhanced pore-DNA interactions will lead to a decrease of DNA translocation velocity [104, 106]. Subsequent experiments were carried out using the hybrid nanopore in "chemical" mode and a large increase of ssDNA translocation time was observed [104].

Moreover, inspired by the process of insects' pheromones detection, Yusko et al. proposed a lipid bilayer coated Si_3N_4 nanopore (Figure 5(c)) with fluid anchored capture sites [107], which can tune the pore size *in situ* with the control of temperature condition. By anchoring the analytes onto the lipid, the dominant factor that influences the translocation behavior changes from the low viscosity aqueous electrolyte to high viscosity of the lipid bilayer coating, prolonging the translocation time. Experimental results showed that the translocation time of individual proteins increased sufficiently [107]. However, more theoretical and experimental research is needed to reveal the mechanism and performance of DNA molecules translocate through those hybrid nanopores.

4.4. Modification of the DNA Molecules. The modification of the DNA strands is another alternative approach to substantially reduce the DNA translocation velocity through the nanopore. By binding enzymes [108, 109] or oligonucleotides [32] to DNA strands, the determinate factor of the translocate velocity changes from the electrophoretic force to the enzyme replicating or unzipping processes, which ratchets the DNA strands one nucleotide at a time and successively acts up to tens of thousands of nucleotides. Unlike decorating a nanopore, these methods reduce the demand of biological compatibility of solid-state nanopores. However, these methods show a higher requirement on the morphology of solid-state nanopores, which means the nanopore should be small enough to hinder/unzip the DNA-enzyme/oligomer complex and meanwhile large enough for ssDNA to pass through.

Benner et al. used a biological nanopore to discriminate the enzymes which bind to corresponding DNA substrates [109]. The experimental results showed a great reduction in translocation velocity of enzyme-bound DNA complex, compared with the unbound DNA. Lots of theoretical and experimental researches have also been done in nanopore unzipping method to slow down the translocation velocity [110–112]. Soni and Meller used SiN nanopores (average 2 nm in diameter) to peel off the fluorescently tagged oligonucleotides which hybridized on the designed DNA polymer and detected the corresponding flashed light using a total internal reflection fluorescence (TIRF) microscope [32]. The DNA translocation speed was determined by the unzipping kinetic event and achieved a readout time of approximately 5 ms/base.

Subsequent research carried out by Cherf's group showed that a forward and reverse ratcheting processes can be used to reduce the translocation velocity of DNA strand coupled with a phi29 DNA polymerase (Figure 5(d), I) [113]. Figure 5(d), II, illustrates the whole forward and reverse processes of the DNA through the nanopore. In the voltage-driven unzipping stage, with the removal of the specially made blocking oligomer, the phi29 DNAP-DNA complex was driven forward though the nanopore by the electrophoretic force; with the exposure of the DNA primer's $3'$-OH terminus in the polymerase active site, the replication process began and ratcheted the complex in the reverse direction with median translocation rate 2.5~40 nucleotides per second. However, the insertion and deletion error rates caused by the DNA back-and-forth moment and readout data acquisition rate still need to be optimized [113].

4.5. Optical and Magnetic Tweezers. The methods mentioned above may share a common problem where the actual position and force exerted on the DNA molecule inside the nanopore are hard to predict, which can only be inferred by MD simulation. The optical tweezers technology may provide a straightforward way to slow down the DNA translocation velocity. This method was initially used in the measurement of the forces exerted on the DNA molecule inside the nanopore during the voltage-driven process and was first published by Keyser's group in 2006 [114]. The DNA molecule is controlled by the tethered bead and laser tweezers, as shown in Figure 5(e). By the control

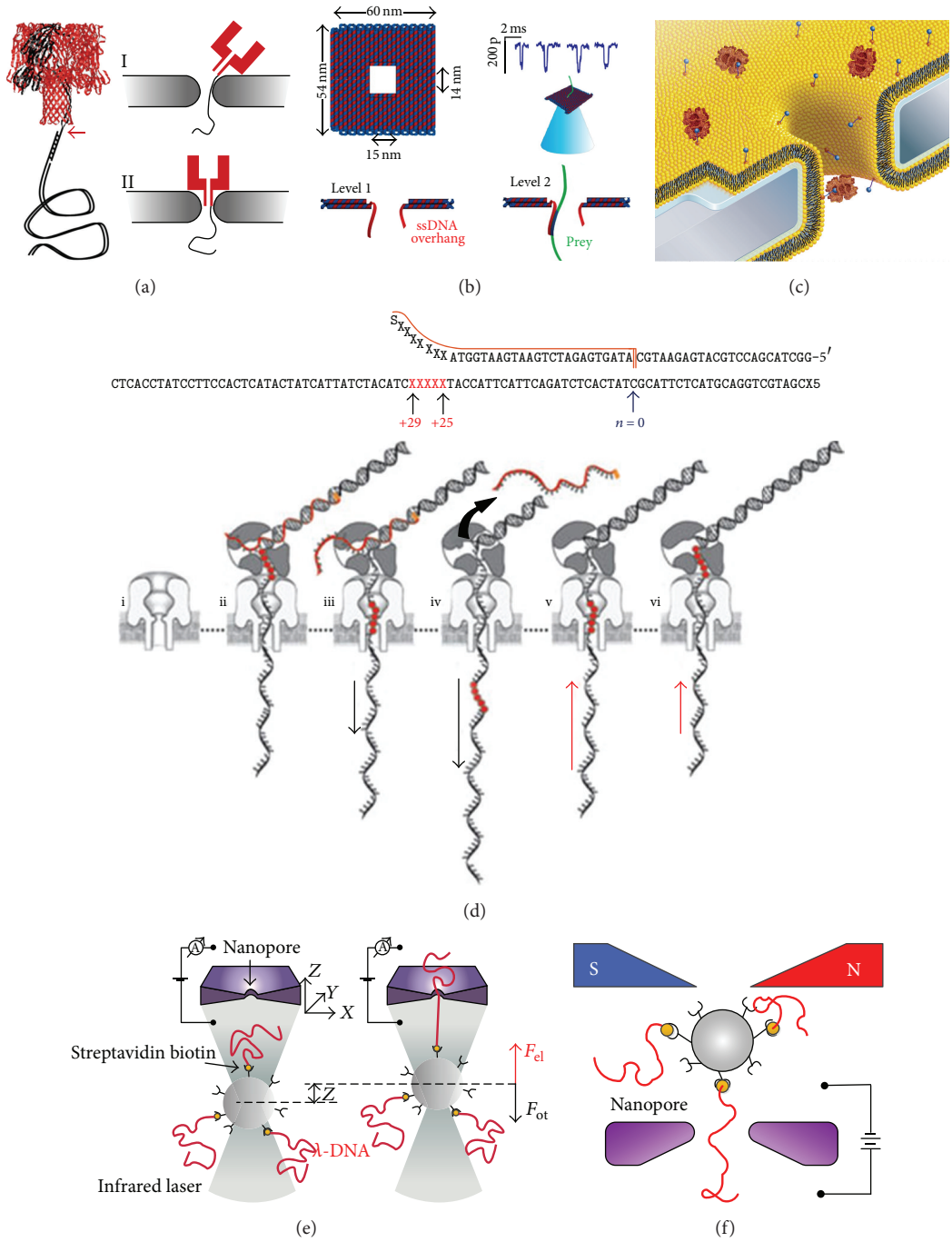

FIGURE 5: Technologies to modulate DNA molecule translocation behavior. (a) Left: α-HL with a 3 kbp guiding-dsDNA attached via oligomer, the red arrow shows the position of the connect point. Right: the "preinsertion" stage (I) and "final-insertion" stage (II) of an α-HL protein pore inserted into a solid-state nanopore [101]. Copyright © 2010, Nature Publishing Group. (b) Top left: schematic representation of a DNA origami structure with a 14 nm × 15 nm nanopore. Top right: a λ-DNA translocates through a 5 nm hybrid nanopore in "physical" mode. Bottom: a DNA molecule translocates through a hybrid nanopore in "chemical" mode [104]. Copyright © 2013, American Chemical Society. (c) The cross-section of a lipid bilayer coated Si_3N_4 nanopore with specific lipid-anchored biotin-PEs (blue circle), which can anchor and translate the complex (large red bunch) through the nanopore [107]. Copyright © 2011, Nature Publishing Group. (d) Top: the DNA structure protected by the blocking oligomer (red line). Bottom: schematic of forward and reverse processes of DNA through a biological nanopore; (i) an open nanopore; (ii) the capture stage of the phi29 DNAP-DNA complex with blocking oligomer; (iii) the unzipping stage of the blocking oligomer (forward); (iv) releasing the blocking oligomer and exposing polymerase active site; (v) replication stage by phi29 DNAP (reverse); (vi) stalling of replication [113]. Copyright © 2012, Nature Publishing Group. (e) Left: a DNA-tethered bead is trapped near the solid-state nanopore by a tightly focused laser beam. Right: the electrical force F_{el} drives the DNA strand through the nanopore and the strand is straightened and controlled by the composite of optical force (F_{ot}) and F_{el} [115]. Copyright © 2006, Nature Publishing Group. (f) Schematic of magnetic tweezers to control the translocation of a DNA-attached colloid by magnetic force [118].

of the speed and direction of the optical tweezers, the DNA translocation velocity can be set at any desired value [115, 116]. Subsequently, Trepagnier et al. reduced the DNA translocation speed to 150 bp/ms and controlled the DNA flossing back and forth through the pore by this technology [117]. This approach can theoretically achieve true three-dimensional spatial control of the DNA molecule and allow massively parallel detections. However, the ionic current is sensitive to the motion of the optical bead due to the absorption of the laser light by the solution, which results in a great influence on the ionic current measurement [114, 115].

Different from the optical one, the magnetic tweezers technology utilizes adjacent magnet to generate a magnetic-field gradient, thus, inducing a constant force to control the DNA molecule attached to the magnetic colloid, as shown in Figure 5(f). Employing this technology, Peng and Ling reversed the DNA translocation and achieved an average speed of 0.0096 bases/μs [118]. The sequencing process can be theoretically operated in massively parallel. However, magnetic tweezers cannot take total control of the DNA translocation behavior and a series of different stages might occur during the translocation process, because the resultant force, which consists of magnetic, electric, and DNA-pore interaction force, is roughly constant [116].

5. Conclusions

Over the past few decades, the solid-state nanopore-based sequencing technology has been promoting the development of single-molecule real-time DNA sequencing field and providing a brand new future in gene detection area. However, there are still several issues that need to be settled, such as how to achieve massive and repeatable fabrication of high-quality solid-state nanopores in low cost, how to improve the electrical detective methods for the high-resolution nanopore-based DNA sequencing, and how to take control of DNA translocation behavior and velocity through the nanopore.

In this review, we focused on the typical and advanced techniques applied in the field of solid-state nanopore-based DNA sequencing technology, involving the detection methods for base-specific signals, the solid-state nanopore fabrication techniques, and the methods to modulate the DNA translocation behaviors. The nanopores with asymmetry or 2D structure seem to be a better choice than the symmetry ones due to the higher spatial resolution. Both biological and solid-state nanopores have their unique advantages in DNA sequencing application; we believe that with the joint efforts of researchers in various fields, the hybrid biological-solid-state nanopores will finally play an important part in this sequencing platform. The field of solid-state nanopores-based DNA sequencing is still in its early stage. With the development of novel fabrication technology and advanced material, and with the combination of the nanofluidic and *in situ* electrical or optical readout devices, which are associated with reliable data processing and calibration methods, the parallel DNA sequencers based on solid-state nanopores will finally become an integrated and efficient nanosystem providing services for personalized medicine fields.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (91023040 and 61273061).

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