Solid-State Nanopores Used for the Detection of DNA Molecules

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Abstract. Solid-state nanopore is crucial in dection of DNA molescules. DNA is made up of two antiparallel strands of polynucleotides. They are joined together by hydrogen bonds between complementary base pairs. The DNA structures are essential for the ability of genetic carrier to control gene expression, promote DNA recombination, and enhance mutagenesis. There are many ways to do DNA testing. Among these methods, nanopore have relatively small size which allows single-molecule detection. It is also less expensive. There are two kinds of nanopores, which are solid-state nanopore and biological nanopore. Solid state nanopore has better capacity to be incorporated into systems and arrays. The channel length and diameter of it can be controlled easily. In addition, its surface properties are also tunable. Therefore, this essay discussed concerning with the different types of nanopore technology: FEB, FIB and wet etching. The mechanism of nanopore was also explained. The existence of nanopores is conducive to a series of research on genes, pharmaceuticals, biology and so on.

Keywords: Solid-State Nanopores, DNA Molecules, Nanopore Mechanism.

1. Introduction

Medical component detection becomes very important nowadays. DNA is a double helix structure comprised of antiparallel strands of polynucleotides connected by hydrogen bonds between complementary base pairs. The idea of double helix structure was suggested by Waston and Crick in the year 1953 [1]. All the nucleotides contain base, phosphate and sugar. There are four bases for DNA: actine, guanine, cytosine and thymine. Actine is complementary with thymine, guanine is conplementary with cytosine [2]. In the genetic carrier, the DNA structures perform crucial roles in the control of expression of gene, recombined DNA, and the promotion of mutagenic activities [1].

The need to lower the cost and accelerate genome sequencing led to the concept of nanopore-based DNA sequencing. Traditional marker-based DNA sequencing requires fluorescent tagging on all the biomolecules that are intended for sequential determination, which makes it more time- and money-consuming. Genome research has been greatly impacted by the dramatic improvements in DNA sequencing technology over the past ten years. The development of novel medical therapies and diagnostics is a result of our improved understanding of the genetic roots of various diseases. Aside from human medicine, genetic research has many other uses in fields including security, biology, and agriculture. However, the cost of the present sequencing technologies, such as the well-known Sanger method is the main issue [1]. In contrast, DNA sequencing using nanopores is substantially less expensive and provides single-molecule detection without the need to modify the sample [3].

There are many ways to do dna testing, like PCR, microfluidics, fluorescence. However, there size is relatively large, but nanopore is small in size. Biological Nanopore, also called polymer Nanopore are made from manufactured nanopores produced by genetic engineering versus protein molecules found in nature. The first polymer nanopore named Staphylococcus aureus α-hemolysin has been developed in the year 1996, which appeared in David Deamer's group's experiment: translocation of nuclei acid through a Nanopore set in a lipid bilayer. However, biological nanopore have so many drawbacks. For example, it was insufficient lifetime, inherent unreliability with rigidt demand of the precise environment to stay. The lipid bilayer and biological pores will be impacted by changes in factors like pH level, concentration of salt, humidity, and mechanical stress [4]. So solid-state nanopore was invented in order to cover the shortage of biological nanopore. In 2001, the first solid

nanopore was used in professor Hagan Bayley's lab in oxford. They have showed the working of Nanopore bio sensing which is an experiment of single-stranded DNA. Nanopipettes, graphene, and silicon materials are used to create solid state nanopores. It can manage diameter and canal's length, has adaptable surface qualities, and is possible to be integrated into devices and arrays [4-6]. It also has a high thermal, mechanical, and chemical stability [7].

In recent years, research about solid-state nanopore has become a hot topic. Therefore, it is necessary to introduce and summarize. Different techniques can be invested to make solid-state nanopores; in this essay, we'll focus more on fabricating electron beams as well as manufacturing ion beams and wet etching.

2. Nanopore Mechanism

Nanopore technology is created by exposing both a positively and negatively charged electron to the fluid on the membrane's front and back sides, respectively. When applied voltage creates an electrophoretic force, single-stranded DNA molecules may be put on the forward side and can flow through membrane holes. The current level of the various molecules that are moving through the pores may be recorded and utilized for further computation with computer-aided tools. Copies of the processive enzyme are bond with the strands of DNA that will be sequenced. The enzyme binds to a single stranded leader at the end of the double-stranded genetic material template, unzips the double strand, and feeds one single strand through the nanopore as the DNA-protein complex approaches the nanopore as the split DNA long strand proceeds to only one base at a time will pass through the channel. One base or molecule that has considerable selectivity may be able to stop the current. Using those current signals, you may identify the type of base. On both sides of the pore, it is broader than average, while the center is smaller. The current breaks when the various bases of single-stranded DNA and nanopores interact, and this break may be measured. The present break is different because the molecules of the four bases—actine, guanine, cytosine, and thymine—are different. We are able to determine the DNA sequence in this manner [8].

An example of nanopore ONT's MinION sequencer. Compared to existing HTS platforms, this relatively new technology has a number of significant advantages. It allows for effective immediate evaluation, data transfer, and long-read output with just a single USB port attached to a usual laptop computer. It is affordable, its startup cost is $1000, and reasonably simple and quick library preparation processes. When identifying an authentication sample is quick and necessary, the simplicity of use and quick processing time are especially advantageous. This platform has a significant error rate in raw reads, ranging from 5% to 25%. Despite this, the rate of making an error is constantly dropping, first because of improvements to ONT's nanopores and updates to the sequencing chemistry, and second because of constantly improving bioinformatic tools created especially to handle the MinION's unique type of sequencing error. As a result, the scientific community tests and evaluates these computational tools. Numerous investigations have shown that the MinION's data are accurate enough to create a sequence that is unanimous from a sample of a single species. With >99% accuracy, it can identify the species [9].

3. FEB

Electron beam methods are appealing for a variety of technical applications due to the high-power density of electron beams and the flexibility of changing the beam parameters over a wide range [21]. With minimal restrictions on the kinds of tools and materials that can be used, the electron beam is a creative alternative for micro hole drilling. The EB is a practical technique for high aspect-ratio hole drilling because of the special energy absorption of electrons traveling faster and more density beam energy. Whether the substrates are nonmetallic or metallic, targeted EB has been employed to create higher aspect-ratio hole arrays. More than 100,000 holes per second of machining ensures significantly more efficiency than other drilling methods as well [10].

A focused electron beam can be used to start a non-self-sustaining discharge in a long, skinny metal tubing [11]. The electron beam in this instance starts the discharge, which has an impact on how the discharge current is distributed down the length of the tube. Fore vacuum-pressure plasma-cathode ion generators are the most ideal device for producing electron beams. It can effectively ionize an operational gas by generating strong electrons in the atmospheric pressure range of 1-50 Pa [12].

4. FIB

A well-developed technique, FIB has been widely applied in the field of materials research [13]. FIBs, or focused ion beams, are regarded as a vital technology. Today, a variety of FIBs, such as standalone FIB systems, gallium FIB/SEMs, plasma FIB/SEMs, and the helium ion microscope (HIM), assist in addressing research concerns and advancing nanofabrication. In situ structure-based biology experiments have been accelerated by the development of cryo-FIB for the production of cryo-lamellae. The source that is used most frequently is gallium ion (Ga+). Other ionized particle sources like Oxygen, Nitrogen, Argon and Xenon. Compared to CEMOVIS, Cryo-FIB is simpler to use while resulting in less artifacts. The quality of cryo-EM pictures rapidly decreases when lamellae are machined thicker because thick lamellae have a larger protein foreground and inelastic electron scattering than thin lamellae. Cryo-EM pictures from a group of 110-nm-thick lamellas, however, showed characteristics resembling those of a 150-nm-thick group, indicating a sizeable amount of the lamella in the 110-nm-thick group may become harmed by FIB. Therefore, a measurable method is needed to determine the precise depth and severity of damage in low-temperature wafer milling, and a strategy to lessen the damage must be investigated [13].

Focused ion beam (FIB) has the capacity to selectively grind the surface of an object using ion sputtering without typical photomasks, etch a sample utilizing the gas injection system, and locally deposit materials. Consequently, FIB has been used frequently in the creation of specimens for TEM. The FIB sampling approach is becoming more important. In particular, in MEMS-based in situ TEM where we may see real-time microstructural changes. It is brought on by external stressors including temperature, mechanical force, and electrical current. For in situ TEM using electric MEMS chips, a connecting medium with low resistance to electrical current must be constructed in order to connect TEM sample and anode or cathode on a MEMS chip together. The conducting pathway in the FIB can be constructed using either the technique of IBID or EBID [14].

5. Wet etching

Since the creation of wet-etched, pyramidal "micromechanical piercing structures" in 1995, a broad variety of silicon-based microneedle shapes have been created. The solid, 0.1-m-radius tip microprobes with an 80-m height were utilized to speed up DNA transfer into tobacco plant cells and nematodes. Reactive ion etching was used by Henry et al in 1998 to demonstrate the first (solid) silicon microneedle. By demonstrating that these 180 m tall devices could penetrate human skin painlessly, they opened up a new pathway for transdermal medication delivery [15].

Chemical materials can be removed by a procedure called wet etching. To remove components from wafer, it either uses dissolved product from a liquid solution or etchings. Although wet etch is also called isotropic, this might not hold true for samples (particularly thin films) that have clearly defined crystallographic orientations. Wet etching can be very selective when it comes to other materials [16]. Aqueous etchants are used in wet etching, such as potassium hydroxide or a solution of hydrofluoric, nitric, and acetic acid (HNA) [15]. The solution was initially tested at a 20% concentration. The action was so powerful that it excessively scratched the entire HZO film, as well as the PR, showing that the highly isotropic in nature etching was frequent. This suggests that the etching process was harsh in addition to being quick. The etching process must be controlled carefully for nanoscale HZO thin films, yet such vigorous etching results in poor control. Therefore, a

concentration of 1% HF etchant was created. It is made from diluted sample of solution in water in order to make sure exact handle over the process of wet etching. Therefore, the etching process is more precise and homogeneous because to the dilution's ability to reduce the etchant's reactivity. The necessary results may be obtained without harming the HZO layer or losing control of the etching manufacture.

Wet etching is simple to use and affordable to implement. It prevents hydrogen incorporation and damage from ion bombardment effects [16]. However, from the standpoint of post-processing, there aren't many studies on improving the surface quality and regulating the size of microstructures [9]. Various ways have been used for finding of DNA bases by using solid-state nanopore.

6. Conclusion

Various ways have been used for detection of DNA molecules by using solid-state nanopore. The nanopore detection DNA sequence, in my opinion, will be crucial for biological medicine, biochemistry, biotechnology, and other fields. Today, nanopores are more widely used in detecting long chains, but detecting short chains is still a shortcoming. I think we forcus more about the enzyme that we could split the long chain into small ones. So, the detection will be more precise.

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