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Nanopore sequencing technology in clinical diagnostics and genomic research: Opportunities and challenges

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Abstract. Nanopore sequencing technology, an advanced third-generation sequencing technology, is a revolutionary sequencing method widely used in clinical diagnosis and genomic research because of its features such as real-time sequencing, direct sequencing, long read length and portability. This paper outlines the basic principles and advantages of the technology, and briefly introduces its applications in clinical medicine such as diagnosis of diseases rare and genetic diseases, detection of infectious disease pathogens, public health emergency response, and cancer genomics screening. In genomics, nanopore sequencing is instrumental in genome assembly, structural variation detection, recovery of DNA from ancient organisms, and microbiological research. It enables direct sequencing and analysis of molecules, allowing for the identification of complex structural variations within the genome. This study finds that the technology also suffers from low accuracy, high cost associated with large data volumes, and significant requirements for data processing capabilities. These limitations can potentially be addressed through innovations such as improved nanopore materials and design, and integration with artificial intelligence. Finally, the latest innovations of the technology are analyzed, and the development trend and application prospects are outlooked.

Keywords: nanopore sequencing, clinical diagnostics, genomic research, development trends

1. Introduction

Gene sequencing technology began in the 1970s, and it is of great significance in discovering human genes, finding out their locations on chromosomes, and deciphering human genetic information. Since Sanger et al. established the first generation DNA nucleotide sequencing method-dideoxyterminal termination method (Sanger method) in 1977 years, DNA sequencing technology has become a key scientific research subject in the field of life science [1]. It has revolutionized the development of life science and medicine, promoted the research of human genomics and offered new perspectives for investigating the etiology and clinical diagnosis of complex diseases [2]. With the in-depth study of DNA structure and sequence, DNA sequencing technology has evolving significantly, undergoning continuous technological changes and iterations. In recent years, the third-generation nanopore sequencing technology based on the principle of single molecule detection has gradually emerged as the most cutting-edge and hottest scientific research topic of DNA sequencing, and has been widely used in clinical medical diagnosis and genomics research. Compared with traditional gene sequencing technology, nanopore sequencing technology has the advantages of real-time efficiency, long read length, portability, low cost, etc., and is the most likely to reduce the cost of sequencing the human genome to the target of less than $1,000 proposed by the National Institute of Human Genetics [3].

The rise of nanopore sequencing technology is conducive to promoting the development of life sciences, bringing a new revolution in medical diagnosis and genomics research, and bringing the clinical diagnosis and treatment of human diseases into a new era. More and more researchers are joining in the research and application of nanopore sequencing technology, inspired by the $1,000 Genome Project of the U.S. National Institute of Human Genetics. Nanopore sequencing technology has become the focus and hotspot of genetic research. This paper introduces the application of nanopore sequencing technology in clinical diagnosis and genomics research from the basic principle of nanopore sequencing technology. It also discusses the shortcomings and innovations, providing prospective information and reasonable insights for the scholars in this field.

2. Overview of nanopore sequencing

2.1. Principles of nanopore sequencing

In 1976, Neher and Sakamann proposed the concept of nanopore sequencing [4]. The main principle is to utilize the nucleic acid molecules to pass through the nanopore under the action of an electric field and cause a change in the electric current in order to obtain information about the structure of nucleic acid molecules. When an electric field is applied in a salt solution cell, the charged ions will flow in a specific direction and produce current. Similarly, the electrically charged molecules of nucleic acids will travel in a specific direction in the electric field. In the salt solution cell, a biofilm or artificial membrane partition with nanopores is added. When nucleic acid molecules traverse nanopores driven by an electric field, they can block the nanopores, which interferes with the flow of other charged ions and leads to changes in the electric current. The four bases that constitute nucleic acids—adenine (A), guanine (G), cytosine (C), and thymine (T)—differ in molecular structure and size. As a result, the changes in electric current associated with each base as it passes through the nanopore are distinct. These characteristic current fluctuations are unique to each base, allowing for real-time detection and analysis of these signals to identify the sequence of nucleic acid molecules.

2.2. Advantages of nanopore sequencing

Nanopore sequencing technology, as a third-generation sequencing technology, has the following features and advantages, compared to the first, second, and other sequencing technologies.

2.2.1. Real-time sequencing

Compared with traditional sequencing technology, nanopore technology can realize dynamic real-time sequencing, output results while sequencing, and monitor dynamic changes in the genome in real time, which makes it possible to track the genome in the process of cellular differentiation, and provides a highly efficient tool for the study of dynamic genomes [5]. Also the length of time required for nanopore sequencing technology is much shorter than other sequencing methods, saving time costs. In the 2019 African foot-and-mouth (FMDV) outbreak, German scientists for the first time used nanopore sequencing to establish a rapid sequencing protocol for FMDV serotyping in a mobile suitcase laboratory, completing sequencing and data analysis within 5h [6].

2.2.2. Long read length

Nanopore sequencing technology is capable of reading information from ultra-long nucleic acid molecules in a single run, theoretically detecting the entire nucleic acid sequence passing through the nanopore. The read length length is limited only by the length of the single-stranded nucleic acid molecule being tested [7]. This capability provides a more complete and contiguous genome assembly, reducing errors and information loss due to short read length splicing. It has significant advantages when sequencing genomes with large structural variants and high levels of repetitive regions [8]. For example, the PacBio RSI single-molecule real-time sequencing system, launched by Biosciences in 2010, has a read length of up to 20 kb and can produce 400 Mb of sequence per cycle. The MinON sequencer, developed by Oxford Nanopore Technologies and released in 2013, has a read length of up to 10 kb [9]. The ONome-9604 nanopore gene sequencer, launched in 2020 by Chengdu QiCarbon Technology Company, can achieve read lengths exceeding 150 kb and maintain a stable output of more than 500 Mb within 8 hours of data collection [10].

2.2.3. Portability

Nanopore sequencing technology does not require biomolecular modification, labeling or surface immobilization, PCR amplification, DNA polymerase or ligase, or deoxyribonucleoside triphosphate (dNTPS), so nanopore sequencers can be made very small. The MinION sequencer is approximately the size of a pen, measuring about 10 cm in length and weighing around 100 g. It connects to a computer via a high-speed USB port, earning it the nickname "USB flash drive sequencer" [11]. At the same time, the real-time, rapid and efficient characteristics of nanopore sequencing technology together with the "USB flash drive sequencer" make it portable. Real-time sequencing can be accomplished in a variety of complex environments, such as the laboratory, the field and even space. The small portable DNA sequencer has a broad application prospect in many fields such as biological research, clinical diagnosis, drug customization, food safety, agricultural research, environmental monitoring, security protection and so on.

2.2.4. RNA direct sequencing

A distinctive feature of nanopore sequencing is its capability for single-molecule sequencing, which allows for direct sequencing without the need for PCR amplification [12]. This approach simplifies the sample preparation process and avoids mismatches that can arise during amplification. Additionally, nanopore sequencing is not affected by the content of the sample, enabling the direct

sequencing of RNA and methylated DNA sequences. This method not only saves time and costs but also preserves the modification information of bases (A, G, C, and T) intact. By eliminating the need for reverse transcription of RNA into DNA, nanopore sequencing avoids the biases and potential introduction of mutations and errors that can result from amplification processes [13]. This makes it a powerful tool for accurate and comprehensive genomic analysis. Therefore, in nanopore sequencing, both DNA sequence and modification can be detected using the original signal information. The methylated cytosine can be read out directly, and the accuracy of detection can be as high as 92%~98% [14].

3. Application of nanopore sequencing

3.1. Application in clinical diagnosis

3.1.1. Diagnosis of rare and genetic diseases

Nanopore sequencing technology plays an important role in the clinical diagnosis of rare and genetic diseases. The key causes of rare diseases and genetic disorders often involve complex structural changes in the genome, such as sequence duplications, ectopic insertions, or deep intronic variants. Traditional sequencing technologies, with their short read lengths, frequently struggle to adequately cover these genomic regions, leading to incomplete data and potential oversight of critical variants. In contrast, nanopore sequencing technology excels in this regard due to its ability to theoretically detect the entire length of single-stranded nucleic acid molecules. This superior long read length capability enables a broader and more comprehensive detection of genomic regions, facilitating a more precise identification of the causative factors behind rare diseases [15]. In addition, the real-time nature of nanopore sequencing technology greatly reduces diagnostic time, providing more time and opportunity for patient treatment.

3.1.2. Infectious disease pathogen detection

Infectious disease detection has also benefited greatly from the use of nanoporous sequencing technologies. It can directly sequence DNAs or RNAs without the need for a number of complicated pre-processing steps like polymerase chain reaction amplification. Its detection equipment is small and portable, adapts to various complex environments, and operates quickly in real time. This features give it a special high efficiency in the identification of infectious diseases pathogen, which greatly aids in the detection of infectious diseases. This advantage was perfectly displayed during the outbreak of Ebola virus. Quick et al. designed a genomic detection system based on the MinION platform, which tested 142 samples of Ebola patients collected during the Ebola outbreak in Guinea, and discovered the spread of the virus between 2 countries, which made a great contribution to the prevention and control of the outbreak [16] .

3.1.3. Public health emergency response tools

Nanopore sequencing platforms are designed to be user-friendly and do not require extensive laboratory space or specialized facilities. They have minimal requirements for cleanliness, temperature, humidity, and other environmental conditions, making them highly versatile. The portability that nanopore sequencing devices can achieve (e.g. Oxford Nanopore MinlON) allows them to be used in resource-limited environments. In addition to this, nanopore sequencing technology can sequence DNA and RNA viruses in environmental water samples for rapid detection and analysis of environmental viruses in the field [17]. Its excellent environmental adaptability makes it a key tool for public health emergency response.

3.1.4. Cancer genomics screening

Nanopore sequencing technology has a wide range of applications in the study of cancer genomics. Similar to the diagnosis of rare diseases, the immediate cause of carcinogenesis is generally mutations in the structure, number, and deep introns of the genome, which are common in cancer and other genetic diseases. Nanopore sequencing technology offers long read length sequencing data, which is essential for comprehensive genome analysis. This capability allows researchers to effectively locate key genomic regions and mutation sites, facilitating the detection and analysis of complex chromosomal structural variations. When integrated with contemporary medical technologies, nanopore sequencing significantly enhances our research and understanding of tumor biology. At the same time, this supports personalized treatment strategies and promotes their development [18].

3.2. Applications in genomic research

3.2.1. Genome assembly and structural variation

Nanopore sequencing technology is excellent in detecting structural variations in genomes. Traditional sequencing technology requires short fragment genome splicing due to the limitation of short read length. Due to the polyploid, highly repetitive, and highly heterozygous nature of some animal and plant genomes, genome splicing is exceptionally difficult and splicing errors may

occur. Nanopore sequencing is characterized by long read length, which can read ultra-long DNA sequences in one go, well avoiding the problem of splicing sequences in traditional sequencing technology, which makes the test results more accurate and complete.

The genome can produce numerous large segment structural variants, such as deletions, inversions, and ectopic insertions, which are often associated with human diseases. Traditional short read sequencing technologies typically struggle to accurately detect these complex variants due to their limited read lengths. Nanopore sequencing is suitable for detecting large segment structural variants and resolving complex structural variants such as genome rearrangements and copy number variations. It presents promising prospects for development in disease research and diagnostics [19]. Meanwhile, the real-time nature of nanopore sequencing technology also gives the technology the opportunity to become a key tool for identifying multiple diseases caused by genomic structural variations, which is of great significance for the study of complex genomic diseases [20].

3.2.2. Paleontological research

Nanopore sequencing technology is an ideal tool for ancient DNA research. Ancient DNA is usually extracted from bones, teeth, plants and other biological remains and analyzed, but these short ancient DNA fragments are usually highly degraded, and sequencing of such DNA fragments usually requires splicing. However, such sequencing requirements are very limited for first and second generation sequencing technologies, while nanopore technology can directly sequence ancient DNA fragments without PCR amplification and reduce detection errors. The HeliScope genetic analysis system based on nanopore sequencing, introduced by Helicos Bioscience in 2008, requires less sample volume and has low requirements for sample quality, making it an ideal tool for the detection of ancient biological information [21]. In addition, the portability of nanopore sequencing can support detectors working in outdoor environments and shorten the cycle time of experiments. This technology provides new perspectives for exploring species evolution and genetic diversity [22].

3.2.3. Microbiological studies

Microbial populations are heterogeneous and complex, including a variety of microorganisms, such as bacteria and fungi, etc., and their genetic studies are usually conducted by macro-genomic studies. At present, the fastest progress in macro-genomic studies is 16SrRNA sequencing. Different highly variable regions in 16SrRNAs present in prokaryotic genomes reflect evolutionary differences among different microorganisms to a certain extent. Thus, 16SrRNAs have been widely used in prokaryotic taxonomic studies [23]. Macrogenomics studies require high read lengths for sequencing platforms, which are difficult to be met by traditional sequencing technologies. The advantage of long read lengths of nanopore sequencing allows sequencing of the entire 16SrRNA gene, which greatly improves the resolution of community structure [24]. At the same time, nanopore sequencing technology allows for the analysis of microbial speciation and metabolites to comprehensively and accurately assess microbial function in the environment.

4. Challenges and innovations

4.1. Shortcomings and challenges

Compared with traditional sequencing technology, nanopore sequencing technology has some shortcomings that limit the application of the technology despite its unique advantages. First, nanopore sequencing technology identifies sequences by analyzing the current changes generated when DNA or RNA molecules pass through the nanopore. However, this process can be affected by noise signals and other factors, leading to sequencing errors and reduced accuracy.

Moreover, although nanopore sequencing has a low cost for a single run, the low throughput of each run can result in high overall costs whenlarge volumes of data are required. Factors contributing to these costs include the materials used in the nanopore technology, disposable chips, sample preparation, data storage, and computational needs.

Furthermore, nanopore sequencing generates a significant amount of current signal data, which necessitates strong data processing and storage capabilities. This requirement poses challenges for the development and implementation of this technology.

4.2. Improvements and innovations

First of all, researchers are focusing on improving nanopore materials. By using more heat and voltage resistant nanopore materials, they can extend the life of nanopores, reduce costs, and accomplish higher quality data reads [25]. Optimizing the sequencing process reduces the use of expensive reagents and lowers operational costs. Meanwhile, in the future, nanopore sequencing is widely used, the corresponding equipment and materials are produced on a large scale, and the cost will be reduced [26].

Additionally, optimized nanopore design can enhance the stability, durability and accuracy of nanopore sequencing technology. Precise nanopore diameters and structures can reduce the influence of signal noise, current signals, and other factors on the results, improve the resolution of base recognition, and make up for the lack of accuracy of the existing protein or solid-state nanopore channels, as aswell improve their stability and durability.

The researchers also combined nanopore sequencing technology with artificial intelligence. By learning the relevant algorithms and models, they can reduce the errors generated in the sequencing process due to base identification, error correction, etc. At the same time, the artificial intelligence handles a large amount of data generated by sequencing, enabling accelerated data processing, which greatly improves the efficiency and the accuracy of the sequencing results. In addition, the combination of cloud computing and distributed computing technology with nanopore technology enables the remote processing of data to reduce the dependence on equipment. Compression algorithms and edge computing technology can also reduce the need for data savings in this technology.

5. Conclusion

Nanopore sequencing technology's real-time, long-length function enables it to read DNA and RNA fragments directly. At the same time, it also possesses high flexibility as well as portability that surpass those of traditional technology. These advantages make it play a great role in medical clinical diagnosis and genomics research, making it a powerful tool for genetic research. Although nanopore sequencing technology has made some breakthroughs by virtue of its advantages, there are also shortcomings such as low accuracy of sequencing results, high cost of acquiring a large amount of data, and the need for large amounts of data processing and storage at a later stage, which have led to certain limitations in the application of this technology. Researchers are working to enhance sequencing accuracy by improving nanopore materials, reduce costs by optimizing the process, and optimize data algorithms by combining post-processing data with artificial intelligence to improve processing efficiency.

However, this paper still has some limitations. For example, it is difficult to obtain access to all the latest advances in the development of nanopore sequencing technology because of limited literature, making it impossible to exhaust all the fields of application.

In conclusion, the emergence of nanopore sequencing technology marks that sequencing technology has entered a brand-new era. Looking ahead, the unending improvement and development of this technology will further promote the research of clinical diagnosis and genomics, and offer a broad application prospect in more fields.

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