

Development and Application of Nanopore Technology in Biosensing and Diagnostics

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Abstract: Nanopore technology has emerged as a powerful tool for biosensing, enabling real-time, single-molecule detection with high sensitivity and specificity. Over the past few decades, this technology has evolved significantly, transitioning from biological nanopores to solid-state nanopores, hybrid nanopores, and two-dimensional (2D) material nanopores, integrating advances in materials science, bioengineering, and computational modeling. The primary advantages of nanopore sensors include their ability to analyze nucleic acids, proteins, and small molecules without requiring labeling, making them ideal for applications such as rapid diagnostics and environmental monitoring. Despite these advantages, challenges such as stability issues, signal noise, and molecular translocation speed control remain critical areas of research. Recent developments, including the use of machine learning algorithms, the integration of 2D material nanopores, and improvements in fabrication techniques, have significantly enhanced the accuracy and efficiency of nanopore sensors. This review explores the historical development of nanopore technology, its advantages and limitations, the latest advancements, and fundamental sensing mechanisms, while also discussing its applications in biosensing, disease diagnostics, and environmental monitoring. Future trends and challenges in nanopore technology are considered, including efforts to improve sensor stability, enhance detection capabilities, and integrate artificial intelligence for real-time data processing.

Keywords: Component, Molecule, Nanopore Technology, Biosensing, Single-Molecule Detection

1. Introduction

Nanopore technology is a highly promising approach in molecular sensing, utilizing nanometer-scale pores to detect and analyze biomolecules by measuring disruptions in ionic current as molecules pass through the nanopore under an applied electric field. This method allows for high-resolution, label-free analysis of DNA, RNA, proteins, and small metabolites, making it an essential tool in genomics, proteomics, and disease diagnostics [1]. The origins of nanopore technology can be traced back to the early 1990s when researchers first demonstrated the feasibility of using biological nanopores, such as α -hemolysin (α -HL), for DNA sequencing. Since then, nanopore technology has undergone significant advancements, leading to the development of solid-state nanopores, hybrid nanopores, and 2D material nanopores, each offering unique advantages in terms of stability, sensitivity, and resolution [2].

Despite its transformative potential, nanopore technology faces several technical challenges, including signal noise, variability in nanopore fabrication, and the rapid translocation of molecules, which can limit detection accuracy. Addressing these challenges requires interdisciplinary research efforts across materials science, biophysics, and computational modeling. In recent years, artificial intelligence and machine learning have been integrated into nanopore sensing to improve data processing, enhance molecular recognition, and minimize errors in detection [3]. As this technology continues to evolve, its applications in biosensing and diagnostics are expected to expand, facilitating real-time, cost-effective molecular analysis for precision medicine and environmental monitoring.

2. Development, advantages, challenges, and mechanisms of nanopore technology

Nanopore technology has undergone several phases of development, beginning with biological nanopores in the 1990s, followed by the introduction of solid-state nanopores in the early 2000s, and more recently, the exploration of hybrid and 2D material nanopores. Biological nanopores, derived from protein channels such as α -HL and Mycobacterium smegmatis porin A (MspA), provide high specificity and uniformity in pore size, making them well-suited for DNA sequencing applications [4]. However, their reliance on lipid bilayers for structural integrity results in limited stability, restricting their use in long-term sensing applications.

To overcome these limitations, researchers developed solid-state nanopores fabricated from materials such as silicon nitride, silicon dioxide, and graphene. Solid-state nanopores offer greater mechanical durability, tunability in pore size, and compatibility with various experimental conditions. Nevertheless, they suffer from high background noise, inconsistent fabrication, and non-specific interactions with biomolecules, which can impact sensing accuracy [5]. Hybrid nanopores, which integrate biological and solid-state components, have been introduced as a potential solution to these challenges, combining the specificity of biological nanopores with the robustness of solid-state materials [6].

Table 1: DNA sequencing comparison.

Nanopore Type	Resolution	Mechanical Stability	Biocompatibility	Advantages	Disadvantages
Biological	High	Low	High	High specificity, suitable for long-read sequencing	Sensitive to environmental factors
Solid-State	Moderate	High	Low	Mechanically robust, suitable for high-throughput screening	High noise, variable pore size affects accuracy
2D Material	Highest	High	Low	Atomic thickness, high spatial resolution	Difficult fabrication, noise issues

As can be seen from Table 1, each of the three types of nanopores has its own advantages: biological nanopores have high sensitivity and good biocompatibility, but their extremely low mechanical strength and excessive sensitivity to the environment prevent them from performing well in various conditions. Solid-state nanopores are durable and robust, but they have low sensitivity and poor compatibility. 2D material nanopores have excellent spatial resolution, but their production is

difficult under current technological, and they cannot be applied on a large scale. Therefore, how to produce 2D material nanopores becomes the key.

The most recent advancements in nanopore technology involve 2D material nanopores, particularly those made from graphene, molybdenum disulfide (MoS₂), and boron nitride. These ultra-thin materials offer atomic-scale thickness, enhancing spatial resolution for detecting single molecules. Challenges related to fabrication precision, pore stability, and ionic current fluctuations remain significant hurdles in the widespread adoption of 2D material nanopores [7].

The fundamental mechanism of nanopore sensing is based on ionic current modulation. When a target molecule through a nanopore, it temporarily disrupts the ionic current, generating a characteristic signal. The magnitude and duration of these disruptions provide information about the molecule's size, shape, and charge, enabling precise identification and characterization. This mechanism underpins various applications, including DNA sequencing, protein analysis, and biomarker detection [8].

3. Applications and future directions

One of the most transformative applications of nanopore technology is DNA and RNA sequencing. Nanopore sequencing has revolutionized genomics by enabling rapid, long-read sequencing, facilitating advancements in personalized medicine, infectious disease diagnostics, and evolutionary biology. Unlike traditional sequencing methods, nanopore sequencing does not require PCR amplification or fluorescent labeling, reducing costs and simplifying sample preparation. The development of portable nanopore sequencing devices has further expanded its accessibility, allowing real-time genomic analysis in clinical and field settings [9].

Beyond nucleic acid sequencing, nanopore sensors are being used for single-molecule protein characterization, including the analysis of protein folding, ligand interactions, and post-translational modifications. These applications are critical for drug discovery, structural biology, and disease research. The ability to detect protein conformational changes in real time provides valuable insights into disease mechanisms and therapeutic interventions [10].

Nanopore technology has also been applied to disease biomarker detection, enabling the identification of cancer-related proteins, infectious disease markers, and neurodegenerative disease-associated proteins. The high sensitivity of nanopore sensors allows for early-stage disease detection, improving the accuracy of diagnostic tests and enabling timely medical interventions. COVID-19 detection systems have demonstrated rapid and reliable viral RNA identification, highlighting the potential of this technology in epidemic and pandemic response strategies [11].

Environmental and food safety monitoring is another growing application of nanopore sensors. Researchers have developed nanopore-based methods for detecting heavy metals, organic pollutants, and microbial contaminants in environmental and food samples. These applications are essential for ensuring public health and regulatory compliance, as nanopore sensors provide a rapid and cost-effective alternative to traditional analytical techniques [12].

Recent technological advancements have further enhanced the capabilities of nanopore technology. The integration of artificial intelligence and machine learning algorithms has significantly improved data analysis, enabling automated molecular recognition and reducing error rates in detection. The application of AI-driven models for nanopore sequencing has enhanced base-calling accuracy, improving the reliability of genomic data interpretation [13].

Another major advancement is the development of multi-modal sensing approaches, which combine nanopore technology with complementary detection methods such as optical and spectroscopic techniques. These hybrid detection systems have improved sensitivity and specificity, making nanopore sensors more versatile for complex biological and chemical analyses. Additionally,

the miniaturization of nanopore sensors has led to the creation of portable, point-of-care diagnostic devices, facilitating real-time medical testing in remote and resource-limited settings [14].

Future research in nanopore technology will focus on several key areas. Efforts to enhance detection resolution through improved nanopore design and signal processing techniques will be crucial for expanding the capabilities of this technology. New nanopore materials and hybrid designs will enable broader biomolecular and chemical analysis, while integration with microfluidics and wearable technology will facilitate real-time, on-site monitoring. Advances in AI-driven data analysis will continue to improve diagnostic accuracy and efficiency, paving the way for more reliable and accessible nanopore-based biosensing solutions [15].

4. Conclusions

Nanopore technology has made remarkable progress in recent years, establishing itself as a highly versatile and powerful platform for molecular analysis in biosensing, sequencing, and diagnostic applications. By enabling the real-time detection of individual molecules as they pass through nanoscale pores, this technology offers significant advantages in terms of speed, cost-effectiveness, and sensitivity, making it increasingly valuable across a wide range of scientific and clinical domains. Although technical challenges such as signal noise, variability in nanopore fabrication, and the complexity of data processing still present obstacles, continuous innovation across multiple disciplines is steadily overcoming these hurdles. Advances in materials science—particularly the development of novel two-dimensional and solid-state materials—are improving the precision and robustness of nanopores, while the integration of artificial intelligence and machine learning is revolutionizing signal interpretation, base-calling, and pattern recognition. At the same time, hybrid nanopore systems that combine the strengths of biological and synthetic components are opening up new possibilities for enhancing selectivity, stability, and control over molecular translocation.

As these technological improvements converge, nanopore sensors are expected to play an increasingly vital role in the advancement of precision medicine, enabling earlier disease detection, personalized treatment strategies, and rapid outbreak surveillance. Furthermore, their applicability extends to environmental monitoring, food safety, and synthetic biology, where real-time, high-throughput molecular detection is equally transformative. Looking ahead, the continued integration of nanotechnology, bioengineering, and computational innovations is likely to unlock the full potential of nanopore platforms, ushering in a new era of accessible, accurate, and efficient molecular diagnostics that will drive forward scientific discovery and improve human health on a global scale.

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