

Nanopore Technology for Future Point-of-Care Testing (POCT): System Integration and Commercialization Prospects

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Abstract:

The potential applications and potential business applications for nanopore sensing systems in point-of-care tests (POCT) will be covered in detail in this paper. With the COVID-19 crisis exposing the limitations of traditional point-of-care testing methods like LFA in terms of awareness, nanopore technology, with its benefits of no tagging, real-time recognition, and single-molecule resolution, has developed into a potent candidate to bridge the gap between POCT and high-sensitivity lab testing. The paper introduces the fundamental principle of nanopore sensing and its two detection strategies, direct detection and carrier-controlled detection, which both apply to various complex sample environments. The article provides further details on the POCT system integration process, including the inclusion of high-speed and low-noise electrical equipment, a smooth link with smartphones and sky platforms, and the design of microfluidic chips for test pretreatment, flexible solid-state nanopore arrays, and system integration. The paper assesses the challenges faced during commercialization, such as sample interference, pore blockage, and technical bottlenecks caused by difficult economic noise. It looks forward to solving these problems through fresh materials, edge AI, and low-power devices. Ultimately, nanopore technology has much potential for quick, sensitive, and multi-target point-of-care testing in neighborhoods, pharmacies, homes, and even far-off places. It provides a crucial technological foundation for marketing distributed open health control.

Keywords: Point-of-care diagnostic testing (POCT); Multiplex detection capability; Nanopore-based sensing; Engineering monetization process

1. Introduction

Later in 2019, the COVID-19 pandemic highlighted the importance of quick, distributed checking on a never-before-seen scale for common wellness. Millions of lateral-flow immunoassays (LFAs) were created worldwide to enable people to test themselves at home or perform decentralized triage. However, many false negatives, particularly early infection, hampered disease surveillance and proper isolation because of their lower sensitivity. Due to the high cost of the necessary equipment and the fact that the operation process relies on specialized technical knowledge, nucleic-acid amplification tests (NAATs), including qPCR, were still gold standard methods that could only be carried out in laboratories. The diagnostic efficacy gap between point-of-care clinical schemes using simple procedures and high-sensitivity laboratory testing methods has grown increasingly important as more powerful but still flawed recognition technologies are being developed. This disparity can only be resolved by using a sequencing diagnosis. When a single molecule traverses nanopore sensing systems, the inorganic recent changes, and the resultant electronic message characteristics, you include information about the molecule's size, cost, and construction. Because of its small, low-power electrical devices and real-time online readings, this single-molecule mode, which is unmarked, is inherently suitable for point-of-care testing (POCT). Nanopore technology has several benefits in relation to POCT. It can work with non-amplification processes, is portable, can quickly produce results, and can quickly enable continuous detection of several targets in a single test [1,2].

Unlike sequencing applications where nanopore platforms have already gained commercial traction (e.g., Oxford Nanopore Technologies' MinION), their adaptation to POCT requires additional integration [3]. The key requirements of this technology include automated sample preparation, robust and scalable solid-state equipment, portable and reliable electronic devices, as well as user-centered industrial design. This paper presents a conceptual system design and commercialization path for an onsite rapid testing (POCT) based on nanopores.

2. Principles of Nanopore Sensing and Biomedical Applications

2.1 Sensing Mechanism

Nanopore sensing systems are a sympathetic recognition technique based on nanoscale pores. In the field of biomedical research, it is frequently used. The main structure of this system consists of two holes filled with electrolyte

solution, and a pretty little pore connects these two holes. A second molecule can simply pass through this pore because its diameter is typically at the nanometer level. Also, the pore's interior has a DNA ring category with an end fixed to the side of the porosity to allow interaction with molecules passing by. Experts will use a specific energy between the two chambers to initiate the electrolyte particles' flow and create a stable atom present during the procedure. When natural substances that target the pore enter, such as DNA, RNA, or proteins, they partially or completely stop the atom movement, changing the current transmission. The natural characteristics of the biological molecules passing through the pore, such as length, cost mass, and three-dimensional architecture, can be inferred from this change in presence, which can be accurately measured and its amplitude, duration, and fluctuation pattern [4]. Nanopore sensing technologies identify the specific molecules' current changes through microscopy structures. It has wide application potentials in different fields, including protein sequencing, the recognition of disease biomarkers, and environmental monitoring due to its advantages, such as the lack of labeling, real-time capability, and higher resolution. There are presently two main types of nanopores used. Solid-state nanopores have attracted attention because of their higher security and scalability, while natural nanopores have attracted attention due to their good stability and tunability [5]. Two main forms of monitoring methods have been developed. The operation of direct detection is easy, but it's sensitive to noise. The other is the carrier-mediated detection, which uses sensor molecules and barcodes to transform specific molecules into recognizable signals and achieves transmission amplification and cinema detection [6].

2.2 Direct and Carrier-Mediated Detection

A chemical identifying technique based on nanopore technology is known as direct detection. The key to its success is that it can detect nucleic acids or proteins, movement patterns in nanopores without modifying them further. There is no need for tagging or a plain procedure with this method. It is also very simple and effective. However, direct detection methods encounter particular challenges when dealing with difficult clinical samples. These parts may cause significant background noise, interfering with the target substances, signal recognition, because clinical samples typically contain many non-target molecules. In some specimens, the target particles, concentration is low, making it difficult to distinguish the signal from the sound, which affects the detection's sensitivity and accuracy [6]. In order to increase their identification performance in difficult situations, direct detection methods

frequently require combining more sophisticated message processing techniques and test pretreatment techniques.

In contrast to direct detection, carrier-mediated detection uses special probes made of synthetic DNA particles or structures. These probes especially bind to the specific substance. The intricate formation during passing through the sequencing undergoes a distinctive electric signal change when the sensor binds to the destination. These signals can be designed using a variety of probe sequences to discover various target molecules and have great programmability. This approach substantially expands the range of sequencing detection applications, enabling several detection options. That is to say, carrier-controlled detection can instantly identify various target molecules in the same experiment, substantially improving detection efficiency and information acquisition ability [7]. Additionally, this approach exhibits good resilience in listening to various monitoring requirements due to the probes, extremely flexible design.

2.3 Biomedical Applications

2.3.1 Nucleic acids

Nanopores enable precise discrimination of nucleic acids at the molecular level, allowing for the identification of viral subtypes, antimicrobial resistance (AMR) genes, and single-nucleotide polymorphisms (SNPs). By discovering certain abnormalities in viral RNA straight from clinical samples without the need for replication in some cases, nanopore sequencing has been used to effectively separate SARS-CoV-2 varieties, such as Omicron sublineages [8]. In order to quickly identify drug-resistant pulmonary infections, it has been able to identify AMR genes in fungal circumstances, like those that confer carbapenem resistance in *Klebsiella pneumoniae*, from breathing tests [9]. By amplifying low-amount targets before sequencing, pairing nanopore technology with isothermal amplification techniques like loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA) significantly increases sensitivity. LAMP-integrated nanopore sequencing has been used in low-virgin-load tests to identify SARS-CoV-2 RNA with better accuracy, reducing false negatives [8].

2.3.2 Proteins and peptides

Aptamers and antibodies: DNA conjugates allow direct protein processing via DNA labels translocated through nanopores, facilitating programs like cytokine profiling and tumor biomarker detection. In tumor biomarker detection, antibody-DNA compounds have been used to identify prostate-specific protein (PSA) in blood, providing early cancer screening with differences down to femtomolar

levels [10]. Another instance involves aptamer-conjugated nanopores for detecting carcinoembryonic protein (CEA), a typical colorectal cancer marker, where the aptamer's structural change upon binding generates specific current blockades for quantification [10]. Nanopores, which can identify post-translational changes like phosphorylation in thioredoxin proteins as indicators of neurodegenerative diseases, have even advanced in this area [11].

3. Toward POCT System Integration

3.1 Workflow and Design Philosophy

An optimized nanopore-based POCT workflow that includes a smartphone (with integrated AI) and microfluidics-based pretreatment, nanopore array sensing, and a portable reading device (for showing and cloud connectivity). Anyone can use the system even if they aren't an expert in this field because the principal design concept emphasizes automation, portability, and ease of use.

3.2 Microfluidic Processing

One of the biggest obstacles to POCT advancement is the preparation of samples. Because of the large number of proteins, salt, and other possible intruding components in clinical samples, such saliva and blood, may significantly impact the stability and precision of sequencing recognition. To solve these challenges, a biodegradable micro device was designed and proposed. Important pre-processing steps, such as trial apoptosis, nucleic acid purification, and cushion replacement, may be automatically completed with this device. The entire procedure runs smoothly thanks to its outstanding automation performance. The device uses blood action or vacuum-aided drive to facilitate smooth flow, making for easy operation and suitability for on-site testing environments. The whole pretreatment procedure can be completed relatively quickly, completely satisfying the scientific diagnosis's requirements and ensuring the accuracy of the evaluation results.

3.3 Solid-State Nanopore Arrays

Unlike those employed in medical research, POCT techniques require multiplexed sequencing arrays for faster detection and reliability. These devices have the following essential characteristics for social operation. Each pore serves as a sensor independently or as a component of the outfit. Antifouling coatings and surface functionalization prevent pore blockage, thus increasing the stability of the equipment and fixing a major issue of niche deployment [5].

3.4 Edge Intelligence and Portable Electronics

Low-noise message synthesis, high-speed automation, and a small battery-powered style are the requirements for the electronics used in sequencing POCT. Integrated edge AI algorithms allow real-time classification of detection events into particular types of targets.

In the presence of sound, CNN processes the latest besieged pulses and identifies the correct targets of attention [12]. Because of the top assumption, data privacy and dependence on fog communication can be reduced.

3.5 Smartphone and Cloud Integration

As a multi-functional gateway in the system, smartphones are crucially important. First, they can provide the exam results in simple, natural language accessible to non-professional customers. Second, they have information management capabilities, which enable the initial signals to be securely encrypted and uploaded to the sky, keeping data transfer private and secure. Epidemiological monitoring is also accomplished through a cloud-based physical screen. The system provides information to support public health responses by quickly analyzing and interpreting the test results from various stations, monitoring the spread pattern of infectious diseases in near-real time, and providing information support for public health messages.

4. Commercialization Prospects and Challenges

4.1 Market Opportunity and Value Proposition

The latest POCT industry is still searching for a comprehensive system to support rapid on-site testing while maintaining laboratory-level detection accuracy. Nanopore sensing systems perfectly meet this prerequisite. It can identify specific molecules without markers, produce digital signals in real time, and be used to identify nucleic acids, proteins, polypeptides, and various targets at once [13]. These characteristics perfectly tackle the shortcomings of conventional detection techniques: some tests are straightforward but not sensitive enough, while others can only be performed in laboratories. Nanopore technology really fills this difference. Thus, it can be used in various applications, including monitoring antimicrobial resistance, supplementary tumor diagnosis, and economic and public health. This platform is especially appropriate for use in community clinics, pharmacies, inside testing points within companies, and even in the future, under governmental requirements, it can also be used in the patient's vicinity or at home because the entire testing process, from sample processing to result output, is integrated very nicely,

including microfluidic pretreatment, solid-state nanopore chips, portable low-noise electronic equipment, edge computing technology, and the ability to connect to mobile phones and the cloud.

4.2 Challenges

Due to its advantages of real-time monitoring and no labeling, nanopore technology has a lot of potential in the POCT industry. This technology has led to identifying novel coronavirus variants, identifying *Klebsiella pneumoniae*'s resistance genes, and the identification of tumor biomarkers like prostate-specific antigen (PSA). However, integrating this technology into a convenient and user-friendly point-of-care testing system also faces many challenges, generally stem from the detection mechanism principle, detection strategy, and the overall integration method. Problems like complex sample interference, solid-state nanopore pore blockage, noise interference, and reference intake may ultimately be the main obstacles to its shift from laboratory to practical use.

First, one of the biggest technical bottlenecks facing today is test processing. Many proteins, salts, and other meddling ingredients are typically present in clinical samples, such as saliva, blood, or breathing fluids, affecting nanopores' stability and reliability. The background noises created by non-target substances in low-virus-load tests may result in false negative outcomes, as illustrated by the direct detection of low-amount RNA of the novel coronavirus. In order to increase the recognition awareness, amplification techniques like LAMP are frequently employed. However, the system pattern has higher requirements because it can achieve automated trial lysis and purification and complete the processing quickly while maintaining the equipment's portability.

Second, in real-world software, solid-state nanopore arrays encounter the issue of pore blockage. Antifouling coatings and surface functionalization techniques are essential for preventing the deposition of biological molecules in various disease detection, such as the coinfection of the novel coronavirus and *Klebsiella pneumoniae*. Long-term contact with intricate samples, such as bronchoalveolar cleansing liquids, may result in irreversible pore blockage, which lowers the detection efficiency and raises the need for equipment replacement. Although natural nanopores exhibit some tunability, they are far less durable than solid-state nanopores. The production difficulty may be considerable, and the cost will increase significantly if they are expanded to various structures to improve stability. Changes in chemical structure can also result in unstable indicators or blockages in protein detection, such as when activated thioredoxin peptides are

detected.

Additionally, complex specimens, high levels of noise interference pose a significant issue. When no replication techniques are available for low-concentration specific substances, such as the mutant places in the RNA of the Omicron variant, their indicators are difficult to distinguish from the background noises in clinical samples. Other factors, such as heat variations in the on-site monitoring environment, may contribute to the accuracy of the recognition results. Eventually, the transition to POCT also requires substantial resources to develop one-time chips and arrays, despite the program design's goal of automation and ease of use. In some regions with limited resources, this partially limits their development and software.

5. Future Perspectives

Major potential exists for POCT thanks to nanopore technology, which has been demonstrated to detect SARS-CoV-2 variants, AMR chromosomes in *Klebsiella pneumoniae*, and indicators like PSA. Coming developments will break down the existing barriers, making it possible to perform diagnostic tests anywhere in the world.

Advanced microfluidic chips will simplify test planning, integrating phagocytosis and replication within a short time for sophisticated examples like the mouth, improving the detection of viral SNPs. A graphene-based sequencing array may increase strength and reduce blocking in multiplexed assays for coinfections like SARS-CoV-2 and flu. By reducing reliance on synthesis, machine learning may increase sensitivity for solutes like CEA. AI-optimized DNA carriers may support syndromic panels that detect pathogens like fungal 16S rRNA with little crosstalk. Blockchain and low-power computing combined with light edge AI and low-power electronics will enable real-time analysis of laptop data for microbiology. Cost-effective designs will make diseases like Ebola more accessible, making it easier for non-experts to observe them.

6. Conclusion

This paper conducts an in-depth discussion on the prospects of nanopore technology in the coming POCT. Traditional rapid diagnostic techniques, like LFA, have considerable sensitivity issues, despite their simplicity of use. They are more vulnerable to producing false negative effects, especially in the early stages of infections with small viral loads, thus affecting the effectiveness of the incident power. In comparison, nanopore technology can be used in rural areas or areas with limited resources, with its distinct advantages including the absence of labeling

and real-time single-molecule recognition. The two major monitoring strategies for specimens with simple works are further explained in this paper. Direct detection is suitable for samples with simple compositions and has the advantage of quick operation, but it is prone to interference from background noise. While carrier-mediated detection can have many monitoring requirements, it demonstrates better performance in difficult clinical samples. It achieves transmission amplification and specificity enhancement through means like DNA probes. In terms of the particular design of the POCT system, this article proposes an integral solution, covering a microsample preprocessing module, a reliable-state nanopore array, an edge AI electronic processing unit, a smartphone display interface, and a cloud data connection, constructing a very automated and user-friendly detection platform. This system enhances data protection and distant epidemiological monitoring functions and significantly enhances detection awareness. Although microscopy POCT techniques have shown tremendous potential for application, their monetization still faces many challenges. High-precision AI engine processing is required to recognize low-concentration specific molecules, proteins, and water components in difficult samples that may interfere with the diagnosis signals. The problem of pore blockage can reduce the equipment's lifespan. Additionally, disposable products research and production costs are relatively higher, limiting their application in resource-dense areas. To break through the existing bottlenecks and advance the development of genuinely diverse and specific point-of-care testing platforms, the potential growth direction should concentrate on integrating and applying new materials, advanced AI algorithms, graphene nanopore technology, blockchain, and other emerging technologies.

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