

Viewpoint

Running Title: Nanopore Sequencing for Personalized Treatment of Oral Cancer

Received: June 29, 2023; **Accepted:** December 10, 2024

Advancing Oral Cancer Detection and Treatment with Nanopore Sequencing Technology

Anitha Pandi, PhD student, Vijayashree Priyadharsini Jayaseelan [♦], PhD

Clinical Genetics Lab, Centre for Cellular and Molecular Research (The Blue lab), Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai, Tamil Nadu, India

♦Corresponding Author

Vijayashree Priyadharsini Jayaseelan, PhD

Clinical Genetics Lab,

Centre for Cellular and Molecular Research (The Blue lab),

Saveetha Dental College and Hospital,

Saveetha Institute of Medical and Technical Sciences [SIMATS],

Saveetha University, Chennai, Tamil Nadu, India

Email: vijayashreej.sdc@saveetha.com

Introduction

Oral cancer is a significant public health issue, with a high morbidity rate and limited treatment options. Traditional methods of diagnosing oral cancer, such as histopathological examination and imaging techniques, have their limitations in terms of accuracy and sensitivity.¹ However, there has been a remarkable advancement in molecular biology and genomics to overcome these limitations, particularly with the emergence of high-throughput technologies, such as nanopore sequencing (NS).² This sequencing procedure is a revolutionary technology that allows for the direct analysis of DNA and RNA molecules, providing a real-time and single-molecule approach to sequencing. NS involves passing single-stranded DNA or RNA molecules through a nanopore embedded in a membrane. An electrical current is applied across the membrane. As the nucleic acid strands pass through the nanopore, they cause disruptions in the current. These disruptions are unique to each nucleotide (adenine, thymine, cytosine, and guanine in DNA; adenine, uracil, cytosine, and guanine in RNA), allowing the sequencer to read the sequence of the molecule in real-time.³ The two primary types of nanopores used are

biological nanopores derived from proteins and solid-state nanopores made from synthetic materials.⁴

Biological Nanopores

Biological nanopores are proteins with transmembrane pores that can be embedded in a lipid bilayer. Various engineered nanopore proteins and strategies are deployed to improve the sensitivity of specific analytes. An exotoxin-based alpha-hemolysin channel is one of the well-studied biological nanopores. Several other pore proteins, such as MspA and Phi29, are also employed for sequencing ssDNA, dsDNA, peptides, and small proteins.⁵

Solid-state Nanopores

Solid-state nanopores are emerging tools in NS, a technique for DNA analysis. These nanopores, made from materials like silicon nitride or graphene, offer robustness and chemical stability compared with biological nanopores. They detect changes in ionic currents as DNA strands pass through the nanopore. Recent advancements have improved fabrication precision, sensitivity, and throughput.⁶ Solid-state nanopores provide high-resolution data and can be engineered for specific sequencing

applications, enhancing their utility in genomics and personalized medicine. Their versatility and durability make them promising for future sequencing technologies, pushing the boundaries of analysis of biomarkers.⁷

Applications of NS

Screening and early detection of oral cancer

NS has shown significant potential in the early detection of oral cancer. Traditional diagnostic methods, such as biopsies and imaging, often detect cancer at later stages. They can identify genetic mutations and epigenetic modifications associated with oral cancer in its early stages using minimally invasive samples like saliva or blood. It can prompt intervention and treatment by detecting oncogenes, tumor suppressor genes, and specific mutational signatures early. Additionally, NS offers a promising approach for liquid biopsy procedures, allowing for efficient and non-invasive detection of genomic alterations in cancer cells. This technology enables the analysis of circulating tumor DNA in blood samples, providing valuable insights into tumor heterogeneity and evolution with its high sensitivity and throughput capabilities (Figure 1).⁸

Genomic profiling for treatment planning

NS provides a significant advantage by generating long reads, enabling a more comprehensive analysis of complex genomic regions. This is especially beneficial in oral cancer diagnosis, as structural variants, gene fusions, and large deletions or insertions are common. A new technology has been developed by combining Oxford Nanopore Technology and CyclomicsSeq to investigate the TP53 mutation in the circulating tumor DNA at a frequency of 0.02%. This technology had a proven advantage of sequencing short read sequences for the detection of single nucleotide variants, structural variants, and methylation profiling of cell-free DNA.⁹ NS can precisely detect these alterations, offering a detailed profile of the genomic makeup of the tumor. This thorough analysis helps identify potential therapeutic targets and provides insights into tumor behavior.¹⁰

Monitoring treatment response

NS has emerged as a promising tool for monitoring treatment response in oral cancer patients. By allowing for real-time, high-throughput analysis of DNA and RNA molecules, clinicians can track changes in tumor genetics over the course of treatment. This technology can personalize therapy and adapt treatment plans based on the molecular profile of the tumor. With its ability to provide rapid and accurate results, NS holds great promise in improving outcomes for patients with oral cancer.¹¹

Pre-symptomatic testing

Several studies have stated a strong correlation between family history of cancer (FHC) positivity and prognosis in oral cancer patients. Therefore, pre-symptomatic testing should be offered to individuals with FHC. A study analyzed the 39 Kb structural variant causing Lynch syndrome (LS). Oxford Nanopore MiniON mapped the inserted sequence and insertion breakpoints, revealing the presence of the same variant in two unrelated Norwegian families with LS, which was seldom detected using targeted exon sequencing or multiplex-ligation-dependent probe amplification.¹² Once the pathogenic variants are detected in high-risk individuals, the results should be clinically correlated by performing additional tests to include or rule out conditions related to a specific disease type. The interpretation of the rare variant/s identified in individuals can be derived by performing computational analysis to deduce the pathogenicity of the variants.

Advantages of NS

The real-time sequencing capability of nanopore technology offers a significant advantage in clinical settings. Traditional sequencing methods often require weeks to process and analyze samples, whereas NS can provide results in hours. This rapid turnaround is crucial for oral cancer patients, allowing for quicker decision-making regarding treatment plans. Early intervention can significantly impact patient outcomes, making NS a valuable tool in clinical diagnostics. This method is highly scalable and capable of analyzing multiple samples simultaneously.

This high throughput is essential for large-scale screening programs and population-based studies.¹³

In addition, the portability of NS devices, such as Oxford Nanopore MinION, enables on-site testing and analysis, making it accessible in various clinical and field settings. It is a cost-effective option compared with other sequencing technologies. Its lower cost per run and the ability to use minimal reagents make it an attractive choice for widespread clinical use. This affordability can facilitate routine screening and monitoring of high-risk populations, contributing to the early detection and prevention of oral cancer. Genetic mutations and epigenetic modifications, such as DNA methylation, are important factors in the development of oral cancer. NS can directly identify these modifications without requiring bisulfite treatment, which is necessary for other sequencing methods. This ability enables a more thorough cancer genome analysis, offering insights into gene regulation and potential biomarkers for early diagnosis.¹⁴

Potential Limitations

Despite its advantages, NS has some technical limitations. While its accuracy is improving, it is still lower than traditional sequencing methods like Illumina. Errors in base calling and difficulties in sequencing homopolymeric regions can impact the reliability of results. Continuous advancements in nanopore technology and bioinformatics tools are needed to address these challenges and enhance accuracy.¹⁵ The large volume of data NS produces poses storage, management, and analysis challenges. It is crucial to have advanced computational tools and reliable bioinformatics pipelines to process and interpret the data effectively. Integrating sequencing data with clinical information necessitates interdisciplinary expertise and seamless collaboration between researchers, clinicians, and bioinformaticians. NS for diagnosing oral cancer in clinical settings needs thorough validation and standardization.¹⁶ It is crucial to develop standardized protocols and quality control measures and obtain regulatory approvals for its routine clinical use. Additionally, systematic workflows have to be developed for

training healthcare professionals to effectively use and interpret NS data to integrate it into the diagnostic module.

Future Perspectives

The future of NS in oral cancer diagnosis is quite promising. Ongoing nanopore technology advancements, including accuracy, throughput, and cost-efficiency, will further enhance its diagnostic capabilities. Integrating NS with other omics technologies, such as transcriptomics and proteomics, can provide a holistic view of cancer biology, paving the way for personalized medicine and targeted therapies. In conclusion, NS represents a transformative approach to diagnosing oral cancer. Its real-time sequencing, comprehensive genomic profiling, and cost-effectiveness offer advantages over traditional methods. While technical limitations and clinical implementation remain, the continuous evolution of nanopore technology holds great promise for improving early detection, treatment, and, ultimately, patient outcomes in oral cancer.

Acknowledgments

None declared.

Funding

None declared.

Authors' Contribution

AP: Manuscript drafting, formatting, editing and preparation of figures; JVP: Conceptualization, manuscript drafting, manuscript review, overall supervision and approval. All authors read and approved the final manuscript version and agree with all parts of the work in ensuring that any queries about the accuracy or integrity of any work component are appropriately investigated and handled.

Conflict of Interest

None declared.

References

1. Abati S, Bramati C, Bondi S, Lissoni A, Trimarchi M. Oral cancer and precancer: A narrative review on the relevance of early diagnosis. *Int J Environ Res Public*

- Health.* 2020;17(24):9160. doi: 10.3390/ijerph17249160. PMID: 33302498; PMCID: PMC7764090.
2. Augustine D, Sowmya SV, Haragannavar VC, Yousef A, Patil S, Gujjar N, et al. Nanopore sequencing technology in oral oncology: A comprehensive insight. *J Contemp Dent Pract.* 2022;23(2):268-75. PMID: 35748459.
 3. Wang Y, Zhao Y, Bollas A, Wang Y, Au KF. Nanopore sequencing technology, bioinformatics and applications. *Nat Biotechnol.* 2021;39(11):1348-65. doi: 10.1038/s41587-021-01108-x. PMID: 34750572; PMCID: PMC8988251.
 4. Haque F, Li J, Wu HC, Liang XJ, Guo P. Solid-state and biological nanopore for real-time sensing of single chemical and sequencing of DNA. *Nano Today.* 2013;8(1):56-74. doi: 10.1016/j.nantod.2012.12.008. PMID: 23504223; PMCID: PMC3596169.
 5. Feng Y, Zhang Y, Ying C, Wang D, Du C. Corrigendum to 'Nanopore-based fourth-generation dna sequencing technology' [GPB 144 (2015) - GPB 13/1 (4-16)]. *Genomics Proteomics Bioinformatics.* 2015;13(6):383. doi: 10.1016/j.gpb.2016.01.001. Erratum for: *Genomics Proteomics Bioinformatics.* 2015;13(1):4-16. doi: 10.1016/j.gpb.2015.01.009. PMID: 31283804; PMCID: PMC4747661.
 6. Wei J, Hong H, Wang X, Lei X, Ye M, Liu Z. Nanopore-based sensors for DNA sequencing: a review. *Nanoscale.* 2024;16(40):18732-66. doi: 10.1039/d4nr01325e. PMID: 39295590.
 7. Zhu L, Xu Z, Gao Y, Sun N, Qiu L, Zhao J. Highly sensitive detection of tumor cell-derived exosomes using solid-state nanopores assisted with a slight salt gradient. *ACS Appl Mater Interfaces.* 2024;16(37):49218-26. doi: 10.1021/acsami.4c14224. PMID: 39240779.
 8. Levkova M, Chervenkov T, Angelova L, Dzenkov D. Oxford nanopore technology and its application in liquid biopsies. *Curr Genomics.* 2023;24(6):337-44. doi: 10.2174/01138920292866322311270557
 9. Huang X, Duijf PHG, Sriram S, Perera G, Vasani S, Kenny L, et al. Circulating tumour DNA alterations: emerging biomarker in head and neck squamous cell carcinoma. *J Biomed Sci.* 2023;30(1):65. doi: 10.1186/s12929-023-00953-z. PMID: 37559138; PMCID: PMC10413618.
 10. Dixon K, Shen Y, O'Neill K, Mungall KL, Chan S, Bilobram S, et al. Defining the heterogeneity of unbalanced structural variation underlying breast cancer susceptibility by nanopore genome sequencing. *Eur J Hum Genet.* 2023;31(5):602-6. doi: 10.1038/s41431-023-01284-1. PMID: 36797466; PMCID: PMC10172360.
 11. Lau BT, Almeda A, Schauer M, McNamara M, Bai X, Meng Q, et al. Single-molecule methylation profiles of cell-free DNA in cancer with nanopore sequencing. *Genome Med.* 2023;15(1):33. doi: 10.1186/s13073-023-01178-3. PMID: 37138315; PMCID: PMC10155347.
 12. Bjørnstad PM, Aaløkken R, Åsheim J, Sundaram AYM, Felde CN, Østby GH, et al. A 39 kb structural variant causing Lynch Syndrome detected by optical genome mapping and nanopore sequencing. *Eur J Hum Genet.* 2024;32(5):513-20. doi: 10.1038/s41431-023-01494-7. Erratum in: *Eur J Hum Genet.* 2024;32(5):601-2. doi: 10.1038/s41431-023-01519-1. PMID: 38030917; PMCID: PMC11061271.
 13. Lin B, Hui J, Mao H. Nanopore technology and its applications in gene sequencing. *Biosensors (Basel).* 2021;11(7):214. doi: 10.3390/bios11070214. PMID: 34208844; PMCID: PMC8301755.
 14. Sakamoto Y, Sereewattanawoot S, Suzuki A. A new era of long-read sequencing for cancer genomics. *J Hum Genet.* 2020;65(1):3-10. doi: 10.1038/s10038-019-0658-5. PMID: 31474751; PMCID: PMC6892365.
 15. Jain M, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, et al. Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nat*

Biotechnol. 2018;36(4):338-45. doi: 10.1038/nbt.4060. PMID: 29431738; PMCID: PMC5889714.

16. Cretu Stancu M, van Roosmalen MJ, Renkens I, Nieboer MM, Middelkamp S, de Ligt J, et al. Mapping and phasing of

structural variation in patient genomes using nanopore sequencing. *Nat Commun.* 2017;8(1):1326. doi: 10.1038/s41467-017-01343-4. PMID: 29109544; PMCID: PMC5673902.

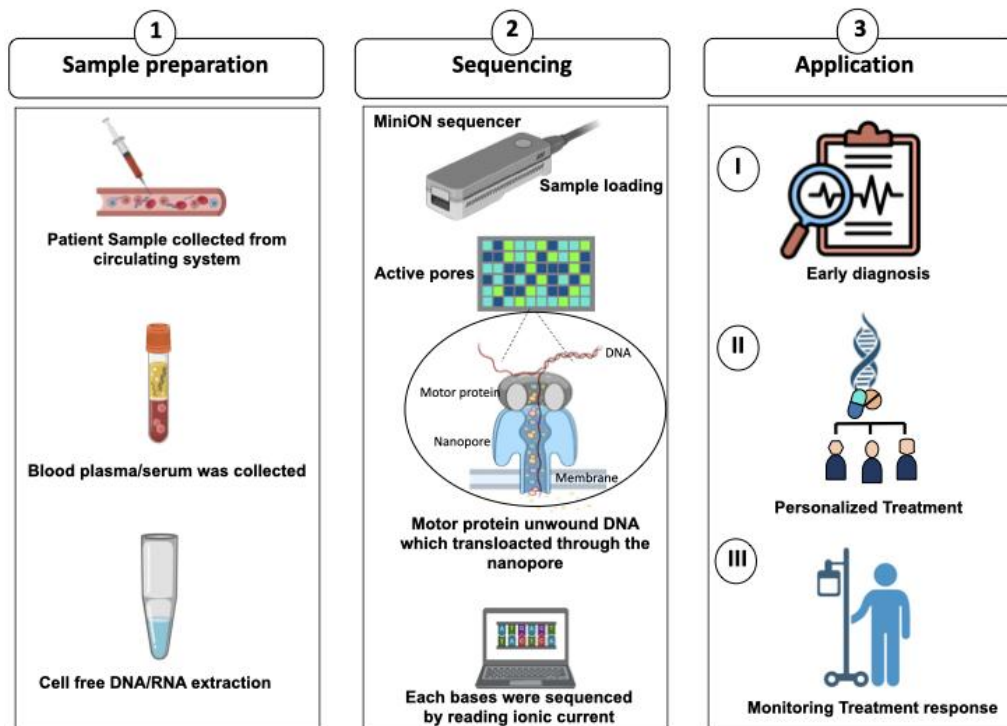


Figure 1. This figure depicts the workflow of circulating DNA/RNA sequencing from sample preparation to application, including early diagnosis, personalized treatment, and monitoring treatment response using nanopore technology.