

NANOPORE TECHNOLOGY FOR DNA SEQUENCING AIDS IN CLINICAL DIAGNOSIS OF GENETIC DISORDERS IN NEWBORN SCREENING: A MINI REVIEW

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Received: March 1, 2017; Accepted: March 20, 2017

Abstract: *Since its inception many modifications have been taken place and now nanopore technology has become technology of choice for DNA sequencing. Since it is simple, quick, accurate and inexpensive, it is very useful for detection of inborn metabolic disorders; sometimes patients of such diseases do not survive for long and clinician needs quick results. Moreover this technique dose not required large sample size.*

Key words: nanopore, dna sequencing, genetic disorders

INTRODUCTION

Deoxyribonucleic acid or DNA is a biomolecule that contains the instructions an organism needs to grow, maintain and reproduce. It is most mysterious biomolecule present inside every cell, and passed down from parents to their children through egg (nucleus and mitochondria) and sperm (only through nucleus). The order of nitrogen bases in a DNA sequence forms genes, which tells cells how to make the gene specific proteins for specific function to perform. Most of us carry a few defective genes, may be due to mutation, which produces defective proteins (enzymes) with no signs of disease and many of these can only contribute to susceptibility; nevertheless, most of the times they are responsible for expressing diseases; many syndromic diseases were identified with specific genomic mutations [1,2]. In addition to human syndromes in solving and management of inborn genetic disorders and childhood cancer were also benefited with recent development of modern technologies in Genome sequencing [3,4].

Impact of Nanotechnology in Diagnosis: Nanotechnology has revolutionized medical laboratory diagnosis [5,6]. Clinical application of molecular technologies to elucidate, diagnose and monitor human diseases is referred as molecular diagnosis [7,8]. Combining advances in related disciplines such as nanotechnology, biotechnology and pharmaceuticals, nano medicine offers the potential move to recognition and characterization of any particular disease at the very early (even pre-symptomatic) stage by non-invasive methods [9]. This technology, at least have broken new grounds in in vivo diagnosis and single cell diagnostics [10].

One of the early applications of nanotechnology in MRI was the use of paramagnetic iron oxide particles [9]. When taken up by healthy hepatocytes, these particles could help to distinguish between normal and cancerous liver cells. Protein nanobiochips can detect traces of proteins in biological fluids that are not detected by conventional immunoassays. Gold particles (~13 nm) attached with small pieces of DNA can detect millions of different DNA

sequences simultaneously.

Management of diseases and healthcare can be more effective if the clinical diagnosis is rapid, accurate, sensitive, non invasive and inexpensive. More than 1,200 genetic disorders have been identified; some of which, now could be diagnosed properly because of improve technologies. Molecular basis of a vast majority of these diseases is not yet clear. Modern genomics has shown that just one mutation can be the difference between successfully treating a disease and having it spread rampantly throughout the body.

Since 1989, when first successful attempt was made to diagnose genetic defects before embryo implantation in human, this diagnostic technique gained lot of importance. After the discovery of complete genome of *Mycoplasma genitalium*, 8% error rate in the annotation for 340 genes was found. If such error rates are extrapolated to the human genome, the outcome and consequences can easily be imagined. In order to avoid such errors, verification of the gene products with protein-based methods is very essential. Generally, genetic defects are tested for by PCR, allows amplification of a selected DNA sequence of interest and chromosomal abnormalities by FISH techniques [11,12].

Sanger's Capillary Electrophoresis DNA sequencing methodology is time consuming, cumbersome, hence become more expensive [13]. Since the 1990s, Church and other researchers have been investigating an alternative method to sequence DNA called nanopore-based sequencing-by-synthesis (Nanopore-SBS). Nanopores are tiny holes within a membrane separating two different electrolyte solutions. By applying a voltage differential, a continuous stream of small charged ion molecules can be made to pass through each pore, from one side of the membrane to the other (see for references) [14].

The new sequencing engine contains seven protein subunits that together build a suitable nanopore complex. Only one of them can be specifically conjugated to a DNA polymerase enzyme that is positioned right at the pore opening.

Being able to multiplex and individually analyze many DNA sequences electronically on the same

chip at the same time, compared with conventional sequencing procedures performed with much less throughput, as well as expensive reagents and machines, has the potential to dramatically lower the costs of sequencing. In the Editorial for *Ind J Clin Biochem* [15] entitled Nanopore Technology: A Simple, Inexpensive, Futuristic Technology for DNA Sequencing, I described that "With the development of Nanopore technology, now it became easy to sequence single strand DNA molecules by passing through the nanopores." Unlike previous technologies in Nanopore technology the probe molecule checks both strands of the target double helix for mutations rather than just one, which explains the increased specificity.

The technique: Most of the current DNA sequencing technologies requires working with short snippets of DNA, typically 50 to 100 nucleotides long. These must be processed by large sequencers in a laboratory. The cumbersome process can take days to weeks to complete. Nanopore technology takes advantage of the small, tunnel-like structures found in bacterial membranes. In nature, such pores allow bacteria to control the flow of nutrients across their membranes. A pore surrounded by proteins or peptides in a solid-state membrane which allows a single-molecule pass through it, is termed as nanopore. With the development of Nanopore technology, now it became easy to sequence single strand DNA molecules by passing through the nanopores. The nanopores were created in an insulating membrane separating two chambers filled with conductive electrolyte. Charged molecules are driven through the pore under an applied electric potential (a process known as electrophoresis) by modulating the ionic current through the nanopore. This current reveals useful information about the structure and dynamic motion of the molecule [16].

When DNA strands are fed through a nanopore with a voltage difference across the pore, each molecule in the DNA strand by the amount of current that flows across the nanopore can be identified. The proper sequence of the bases adenine, thymine, guanine, and cytosine which connect single strands of DNA into a double helix can be ascertain. The main problem with this method is that the distance between bases in the DNA strands are about half a nanometer. Therefore, if the nanopore is thicker than half a nanometer, individual bases in the

DNA strand cannot be measured for that reason, researchers must use material that is only one atom thick to make up the nanopore. Graphene sheets come to the rescue. These sheets are only one carbon atom thick, so nanopores made of graphene are thin enough to resolve individual bases in DNA strands.

Detection of DNA Mutations: The probes are designed to bind with a sequence of DNA that is suspected of having a mutation. This can be done by creating a complimentary sequence of DNA to the double-helix strand in question. Then allow molecules containing both sequences to mix in a test tube in salt water, where they naturally will match up to one another if the base pairs are intact [17]. Unlike previous technologies, the probe molecule checks both strands of the target double helix for mutations rather than just one, which explains the increased specificity. The probe is engineered to emit a fluorescent glow if there's a perfect match between it and the target. If it doesn't illuminate, that means the strands didn't match and there was in fact a mutation in the target strand of DNA.

The probe is engineered to emit a fluorescent glow if there's a perfect match between it and the target. If it doesn't illuminate, that means the strands didn't match and there was in fact a mutation in the target strand of DNA. Identification of Methylated DNA Two DNA bases, 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), marks epigenetic modification, are recognized in immobilized DNA strands and distinguished from G, A, T and C by nanopore current recording, the approach will provide epigenetic modifications in unamplified genomic DNA [17,18].

Decoding long DNA strands: Recently developed nanopore DNA sequencing technology could possibly be used to create 'tricorder'-like devices for detecting pathogens or diagnosing genetic disorders rapidly and on-the-spot, since it is capable of reading long sequences of DNA far more quickly than any existing techniques. Laszlo et al. [19] used *Mycobacterium smegmatis* porin A (MspA). This bacterial pore has been genetically altered so that the narrowest part of the channel has a diameter of about a nanometer, or 1 billionth of a meter. This is large enough for a single strand of DNA to pass through. The modified nanopore is then inserted into a membrane separating two salt solutions to create a channel connecting the two solutions and a small

voltage is applied across the membrane to make the ions of the salt solution flow through the nanopore. The ion flow creates a measurable current. If a strand of DNA is added to the solution on one side of the membrane and then enters a pore, the bulky DNA molecules will impede the flow of the much smaller ion and thereby alter the current. How much the current changes depend on which nucleotides — the individual molecules adenine, guanine, cytosine and thymine that make up the DNA chain — are inside the pore. Detecting changes in current can reveal which nucleotides are passing through the nanopore's channel at any given instant.

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