OXFORD NANOTECHNOLOGIE: A NEW ERA FOR GENOME SEQUENCING AND PRECISION DIAGNOCTICS

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Abstract

The advancement made on sequencing technology over the last years has been impressive. However, a number of new instruments were commercialized, the most attractive and promising one was the MinIon from Oxford Nanopore technology, UK. It is a small USB device using the nanopore technology to sequence more than 100kbp of DNA single stranded in a short time without pre-amplification or optical steps. This review focusses on the use of the new sequencing technology to improve the molecular and the precision diagnostic. Herein, we expose the employment of MinIon device for characterization, monitoring and detection of mutations in infectious agents but also its application in precision diagnosis and mutation analysis in clinical oncology and immunologic research.

Keywords : oxford, nanotechnologie, genome sequencing, precision diagnoctics.

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INTRODUCTION

For a long time medicine prescribes the same drugs to treat all patients that have the same disease. These drugs have shown efficacy in some patient but they have different side effect in other. Recently, research has shown that the efficacy and the metabolism of drugs differ from an individual to another depending on its genetic composition and environmental factors (Nebert et Zhang, 2019). Hence, nowadays the improvement of molecular diagnostics in routine clinical care is needful.

Gene sequencing has played an integral role in the advancement and understanding of disease pathology and treatment. A decade ago, a sequencing revolution was born with the advent of second-next generation sequencing (NGS). The most sold instruments are Illumina and Ion Torrent. The NGS technology works by detecting the incorporation of the labelled nucleotides directly without separation of DNA in a gel (Steinbock and Radenovic, 2015). Therefore, these technologies rely on multiple manipulation steps to covert native DNA in a form that can be detected using electrical or chemical signals by various sensing mechanisms. It is now clear that DNA manipulation can cause artifacts and inaccuracies in DNA measurements (Ozsolak,2012). In addition, each step limits them to short 100–400 bp read lengths due to inevitable phasing issues (when templates in a polymerase colony lose synchronicity). These shorter reads make genome, transcriptome, and metagenome assembly more challenging and leaves some areas of the human genome unresolvable (Leggett and Clark, 2017).

On the other hand, the established of these platforms are very expensive, immobile, and require regular maintenance, making them a costly inclusion on a research proposal or programmatic intervention grant in the developing countries.

The increasing demand for faster and cheaper genome sequencing results in the development of advanced sequencing technologies (Chaisson et al, 2015). Nanopore sequencing is belived to be one of the most promising sequencing technologies to reach foor gold standards set for the "$1000 Genome" project; targeted prevention, effective therapy, better vaccines, lower costs (wang et al, 2015). Effectively, nanopore sequencing has changed the NGS landscape with cheap portable sequencers, rapid simple library preparation (15 min), and automated real-time analysis. Those methods are valuable tools for clinical testing and could possibly enable small/mid-scale research centres and hospitals to conduct research studies by genotypic driver genes and selecting suitable therapeutic approaches (Norris, 2016).

This review is an overview of the new genomic sequencing instrument "oxford nanopore technology" and its clinical employment for microbiology and precision diagnostic in cancer and immunology research.

2- NANOPORE SEQUENCING

In 2003, the first complete inventory was taken of the building blocks of the human genome. Since then, scientists have worked to develop a cheap method to quickly and reliably sequence an individual's entire genome and have launched the international project "the 1000$ genome" (Dondorp and Wert, 2013). The project led to the appearance of the next-generation sequencing instruments.

Over the past two decades, it was shown that polymers and other analytes could be used to estimate the size of nanometer-scale features in protein ion channels for exemple, water-soluble polymers were used to physically characterize geometric featureswithin bacterial pore-foring toxins, including the dimeter, location of the

limiting aperture and pore length (Kullman et al, 2002; Purnell and Schmidt, 2009).

Recent advances suggest that these same nanometer-scale pores may become useful for the detection, identification, and characterization of a wide range of analytes, including polymers like DNA (Wang et al, 2018).

This new wave of technologies is led by Pacific Biosciences (PacBio) of Menlo Park, CA, USA and by the relative newcomer, Oxford Nanopore Technologies (ONT), of Oxford, UK. Pacific Biosystems (Norris, 2016). Both technologies analyze individual molecules of DNA with no need for artificial amplification, and generate longer reads than second-generation technologies, but both platforms have a relatively high error rate compared with Illumina's <1% error rates (Leggett and Clark,2017) .

In 2014, Oxford Nanopore Technologies (ONT) released a new third generation sequencing platform. The MinION is an USB-powered device, measuring 4 inches and weighting only 90g, commercialized together with two flowcells and reagents that cost only US$1000. ONT's technology has already begun to universalize sequencing, giving to scientists the opportunity to acquire their own sequencer and to use genomics in their research. The ONT permit sequencing of none amplified native DNA of more than 100 kbp in a short time (2-10h) with an error rate varying between 3-15%.

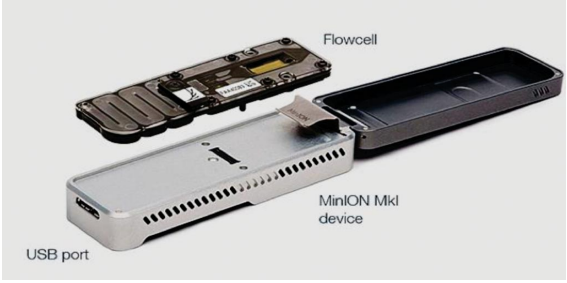

Figure 1: MinIon device (from oxford nanopore technologies).

Nanopore sequencing has been shown to be able to discriminate individual nucleotides by measuring the change in electrical conductivity as DNA molecules pass through the pore. The most nanopores used are made by a single ion channel formed by the *Staphylococcus aureus* endotoxin α-hemolysin (Celaya et al, 2017), or Mycobacterium smegmatis porin A (MspA). These nanopores are narrow channels of 1 nm that only single stranded DNA or RNA chains can pass through them (Duan et al, 2016). To investigate double stranded DNA chains, it was proposed recently to explore the engineered bacteriophage phi 29 protein channel. It has a larger diameter, closer to 3.6 nm and higher conductance than other biological nanopores (Wang et al, 2018a).

To replace the protein nanopores new solid-state nanaopres have been developped, they are more robust durable and mechanically more stable. However, solid-state nanopores have not yet achieved the degree of precision in analyte physical characterization that their protein counterparts have demonstrated (Kasianowicz, 2012).

3- Application of ONT to analyse genome of infectious agents:

The MinIon technology has been applied to sequence genome and to detect mutation in infectious microorganisms (table 1). The device has been used to analyse resistant genes of tuberculosis stains in sub Saharan Africa (Bates et al., 2016) and in diagnostic and analysis of the Ebola virus in West Africa (Kilianski et al, 2015). Another demonstration of the sequencing capabilities of MinION is provided by Quick et al, 2016 that report it uses to monitor Ebola spread, to detect mutation sub-lineages and to evaluate patient's response to vaccination. Similarly, the origin and spread of the Zika virus were analysed using the MinION sequencer in South America, the results were important for interpretation of the birth defects associated with Zika infection (Quick et al, 2017).

Besides, 6 hours sequencing run time, were sufficient to identify *E. coli* genome. Three poxviruses (cowpox, vaccinia-MVA, and vaccinia-Lister) were also identified and differentiated down to the strain level, despite over 98% identity between the vaccinia strains. The ability to differentiate strains by amplicon sequencing on the MinION was accomplished despite an observed per-base error rate of approximately 30% (Laver et al., 2015). A complete influenza virus genome was also obtained by the new sequencer and results shared greater than 99% identity with sequence data obtained from Illumina MiSeq and traditional Sanger-sequencing (Wang et al, 2015; Imai et al, 2018)

Runtuwene et al., 2018 have described the application of the portable sequencer, MinION, for genotyping nine genes causing resistance to the malaria parasite *Plasmodium falciparum*. The study concluded that MinION could generate reads with long sequences and acceptable quality with sequence accuracy was less than 90%. The ONT has also used to sequence the complete genome of *Fusobacterium nucleatum*, an oral bacteria that are associated with human pathologies as diverse as periodontitis, preterm birth, and colorectal cancer (Todd et al, 2018).

Table 1: application of MinIon technology for genome sequencing of some infectious agents

Infectious agent	Sequencing	Reference
Streptomyces avermitilis	Complete genome	Laver et al, 2015
Borrelia burgdorferi	Complete genome	Laver et al, 2015
E. coli K-12	Complete genome	Laver et al, 2015 Kilianski et al, 2015
Hepatite B virus	Complete genome	Sauvage et al, 2018
Plasmodium falciparum	9 genes : - Mitochondrial apocytochrome B (*CYTB*) - Sarcoplasmic/endoplasmic reticulum Ca2+-ATPase6 (*PfATPase6*). - Multidrug resistance protein 1 (*PfMRP1*). - Dihydrofolate reductase-thymidylate synthase (*PfDHFR*). - Dihydropteroate synthase (*PfDHPS*) - Translationally controlled tumor protein (*TCTP*). - Chloroquine resistance transporter (*PfCRT*) - Multidrug resistance protein 1 (*PfMDR1*) - Kelch protein gene (*K13*)	Runtuwene et al, 2018
Infuenza virus	Complete genome	Wang et al, 2015
Zika virus	Complete genome	Quick et al, 2017
Ebola virus	Complete genome	Hoenen, 2016

4- Application of ONT in cancer research and diagnostic:

Cancer is a heterogeneous disease that results from accumulation of mutations and epigenetic modifications in somatic cells. In last decade, researches have developed new anticancer drugs with a higher precision of molecular targeting. The cellular targets are genetically modified in cancer cells and are essential for tumor development and survival. Oncoprotein or oncogenes targets, which are mainly involved in various signaling pathways, are primarily products of gene fusions, obtained or functional mutations or overexpressed oncogenes (Ke and Shen, 2017). The use of a targeted therapy is restricted to patients whose tumor has a specific gene mutation that codes for the target. However, precision medicine aimed to identify patients most likely to benefit from treatment (Tsimberidou et al, 2014). For this reason, genomic sequencing is required nowadays to better manage patients health and targets therapies to achieve the best outcomes in the management of cancer disease. In this context, ONT technonology has been used to detect DNA structural variant of tumor suppressor genes *CDKN2A/p16* and *SMAD4/DPC4* in pancreatic cancer. Results show that nanopore sequencing can detect large deletions, translocations and inversions at dilutions as low as 1:100, with as few as 500 reads per sample (Norris et al., 2016).

De Jong et al., 2017 shows that MinION nanopore sequencing of long-range PCR amplicons is able to resolve the exon structure of whole BRCA1 transcripts. The study has identified 20 novels BRCA1 isoforms, 18 of which contained multiple individual splicing events. The study was successful in demonstrating the capability of the MinION device to characterize the exon structure of whole BRCA1 transcripts and proved that MinION technology overcomes limitations of traditional PCR-based techniques.

In lung adenocarcinoma, a number of molecular-targeting medicines are available, such as gefitinib, erlotinib and afatinib for EGFR;34 crizotinib, ceritinib and alec tinib for ALK;35 and vandetanib and cabozantini. The drug prescription requires molecular characterisation and mutation detection. The study of Suzuki et al., 2017 has reported the use of MinION to detect various types of mutations in cancer-related genes like EGFR, KRAS, NRAS and NF1 in lung cancer but regardless of the error-prone nature of the sequence data of MinION, in the case of homozygous mutant alleles, the cancerous mutations could be robustly detected.

The 2016 WHO (world health organisation) classifications of central nervous system tumors require molecular profiling for final diagnosis. Common genes that delineate this classification include IDH, 1p/19q, SHH, WNT, TP53, and RELA. To date, only few studies has been published using the MinION to support molecular diagnosis of central nervous system tumor tissue. Despite a small sample size, the study of Patel et al., 2018 demonstrated that the MinION could provide critical diagnostic information regarding SNPs (single nucleotides polymorphism), copy number variations, and methylation patterns within a single workday.

Table 2: some cancer related genes sequencing by Min Ion technology.

Cancer type	Samples size/cell line	genes	Reference
Brain tumor	28 patients	TP53, IDH1, TERT	Euskirchen *et al, 207*
Leukemia	24 patients	BCR-ABL1	Minervini, 2017
Lung cancer	8 patients	EGFR, KRAS, NRAS and NF1	Suzuki, 2017
Lang cancer	lung adenocarcinoma cell lines: PC-9, LC2/ad, PC-7, RERF-LC-Ad2, H1437, H1975, H2228, H2347, A549 and H322	EGFR, KRAS, NRAS and NF1	Suzuki, 2017
Pancreatic cancer	PDAC cancer cell lines	CDKN2A/p16 and SMAD4/DPC4	Norris et al, 2017
Breast cancer	Human lymphoblastoid cell line	BRCA1	De Jong et al, 2017

5- Application of ONT for HLA typing and immunogenetic clinical research:

The human genome contains many regions of high and low complexity that have relevance to an individual's health. Some of the most complex regions of the genome are those that encode the human leukocyte antigen (HLA) and KIR (Killer-cell immunoglobulin-like receptors). The nano sequencer MinION is a potential device to sequence the HLA allele's frequencies and KIR (killer-cell immunoglobulin like receptor) genes analysis (Ma et al., 2015; Ton et al., 2018). Another study realized by Liu et al, 2018 have reported the use ONT for HLA typing to assess the immunologic compatibility between organ donors and recipients, their study report that the platform's high error rate makes it challenging to type alleles with accuracy.

Likewise, Deutekom et al, 2017 have announced that the current status and data quality of MinION cannot yet be applied for routine HLA typing.

The application of MinION for sequencing ABO genes, revealed that the new sequencer can be regarded as a novel platform for high throughput ABO genotyping, very suitable in cases where serology is unavailable (Matern et al, 2017). This technology has been also applied for the studies of polymorphisms in Alzheimer related gene and the results showed that the device can detect genetic variation but the high rate of error makes polymorphism determination so difficult (Brooks et al., 2016;Ton et al., 2018).

Furthermore, the ONT long read is a promising technology that can be applied for diagnostics of rare diseases like with ataxia-pancytopenia syndrome and severe immune dysregulation (Boweden, 2019)

6- CONCLUSION:

Regarding the speed and the low capital cost, the ONT is a promising tool that opens new era for scientific research, molecular diagnostic and personalized medicine, especially in developing countries where access to sequencing technology is so limited. This new technology has successful applications within clinical microbiology, human genome sequencing, and cancer genotyping across multiple specialties. The MinIon device is also a new instrument that has the ability to advance our understanding of biological pathways and disease etiology.

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