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Review

Nanodevices for Biological and Medical Applications: Development of Single-Molecule Electrical Measurement Method

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Featured Application: Personalized medicine.

Abstract: A comprehensive detection of a wide variety of diagnostic markers is required for the realization of personalized medicine. As a sensor to realize such personalized medicine, a single molecule electrical measurement method using nanodevices is currently attracting interest for its comprehensive simultaneous detection of various target markers for use in biological and medical application. Single-molecule electrical measurement using nanodevices, such as nanopore, nanogap, or nanopipette devices, has the following features:; high sensitivity, low-cost, high-throughput detection, easy-portability, low-cost availability by mass production technologies, and the possibility of integration of various functions and multiple sensors. In this review, I focus on the medical applications of single- molecule electrical measurement using nanodevices. This review provides information on the current status and future prospects of nanodevice-based single-molecule electrical measurement technology, which is making a full-scale contribution to realizing personalized medicine in the future. Future prospects include some discussion on of the current issues on the expansion of the application requirements for single-mole-cule measurement.

Keywords: single-molecule; electrical detection; nanopore; nano-gap; nano-device

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1. Introduction

Personalized medicine is known as tailor-made medical care and healthy life system for each person by providing appropriate treatment and medication. In order to realize these personalized medication systems, the development of sensing technologies, which can detect detailed personal health status and information by monitoring various medical sensors, is essential. Single-molecule electrical measurement using nanostructure integrated devices, called "nanodevice", is one of the candidates to addressing the issues in this realization of personalized medicine (Figure 1). Compared to conventional analytical techniques, there are several advantages of single-molecule electrical measurements using nanodevices: high sensitivity, which enables early diagnosis and monitoring, the simplicity and compactness of the measurement system, which enables easy-portability in field works and research; and the possibility of the integration of various functions and multiple sensors such as separation and purification.

Nanopore based single molecule measurement is one of the most attractive single molecule measurements using nanodevices [1,2]. The detection principle of nanopore based single molecule measurement methods is the same as the Coulter counter detection principle. The principles are herein briefly described. The nanopore device has two solution chambers separated by a membrane with a single nanopore, and the chambers are filled with an electrolyte solution. When a single molecule/particle flows into the nanopore, the electrical conductivity of the electrolyte solution in the pore decreases due to the excluded volume of the particle, resulting in a decrease in ionic current. From the magnitude of the

electrical signal of the current decrements during the pore translocation, it is possible to analyze physical quantity such as the volume of the sample.

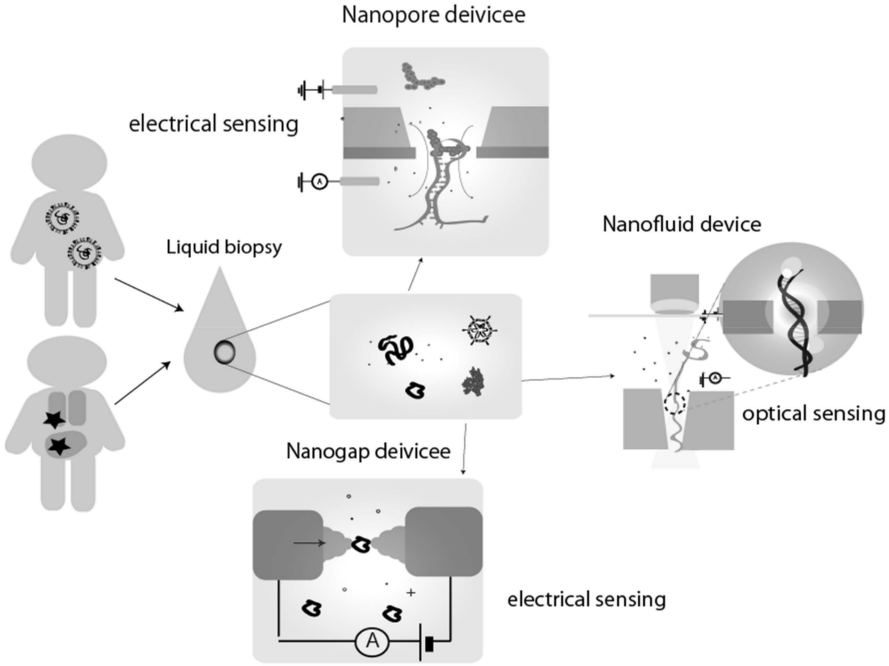

Figure 1. Schematics of single-molecule detection by nanodevices for biological/medical application. In personalized medicine, non-invasive liquid biopsy (urine, blood, etc.) is expected to be used for the application of sampling. The samples include miRNAs and proteins released from the liposomes of cells, as well as the virus itself in case of infection. The target markers in these samples are electrically measured at the single molecule level using nanodevices, such as nanopores, nanogap electrodes, and nano fluid/channels. As single-molecule detection methods, electrical and optical and its combination measurements have been reported.

Nanopipette based single molecule measurement is also one of the important methods for single molecule electrical measurements. A nanopipette device is defined as a pipette with a very fine tip that has a nanoscale opening. Similar to nanopore, the detection principle of nanopipettes is done by blockage of the ionic current through the pipette by the passage of the target samples. Nanopipettes have been widely applied from cells to ions due to their ease of preparation, which allows the size of the pipette to be controlled according to the target sample [3–5].

Nanogap based single molecule measurement is also used for nanodevices for single molecule electrical measurement [6]. The measurement principle is to measure the electrical conductivity of individual molecules by using around 1 nm gaps, which are formed by using a mechanically controllable or electrical break junction method. Sample molecules enter and translocate through the nanogap-electrodes, ensuring the facilitation of the tunneling current via the nucleotide molecules. The tunnel-current intensity is related to the molecular energy level so that signal intensity represents the electrical conductivity of individual molecules. This method enables the analysis of chemical bonds, steric structure, molecular interactions between target molecules and gold electrodes, and the polarity of sample molecules.

With the recent development of the nanodevice fabrication by semiconductor mass-production technique, the fabrication cost of the devices has rapidly decreased so that the nanodevice sensors are able to be utilized for biological/medical applications. In this review, we introduce the cutting-edge medical applications that have been made possible by using nanodevices.

2. Single Molecule Electrical Measurements: Biomolecule Detection

The first step for disease diagnosis and health status is the detection of biomolecules. The detection methods are roughly separated into two categories: direct sensing and indirect sensing for single molecule measurement using nanodevices.

2.1. Direct Sensing by Single Molecule Electrical Measurements

Direct sensing involves directly reading physical properties from the target molecule. Physical properties include the electronic state, the physical volume, which include the solvation shell of the molecule/ions, and structural differences, which are induced by conformational change of the molecule via inter/intra molecular interaction (Figure 2). These direct sensing methods are applied to single-molecule sequencing for biological polymers such as DNA, RNA, and peptides. These direct sensing methods potentially contribute to the detection of rare target markers and the discovery of new target markers.

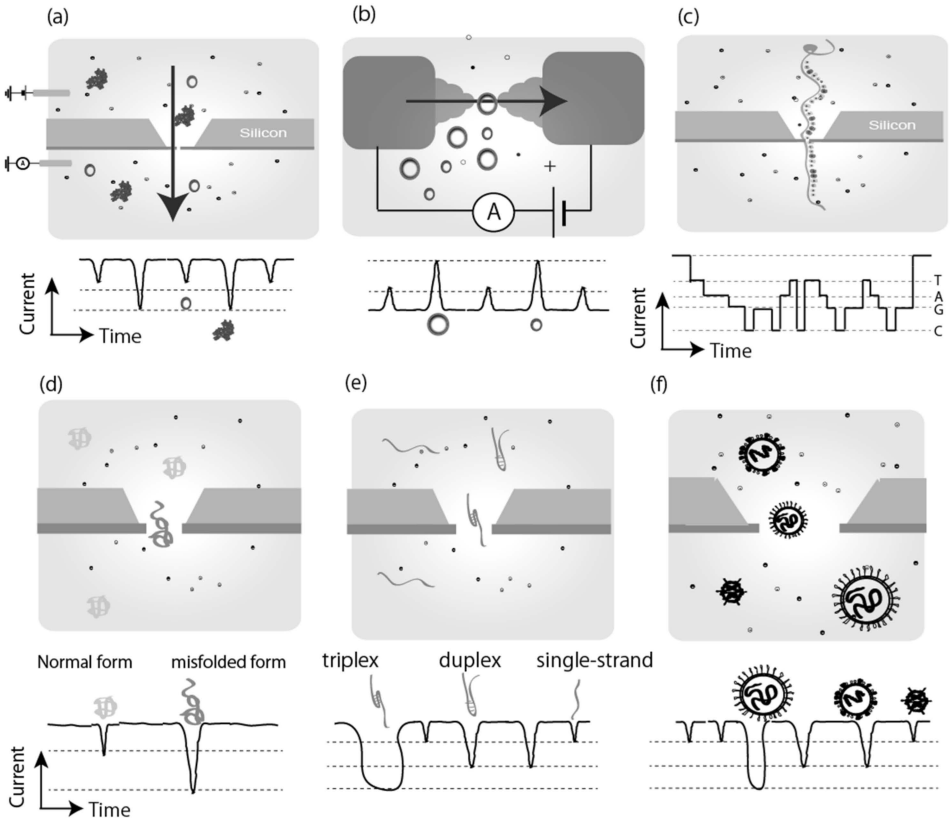

Figure 2. Direct sensing of target molecule/particle by using nanodevices. In single molecule/particle measurement using nanodevices, an electrical signal for a single molecule is observed as a current-time profile when the molecule passes through the sensor device. In the case of nanopore devices, the intensity of decrement in ion current during passage represents the volume of the molecule (**a**), and in the case of nanogap devices, the signal intensity of the tunnel-current represents the electronic conductivity of the molecule (**b**). When a single biopolymer passes through the sensor, each unit of the polymer (nucleotide for DNA and RNA, amino acid for peptide and protein) passes through the sensor, and the electrical signal reflects the sequence of the biopolymer (**c**). Detection of different molecular signals occurs even for the same target molecule. If a structural change has occurred, different electrical signals reflecting the different structures would be observed (**d**–**f**). In fact, signals reflecting differences in the intramolecular hydrogen bond morphology of proteins (d:prion) and nucleic acid base chains (DNA single-strand, duplex, and triplex) have been observed. In addition, differences of shapes in the nanoparticles and viruses have also been identified by electrical signals reflecting the shape of the particles (**f**).

2.2. Indirect Sensing by Single Molecule Electrical Measurements

Indirect sensing involves detecting the specific molecular marker by using analytical probes, which are designed to selectively bind to the target marker and serve to amplify the electrical signal by nanodevices (Figure 3). By using probe molecules, target marker molecules specifically form a host (probe)—guest (target) conjugate, resulting in the signal amplification of target molecules so that they are easily discriminated from the host only (probe) and no-target other molecules. In addition to signal amplification, some probe molecules work as chemical scavengers in order to purify or exclude molecules that interfere with detection other than guests. The use of these probe molecules enables the specific and selective detection of guests, resulting in an improvement of the sensing accuracy and sensitivity.

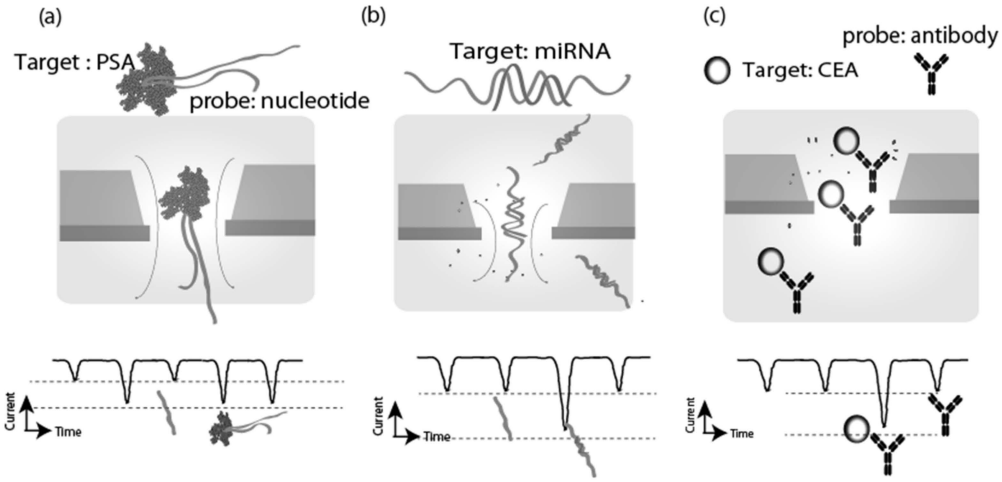

Figure 3. Indirect sensing of target molecule/particle by nanodevices. In this sensing mode, a probe molecule that selectively binds to the target molecule is required. DNA aptamer (**a**) and complementary nucleotides (**b**) are used as probe molecules. In addition to nucleotide probe molecules, peptide chains and monoclonal antibodies (**c**) are used to measure the binding form of target molecules and particles using nanodevices, and identify the differences in electrical signals.

Up to now, there have been the reports on identifications of various chemical species, including nucleobases and nucleotides such as DNA and RNA [7–24], amino acids and peptides [25–29], proteins [30–33], carbohydrates [34–36], biocompatible polymers [37–40], second messenger molecules and organic molecules [41–45], ions [46–52], toxic nanoparticles [53], and viruses [54,55]. Among these bimolecular sensing, based on the DNA and amino-acid identification method, single-molecule electrical sequencing methods are developed [56–62] (Table 1). These molecules are important target biomolecules for medical applications and thus detailed studies on these target groups are described in the following sections.

Table 1. Biomolecule-sensing species: device, target, and tools for single-molecule detection.

Target Sample	Device	Detection	Sample	Reference
Nucleotide (DNA)	Nanopore (Bionanopore)	Ion current signal characterization	DNA	Review: [7–11] [12]
Nucleotide (epi DNA, 5 mC, 5 hmC)	Nanopore (Bionanopore)	Ion current signal characterization	DNA	[13–15]
Nucleotide (DNA)	Nanogap	Tunnel current signal characterization	DNA	[16–19]

Table 1. *Cont.*

Target Sample	Device	Detection	Sample	Reference
Nucleotide (epiDNA, /5 mC, m6A, N2-Et-dG)	Nanogap	Ion current signal characterization	DNA	[20–23]
Nucleotide (DNA)	Nanopipette	Ion current signal characterization	DNA	[24]
Amino acid	Nanopore (Aerolysin bionanopore)	Ion current signal characterization	13 of the 20 natural amino acids	[25]
Amino acid, Peptide	Nanopore (Alpha-haemolysin bionanopore)	Ion current signal characterization	9 mutants in peptide	[26]
Amino acid, Peptide	Nanogap	Tunneling current signal characterization	Amino acids, peptide	[27,28]
Peptide	Nanopore (Aerolysin bionanopore)	Ion current signal characterization	Peptide	[29]
Proteins	Nanopore	Ion current signal characterization	Amino acids, Peptide, Protein	Review: [30]
Proteins	Nanopore (Bioenginered Bionanopore)	Ion current signal characterization	Streptavidin	[31]
Proteins	Nanopore (Alpha-haemolysin bionanopore)	Ion current signal characterization	Maltose binding protein of Escherichia coli (MBP)	[32]
Proteins	Nanogap	Ion current-voltage response	Antigen-DNA/Gold nanoparticle	[33]
Polymer (Carbohydrates)	Nanopore (Aerolysin bionanopore)	Ion current signal characterization	Glycosaminoglycans	[34]
Polymer (Carbohydrates)	Nanopore (Maltoporin bionanopore)	Ion current signal characterization	Maltoheptaose (m7), Maltohexaose (m6), Maltopentaose (m5), Maltotetraose (m4), Maltotriose (m3)	[35]
Polymer (Carbohydrates)	Nanopore (Alpha-haemolysin bionanopore)	Ion current signal characterization	Oligosaccharides	[36]
Polymer (Neutral polymer)	Nanopore (Alpha-haemolysin bionanopore)	Ion current signal characterization	Ethylene glycole	[37–39]
Polymer (Polyelectrolytes)	Nanopipette	Ion current signal characterization	Poly-L-lysine (PLL)	[40]
Second messenger	Nanopore (Alpha-haemolysin bionanopore) with ATP aptamer ligand probe	Ion current signal characterization	ATP	[41]
Second messenger	Nanogap	Tunnel current signal characterization	Cyclic AMP, AMP, ADP, ATP	[42]
Second messenger	Nanogap	Tunnel current signal characterization	Dopamine, Norepinephrine, Serotonine	[43]
Organic molecule	Nanopore (Alpha-haemolysin bionanopore)	Ion current signal characterization	Inositol 1,4,5-trisphosphate (IP3)	[44]

Table 1. *Cont.*

Target Sample	Device	Detection	Sample	Reference
Organic molecule	Nanopore (β-CD adapted alpha-haemolysin bionanopore)	Ion current signal characterization	Adamantan amine	[45]
Ion	Nanopore (Bionanopore)	Ion current signal characterization	Divalent ions (Zn^{2+}, Co^{2+}, Cd^{2+})	[46]
Ion	Nanopore (Polymer modified Solid state nanopore)	Ion current-voltage response	Proton (H^+)	[47]
Ion	Nanopore (Alpha-haemolysin bionanopore) with DNA ligand probe	Ion current-voltage response	Proton (H^+)	[48]
Ion	Nanopore (Bionanopore) with 14-amino-acid peptide ligand probe	Ion current-voltage response	$UO_2{}^{2+}$ ions	[49]
Ion	Nanopore (Bionanopore)	Ion current signal characterization	Hg^{2+}	[50]
Ion	Nanopiptte	Ion current-voltage response	Proton (H^+)	[51]
Ion	Nanopiptte	Ion current-voltage response	Reactive Oxygen Species (ROS: $O_2{\cdot}^-$)	[52]
Nanopaticle	Nanopore (Solid state nanopore)	Ion current signal characterization	Particulates (Atmospheric particulate matter)	[53]
Virus (Influenza A, B, Coronavirus, Adenovirus, Respiratory Syncytial virus)	Nanopore (Solid state nanopore)	Ion current signal characterization by machine learning	Virus sample	Review: [54]
Virus (Coronavirus)	Nanopore (Solid state nanopore)	Ion current signal characterization by machine learning	Virus sample	[55]
Sequencing (Nucleotide/Peptide)	Nanopore/Nanogap	Ion /Tunnel current signal characterization	DNA	Review: [56]
Sequencing (Nucleotide)	Nanopore (Bionanopore)	Ion current signal characterization	RNA	[57]
Sequencing (Nucleotide/Peptide)	Nanogap	Tunnel current signal characterization	DNA	Review: [58], [6,59]
Sequencing (miRNA)	Nanogap	Tunnel current signal characterization	RNA/DNA	[60]
Sequencing (Peptide)	Nanopore/Nanogap	Ion current/Tunnel signal characterization	Peptide	Review: [61]
Sequencing (Peptide)	Nanopore	Ion current signal characterization	Peptide	Review: [62]

2.3. Nucleotide Sensing

Nucleic acid and nucleotides, such as DNA and RNA, are biologically important targets for understanding diseases and health conditions caused by genetic abnormalities and abnormal expression, as DNA is the main body of genes, and RNA is a substance that is translated from DNA during gene expression. Recently, in addition to the natural nucleobases [7–12,16–19,24], the detection of post-translational modifications has become important and interesting as epi-transcriptome research fields because these nucleotide and nucleobase modifications play roles in gene expression and suppression, which are

closely related to various biological malfunction such as disease and aging. Since there are about over one hundred types of known base modifications in eukaryotic cells, some of the epi-modification, such as methylated nucleobases (e.g., methyl cytosine, methyl adenine) and oxidized guanine are detected by these nanodevice detection methods [13–15,20–23].

2.4. Amino Acids, Peptides and Proteins Sensing

Amino acids, peptides and proteins are also important target molecules because they perform various biological functions such as catalyzing metabolic reactions, DNA replication, signal transduction and metabolism thus abnormalities are closely related to diseases. Up to now, there have been reports on the detection of the twenty amino acids, and post-translational modifications of amino acids such as methylation, acetylation, and phosphorylation have also been detected [25–28]. Peptide and proteins, which are polymers of amino acids, are also important targets. Besides reading sequences or direct sensings by nanodevices, the indirect sensing method is often utilized for the detection of proteins such as streptavidin, maltose-binding protein (MBP), etc., by using probes such as nucleic acid aptamers that bind to the host molecules of specific target molecules [29–33].

2.5. Glycans, and Biocompatible Polymer (PEG) Sensing

Glycans are an important biopolymer, belong to group of compounds consisting of various sugars connected by glycosidic bonds. Glycans can bind not only to other sugars but also to proteins, lipids, and other small molecules to produce a variety of molecules. These glycoproteins and glycolipids are important biopolymers that play important physiological roles in living organisms, and their detection has attracted much attention. Such glycosides and its related molecules have been reported by single-molecule detection [34–36]. Besides this, biocompatible polymers have attracted interest as nanodevice modification substances. Among them, polyethylene glycol (PEG) is often used in the chemical modification of nanodevices for avoiding the non-specific sticking of protein on the devices. In the reports of single-molecule measurements, the degree of polymerization of PEGs have been detected [37–39].

2.6. Second Messengers, Ion Sensing

Second messengers and ions, which play an important role in the transmission of information in the body, are also important targets. Among the second messengers, the detection of cAMP and neurotransmitters such as adrenaline, a neurotransmitter are reported [41–45]. In these reports, direct sensing of these molecules were successfully achieved by optimizing the size of nanopores and nanogap for nanodevices because the nanodevice sensors are sensitive to the size of molecular volume, three-dimensional structure, and hydration radius of the targets. In the case of indirect sensing, probe molecules, which selectively interact with the target molecules, are utilized for detection of the target ion [46–52]. For instance, this method has successfully detected harmful ions such as uranium [49] and mercury ions [50]. The strategy of size optimization for nanodevices also works for detection of viruses, cells, particulate matter (PM), etc. [53–55].

2.7. Single-Molecule Electrical Detection Based Sequencing

Based on the sequential identification of chemical species such as nucleotides and amino acids by single molecule electrical measurements, single-molecule electrical sequencing was developed to read sequences for biopolymers of DNA, RNA, and peptides (Figure 4) [56–64]. Nucleic acid sequencing [56–60] and peptide sequencing [61,62] methods have been proposed and recently developed. Compared to conventional sequencers, this single molecule electrical sequencer has the following features: first, this sequencing methodology does not need any amplification process, thus it can reduce the analytical time and cost of reagents for amplification, and allows the equipment to become smaller; second, this electrical sequencing methodology can read DNA information faster than the conventional optical probe based sequencing technologies as optical probe-based se-

quencing needs a DNA elongation reaction; third, epigenetic information can be detected as the native sample molecules are directly observed without amplification. Particularly, single-molecule DNA sequencing is increasingly utilized for on-site portable sequencing because it is cheaper and more compact than conventional DNA sequencers.

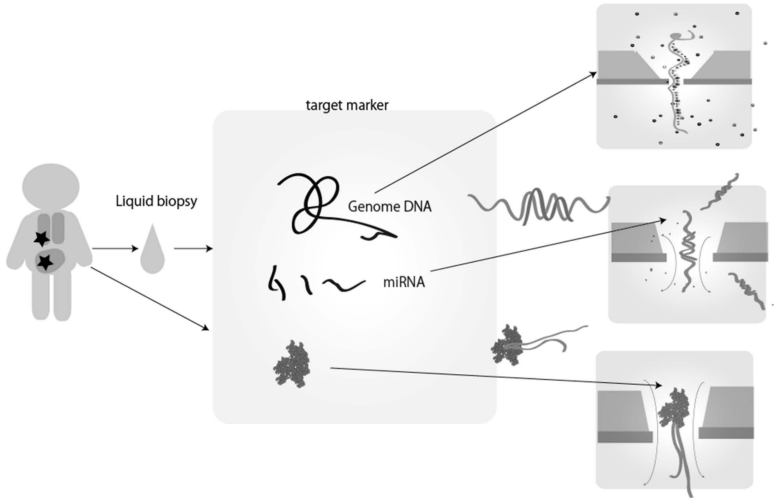

Figure 4. Cancer diagnosis by single-molecule electrical detection of cancer marker molecules and counting of miRNA molecules. Tumor-specific marker molecules in liquid biopsy samples, such as blood, urine, and saliva obtained from patients, are measured. After these samples are pretreated by purification, amplification, and chemical treatment, single-molecule measurements are performed using nanodevices to distinguish between patients and healthy individuals. There are several reports of the detection of genes and miRNAs by sequencing, and/or detection using probe molecules that selectively bind to the target.

3. Applications of Single Molecule Measurement: Disease Diagnosis, Monitoring

As examples of single-molecule measurement applications (Table 2), we focus on the diagnosis of cancer diseases [63–88], viral infections [55,64,89–95], drug detection and screening [96–101], dementia, brain and nervous system diagnoses, and monitoring of other dangerous substance epidemics [102–111].

Table 2. Biological/medical application by single-molecule electrical measurement using nanodevices.

Detection Purpose	Target Marker	Detection Method	Detection Device	Sample Source	Reference
Cancer (pancreatic cancer)	DNA: Tumor suppressor genes (CDKN2A/p16 and SMAD4/DPC4)	Sequencing	bionanopore (Minion)	pancreatic cancer cell lines	[63]
Cancer (cervical cancer)	DNA: human papillomavirus (HPV), which is integrated into the human genome,	Sequencing	bionanopore (MinION)	patient tissue	[64]
Cancer (prostate cancer)	DNA: tumor-specific genomic structural variants	Sequencing	bionanopore (MinION)	blood (circulating tumor DNA)	[65]
Cancer (cervical cancer)	DNA: human papillomavirus (HPV)	Sequencing	bionanopore (MinION)	exfoliated cervical epithelial cell	[66]

Table 2. *Cont.*

Detection Purpose	Target Marker	Detection Method	Detection Device	Sample Source	Reference
Cancer (chronic lymphocytic leukemia)	DNA: TP53 gene mutation	Sequencing	bionanopore (MinION)	peripheral blood	[67]
Cancer (lung cancer)	miRNA (miR-155)	Binding by complementary probe	bionanopore (alpha-haemolysin)	blood	[68]
Cancer (colorectal cancer)	miRNA (miR-21)	Binding by complementary probe	bionanopore	serum	[69]
Cancer (lung cancer)	miRNA (miR-155, miR182-5p, miR-210, miR-21)	Binding by complementary probe	bionanopore	synthesized nucleotide	[70]
Cancer/HIV	miRNA (let7b, miR155, miR21), protein (HIV-TAT)	Binding by complementary probe	bionanopore	synthesized nucleotide/peptide	[71]
Cancer	IgG antibody (HED10)	Binding by poly $(dT)_{45}$ DNA ligand	bionanopore (alpha-haemolysin)	-	[72]
Cancer	DNA:5-methylcytosine (5 mC) in DLX1	Binding by MBD1 (MBD-1x) proteins ligand	solid state nanopore	-	[73]
Cancer	DNA: Target DNA extracted from cell	Binding by complementary probe hybridization	bionanopore (Aerolysin)	cell culture (Ramons, A549, Jurkat, MCF-7, Hela)	[74]
Cancer (breast cancer)	Circulating tumor cells (CTC)	Binding by breast cancer cell-specific aptamer probe	bionanopore (MspA)	blood: tumor cells (CTC) from blood, cell (MCF-7, HpeG2, U87)	[75]
Cancer	Cancer marker proteins (carcinoembryonic antigen, α-fetoprotein antigen, and human epidermal growth factor receptor-2)	Binding by target proteins corresponding monoclonal antibody ligand	solid-state nanopore (multiplex pore)	-	[76]
Cancer (colon cancer)	EpCAM antibody	Binding by/Colon Cancer EpCAM Antigen peptide ligand	bionanopore (engineered EpCAM phi29 connector protein)	antibody from mice blood serum	[77]
Cancer (prostate, colon, lung, liver, breast cancer)	Mehtylated CpG DNA	Binding by methyl-binding protein ligand	solid-state nanopore (SiN)	synthesized DNA	[78]
Cancer (prostate cancer)	Cancer biomarkers: Prostate-specific antigen (PSA)	Binding by DNA aptamer probe	solid-state nanopore (SiN)	PSA in serum	[79]
Cancer	Vascular endothelial growth factor (VEGF), matrix metallopeptidase-9 (MMP-9)	Binding by DNA aptamer probe	bionanopore (α-haemolysin)	-	[80]

Table 2. *Cont.*

Detection Purpose	Target Marker	Detection Method	Detection Device	Sample Source	Reference
Cancer	CarcinoembryonicAntigen (CEA)	Binding by DNA aptamer probe	solid-state nanopore (Nanopipettes)	-	[81]
Cancer	Cancer biomarkers: PSA, CEA, AFP, NSE, CA19-9	Binding (Multiplex assay) by antigen/Barcode probe DNA	bionanopore (α-haemolysin)	bovine blood serum	[82]
Cancer (CpG related disease)	Target/probe mismatch DNA	Chemical Reaction (difference between U-T mismatch, and mC-T mismatch in Hg^{2+})	bionanopore	synthesized DNA	[83]
Cancer (lung cancer, liver cancer, and prostate cancer)	ADAM-17	Chemical Reaction (reacted target:ADAM-17/ no-reacted molecules: ADAM-9, 12)	bionanopore (α-haemolysin)	synthesized peptide	[84]
Cancer (thyroid cancer)	BRAF V600E mutation	Chemical Reaction (The mutant allele/probe duplex can form a mutation sequence-specific nanolock with Hg^{2+})	bionanopore	tumor tissues of thyroid cancer patients	[85]
Cancer (breast Cancer)	mucin 1 protein (MUC1)	Chemical Reaction Mediated signal Conversion (DNA hydrogel Breakdown by biomarker)	bionanopore	CTC	[86]
Cancer (reast, lung, cervical, bladder, esophageal and ovarian cancer)	Cancer biomarker: EGFR protein	Binding by RNA aptamer probe	nanogap (RNA aptamer modified)	EGFR protein	[87]
Virus infection	Influenza A, B, Coronavirus, Adenovirus, Respiratory Syncytial virus	Ion current signal characterization by machine learning	solid state nanopore	virus sample	[88]
Virus infection	HCoV-229E, SARS-CoV, MERS-CoV, and SARS-CoV-2. Detection of SARS-CoV-2	Ion current signal characterization by machine learning	solid state nanopore	virus sample	[53]
Virus infection	Influenza A	Sequencing	bionanopore (MinION)	virus sample	[89]
Virus infection	Ross River virus	Sequencing	bionanopore (MinION)	virus sample	[90]
Virus infection	chikungunya virus (CHIKV), Ebola virus (EBOV), and hepatitis C virus (HCV)	Sequencing	bionanopore (MinION)	virus sample	[91]

Table 2. *Cont.*

Detection Purpose	Target Marker	Detection Method	Detection Device	Sample Source	Reference
Virus infection	HPV	Sequencing	bionanopore (MinION)	virus sample	[64]
Virus infection	NCp7, a protein biomarker of the HIV-1 virus,	Binding	solid state nanopore	virus sample	[92]
Virus infection (miR122)	Hepatitis C virus replication	Binding (miRNA)	solid state nanopore	rat liver	[93]
Virus infection	Neuraminidase (NA),biomarker for influenza A virus (IAV) detection	Binding	bionanopore (cytosine A bionanopore)	neuraminidase (NA), biomarker	[94]
Drug detection	R-ibuprofen, S-ibuporofen/ (M113F/K147N)73βCD complex	Binding	bionanopore	R-ibuprofen, S-ibuporofen	[95]
Drug detecti	Cocaine	Binding by Complementary DNA in DNA oligami	solid state nanopore	synthesized DNA	[96]
Drug detection	DNA Intercalator molecules	Binding by DNA probe	solid state nanopore	ethidium, propidium, ethidium homodimer	[97]
Drug detection	Adenine	Binding by adenine-sensing riboswitch DNA aptamer probe	bionanopore (alpha-haemolysin)	adenine	[98]
Drug detection	Doxorubicin	Binding by DNA probe	bionanopore (alpha-haemolysin)	doxorubicin	[99]
Drug detection	Target DNA analog drug	Sequencing	nanogap	FTD	[100]
Alzheimer's disease (AD)/Parkinson's disease (PD)	APP and SNCA, genes	Sequencing	bionanopore (MinION)	blood	[101]
Alzheimer's disease	Tau 381, AAT, BACE1	Binding By DNA aptamer probe	bionanopore (aerolysin)	blood: serum	[102]
Alzheimer's disease	β-Amyloid peptide	Self-aggregating	bionanopore	-	[103], Review: [104]
Prion Diseases, Transmissible spongiform encephalopathies (TSEs)	Prion peptide (PrP(143-169))	Binding by monoclonal antibody M2188	bionanopore (alpha-haemolysin)	synthesized peptides	[105]
Alzheimer disease, Parkinson disease, Prion Diseases, Huntington disease	β-Amyloid, α-synuclein, Bovine/Human PrP	Self-aggregating, self-folding	bionanopore (alpha -haemolysin)	-	Review: [106]

Table 2. *Cont.*

Detection Purpose	Target Marker	Detection Method	Detection Device	Sample Source	Reference
Bacterial lower respiratory infections (LRIs)	MecA blaTEM, sul1 and dfrA17,gene, E.coil, MRSA	Sequencing (metagenome)	bionanopore (MinION)	respiratory samples from patients	[107]
Polyglutamine diseases (e.g., spinal and bulbar muscular atrophy, Huntington's disease)	Tandem repeat regions (CAG, CAA, GGGGCC and iCCTG)	Sequencing (Tandem genotyping)	bionanopore (MinION)	-	[108]
Rare Mendelian disorders	Structural variants of genes DNA	Sequencing	bionanopore (Minion)	-	[109]
Lassa fever (Lassa virus)	Lassa virus	Sequencing	bionanopore (Minion)		[110]
Volatile organic compounds (VOCs)	Acetone	capacitance signal characterization	nanogap	-	[111]
TNT	Nitroaromatics	Binding	bionanopore (bio engineered nanopore)	TNT	[112]

3.1. Cancer Diagnosis

One of the most widely reported studies is cancer diagnosis. It is generally known that cancer is caused by genetic abnormalities in cells, but there are many possible causes, and the corresponding diagnostic markers are different for each of the causes. In the application of cancer diagnosis for personal medical care, it is necessary to find the cause of each cancer individually and to administer the corresponding drug appropriately. Therefore, the simultaneous detection of various diagnostic marker molecules is required.

Diagnostic marker molecules for cancer diagnosis include specific DNA gene sequences, specific genomic sequence duplications such as specific short tandem repeats (STRs), overexpression or suppression of miRNAs, CpG methylation, and tumor marker proteins and so on.

Sequencing methods by nanodevices are utilized for the detection of cancer related gene sequences such as genetic mutations and polymorphisms [63–67]. For example, the genetic abnormality of TP53, a specific oncogene, was detected in blood samples [67]. It is possible to perform the detection by the Illumina and Sanger sequencing method but the nanodevice based single-molecule sequencer can detect them cheaper and faster.

Indirect detection methods are utilized for the detection of cancer markers by using nucleotide probes, which are designed to selectively bind to the target cancer marker. For instance, by using a nucleic acid probe with complementary base pairs and a target miRNA, the detection of cancer-related miRNAs was successfully achieved [68–75]. In this method, a specific miRNA is detected by the probe molecule bound to the miRNA conjugates in blood. In addition, by using multi targeting probes, which can be hybridized with several types of miRNAs, multiple target miRNAs were detected simultaneously [70,71]. For no nucleotide type target markers, detection by using the nucleotide probe, which can interact with target markers, was successfully achieved [79,81]. For instance, using a nucleic acid aptamer probe, prostate-specific antigen (PSA) was detected [79]. The development of the evolutionary engineering method using nucleic acids and peptides can produce probe molecules with high selectivity so that the nanodevice detection method using probe molecules can become more accurate in the future. CpG methylation has been reported in various cancers such as colorectal cancer and lung cancer, and has attracted attention

as target cancer markers. By using single-molecule detection with nanodevices, such epi-genetic detection was also reported [83].

3.2. Alzheimer's Disease, Huntington's Disease, and Prion Diseases Detection

In addition to cancer diagnoses, there have been reports on the detection of Alzheimer's disease, Huntington's disease, and prion diseases related to target markers [101–106]. These diseases are known to be induced by abnormal protein aggregation, thus the detection of the protein aggregation is the first step for the disease diagnosis. For instance, single-molecule detection method by nanodevice succeeded in detecting differences in the aggregation structure of amyloid-β [103,104] and prion [105]. Therefore, these methods are expected to contribute to the understanding of the cause of the disease, early detection, and the development of drugs.

3.3. Virus Detection

It has become an important target to diagnose the presence or absence of viral infection cheaply, quickly and accurately (Figure 5) due to the recent COVID-19 pandemic having raised interest in on-site sequencing to monitor mutant strains. Up to now, there have been several reports on virus detection by single-molecule detection methods [53,64,88–94]. They are mainly categorized into two methods. The first is the detection of viral RNA sequences, and the second is the detection of viral particle shapes. For instance, the first method successfully detected influenza A [89], HPV [64], Lhasa fever [110], and Ebola [85] by on-site sequencing with Minion. The second method is the direct detection of differences in the three-dimensional structure and shape of viruses. Using this method, various types of viruses, such as Influenza A and B, Coronavirus, Adenovirus, and Respiratory Syncytial virus [88], along with HCoV-229E, SARS-CoV, MERS-CoV, and SARS-CoV-2 [53], have been successfully identified.

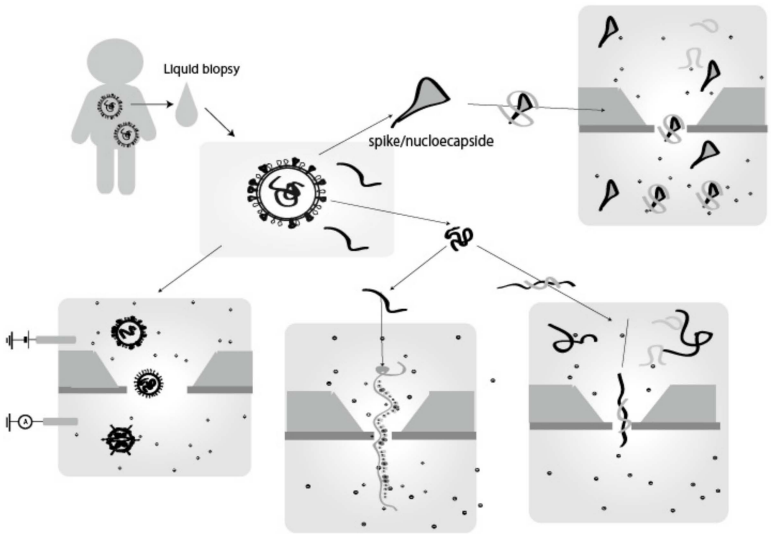

Figure 5. Virus detection by single-molecule/particle electrical detection with a nanopore device. Sample viruses are collected from liquid biopsy samples, such as nasal swabs, saliva, blood, and urine, etc. As targets, there are virus whole-particles, RNA and/or its cDNA converted from the virus RNA, and parts of the virus particles and its proteins such as spikes that constitute the virus. The presence of viruses has been detected by identifying the shape of the virus, sequencing nucleic acid base chains, detecting known target nucleic acid base sequences by complementary strand sequences, and detecting target markers such as proteins by using probe molecules that selectively bind to them.

3.4. Drug Screening and Environmental Monitoring

Single-molecule drug screening is one of the major targets due to its featuring inexpensive measurement systems and rapid evaluation of various candidate chemical substances.

The detection of drugs by single-molecule detection methods has been reported [89–94]. For example, drugs such as ibuprofen [95], doxorubicin [99], and trifluridine (FTD) [100] were detected. It is expected that further integration of these single-molecule detection technologies enables comprehensive drug screening by using the Total Analysis System (TAS), which consists of various kinds of probe molecules immobilized sensor array.

In the application of environmental monitoring, these single-molecule measurements by nanodevices are expected to provide stable, long-term measurements of a wide variety of target molecules with low costs. The application of environmental monitoring includes the constant monitoring of pollutants in the air, aqueous solutions, and soil, such as toxic and hazardous substances. Among them, metagenomic sequencing has recently become interesting [107,110]. Metagenomic sequencing does not target a single microorganism or bacterium, but a mixture of genomes from various microorganisms or bacteria. For example, it can be used to classify bacterial populations in clinical samples of patients without purification. In addition to such medical applications, the environmental impact of genetically modified organisms (GMOs) by the monitoring of the rate of genetic modification in plants is a potential important target. The monitoring of hazardous substances such as bacteria, viruses, and explosives are also important targets. There have been reports on the detection of harmful ions [44,47,48] and explosives such as TNT [112].

4. Discussion and Future Prospects

In order to increase applications of single-molecule measurement by nanodevice, further integration of functional nanostructures and improvements and the simplification of analysis methods are required. Some approaches to these issues are described in the following paragraphs.

The first approach is the integration of various functional nanostructures on nanodevice sensors. For instance, nanochannels have made great achievements in sample control and transport by electrophoresis in sensor devices [113–115] and nanopillar structure serve as purification, separation, and transportation [116,117]. Along with the integration of nanodevices, the development of fabrication techniques for integrated nanodevices is an important issue. In nanodevices, if the shape of the fabricated device can be parallelized with high accuracy, the throughput of measurement can be expected to be dramatically improved. So far, parallelization has been reported for nanopore and nanogap devices [118,119].

The second approach is to use nanochannel or nanowell structures for optical sensor detection. For example, a single molecule in a nanochannel can be detected by optical microscopy with high sensitivity [120–123]. There are also zero-mode waveguides using the near-field effect and Raman spectroscopy using the plasmon phenomenon in the nanogap [124–128]. These nano-optics/electronics hybrid devices would improve the sensing selectivity.

The third approach is the development of bioinformatics analysis methods using artificial intelligence (AI). In single-molecule measurements, the data volume is exponentially increased because of the high-speed data acquisition and large number of detected molecular signals in sample solutions, compared to conventional analytical methods. Moreover, the detected signal shape, i.e., electrical current-time profile, become more complex. Therefore, AI-based informatics analysis methods are suitable for sample identifications as the method enables the extraction of characteristic parameters from complex signals. In recent years, AI-based analysis has made it possible to identify target molecules among similar structural molecules [10,111,129–131]. Thus, it can be said that the evolution of bioinformatics analysis is essential for single-molecule measurement by nanodevice.

In addition to these developments of nanodevice based technologies and methods, there are several steps that precede real clinical practice use. The first step is the standardization of measurement systems, the nanodevices, and the data-analysis methods. The next step is to obtain medical approval from the government in each country. In the current stage, nanodevice methods are currently taking these steps towards application in the medical field. For example, the Food and Drug Administration (FDA) has issued

guidelines for obtaining medical approval for next-generation sequencing nanodevices such as MinION [132–134]. Furthermore, the recent COVID-19 pandemic has accelerated the movement to detect viruses (SARS-CoV-2) and virus mutants by nanodevices. It is expected that the application of this technology will expand as this trend becomes more active in the future.

In summary, the single-molecule electrical measurement by nanodevices is an interdisciplinary field that is currently undergoing development while incorporating new technologies. I believe that single-molecule measurement methods using nanodevices will greatly develop as a key technology for realizing personal medical care that enables point-of-care, which is considered a dream come true, while expanding the range of its applications.

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