Nanomaterial-based Clinical Testing and Diagnostic Technologies

Ruiqi Jiao *

School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, China

* Corresponding author: 19000776@ecust.edu.cn

Abstract. With the development of medical and health care, clinical medical practice has put forward higher and higher requirements on the performance indicators such as sensitivity, detection limit and analysis speed of in vitro diagnostic technologies. While traditional in vitro diagnostic techniques are increasingly unable to meet the demand, in vitro diagnostic techniques based on nanomaterials have attracted more and more attention and research in combination with their high sensitivity and high analytical speed. This review summarises the commonly used nanomaterials, such as AuNPs, MNPs and QD, and their applications in the in vitro detection and analysis of proteins or nucleic acids. The methods mentioned in the text are proximity ligation assay, BCA, nanochromatography, nanopore sequencing and so on. In order to provide an introduction to nanomaterial-based clinical testing and diagnostic techniques, and new ideas for improving the use of nanomaterials in disease diagnosis. Due to their high sensitivity and quick detection, nanomaterial-based in vitro diagnostic methods offer a lot of potential for clinical use. But it still faces certain barriers to entry into clinical applications. It is foreseeable that these technologies will to enter clinical applications in the near future continuously and will play to their strengths to reach various clinical demands and better serve humanity. This is due to the growth of nanoscience and medical test science, as well as the appearance of diagnostic companies and regional test centres in vitro.

Keywords: Nanomaterials, application, clinical testing, diagnostic.

1. Introduction

Nanomaterials and their technology are another focus of attention after cyber technology and genetic technology. With the continuous development and progress of modern technology, nanomaterials and their technologies have been known to more and more people. Nanomaterials and their technologies are affecting every aspect of people's life, work and study. In the vibrant 21st century, nanomaterials and their technologies have become a hotspot for research, and the research on their applications is becoming more and more extensive.

In medicine, the use of nanomaterials and their technology is much more common. The addition of nanotechnology has made the process of drug production more refined, and the direct use of atomic and molecular arrangement manufacturing allows the creation of drugs with specific functions. Many minimally invasive nano-surgeries not only help patients to recover, but also greatly reduce the pain of patients during and after surgery.

As health care grows, the early diagnosis of various diseases in clinical medical practice has put forward higher and higher requirements on the sensitivity. Therefore, nanomaterial-based-in-vitro diagnostic techniques were born to satisfy demands, such as high sensitivity and analysis speed and having great potential for early diverse diagnosis. For example, the use of AuNPs for localised lesion treatment can reduce the occurrence of side effects.

Nanomaterials are substances with unique size-dependent physical and chemical characteristics, such as electrical, magnetic, mechanical, optical, thermal, etc., that have at least one dimension in three dimensions and are at the nanoscale size (0.1–100 nm) or are made of them as basic units. as shown in Table 1.

Table 1. Main properties of nanomaterials [1].

Properties	Characteristics
Electrical	Nanomaterials can be fabricated as nanoelectronic devices with ultra-high speed, ultra-capacity, ultra-miniature and low energy consumption.
Thermal	The melting point of nanometals is several hundred degrees Celsius lower than that of ordinary metals.
Magnetic	The magnetic recording density of nanomagnetic materials can be increased by a factor of 10 compared to ordinary magnetic materials.
Mechanical	Gases diffuse thousands of times faster in nanomaterials than in ordinary materials; nanomaterials are more ductile, stronger and more rigid.
Optical	Nanocomposites reflect very little light but absorb electromagnetic waves extremely well and are a breakthrough in stealth technology.

2. Nanomaterials

The use of nanomaterials in the field of in vitro diagnostics has drawn increasing attention as nanotechnology has advanced. Nanomaterials can be targeted to certain detecting items depending on their own qualities by altering antibodies, probes, or other bio-recognition components on their surface. Among the many nanomaterials, magnetic nanoparticles (MNPs), quantum dots (QDs) and gold nanoparticles (AuNPs) have been used to develop a wide range of nanoparticles. QDs and AuNPs are more commonly studied in the field of in vitro diagnostics.

2.1. MNPs

MNPs are a nanomaterial with superparamagnetic properties, i.e., they are magnetic in the presence of an external magnetic field and not magnetic when the external magnetic field is withdrawn. After attaching monoclonal antibodies, nucleic acid probes, etc. to the MNP, targeting and capturing the target substance, the target substance is adsorbed and retained in the magnetic field by an applied magnetic field, while other substances are separated. With this magnetic separation technique, separation and enrichment can be achieved in a short time, thus increasing the detection rate [2-4].

2.2. QDs

Quantum dots are inorganic semiconductor nanocrystals that can produce fluorescent spherical semiconductor nanocrystals when exposed to excitation light. They are made of group II-VI (CdSe, CdTe, CdS, ZnSe, etc.) or group III-V (InP, InAs, etc.) elements and have a size limit of 1–10 nm in three dimensions. The wavelength of emission of quantum dots can be adjusted by changing the particle size. In addition, because their multi-electron system, fluorescence lifetime, quantum yield and optical stability are better than fluorescent dyes and fluorescent proteins, plus their multi-colour nature, excellent optical properties and long observable time, QDs are widely investigated for biomedical applications in labeling, imaging, targeted drug delivery, sensing, and therapy [5-7].

2.3. Au NPs

AuNPs appear to be the most efficient, with minimal systemic toxicity of all the nanoparticles. Gold at the nanoscale level also exhibits easy surface modification, high biocompatibility and unique optical properties, especially has a strong local surface plasmon resonance (LSPR) effect and surface enhanced Raman scattering (SERS) properties. When gold nanoparticles are irradiated by incident light electromagnetic waves, the gold nanoparticle surface electrons will resonate with the light of that wavelength at a certain frequency, thus making its nanoparticle solution show a certain colour at a specific absorption wavelength. With the variation in nanotechnology, gold turns from red to purple. As a result, it is used in many in vitro diagnostic testing systems [8-13].

3. Protein detection

3.1. Proximity ligation assay (PLA)

A specialized immunoassay technique called PLA was created recently to detect target proteins, protein interactions, etc. Using a pair of monoclonal or polyclonal antibodies that have been individually or collectively labeled with an oligodeoxyribonucleotide (single-stranded DNA), i.e. PLA enquires. As illustrated in Figure 1, the target protein may be detected when the pair of antibodies attach to separate epitopes on the target protein to recognize it. This proximity effect occurs when the two probes are close to one another and results in a specific signal output. It is a method that changes a protein's detection into a nucleic acid's detection.

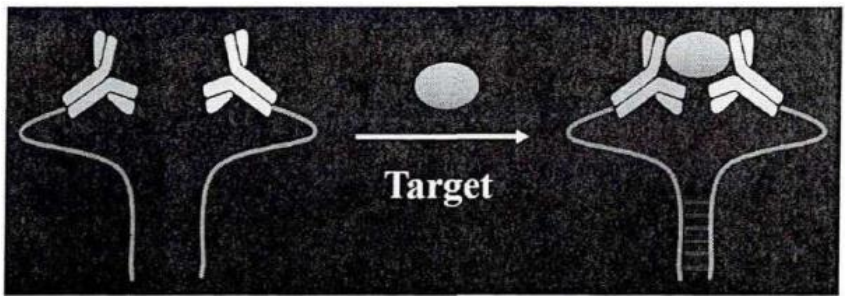

Figure 1. Schematic of proximity ligation assay [14]

Zhang et al. selected several major allergens in peanut, hazelnut and soybean foods, designed specific primers and padlock probes, and established an efficient and accurate method for the detection of important allergen proteins in foods using PLA, and improved the sensitivity and specificity of allergen detection by changing the real-time fluorescence polymerase chain reaction (PCR) reaction conditions to determine the optimal reaction conditions [15].

Jiang used immune microspheres as a medium to detect prostate-specific antigen (PSA) using solid-phase neighbor-joining (spPLA) technology having a minimal detection threshold of 0.1 pM and better reproducibility than immuno-PCR [16]. The combination of spPLA with ring-mediated isothermal amplification (LAMP) allowed the finding of PSA at a minimum concentration of 0.001 pM, which is 100 times higher than conventional immunoassays.

In order to make the assay easier to perform and to avoid experimental errors caused by washing microspheres, they used glutaraldehyde-treated quantitative PCR tubes instead of immune microspheres and developed a more efficient and sensitive method for protein detection using quantitative PCR tubes as stationary phase. The method is easy to wash, stable in antibody fixation, and has a sensitivity of 0.001 pM for PSA and wild-type p53, and can achieve a detection range of 7 orders of magnitude. Furthermore, he encapsulated the mutant p53-specific antibody PAb240 on the wall of the tube and applied the wall spPLA to detect mutant p53 in serum at a minimum concentration of 0.01 pM, which is approximately 500 times more sensitive than the conventional ELISA assay.

3.2. Au NPs for marker detection

As mentioned above, AuNP is a non-toxic, easily surface-modified nanomaterial with high biocompatibility and unique optical properties. The application of AuNP in protein detection is discussed here.

Xue et al. constructed a novel assay for the detection of bovine milk casein and its hydrolysates based on the antigen-antibody specific reaction and the nanogold fluorescence enhancement effect [17]. The experiment used nanogold as the carrier, firstly, the antibody was bound to the surface of nanogold with non-covalent bond, and then combined with fluorescein isothiocyanate (FITC) labelled protein according to the antigen-antibody specific reaction, based on the nanogold fluorescence enhancement effect to maximise the fluorescence of the labelled system, the protein to be measured and the FITC labelled protein competitively bound to the gold labelled antibody, and the protein content was indirectly determined by detecting the fluorescence intensity. The principle is shown in

Figure 2, and the assay system was then established through a series of optimisation experiments. The results showed that the fluorescence labelling rate of casein was 1.35, which was adequate, and the optimum amount of antigen added to the system was 30 μg/mL. The linear range of the method was 0.2-1 μg/mL, and the minimum detection limit was 0.2 μg/mL. The coefficients of variation of the reproducible tests were less than 6%. The results showed that the developed nanogold fluorescence-enhanced assay is simple, rapid, easy to operate and reproducible, and can be used for the quantitative determination of bovine casein and its hydrolysates.

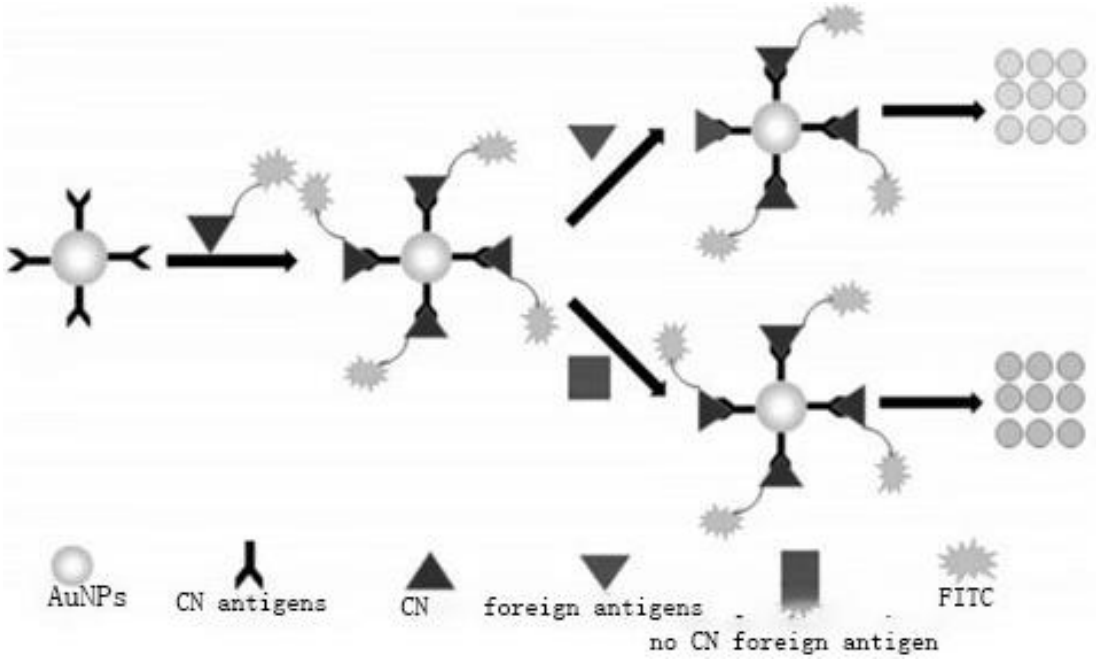

Figure 2. Nanogold fluorescence enhancement method for CN [17]

In these approaches, non-amplifiable protein detection is transformed into nucleic acid detection by NP-PLA and Bicinchoninic Acid Assay (BCA), providing very sensitive protein identification. Depending on the antibodies employed in the mechanism of reaction, NP**-**PLA and BCA can detect a wide range of targets, but also mean that their specificity is influenced by the specificity of the antibodies used. Protein detection techniques with AuNP as a marker use the FERT or NSET properties of AuNP to construct a fluorescent signal system similar to that of fluorescent probes in real-time fluorescent PCR, and to reflect the interaction between substances in a certain linear range of fluorescence intensity increment or decrement, thus achieving ultra-low level protein detection.

Qi used the thiolated aptamer DNA1 and its complementary short-stranded DNA2 to modify nanogold to construct AuNPs-DNA1 and AuNPs-DNA2 probes, respectively, and hybridized with the third fluorescently labeled aptamer complementary short-stranded DNA3 to form the assemblies to establish a biosensing platform with visible and fluorescent dual-mode signals [18]. When fish microalbumin was present in the sample, it competed for the aptamer-bearing AuNPs-DNA1 in the nanogold assemblies, leading to the unassembly of the nanogold assemblies, which brought about a change in the visible light signal on the one hand, and restores the fluorescence signal quenched by the FRET effect through the release of the fluorescently labelled complementary DNA3 on the other. The results show that this biosensor can be used for qualitative and quantitative dual-mode detection based on visible and fluorescent signals and has great potential for the detection of food allergens.

3.3. BCA

BCA technology is a new molecular diagnostic technique based on "signal amplification" that emerged at the beginning of the century. The detection system consists of two types of nanoparticles, AuNPs and MNPs, of which MNPs with monoclonal antibodies (or single-stranded DNA) on the surface are mainly used for the isolation of the test substance, while AuNPs with polyclonal

antibodies (or single-stranded DNA) and a large amount of "barcode DNA" on the surface are used to capture the test substance. In the presence of the target material, the two nanoparticles form an "AuNP **-**target material **-**MNP" sandwich structure (Figure 3). This technique was first reported in 2003 for the successful detection of prostate-specific antigen (PSA) due to the large amount of DNA bound to the AuNP surface. The AuNP surface is bound to a large amount of biocoded DNA, which increases the sensitivity of the assay by 2 to 3 orders of magnitude.

Butruk-Raszeja et al. studied polyurethane samples chemically modified with acrylic acid and polyurethane-grafted peptide (GSGREDVGSG) and developed a simple, rapid, and accurate method for calculating the surface density of a short peptide sequence bound to a polymeric substrate. The solid-supported peptide's surface density was successfully measured with the BCA assay.

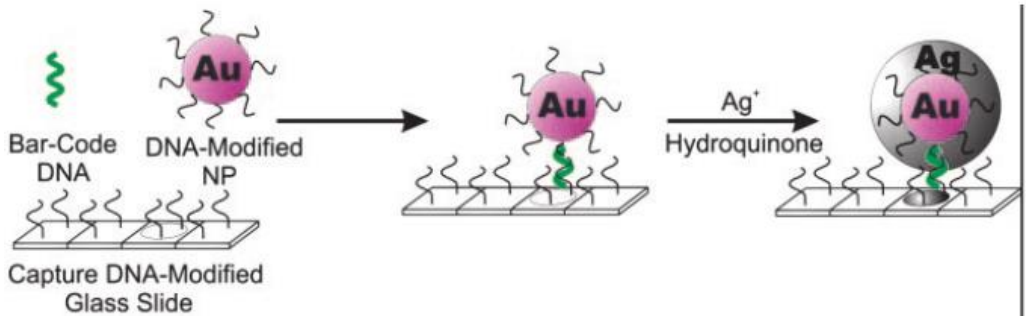

Figure 3. Sandwich structures formed in protein detection using BCA [19]

Miao et al. purified high purity FMD virus by discontinuous sucrose density gradient centrifugation and prepared several different dilutions of albumin from 0 to 200 μg/ml, which were simultaneously assayed by the BCA protein quantification method and successfully determined the content of virus protein [20]. In addition, Georganopoulou et al. used BCA to specifically detect a soluble pathogenic biomarker for Alzheimer's disease [19].

4. Nucleic acid detection

4.1. Nanochromatography

Nanochromatography strips are a class of devices based on the lateral flow assay (LFA) that can be used for the rapid validation of nucleic acid products obtained by PCR amplification, hybridisation chain reaction (HCR), loop**-**mediated isothermal amplification (LAMP) and other amplification techniques. Compared to traditional methods such as electrophoresis and imaging, the use of nanochromatography based on nanochromatographic strips for the validation of nucleic acid products does not require expensive instruments, is easy to operate and has the advantages of being short and specific, which makes it suitable for application in areas with limited equipment and can also meet the needs of POCT.

Lu constructed a rapid and low-cost lateral flow test strip biosensor for the genetic detection of hepatitis B virus [21]. The principle of the assay is based on the nucleic acid hybridisation reaction (Figure 4). In view of the time-consuming nature of the traditional DNA modification of gold nanoparticles, this experiment used a freeze-thaw method to rapidly modify the sulfhydryl DNA on the surface of the gold nanoparticles and add it to the strip sensor together with the target. The signal intensity over a given area of the test line was then read by a portable stripe reader as a basis for quantitative analysis. The method had a response span of 0.5-50 nM target DNA, with a detection threshold of 0.46 nM. It is promising for clinical applications and biomedical diagnostics, especially in resource-limited environments, saving cost and time.

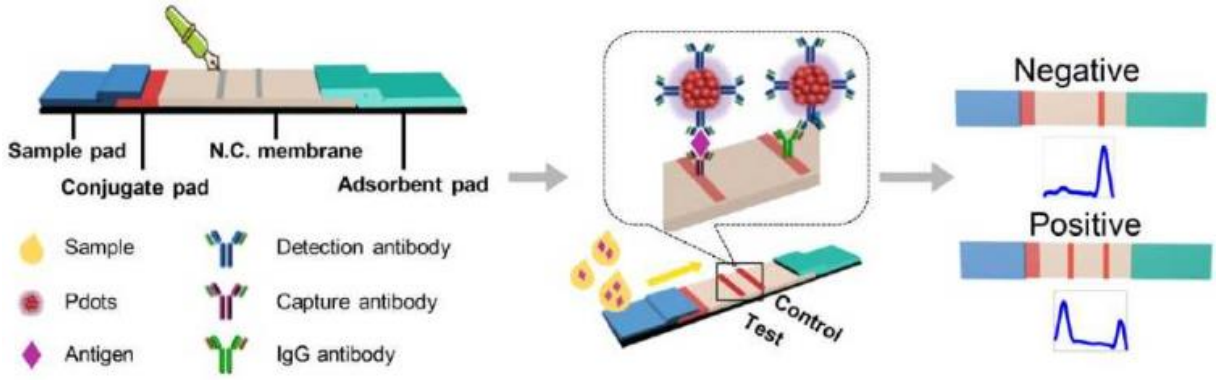

Figure 4. Schematic of Nanochromatography [21]

Zhao et al. used the principle of nucleic acid base complementary pairing to design the detection line and control line of nanogold flowmetric chromatography strips as above, and established the detection system for DENV [22]. The findings demonstrated the sensitivity of the isothermal chain substitution amplification was 10 fmol/L, and the results were visible to the naked eye with high sensitivity and specificity, and there was no crossover with other controls. A visual test for Salmonella spp. was created by Ying et al. For the extremely perceptive and reasonably priced visual detection of nucleic acids, 16S rRNA hybridization chain reaction (HCR) was created [23]. It is appropriate for non-specialist individuals and point-of-care (POC) diagnostics in low-resource settings due to the detection limit of 3 103 CFU/mL.

4.2. As an auxiliary to PCR

Since the advent of PCR, the ability to detect and analyse DNA and RNA has advanced and is now an important tool in the diagnosis of disease. PCR is one of the most fundamental and popular techniques in modern biology and in vitro diagnostics, amplifying very small amounts of DNA millions of times to detectable levels, and is used in a wide range of applications. Since its inception, a large number of researchers have improved and optimised the technique at the level of reagents, instruments and PCR procedures, greatly improving the amplification efficiency and gradually developing methods such as nested PCR, multiplex PCR, RT**-**PCR, fluorescent quantitative PCR and digital PCR that are suitable for different purposes and high sensitivity. However, the specificity of PCR techniques is still not up to standard compared to the increased sensitivity, and there are certain problems in the analysis of GC-rich fragments and amplification of long DNA fragments. Although it has been found that the addition of such as dimethyl sulfoxide, BSA, single strand DNA **-**binding protein (SSB) to PCR systems as enhancers can facilitate PCR amplification, the overall effect is still not satisfactory.

Nanomaterial-assisted PCR (nanoPCR) technology has drawn a lot of attention as nanotechnology has gradually permeated the biological sciences. According to previous research, there are three basic ways that nanoparticles play a role in PCR reaction systems. (1) Binding to DNA molecules: By binding to single-stranded DNA, nanoparticles (such as AuNPs, carbon nanotubes, etc.) might lessen mismatches between primers and single-stranded DNA templates. Additionally, certain nanoparticles can help the DNA template's double strands separate during the PCR process, which lowers the unstranding temperature. (2) Interaction with DNA polymerase: Some nanoparticles can boost DNA polymerase activity, while excessive nanoparticle concentrations can limit DNA polymerase activity. This impact can be somewhat countered by using more DNA polymerase. (3) Because of their great thermal conductivity, nanoparticles help PCR reaction systems achieve the target temperature more rapidly, which increases amplification efficiency and makes primer and template pairing more effective. [24].

Using nanoparticles to amplify low-copy gene templates from the human genome, Li et al. achieved the amplification of low-copy genes in complex systems, improved the yield of specific amplification and established a nano-PCR assay for Streptococcus suis type II and Haemophilus

parvum [25]. The sensitivity of nano-PCR can be anywhere from 10 to 1000 times higher than that of the normal PCR technique. In addition, gold nanoparticles can also be used for real-time fluorescent quantitative PCR to accelerate the reaction speed and improve the sensitivity of the assay. The products amplified by PCR using nanoparticles are easy to purify and facilitate subsequent molecular biology operations.

Qing et al. developed a new label-free, low-cost and environmentally friendly method for nucleic acid amplification detection using double-stranded DNA-specific copper nanoparticles as a "nanodye" in combination with PCR [26]. When the target DNA is not present, the single-stranded primers in the system do not mediate the formation of copper nanoparticles and thus no fluorescence is observed. However, the introduction of target DNA can trigger primer extension of the template-primer complex, which, through PCR amplification, can generate exponentially amplified double-stranded DNA, thereby mediating the formation of fluorescent copper nanoparticles.

A nanoPCR assay for brain-eating amoebas was reported in a study by Liu et al. [27]. They incorporated three nanomaterials, graphene oxide, copper oxide and aluminium oxide, into a common PCR system to achieve rapid diagnosis of several amoebas, and increased amplification yields were seen with electrophoretic band grey values of nucleic acid amplification products that were 1.25-2 times more than those of the no-gami-particle control.

4.3. Nanopore sequencing

The first generation of sequencing technology, represented by the double deoxygenated chain termination method (Sanger method), emerged in the middle of the 1970s, sparking interest in the human genome. The human genome project (HGP) from 1990 to 2003 directly contributed to the development of sequencing technology, and since then, sequencing technologies have developed rapidly, with 2nd and 3rd generation sequencing technologies coming into being.

Nanopores are microscopic holes created either physical drilling on a solid substrate or by the self-assembly of biomolecules. There are three types of nanopores: biological, solid-state, and composite nanopores, which are made up of both types. The fundamental idea behind nanopore sequencing is to transfer genetic material through a tiny molecular pore after passing an electric current across it. Because the bases that make up genetic material range in size and chemical structure, different bases cause different changes in the electric current when they pass through the nanopore, which results in the sequence information of the DNA or RNA being measured (Figure 5).

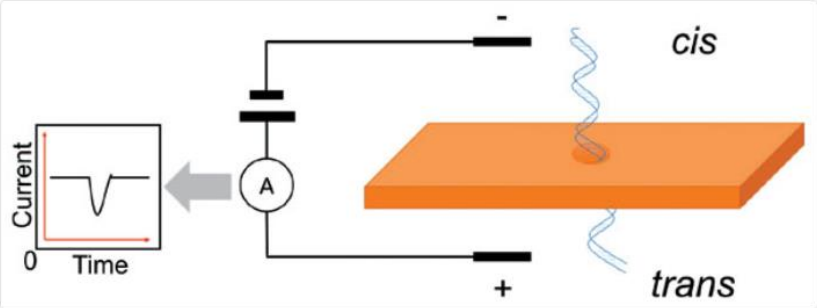

Figure 5. Schematic of Nanopore Sequencing [28]

Nanopore sequencing technology is regarded as a significant advancement for third generation sequencing technology since it is a single-molecule sequencing technology providing real-time data production, fast read throughput, and read length. In addition, nanopore sequencing technology has been selected as one of the National Institutes of Health's (NIH) "$1,000 Genome Initiative" because it does not require the involvement of various enzymes, biomolecular modification markers or surface immobilisation techniques, and has low cost. Several generations of products produced by Oxford Nanopore Technology (ONT) are now being utilized in applications such as public health and in vitro testing.

Because of its comparatively high error rate and the need for further processing to remove sequencing mistakes, nanopore sequencing technology formerly competed with next-generation sequencing technology (NGS).

DNA methylation is one of the most common types of epigenetic modifications, with 5-methylcytosine (5mC) methylation modifications, the most common in the mammalian genome, being involved in many important life events, and abnormalities in its levels being strongly associated with the development of disease, particularly cancer. Gigante et al. used MinION and PromethION and combined with Nonopolish to generate an embryonic placental whole-genome haplotype methylome from C57BL/6 and Cast-type hybrid mice (Mus musculus) [29]. With an average sequencing depth of 10×, they successfully identified known DMRs and novel DMRs, as well as imprinted genes with parental bias. By comparing with data obtained from Illumina-based sequencing platforms, the length-long nanopore gene sequencing technology is not only comparable in accuracy, but can also generate haplotype mammalian methylomes more efficiently.

Nanopore sequencing has also been used for the simultaneous study of methylation patterns and chromatin accessibility. NanoPore sequencing of nucleosome occupancy and methylome was developed by Lee et al. using CpG dinucleotide exogenous methyltransferase for the labelling of genomic open chromatin developed nanopore sequencing of nucleosome occupancy and methylome (NanoNOMe) [30]. They examined four different human cell lines and mapped the human epigenome including information on CpG methylation and chromatin accessibility in a fully staged manner. The results showed an inverse correlation between chromatin accessibility and methylation levels, suggesting that NanoNOMe not only reveals the potential role of DNA methylation in chromatin accessibility, but also allows for an assessment of the allele-specific epigenetic status of the entire genome.

5. Discussion

It is worth noting that nanomaterial-based tests often require characterisation of nanomaterials and the development of reproducible procedures to ensure the accuracy and reproducibility of the tests, which is less feasible in clinical practice. As a result, most nanoassay technologies still rely on the investment of in vitro diagnostic companies and the development of the nanotechnology industry to enter clinical applications; at the same time, despite the many advantages of such technologies, a certain amount of investment in human and material resources and the training of appropriate technical personnel is required before they can enter clinical applications, which also makes nanomaterial-based testing technologies still a long way from large-scale clinical applications. Nanomaterials that enter the environment during production, use and disposal can cause certain ecological effects and population exposure. According to nanotoxicological studies, nanomaterials can have toxic effects on cells and the body by causing oxidative stress and inflammation in humans, inducing or inhibiting cellular autophagy, and adsorbing a range of proteins in the physiological environment to form 'protein crowns' [31,32]. Therefore, while applying nanomaterials, the development of perfect nanotechnology standards should also accelerate to keep up to avoid the long-standing issues of "pollution first and treatment later".

6. Conclusion

In conclusion, due to their excellent sensitivity and quick detection, nanomaterial-based in vitro diagnostic technologies have a great deal of potential for clinical application. However, because traditional testing methods can still handle the majority of the demands of daily work and because nano-testing methods haven't yet found a market, the immaturity of the industry chain, the imperfection of their own technology and the need for appropriate technical personnel, such technologies still face certain obstacles in entering clinical applications. Future clinical uses of these technologies are anticipated, and they will build on their capabilities to address various clinical

demands and benefit both clinical medicine and humanity as a whole. This is a result of the advancement of nanoscience and medical laboratory science, as well as the growth of in vitro diagnostic businesses and local testing facilities.

References

[1] Ding Zehao. Research on the application and development of nanomaterials and their technologies. Materials and Applications, 207 (6) (2022) 52 - 54.

[2] Hira Fatima and Kyo-Seon Kim. Magnetic nanoparticles for bioseparation. Korean J. Chem. Eng., 34 (3), 589 - 599 (2017).

[3] Evgeny Katz. Magnetic Nanoparticles. Magnetochemistry, 6 (2020) 6.

[4] Su Zhiwen, Wu Deguang, Li Dongtai, Wu Youhui, Yu Bo. Magnetically responsive nanomaterials in bone tissue engineering. New Clinical Medicine in China, 15 (6) (2022) 557 - 562.

[5] Weidong Zhou, James J. Coleman. Semiconductor quantum dots. Current Opinion in Solid State and Materials Science, 20 (2016) 352 - 360.

[6] Li Shiguo, Wang Xinzhong, Fan Jinping, Xia Linzhong, Zhang Chunxiao, Du Jun. Overview of semiconductor quantum dots and their applications. Science&Technology Information, 29 (2011) 439, 454.

[7] Wei Xiaolei. Preparation of water-soluble quantum dot fluorescent probes and their biological applications. Huazhong Agricultural University, MA thesis (2011).

[8] Abdou Mohamed M A, Kozlowski H N, Kim J, et al. Diagnosing Antibiotic Resistance Using Nucleic Acid Enzymes and Gold Nanoparticles. ACS Nano, 15 (6) (2021) 9379 - 9390.

[9] Fan M, Han Y, Gao S, et al. Ultrasmall gold nanoparticles in cancer diagnosis and therapy. Theragnostic, 10 (11) (2020) 4944 - 4957.

[10] Ko W C, Wang S J, Hsiao C Y, et al. Pharmacological Role of Functionalized Gold Nanoparticles in Disease Applications. Molecules, 27 (5), (2022).

[11] Milan J, Niemczyk K, Kus-Liskiewicz M. Treasure on the Earth-Gold Nanoparticles and Their Biomedical Applications. Materials (Basel), 15 (9), (2022).

[12] Peng G, Tisch U, Adams O, et al. Diagnosing lung cancer in exhaled breath using gold nanoparticles. Nat Nanotechnol, 4 (10) (2009) 669 - 73.

[13] Fang H. Study on two signal amplification strategies based on gold nanoparticles to improve the sensitivity of immunological detection. Nanchang University, MA thesis (2021).

[14] Gao Hongfang. Research on new substances for luminescent chemical sensing and new methods for electrochemical luminescent biosensing. Shaanxi Normal University, PhD dissertation (2020).

[15] Zhang Xia, Zhao Liangjuan, Gao Lin, Wen Huawei, Wang Feixue, Dong Yan, Liu Doumeng, Liu Yin, Zheng Wenjie. Detection of peanut allergen components using neighbour-joining techniques. Food Research and Development, 37 (9) (2016) 148 - 151.

[16] Jiang, Xuecheng. Novel neighbor-joining technique for high sensitivity detection of biomolecules. Zhejiang University, PhD dissertation (2015).

[17] Xue Haiyan, Xue Lihuan, He Baoyuan, Fan Jiaojiao, Li Jingying. Nanogold fluorescence-enhanced detection of bovine milk casein and its hydrolysates. China Dairy Industry, 48 (6) (2020) 46 - 51.

[18] Qi Qinqin. Rapid detection of fish microalbumin based on aptamer-nanogold dual-mode biosensor. Zhejiang University of Technology and Industry, MA thesis (2020).

[19] Dimitra G. Georganopoulou, Lei Chang, Jwa-Min Nam, C. Shad Thaxton, Elliott J. Mufson, William L. Klein, Chad A. Mirkin. Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. PNAS, 102 (7) (2005) 2273 - 2276.

[20] Miao Haisheng, Li Le, Liao Defang, Li Huachun. BCA method for the determination of proteins and foot-and-mouth disease antigens. China Veterinary Husbandry, 35 (4) (2008) 84 - 86.

[21] Lv Sijia. Visualization of disease-associated genes by chromatographic test strips based on nanogold and nucleic acid probes. Hubei University, MA thesis (2019).

[22] Zhao J, Xia HX, Liu YJ, Ravinder Pal, Fan ANR, He ZX, Zeng LW. Detection of dengue virus using isothermal chain displacement amplification combined with nanogold flowmetric chromatography strips. Journal of Zhejiang University (Medical Edition), 47 (04) (2018) 405 - 412.

[23] Na Ying, Chuanjing Ju, Zhongyi Li, Wensen Liu, Jiayu Wan. Visual detection of nucleic acids based on lateral flow biosensor and hybridization chain reaction amplification. Talanta (IF 6.556) (2017).

[24] ALI Z, JIN G, HU Z, et al. A review on NanoPCR: history, mechanism and applications. J Nanosci Nanotechnol, 18 (12) (2018) 8029 - 8046.

[25] Li He, et al. Establishment and application of nano-PCR detection technology for two porcine bacterial diseases (2016).

[26] QING Taiping, QING Zhihe, HE Xiaoxiao, WANG KeMin, HE Dinggeng, SHI Hui, MAO precious. DNA amplification detection based on double-stranded DNA-stabilized fluorescent copper nanoparticles and PCR technology. Proceedings of the 29th Annual Meeting of the Chinese Chemical Society - Session 04: New approaches to nanobiosensing (2014).

[27] Liu QD, Wang JJ, Wang Y. Advances in nanoparticle PCR research. Biology Bulletin, 45 (2) (2010) 1 - 3.

[28] Abedini-Nassab Roozbeh, Nanotechnology and Nanopore Sequencing. Recent Patents on Nanotechnology, 11 (1) (2017).

[29] Gigante S, Gouil Q, Lucattini A, Keniry A, Beck T, Tinning M, Gordon L, Woodruff C, Speed T.P, Blewitt M.E, and Ritchie M.E. Using long-read sequencing to detect imprinted DNA methylation. Nucleic Acids Res, 47 (8) (2019) e46.

[30] Gilpatrick T, Lee I, Graham J.E, Raimondeau E, Bowen R, Heron A, Downs B, Sukumar S, Sedlazeck F.J, Timp W. Targeted nanopore sequencing with Cas9-guided adapter ligation. Nat. Biotechnol, 38 (4) (2020) 433 - 438.

[31] Thaxton C S, Elghanian R, Thomas A D, et al. Nanoparticle**-**based bio**-**barcode assay redefines “undetectable” PSA and biochemical recurrence after radical prostatectomy. Proc Natl Acad Sci USA, 106 (44) (2009) 18437 - 18442.

[32] YIN HQ, JIA MX, SHI LJ, et al. Nanoparticle**-**based bio**-**barcode assay for the detection of bluetongue virus. J Virol Methods, 178 (1/2) (2011) 225 - 228.