Chapter 9
Sandwich Assays Based on QCM, SPR, Microcantilever, and SERS Techniques for Nucleic Acid Detection

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Abstract Signal transducers which can read the signal toward targets are widely used for nucleic acid assay. Typically, the signal transducers based on quartz crystal microbalance (QCM), surface plasmon resonance (SPR) sensor, microcantilever, and surface-enhanced Raman scattering (SERS) play a significant role in the development of techniques for the detection of nucleic acid. The combination of these techniques with sandwich assay has received extensive attention due to the advantages of sensitivity and specificity. In this chapter, we summarized the recent development of the nucleic acid sandwich assay based on QCM, SPR sensor, microcantilever, and SERS. Additionally, the advantages and disadvantages of these sandwich assays along with the challenges and prospects are also presented, devoting to guide researches to design more of robust sandwich assays for nucleic acid assay.

Keywords Sandwich assay · Nucleic acid · Detection · Quartz crystal microbalance · Surface plasmon resonance · Microcantilever · Surface-enhanced Raman scattering

9.1 Sandwich Assays Based on QCM

QCM is a potential method to assess the surface phenomena of layers, such as antigen–antibody recognition [1, 2]. It is based on the mass change and the consequent change of piezoelectric crystals resonance frequency. Detailedly, functionalized crystal surface selectively captures the analyte, which results in the increase of the effective surface mass and the ensuing decrease in resonance frequency. Thus,

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the measurement of the binding event is achieved. This measurement is simple, robust and allows real-time detection. In earlier studies, Ward et al. coupled amplified mass immunosorbent assays with QCM to detect the adenosine 5′-phosphmulfate reductase and human chorionicgonadotropin. The measurement was based on the sandwich structure among anti-hCG, hCG, and anti-hCG/HRP [3].

However, it is known that the signal response of QCM-based assays in a very low target concentration is unstable, thus resulting in a low sensitivity. One way to increase the sensitivity is increasing the mass on the surface. The involvement of nanoparticles has been regarded as one of the methods. Based on this, Zhou's group developed a method for gene detection [4]. The oligonucleotide 1-functionalized QCM was hybridized with part of the target DNA 2 to form the dsDNA complex. The interaction between them resulted in the frequency decrease because of the increased surface coverage of the sensing interface. By adding the Au nanoparticle-modified oligonucleotide 3, the resulting frequency decrease was enhanced. The frequency signal was amplified because of the formation of a sandwich-type ternary complex, which consisted of an oligodeoxynucleotide immobilized on a QCM electrode, the target DNA, and an Au nanoparticle-modified oligonucleotide. Compared with the masses of the binding pair members, the mass of each nanoparticle was relatively large.

Another way to improve sensitivity is to change surface properties; Fan's group studied how single-stranded DNA (SH-ssDNA) and non-SH-ssDNA changed frequency in the presence of target DNA [5]. The result showed that the surface with thiolated SH-ssDNA attached demonstrated a sharp decrease of frequency, indicating the rapid occurrence of hybridization. However, the surface with non-SH-ssDNA attached showed no significant change in frequency.

By doing so, Su's group first developed a DNA sensor based on QCM for detection of pathogenic bacteria [6]. A thiolated single-stranded DNA (ssDNA) specific to *E. coli* O157:H7 eaeA gene self-assembled on the surface of QCM sensor. Then biotinylated target DNA was captured by ssDNA. The hybridization between the ssDNA probe and target DNA resulted in the mass change and consequent frequency change of the QCM. Moreover, the "mass enhancers" used in their assay was Fe_3O_4 nanoparticles, which amplified the frequency change. Their assay could sensitively detect 267 colony-forming units (CFU)/mL *E. coli* O157:H7.

Although the above assay improved the sensitivity and detection limit to some extent, it is still far from satisfactory compared to the traditional culture plating methods. Recently, Sandhyarani et al. developed a genosensor based on QCM and modified traditional sandwich assay [7]. In this work, to improve the sensitivity, gold nanoparticle was replaced by gold nanoparticle cluster (AuNPC), which conjugated with reporter probe DNA (DNA-r) for the hybridization with target DNA (DNA-t) (Fig. 9.1). The efficient immobilization of capture DNA (DNA-c) on the surface is necessary for a sensitive sensor. The surface of their sensor was modified with mercaptopropionic acid self-assembled monolayer in order to avoid the non-specific binding of the DNAs on the gold surface. The DNA sensor is based on the traditional sandwich assay. DNA-c was first immobilized on the SAM through EDC/NHS chemistry. Part of the DNA-t on the surface was complementary to DNA-c,

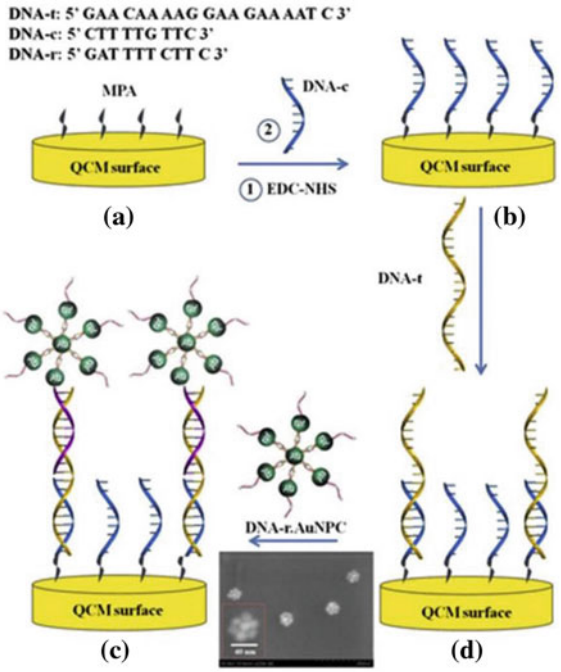

Fig. 9.1 Capture DNA (DNA-c) is immobilized on the QCM surface. On treating the surface with target DNA (DNA-t), reporter probe DNA (DNA-r) conjugated gold nanoparticles cluster (DNA-r.AuNPC) can hybridize to the DNA-t, which can be monitored as a function of DNA-t concentration using frequency change of the crystal (Reprinted from Ref. [7]. Copyright 2016 Elsevier)

which was hybridized to the immobilized DNA-c. Then, the open part of DNA-t hybridized to the DNA conjugated with AuNPC (DNA-r.AuNPC). The hybridization of DNA-r with the DNA-t produced a large increase of mass on the surface even at ultralow concentration of DNA-t. With this method they achieved the detection of 10 aM target DNA, which enhanced the sensitivity to few orders of magnitude.

QCM was also used to monitor the multiple (re)programming of protein–DNA nanostructures. Sánchez et al. studied the binding affinity of the multi-ligand-binding flavoprotein dodecin on flavinterminated DNA monolayers based on quartz crystal microbalance with dissipation (QCM-D) measurements (Fig. 9.2) [8]. A single apododecin–flavin bond was relatively weak, and stable dodecin monolayers were formed on flavin-DNA-modified surfaces at high flavin surface coverage due to multivalent interactions between apododecin bearing six binding pockets and the surface-bound flavin-DNA ligands. If bi- or multivalent flavin ligands were adsorbed on dodecin monolayers, stable sandwich-type surface-DNA-flavin-apododecin-flavin ligand arrays were obtained. The research showed how protein-DNA nanostructures could be generated, deleted, and reprogrammed on the same surface by exploiting multivalency and the redox properties of dodecin on the same flavin-DNA-modified surface.

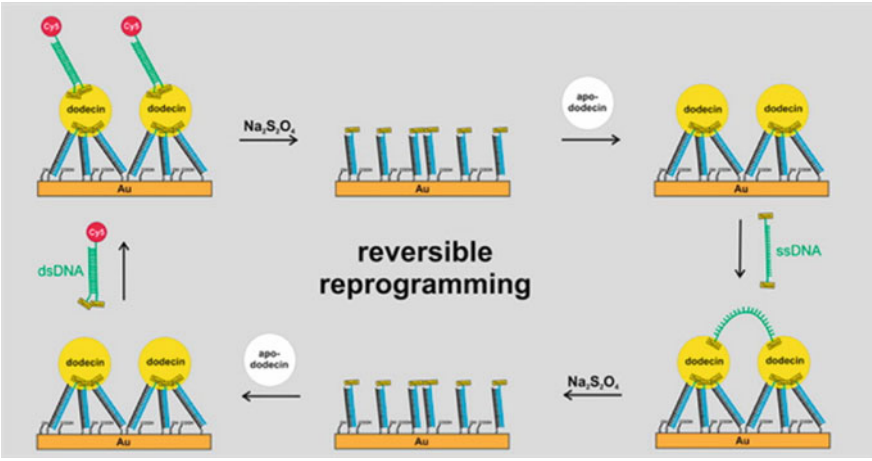

Fig. 9.2 The binding of apododecin and bidentate flavin-DNA ligands (writing) is made of the flavin-modified dsDNA layer. After the disassembly of the surface architecture by chemical flavin reduction (erasure), dsDNA layer modified with flavin can be used for further writing and erasure cycles. Multiple reprogramming with different bidentate flavin-DNA ligands is possible (Reprinted from Ref. [8]. Copyright 2015 American Chemical Society)

9.2 Sandwich Assays Based on SPR

SPR sensors possess unique ability for real-time monitoring the interaction of chemical and biological analytes through measuring the refractive index changes at the SPR sensing surfaces [9–11]. In order to detect low molecular weight biological analytes (DNA) under extremely low concentration conditions, improve the specificity as well as decreasing the limit of detection, sandwich format based on nanoparticles- or/and enzymes-enhanced SPR is usually employed. Typically, the SPR sensing film is modified with capture sequences at first. Then the targeted DNA sequences are flowed onto the film and bind to the capture sequences through sequence-specific hybridization. Following, the nanoparticles tags bind to the targeted sequences through sequence-specific hybridization or/and enzymes bind to the part of the DNA-DNA/DNA-RNA sequences catalyzing the downstream reaction. Therefore, the sandwich-like structure is formed and the enhanced SPR signal can be obtained.

Gold nanoparticles (Au NPs) are one of the most commonly used signal amplification labels in nanoparticles-enhanced SPR. Duo to efficient electromagnetic coupling between Au NPs and Au sensing film, a higher sensitivity can be obtained. The detection of nucleic acids based on Au NP-enhanced SPR has attracted much attention in the past decades [12–23]. Keating et al. reported the first demonstration of DNA hybridization sensing based on Au NP-enhanced SPR [12]. The sensing film was initially functionalized with the capture sequences. Then the oligonucleotide sequence-functionalized Au NPs and the targeted DNA sequences

were exposed to the SPR surface. A sandwich-like structure was formed through capture sequence-targeted DNA hybridization and targeted DNA-oligonucleotide sequences hybridization. A detection limit of 10 pM for 24-mer oligonucleotides was achieved, and such sensitivity for the target DNA has been significantly improved by more than 1000-fold compared to the unamplified binding detection method. This pioneering work demonstrated the potential of Au NP-amplified SPR for ultrasensitive detection of oligonucleotides. However, such SPR method is faced with a problem, non-specific absorption, when used for complicated samples. Misawa et al. coated a mercapto-acetic-acid (MAA) layer on the Au film to prevent undesirable DNA absorbing and optimized the length of Au NP-attached probe DNA to improve the performance of Au NP-enhanced SPR [13]. The DNA sequences with different chain lengths (15-mer, 30-mer and 60-mer) were tested, and the best sensitivity was obtained with the 30-mer DNA-functionalized Au NPs probe. With such optimized probe, target DNA within a large dynamic detection range of 1 pM to 10 mM can be detected. Zhou et al. reported that 39-mer target DNA as well as p53 cDNA can be detected with high specificity and reproducibility by combining oligonucleotide-capped gold nanoparticle with a microbore flow injection (FI) SPR setup [14]. Specifically, a carboxylated dextran film was immobilized onto the sensing film so that non-specific adsorption of oligonucleotide-capped Au NPs can be eliminated. Using this sensing strategy, 1.38 fM of 39-mer oligonucleotides and 100 fM of p53 cDNA can be detected with a remarkable sensitivity.

It is known that the size, shape, morphology of Au NPs play an important role in increasing the sensitivity of SPR [9]. Haam et al. further used a series of spherical Au NP to investigate the sensitivity enhancement properties for DNA hybridization detection [19]. In their work, Au NPs with different sizes (12–20 nm) and Au nanograting patterned sensing film were employed. The signal enhancement factor increased from 6.6-fold to 11.6-fold, and the maximum 18.2-fold was achieved with the 20 nm Au NPs coupling with the nanograting patterned sensing film. Minunni et al. used star-shaped gold nanoparticles as the nanoparticle tags and 6.9 aM of human genomic DNA can be detected [23]. Such high sensitivity was achieved thanks to the enhanced-plasmon coupling between the stars and the sensing film. In addition to DNA sequences, peptide nucleic acids (PNAs) are proposed as valuable alternatives to oligonucleotide as capture probes. Kim and co-workers utilized peptide nucleic acid as the capture probe and cationic Au nanoparticle for signal amplification by ionic interaction [18]. This method resulted in a detection limit of 58.2 ± 1.37 pg mL^{-1}. Similarly, Spoto and co-workers used PNA as the capture probe and achieved a detection limit of DNA sequences as low as 1 fM [17]. Besides, this sandwich format can remain highly sensitive in single-nucleotide mismatched recognition.

Other kinds of nanoparticles, such as hydrogel nanospheres [24], Fe_3O_4 nanoparticles [25, 26], and silica nanoparticles [27], have been employed for signal amplification of SPR sensors as well. Enzyme-enhanced SPR is another way to improve the sensitivity with enhancement factors compared to that of Au NP-enhanced SPR [28–33]. Gao et al. reported a signal amplification strategy with the assistance of DNA-guided polyaniline deposition [30]. The target DNA

hybridized with the PNA probe first and DNA-templated polyaniline deposition was followed in the presence of H_2O_2 and horseradish peroxidase. The in situ polymer chain growth along DNA strands contributed to the sensitivity improvement and 50-fold improvement of the limit of detection was achieved. Corn and co-workers used RNase H that can specifically digest RNA oligonucleotides in RNA-DNA heteroduplex to gain significantly enzymatic amplification [28]. When the target ssDNA binding to the ssRNA capture probe immobilized on the sensing microarrays to form a heteroduplex, RNase H can digest the ssRNA and the target ssDNA can be released. The released DNA was further hybridized with another ssRNA and induced another enzymatic hydrolysis. Such repeatable cycle resulted in enzymatic amplification sensitivity by 6 orders of magnitude. Therefore, DNA targets with concentration down to 10 fM can be detected.

By combing signal enhancement based on the enzymatic amplification and nanoparticles, the sensitivity can be further improved [11]. In 2006, Corn et al. put forward a novel approach to detect multiple microRNAs by combining a surface enzyme reaction with nanoparticle-amplified SPR imaging (SPRI) [34]. In their work, three kinds of miRNAs (miR-16, miR-122b, miR-23b) obtained from mouse liver tissue were detected and locked nucleic acids (LNAs) were specifically used as the capture probe for these miRNAs. The proposed method includes three steps. The LNAs were initially immobilized onto a microarray format and the targeted miRNAs hybridized with the complementary LNAs. Then poly(A) polymerase was added into the array to form a poly(A) tail on the miRNAs. Finally, the solution of T_{30} functionalized Au NPs was flowed onto the sensing surface and hybridized with poly(A) tails. Through such ultrasensitive NPs-amplified SPRI methodology, a detection limit of 10 fM was gained. In 2011, Corn et al. proposed a signal amplification strategy by coupling a surface RNA transcription reaction to nanoparticle-enhanced SPRI (Fig. 9.3) [35]. In their design, two kinds of adjacent microarrays, one called generator for RNA transcription and the other called detector for Au NP-enhanced detection of the transcribed RNA, were employed. The capture DNA sequences containing T7 promoter sequence was modified on the generator microarray and then target DNA sequences hybridize with the capture DNA to form dsDNA templates. In the presence of T7 RNA polymeras, numerous ssRNA copies were synthesized. Such transcribed ssRNA can diffuse to the detector microarray and be captured by the second ssDNA immobilized on the detector. ssDNA-functionalized AuNPs were added into the system and absorbed on the detector through hybridizing with the transcribed ssRNA. This dual amplification method can be used to detect ssDNA down to 1 fM.

In addition to the methods mentioned above, there are other types of amplification strategy. Szunerits and co-workers demonstrated that DNA in the attomolar concentration range can be detected with SPR by non-covalent coating graphene layers on gold sensing film [36]. Recently, Wang et al. proposed a multiple signal amplification strategy for miRNA detection (Fig. 9.4) [37]. In their strategy, a hairpin probe was employed as the capture probe and immobilized on the Au film first. Then target miRNA was hybridized with the hairpin probe; therefore, the stem-loop was unfolded and the DNA-functionalized Au NPs can hybridize with

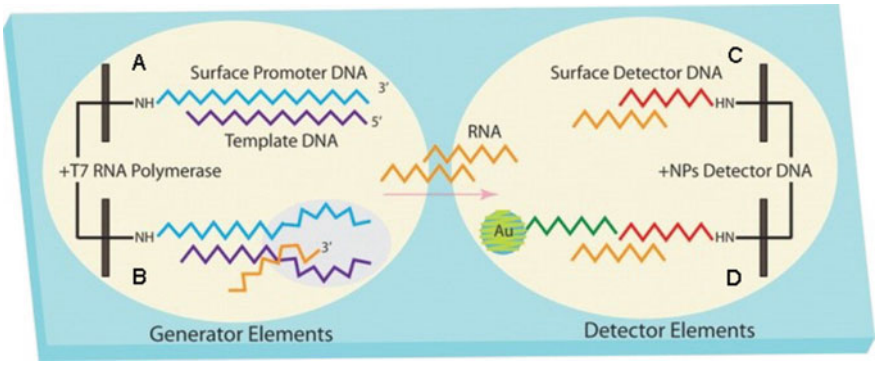

Fig. 9.3 Schematic illustration of the signal amplification strategy. **a** On the generator elements a surface promoter DNA is covalently attached to the gold surface and then hybridizes with template DNA from solution. **b** An in situ surface RNA polymerase reaction is used to transcribe numerous ssRNA copies. **c** The ssRNA is base-paired with the surface detector DNA. **d** DNA-modified AuNPs can bind to the detector elements via RNA hybridization and the ssRNA is detected with nanoparticle-enhanced SPRI (Reprinted from Ref. [35]. Copyright 2011 American Chemical Society)

the terminus of the unfold hairpin. Subsequently, two kinds of report DNA sequences were added into the above system and DNA supersandwich structure was formed. Finally, numerous positively charged Ag NPs were added and absorbed onto the long-range DNA supersandwich. Through such strategy, 0.6 fM of miRNA-21 can be detected and single-base mismatch can be sensitively recognized. The multiple signal amplification was achieved through three main factors: (1) enhanced-electronic coupling between localized plasmon of the Au NPs and surface plasmon of the sensing film; (2) enhanced-refractive index of the medium induced by DNA supersandwich structure; (3) enhanced-electronic coupling between localized plasmon of the Ag NPs and surface plasmon of the sensing film.

9.3 Sandwich Assays Based on the Microcantilever

Microcantilever has recently been emerging great attention in the field of chemical, physical, and biological detection [23–26, 38–43]. The fundamental principle for microcantilever-based assay is that the adsorption of molecular on one cantilever surface can change the surface stress that causes the mechanical bending deflection motion of the cantilever [44–47]. By selecting adsorbed probe that can recognize the specific molecular, it has the possibility to detect various targeted molecules. Like QCM and SPR, microcantilever can directly transfer the molecular recognition into nanomechanics and does not need the labeling of targets. Serving as an upcoming sensing technique, microcantilever exhibits many advantages such as high sensitivity, potential low cost, and faster response time. It has broad application in chemical, physical, and biological detection. For instance, Gerber's group developed

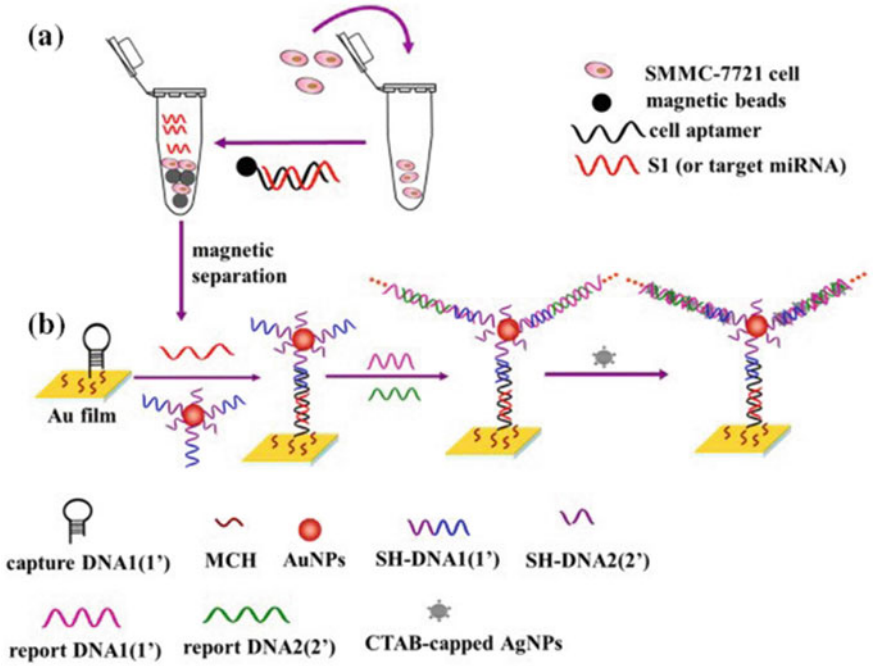

Fig. 9.4 Schema of SPR biosensor. **a** The SMMC-7721 cell aptamer-modified magnetic beads were hybridized with the complementary sequence, and then were incubated with cells. S1 probe was released through magnetic separation, and the S1 probe could act as the target. **b** The stem-loop structure was unfold in the presence of S1 probe, and then DNA-linked gold nanoparticles was hybridized with S1 probe. DNA supersandwich structure was formed upon introducing report DNA1 and report DNA2. Numerous positively charged silver nanoparticles (AgNPs) were bound to the DNA supersandwich, resulting in a increase of resonance angle shift (Reprinted with permission from Ref. [37]. Copyright 2017 Elsevier)

a method based on microcantilever arrays to detect the mutation in total RNA samples extracted from melanoma cells [40]. The BEAF-specific oligonucleotide probe was linked on one cantilever surface. The target DNA or RNA containing the matching sequence and the other non-related sequences was injected to the microcantilever arrays. On hybridization, only the probe cantilever bended, but no binding occurred on the reference cantilever, thus giving a differential deflection (Fig. 9.5). They detected the mutant BRAF at a concentration of 0.5 nM in a 50-fold excess of wild-type sequence. This method had an ability to distinguish melanoma cells with mutation BRAF using the RNA concentration as low as 20 ng μL^{-1}, without using PCR amplification. Gerber et al. reported microfabricated cantilevers for DNA hybridization assay [23]. They immobilized a selection of receptor molecules on one cantilever and then detected the mechanical bending induced by the ligand binding. The differential deflection of the microcantilevers provided a true molecular recognition signal, and they monitored the bending of each cantilever in real time by

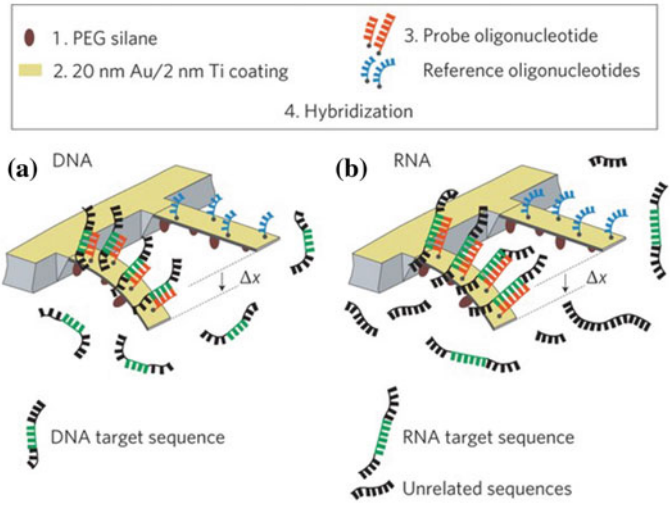

Fig. 9.5 Principle of microcantilever array for the detection of a BRAF mutation in total RNA. The cantilevers were modified with a probe oligonucleotide (in red) and a reference oligonucleotide (in blue). On treating with target DNA or RNA, only the probe cantilever bends, and then the differential deflection Δx increased (Reprinted with permission from Ref. [40]. Copyright 2013 Nature Publishing Group)

investigating changes in the optical beam deflection. They have shown that a single-base mismatch between two 12-mer oligonucleotides could be clearly detected. In the opposite way, the thermal dehybridization of double-stranded DNA on the cantilever surface was investigated by Majumdar's group [46]. They used heat to separate the double-stranded DNA into two single strands. The dehybridization of double helix on one microcantilever beam could lead to one complementary DNA diffused away from the other DNA strand. Therefore, the change of surface stress was observed. They have successfully distinguished the changes in the melting temperature of double-strand DNA on the basis of salt concentration and oligomer length. Interestingly, McKendry et al. reported that the force generated by an i-motif conformational change could be probed using the micromechanical cantilever arrays coated with a non-specific sequence of DNA [48].

Improving detection sensitivity is of significance for clinical diagnosis and various genome projects. In the previous study, Dravid's group has developed a sandwich-based microcantilever for DNA detection using gold nanoparticle-modified probes [45]. The capture DNA was firstly linked on the cantilever, and then the target DNA was hybridized with the capture DNA. The gold nanoparticle-labeled DNA strand was integrated into the capture DNA–target DNA complex through the complementary interactions. Gold nanoparticles served as a nucleating agent for the growth of silver, which could lead to a detectable frequency shift due to the increasing of the mass of the microcantilever. The core strategy of this idea is that the DNA hybridization can cause the mass change of a microfabricated cantilever, and the

signal can be amplified by gold nanoparticle-catalyzed nucleation of silver. They can detect the target DNA concentration down to 0.05 nM. In addition, a single-base mismatch can be discriminated.

According to recent studies, dynamic-mode millimeter-sized cantilevers can detect the oligonucleotides at extremely lower concentration in comparison to the static-mode microcantilevers. Kim et al. have developed a silica nanoparticle-enhanced dynamic microcantilever biosensor for Hepatitis B Virus (HBV) DNA detection (Fig. 9.6) [49]. The capture DNA was immobilized on the microcantilever surface. Then, the HBV target DNA could hybridize with the capture DNA, and the silica nanoparticle-labeled probe DNA was conjugated with this capture DNA-probe DNA complex. To make the silica nanoparticle efficiency to enhance the detection sensitivity, they optimized the size of the silica nanoparticle and the dimension of the microcantilever. Without nanoparticle enhancement, the HBV target DNA was detected up to pM level. When the silica nanoparticle-based signal amplification process was applied, they could detect the concentration of HBV target DNA at fM level.

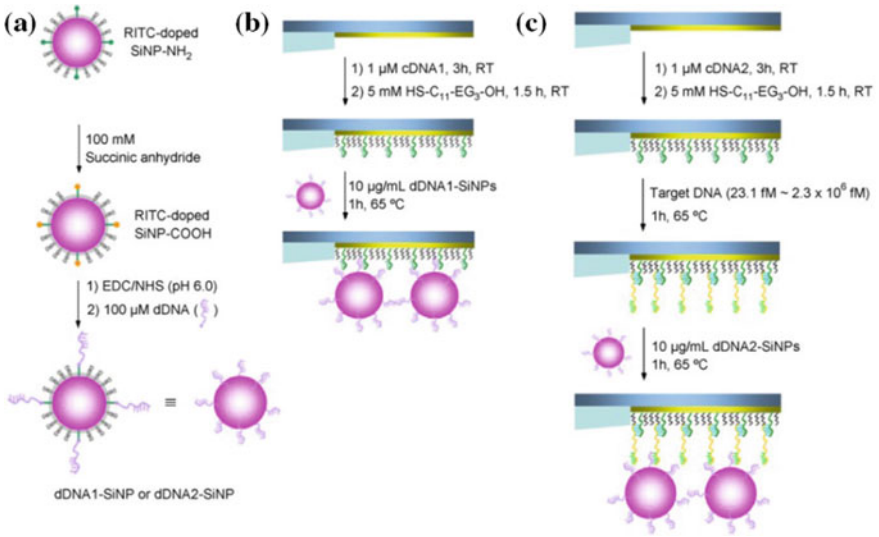

Fig. 9.6 Schematic illustration of DNA assay using the silica nanoparticle (SiNP)-enhanced microcantilever. **a** SiNP was modified with detection DNA. **b** dDNA was hybridized with cDNA on the microcantilever to optimize conditions of the SiNPs-enhanced DNA detection. **c** In the presence of the target DNA, the nanoparticle-based sandwich assay was formed. The concentration for the target DNA was monitored by the resonant frequency shifts (Reprinted with permission from Ref. [49]. Copyright 2009 Elsevier)

9.4 Sandwich Assays Based on SERS

SESR is a surface-sensitive technique that can enhance the Raman scattering by absorbing molecules on rough metal surfaces or by nanostructures [50]. The phenomenon of SERS is commonly explained by combining an electromagnetic mechanism (reflecting the surface electron movement in the substrate) and a chemical mechanism (relating to charge transfer between substrate and target molecules) [42, 51–53]. Mirkin's group reported a sandwich assay strategy based on the Raman spectroscopic fingerprints for multiple DNA and RNA detection [38]. They designed the nanoparticle probes by labeling the gold nanoparticles with specific oligonucleotides and Raman dyes. The silver-coated gold nanoparticles were employed as a surface-enhanced Raman scattering promotor. By integrating with SERS spectroscopy, the Raman spectroscopic fingerprint could be identified by scanning Raman spectroscopy. A series of Raman scattering response could be obtained. Therefore, a large number of oligonucleotides with different sequences could be detected. The detection limit of this strategy was 20 fM.

To make the nucleic acid detection more sensitive and stable, Yang et al. found that the semiconductor nanoparticles which have Raman signal were more applicable for achieving the high sensitivity detection than the dye molecules [54]. Since ZnO quantum dots (QDs) are capable of transferring electrons to gold nanoparticles, ZnO/Au nanocomposites can achieve the electromagnetic-field enhancement. They functionalized the thiol-oligonucleotides with ZnO/Au nanocomposites as Raman labels. The capture DNA was firstly immobilized on the gold film, and the target DNA and the ZnO/Au functionalized probe DNA could be hybridized with the capture DNA to form a stable sandwich structure. With a strong resonance Raman scattering signal output, the target oligonucleotide strand could be detected with extraordinary sensitivity and selectivity (Fig. 9.7).

A SERS "hot spot" is predicted to be created by forming a junction between nanoparticles and smooth surface. Reich and Moskovists developed a versatile SERS biosensor by assembling the probe DNA-tethered Ag nanoparticles to the smooth Ag surface due to the hybridization of the target DNA with the capture DNA and probe DNA [48]. The "hot spot" was thus created to enhance the Raman signals. The intense and reliable SERS signals could be obtained at near-single particle level. Furthermore, it was indicated that the decrease of the distance between the nanoparticle and Raman molecule can enhance the Raman signal. Based on this principle, by forming a SERS "hot spot" between the nanoparticles or between the nanoparticles and surface, the sandwiched structure-based DNA biosensor has been developed for HIV-1 DNA detection by Liu's group [55]. The target DNA triggered the formation of sandwich structure of capture DNA–target DNA–probe DNA. Then, the probe DNA was further recognized by another Raman tag-labeled probe DNA. The multi-metal-molecule-metal-sandwiched structure was finally constructed, which created a SERS "hot spot" and further decreased the distance between the gold nanoparticles and Raman tags. Therefore, the Raman signal has been largely enhanced and this sandwich-based platform could detect

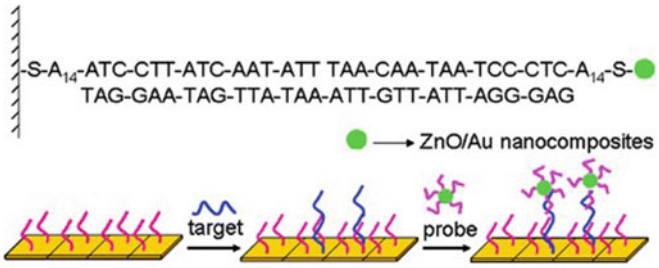

Fig. 9.7 Capture DNA was attached to the Au film. In the presence of target DNA, ZnO/Au nanocomposites-based sandwich assay was formed, and the ZnO/Au nanocomposites were used as Raman labels (Reprinted with permission from Ref. [54]. Copyright 2008 American Chemical Society)

DNA concentration down to 10^{-19} M with the capability for distinguishing the single-base mismatch.

To control the assembly of nanoparticles to turn on the Raman signal enhancement in a reproducible manner, Faulds developed a DNA-based assembly process to control the enhanced Raman scattering [56]. They applied the DNA hybridization to control the enhancement of Raman scattering from Ag nanoparticles labeled with a Raman dye. Here, two mechanisms were involved to be responsible for the signal enhancement, which were chemical and electromagnetic. A monolayer dye was firstly modified on the Ag nanoparticles; two different oligonucleotide probe sequences were then linked on the surface of Ag nanoparticles, respectively. The target DNA sequence finally integrated with these two different Raman dye-labeled Ag nanoparticles to form a controlled assembly. The signal was only capable of being obtained when the target recognition event of DNA hybridization taken place. Similar to the strategy reported by Faulds, Graham et al. precisely controlled the assembly of dye-coded and oligonucleotide-modified Ag nanoparticle conjugates [49]. This strategy could discriminate the single mismatched base in an unmodified target oligonucleotide.

To achieve the simultaneous multiple detection of nucleic acid, Song and Wen developed a SERS-based sandwich method using the mixed DNA-functionalized Ag nanoparticles [57]. Three kinds of probe DNA strands were co-assembled at the surface of the Ag nanoparticles at equal molar ratios to form conjugate 1. In addition, they prepared three kinds of stable Ag nanoparticle-oligonucleotide conjugates based on the Raman dyes and triple-cyclic disulfide-modified DNA strands. The targeted DNA could be hybridized with the conjugate 1 and the corresponding probe conjugate 2, and the electromagnetic enhancement of Raman dyes labeled on these nanoparticles is therefore enhanced (Fig. 9.8). Thus, the specific detection of multiple target DNA could be achieved.

Wang showed that enriching the target-mediated Raman tags aggregation is a promising strategy to improve the detection sensitivity [58]. They attached the probe DNA to the silica-Ag nanoparticles composite and labeled the magnetic

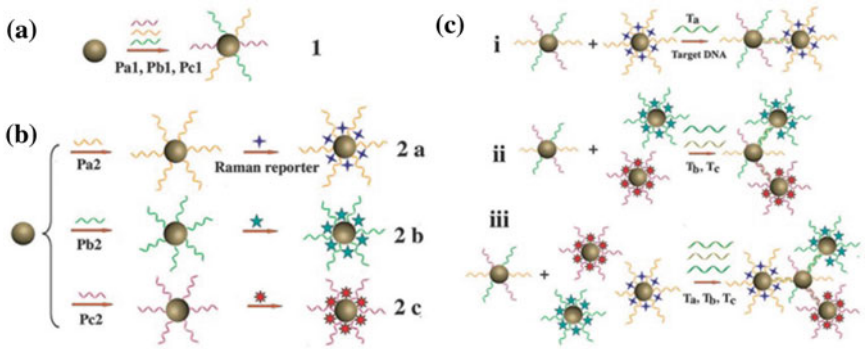

Fig. 9.8 **a** and **b** A scheme of the preparation of the mixed DNA-modified AgNPs 1 and Raman dye and DNA-modified AgNPs 2, respectively. **c** The SERS-based sandwich detection system for one (I), two (II), three (III) target DNA detection (Reprinted with permission from Ref. [57]. Copyright 2011 The Royal Society of Chemistry)

nanospheres with the capture DNA. The target DNA was first allowed to hybridize with the SERS tag-labeled probe DNA. With the addition of the capture DNA-linked magnetic nanospheres, it could be integrated with the above composite. When the external was applied to the reaction solution, the nanocomposites were deposited together and then could be separated and analyzed by SERS (Fig. 9.9). They realized the quantitative detection of target DNA in the range of

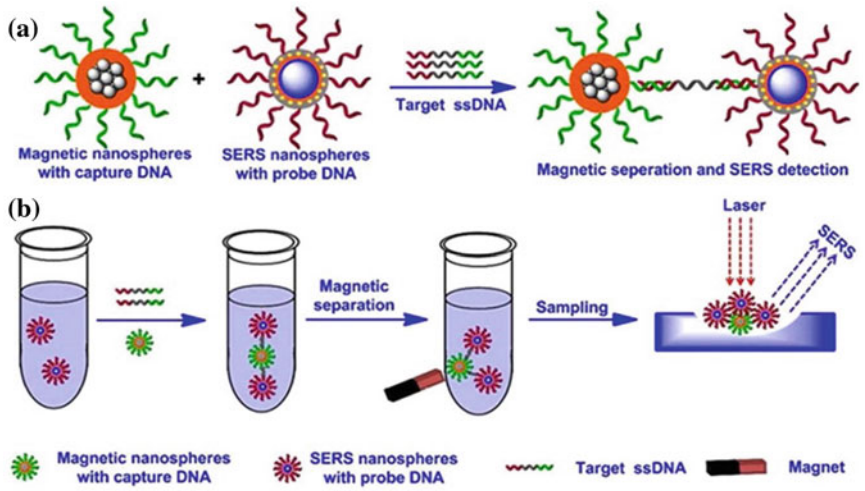

Fig. 9.9 **a** Schematic representation of a three-component-based sandwich assay in the presence of target ssDNA. **b** Scheme of the experimental procedure for the detection of target ssDNA (Reprinted with permission from Ref. [58]. Copyright 2013 American Chemical Society)

10 nM to 10 pM. In addition, they achieved the multiplexed detection of up to three different target DNAs.

9.5 Conclusion

The analysis of nucleic acids is important in the research of their fundamental functions. Developing sensitive, simple, and specific detection techniques for DNA detection is of great significance for the development of molecular diagnostics. In this chapter, we have described the recent developments in the detection of nucleic acid by the sandwich assays based on QCM, SPR sensor, microcantilever, and SERS. Sandhyarani et al. achieved the ultrasensitive detection of DNA by using the QCM-based sandwich assay. For SPR-based sandwich assay, various nanoparticles such as AuNPs, hydrogel nanospheres, Fe_3O_4 nanoparticles, and silica nanoparticles have been used as signal amplification labels for signal amplification of SPR sensors. This amplification method can be used to detect ssDNA down to 1 fM. In addition, by applying the silica nanoparticle-based signal amplification process to the microcantilevers-based sandwich assay, Kim et al. achieved the detection of HBV target DNA at fM level. The simultaneous multiple detection of nucleic acid was achieved by Song and Wen by using the SERS-based sandwich assay. The large achievement of the sandwich assay was realized in recent decades, but there are still some problems such as complex process, non-specific adsorption, and stability need to be solved. This relies on the further development of sandwich assay to resist these problems.

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