OPEN ACCESS

Nano Biomed Eng

ISSN 2150-5578 http://nanobe.org

Review

Nanoparticle-based Optical Detection of MicroRNA

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Abstract

MiRNAs are valuable biomarkers for diagnosis and prognosis of cancers, so it is imperative to develop rapid, sensitive, high throughput miRNA detection methods. In this review article, the current nanoparticle-based miRNA detection by optical technology was summarized from the following aspects about fluorescence, scattering and colorimetry. And the emphasis is laid on employing quantum dots (QDs) and silver nanoclusters (AgNCs)-based fluorescence and silver nanorod-based surface enhanced Raman scattering (SERS) to detect miRNAs. Nanoparticle-based optical detection is prospective for multiplexed miRNA detection or in vivo imaging.

Keywords: Colorimetric detection, MicroRNA detection, Quantum dots, Silver nanoclusters, Surface enhanced Raman scattering

Citation: J.P. Zhang and D.X. Cui. Nanoparticle-based optical detection of microRNA. Nano Biomed. Eng. 2013, 5(1), 1-10. DOI: 10.5101/nbe.v5i1.p1-10.

1. Introduction

MicroRNAs (miRNAs) are endogenous noncoding RNAs with a length of ~22 nt [1]. It was first found in C. elegans by Victor Ambros, Rosalind Lee and Rhonda Feinbaum in 1993 [1]. Until the year of 2010, the registered miRNAs in the miRSanger Base reached up to more than 10,500 from 115 species [2]. MiRNAs are involved in the control of development, differentiation, proliferation, apoptosis, the stress response and other cell processes through the cleavage of target mRNA or repressing its expression. One mRNA is regulated by multiple miRNAs, in turn, one miRNA is involved in regulations of various mRNAs, thus forming a complicated miRNA functional network [3]. More importantly, most of human miRNAs are closely correlated with human diseases, including cancers. MiRNAs are usually located in cancer-associated genomic regions (CAGRs) [3], such as minimal regions of LOH (loss of heterozygosity) and amplification, common breakpoint regions and fragile sites (FRA), and thus frequently dysregulated in cancers [4]. MiRNAs have been proposed to contribute to oncogenesis because they can function either as tumour suppressors (as is the case for miR-15a and miR-16-1) or as oncogenes (as is the case for miR-155 or members of the miR-17~92 cluster) [3]. Compared to mRNA expression profiles miRNA expression profiles may be more accurate in cancer classification [5]. Therefore, miRNAs are potent to be valuable biomarkers for diagnosis and prognosis of cancers.

MicroRNAs from both the solid tissues and the body fluids are capable of being predictive biomarkers. MiRNAs can be reliably extracted and detected from frozen and paraffin-embedded tissues [4], cultured cell-lines [6] and even circulating cells in peripheral blood [7]. Tissue miR-21 is an anti-apoptotic and pro-survival factor for different types of tumors, including breast, lung, gastric and prostate, by targeting PTEN (phosphatase and tensin homolog deleted on chromosome ten) [3]. Wu and Lee etc. summarized that a substantial number of miRNAs associated with cell cycle, apoptosis, metastasis and transcriptional regulation show differential expression in gastric cancer tissues [8]. Additionally, miRNAs are shown to present in human blood (including whole blood, plasma and serum) in a remarkably stable form that is protected from endogenous RNase activity [9]. MiRNAs are also found in other body fluids, such as saliva, human breast milk [10], circulating exosomes and even urine [4]. Excitingly, Mitchell and his collegues employed TaqMan RT-PCR detecting the serum levels of miR-141 and demonstrating miR-141 from serum as a biomarker to distinguish patients with prostate cancer from healthy controls [9]. Nearly at the same time, serum miR-25 and miR-223 were found as non-small cell lung cancer-

specific biomarker by Solexa sequencing, and this fact was further validated by quantitative reverse transcription polymerase chain reaction assays [11].

For the purpose of developing miRNAs as reliable diagnostic and prognostic biomarkers, it is primarily important to develop or optimize the efficient, sensitive and reproducible detection methods [4]. Different methods and technologies for miRNA detection have different developmental levels from a single miRNA detection to mass population or multiplexed miRNAs profiling. Currently widely used methods are northern bloting, qRT-PCR (quantitative reverse transcription-polymerase chain reaction), and microarray for miRNAs with the already-known sequences, and next generation sequencing for miRNAs with unknown sequences. As the conventional standard method, northern bloting bears the following defects: the huge time requirement, necessity for large RNA samples and potentially hazardous radioactive labels, making it somewhat unsuitable for expression level studies [12]. MiRNAs detection by RT-PCR not only needing the prior reverse transcription with low efficiency easily makes underestimation or even false negative PCR reactions, but also needing dedicated design of primers, specified instruments, difficult normalization methods and complex data analysis makes it time consuming and costly per sample [13]. The microarray methods-which hybridize all the target miRNAs or cDNAs to the probes under the same condition, are prone to cross-hybridization, thus result in false positives or negatives [14]. Next generation sequencing could discover novel miRNAs with unknown sequences with high sensitivity, specificity and throughput, but the large quantity of data to analysis and the highly-cost reagents and instruments limited its wide use [14]. Microarray and deep sequencing with higher throughput and RT-PCR with higher sensitivity and specificity are usually combined to screen the miRNA expression differences between normal and abnormal (such as samples with cancer and other diseases) samples and confirm microRNA biomarker for disease diagnosis, prognosis, and even therapy [15,16].

Recently, with the development of nanotechnology, emerging miRNA detection methods, which exploit properties including electrics [17], electrochemistry [18], mechanics [19], and optics [20] of nanomaterials such as nanopore [21], nanowires [22], and nanoparticles [23], are booming. The electrical, electrochemical and mechanical detections of miRNAs are limited for further in vivo or in situ detection because of the prior miRNAs isolation step and the high susceptibility to the environment resulting in too many background signals and other noise present in the detection system [17]. Optical detections of microRNAs based on nanoparticles translate the miRNA hybridization, adsorption, or binding events into measurable optical signals including fluorescence, scattering, colorimetric signals, and so on. Compared to other properties, miRNAs optical detection has greater potential for further biological samples detection, in vivo detection, or even in-situ imaging, as well as achieves high sensitivity and specificity within less time by less labor. In the present paper emphasis is laid on employing quantum dots (QDs) and silver nanoclusters (AgNCs)-based fluorescence and silver nanorod-based surface enhanced Raman scattering (SERS) to detect miRNAs.

2. Characteristics of microRNA detection

Both the single molecule detection and multiplexed detection of miRNAs encounter the following challenges due to the intrinsic characteristics of miRNAs [24,25]. Firstly, miRNAs with ~22 nt are too short to label and amplify, and thus hard for probe design. Secondly, miRNAs from different families show great differences in GC content, expression levels in biological samples, hybridization efficiency with complementary DNAs, which leads to difficulty in normalization of the hybridization efficiency for each microarray element. Moreover, miRNAs within the same family may differ by a single nucleotide (eg., Let-7 family). The lack of a common sequence among miRNAs also makes them difficult to selectively purify. Finally, the primary transcripts (pri-miRNAs), precursors (pre-miRNAs) and other non-coding short RNAs may affect the detection signals from mature miRNAs.

To solve the above problems in miRNAs detection, various improvements are made for the specific detection methods. In miRNAs profiling with probes, LNA and PNA are usually introduced into the probes to obtain excellent detection results. LNAs comprise a class of bicyclic high-affinity RNA analogues in which the furanose ring of LNA monomers is conformationally locked in an RNA-mimicking C3'-endo/N-type conformation [26]. LNA exhibits the following advantages as hybridization probes. On the one hand, LNA mediates high-affinity hybridization without compromising base pairing selectivity, and excellently distinguishes single base mismatching. On the other hand, LNA incorporations display high stability and low toxicity in biological systems show efficient transfection into mammalian cells, good aqueous solubility and potent antisense activity in vivo [26]. LocMeCytidine-5'-triphosphate (LNA-mCTP) was synthesized by Kore and his colleagues and further used to transcribe into the short ^{33}P-LNA RNA probes, which exhibit strong binding affinity to target oligonucleotides in experiments to detect miR-124 within different tissue regions from mouse and let-7C in human cancer tissues by in-situ hybridization [27]. The PNA is DNA analog in which the negatively charged deoxyribose phosphate backbone is replaced by an electroneutral peptide like backbone consisting of N-(2-aminoethyl)-glycine scaffold [29]. The PNA probe exhibits superior hybridization characteristics, improved chemical and enzymatic stability and unprecedented thermal stability relative to natural nucleic acids [28]. On the one hand, the uncharged backbone of PNA exerts no internal repulsion to the complementary target oligonucleotide, enabling highly specific, rapid binding at low ionic strength. On the other hand, PNA increases melting temperatures by

approximately 1.0 °C per base pair for PNA-DNA duplex, and thus contributes to distinguish single base mismatch [28-30].

As to miRNA profiling without probes, a variety of chemical and enzymatic methods to directly modify miRNAs through attaching labels or tags allow sensitive detection [24]. More importantly, label-free miRNA detection with the help of wonderful optical technologies emerges as the novel miRNA detection methods characterized by no need for sample preparation and microRNA amplification, small amount of crude sample in demand, simple detection methods, less time, high specificity and sensitivity, and stringent discrimination between miRNAs and other RNAs [12].

According to the environment for the binding or hybridization between the miRNA targets and signal molecules, the optical detection of miRNAs can be classified as solid-phase or surface-based detection and solution-phase or solution-based detection [24,31]. Solid-phase detection can be further developed into in vitro non-invasive detection, such as detecting miRNAs in blood, plasma, serum [32] or other kinds of body fluids despite needing post-hybridization wash procedure. Solution-phase detection has the potential for further in vivo or in situ detection [31] as well as enables directly detecting miRNAs in complex matrices [12].

3. Fluorescence-based microRNA detection

Recently in addition to conventional fluoroscopic technologies based on fluorescence emission and quenching, various improved fluorometric analysis methods are employed to achieve sensitive detection of miRNAs, such as total inner reflection fluorescence [33], time-resolved fluorescence [34,35], fluorescence correlation spectroscopy [36] and fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET) [37] or chemiluminescence resonance energy transfer (CRET) [38]. Correspondingly, the fluorescent materials for miRNAs detection cover from organic dyes (eg. 6-carboxyfluorescein, Texas Red fluorophore, Cy3 and Cy5) [39,40] and fluorescent proteins (eg. Renilla luciferase) to inorganic nanostructures(eg. QDs and AgNCs) and other non-fluorescent nanomaterials conjugated or encapsuled with the fluorescent dyes [41-44].

3.1 QDs

QDs are superior to organic fluorophores in following: Firstly, size-tune fluorescent emission. Secondly, the broad excitation and narrow emission spectra, which allow excitation of mixed QD populations at a single wavelength far removed (>100 nm) from their respective emissions [45]. Finally, high quantum yield and good photo stability [46]. All of these advantages of QDs play important roles in miRNA detection.

Song and his co-workers established a ligase-free sensor with one-step hybridization based on quantum dots functionalized reporter DNA for the specific detection of miRNA mimics (the dual short RNA), and demonstrated that quantum dots-modified reporter probes could increase the melting temperature [42]. In the present paper, two sizes of QDs with emission wavelengths at 605 nm and 655 nm functionalized respectively by SA and anti-FAM (denoted as SA-QDs and anti-FAM QDs) were bound to biotinylated oligonucleotides reporter A and FAM-reporter B, in which one end was complementary to the capture probe immobilized on 96-well plate, and the other end was complementary to the short RNA target (Fig. 1). The reporter-functionalized QDs and appropriate amounts of target RNAs were mixed in a reaction volume, and then added into the capture-probe modified microwells. After fully hybridization and washing procedures, the fluorescence signals excited at 355 nm can be obtained on a fluorescence spectrophotometer.

The present sensor was shown to detect as low as 10 pM in 100 μL of solution and span 3 orders of magnitude. Because the applied QDs with modulated sizes exert advantages on wide excitation and narrow emission wavelength ranges, the proposed method is capable to detect the dual RNAs simultaneously in a single sample at one excitation wavelength without interference, and is prospective for multiplexed miRNAs detection. Moreover,

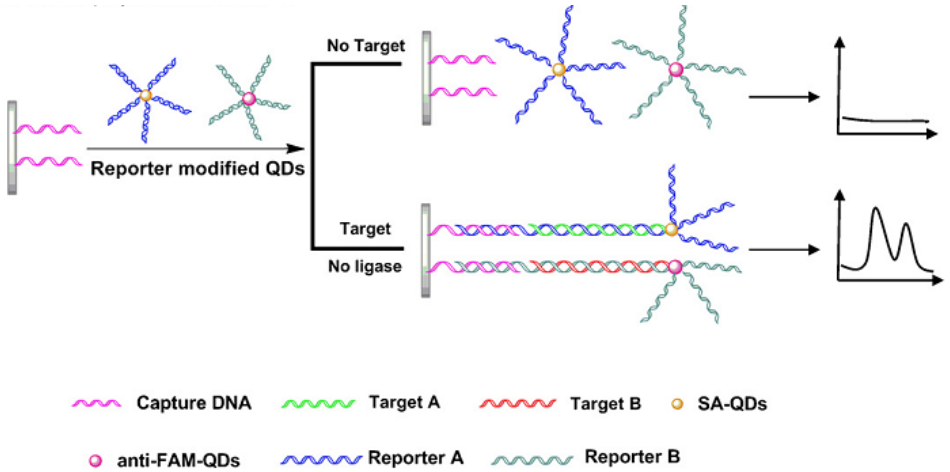

Fig.1 Scheme of the QD-enhanced detection of multiple short RNA targets via one step template-dependent surface hybridization. Copyright permission from ref. [42].

the QDs-modulated reporter probes enhance the binding affinity among the ternary oligonucleotides, eliminating the use of enzymatic ligation, and thus reducing the costs. However, it should be noted that the length of capture probes differently affected the different targets, making the design of the capture probe and reporter probes more sophisticated. In addition, the present method was no further applied to real samples, reducing its robustness validation.

In addition, the particular properties enable QDs as good energy donor or acceptor in QD-based FRET [47], CRET [46] and BRET [48]. Recently, a rapid, sensitive and specific miRNA detection method was developed by combining the two-stage isothermal exponential amplification reaction (EXPAR) with the single-QD-based nanosensor [47]. The principle of the present miRNA assay is shown in Fig. 2. The target miRNA is first exponentially amplified through the first-stage EXPAR and then linearly converted into reporter oligonucleotide Y in the second-stage EXPAR. The reporter oligonucleotide Y then hybridized with the biotinylated capture probe and Cy5-labeled reporter probe to form the sandwich hybrid which were then caught and assembled on the surface of 605QDs through specific streptavidin-biotin binding. The consequent 605QD/reporter oligonucleotideY/Cy5 complexes were detected by nanosensor in a microfluidic flow at an excitation wavelength of 488 nm.

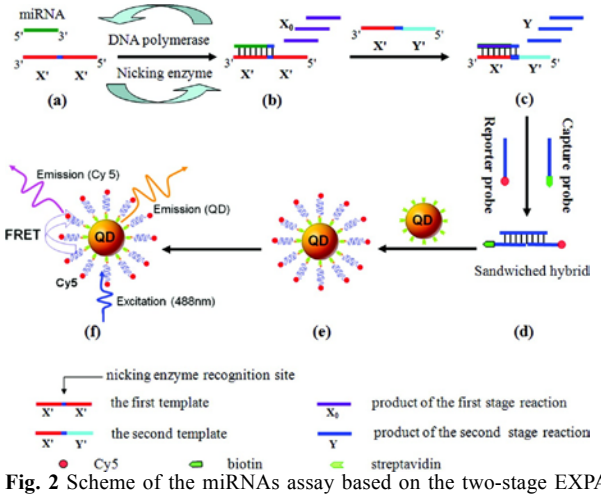

Fig. 2 Scheme of the miRNAs assay based on the two-stage EXPAR and single-QD-based nanosensor. Copyright permission from ref. [47].

The promising assay in the paper was applied to detect let-7a miRNA with the limit of detection (LOD) as low as 0.1aM. More excitingly, the single-QD-based nanosensor can discriminate single-nucleotide differences between let-7 miRNA family members including let-7a, b and c. What is more important, miR-21 and let-7a was detected simultaneously by designing two sets of corresponding templates with the same capture and reporter probes. Undoubtedly, attributed to the combination of high amplification efficiency of EXPAR, high sensitivity of single-particle detection, and near-zero background noise of the single-QD-based nanosensor, the present method exhibits highly improved sensitivity, specificity and bright promise for further multiplexed miRNA detection. However, before the clinical application, the method there should seek further improvement on miRNA detection in real samples without interference from the complex environment. And it is worthwhile to develop a microfluidic setup capable of simultaneously manipulating massive samples detection.

3.2 AgNCs

DNA strands with specific sequences were used as templates to create the time-dependent and size-specific AgNCs that follow the contour of the DNA templates [49]. As the promising substitutes for organic dyes and QDs, these silver nanoclusters encapsulated in single stranded DNA (ss-DNA) do well in high emission rates throughout the visible and near-IR, photostability [50], biocompatibility, small size (<2 nm) [51] and water solubility [52], thus exhibiting great potential for nontoxic, sensitive high-resolution biological imaging and chemical/biological detection by enhancement or quenching of the emission intensity and/or by shifting the emission wavelength [52,53].

Yang *et al.* developed a DNA/AgNCs probe to detect target miRNA using the fluorescence dropping of the probe (Fig. 3) [54]. In the present paper, the DNA-12nt-RED, a DNA sequence capable of creating red emitting AgNCs, was attached with DNA -160, a DNA sequence complementary to miR-160, to obtain the final DNA template DNA-12nt-RED-160. After that, the DNA-12nt-RED-160 probe and miRNA target were mixed together, followed by addition of $AgNO_3$ and reduction

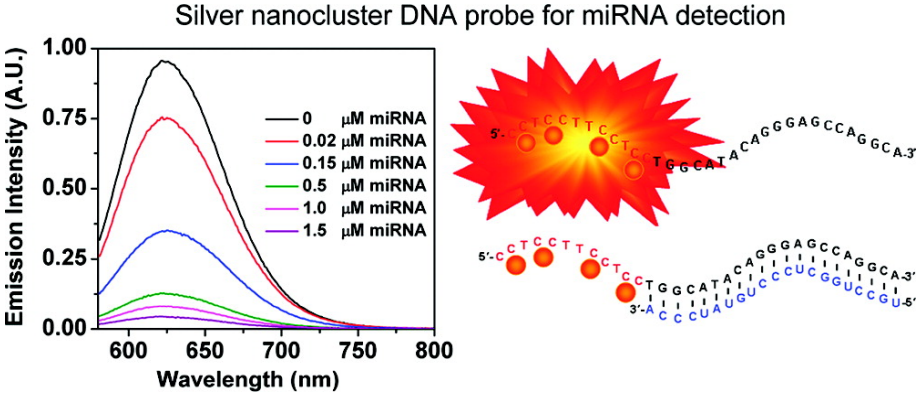

Fig. 3 Scheme of DNA/AgCNs probe for miRNA detection. Copyright permission from ref. [54].

by $NaBH_4$ to form AgNCs and monitoring the changes on its red emission after 1 h. The AgNCs-based fluorescence method has great prospective for picomole level detection of miR-160, and the capacity to detect miR-160 in RNA extracts from plants.

In the later work by the above team, they perfomed a series of experiments to illustrate the formation of bright red emissive AgNCs in a DNA sequence (DNA-12nt-RED-160), demonstrating that the mismatch self-dimer formation of the DNA probes contributes to the bright red emission. And based on the mechanism a novle DNA/AgNCs probe for miR-172 detection was successfully developed [53].

Undoubtedly, the present method paves the way for the application of fluorescence properties of DNA/AgNC to detect miRNAs, which integrates the probe design and target detection into one step. However, lack of fully understanding of the mechanisms underlying the AgNC-based fluorescence dropping, it is difficult to eliminate the effects of non-miRNAs on fluorescence changes. Additionally, the synthesis of AgNCs by DNA template containing the relevant target sequences makes it necessary to design the specific DNA/AgNCs probe to detect one target miRNA sequence. Therefore, the method encounters great challenges for multiplexed miRNA detection.

3.3 Magnetic nanoparticles and others

Recently, magnetic beads conjugated with molecular beacons [55] and magnetic fluorescent (MF) nanoparticle-based molecular beacon (MB) [44,56] have been developed as multimodal miRNA detecting or imaging probes for miRNA-based diagnosis and even therapy. In the specific probe, magnetic particles are exploited to facilitate the separation or delivery. And to introduce stable fluorescence sources, such as organic dyes, silica and MB are widely used as the scaffold or linker.

Utilizing the array surface provided by magnetic beads conjugated with molecular beacons, the cation exchange-based fluorescence amplification (CXFluoAmp) method to detect miRNA was developed [55]. As shown in Fig. 4, the molecular beacon bound on magnetic beads worked as capture probe to hybridize with the target miRNA and link with detection probe containing nonfluorescent ionic nanocrystals (NCs) CdSe after ligation, thus triggering the release of Cd^{2+} by adding Ag^{+} to the above complex and finally turning on the fluorescence response of the dye Rhod-5N. The promising assay enabled detecting synthesized miR-21 with the LOD at 35 fM and dynamic range spanning 7 orders of magnitude and discriminated the different miR-21 levels in the total RNA extracts from the breast cancer tissue and normal breast tissue. In addition, the method showed high specificity to distinguish one or few nucleotides mutation. The ligation reaction by T4 DNA ligase contributed to the prominent sensitivity and specificity. However, it should be noted that the self-release of Cd^{2+} in nanocrystals may adversely affect the assay.

Do Won *et al.* first reported the application of magnetic fluorescent (MF) nanoparticle- conjugated molecular beacon to image intracellular miRNAs [44]. As seen in Fig. 5, the MF nanoparticle which was composed of cobalt ferrite in the central core and a rhodamine fluorescent dye coated with a silica shell was linked with miR124a-specific molecular beacon with a partially double-stranded DNA including aptamers that contain nonfluorescent dye, a black hole-quenching (BHQ) molecule. In the absence of the target miR-124a, the quencher close to the MF nanoparticles makes the fluorescence signal off, while in the presence of the target miR-124a, the stronger complementary pairing between miR-124a and its binding sequence in MB leads to the quencher detached from the MF nanoparticles and consequently the signal is on. Based on the MF particle-conjugated miR-124a beacon, the present method achieved specific miR-124a detection in microtube, in vitro intracellular miR-124a imaging in P19 cells induced and cultured as neuronal differentiation of cells, and in vivo miR-124a imaging in mouse.

In addition to magnetic particles-based fluorophores, metal nanoparticle fluorophores have emerged as attractive probes in miRNA detection and imaging in the past decades [43]. Take silver shell/silica core fluorophore for example. It was employed to detect miR-486 in lung cancer cell by fluorescence in situ hybridization (FISH). As shown in Fig. 6, the probe was composed of silica core (50 nm) encapsulated with the dye $Ru(bpy)_3^{2+}$ and the silver nanoshell (10 nm) with the single-strand oligonucleotide complementary to the target miRNA. Based on the metal-fluorophore interaction resulting in the enhanced intensity and shortened lifetime of fluorescence, the probes indicated the location of single

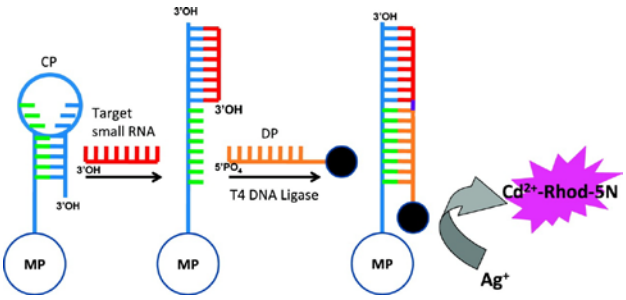

Fig. 4 Scheme of the QD-enhanced detection of multiple short RNA targets via one step template-dependent surface hybridization. Copyright permission from ref. [42].

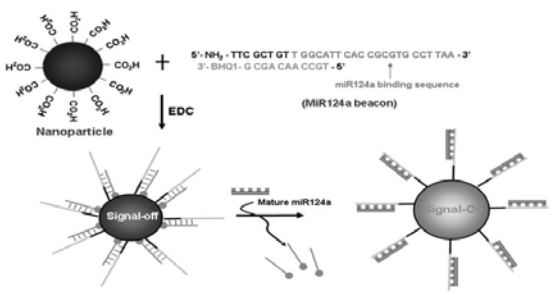

Fig. 5 Scheme of the QD-enhanced detection of multiple short RNA targets via one step template-dependent surface hybridization. Copyright permission from ref. [42].

miR-486 in lung cancer cells by counting the numbers of spots with certain fluorescence lifetime. The metal nanoparticle fluorophore-based FISH assay provided prospect for cell-to-cell variations in miRNA expression including quantity and location. However, it is strange that the assay selected Cy3 covered by silver nanoshell with the already shortened fluorescence lifetime to image the miRNAs in cells with shorter fluorescence lifetime than the former one, thus resulting in the question on the use of the silver nanoshell.

4. Scattering-based microRNA detection

4.1 SERS

SERS technique, with huge enhancements of adsorbate Raman signals and a drastic quenching of fluorescence [57], shows great strengths in sensitivity to detect extremely low levels of analyte and specificity to provide the molecular fingerprint of the analyte in near-real time [58,59], thus providing a prominent pathway for miRNA detection. However, the practical application of SERS-based sensing suffers from the difficulty in fabrication large area, uniform and high enhancement substrates [60]. In the current reports on SERS-based miRNA detection, the substrates with aligned silver nanorod arrays (AgNR) prepared by oblique angle vapor deposition (OAD) were proposed to have prospect for routine miRNA expression profiling in future clinic laboratories [59].

Exploiting OAD-fabricated AgNR and affinity differences between samples and probes on the substrates, the SERS-based miRNA detection method was developed by Driskell and his coworkers [61]. In the detection (Fig. 7), after the hybridization of small RNAs including the target RNAs with the thiolated complementary probes, oligo samples were incubated with the AgNR substrate, rinsed, and dried, resulting in the merely adsorption of the

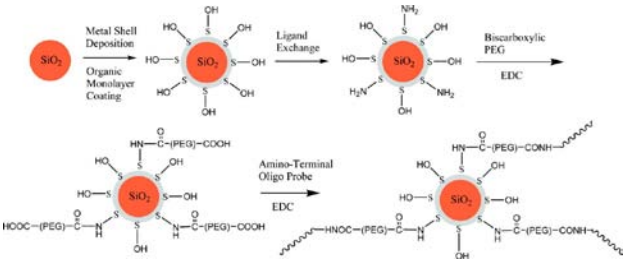

Fig. 6 Scheme of preparation metal shell/silica core structure bound with the single-strand oligonucleotide probe. Copyright permission from ref. [43].

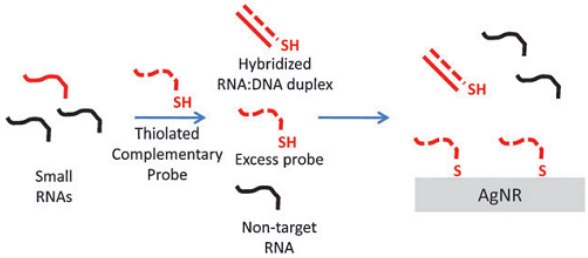

Fig. 7 Scheme of label-free SERS detection of miRNA based on affinity. Copyright permission from ref. [61].

excess thiolated probes and the corresponding production of SERS signals because only thiolated ssDNA showed strong adsorption on the substrate while the dsRNA: DNA and non-target RNA exhibited negligible adsorption. The present method opens possibility for multiplexed label-free miRNA detection. However, with LOD of 28 nM, the sensitivity should be further enhanced to compete with other optical miRNA detection methods, as well as the specificity must be validated by detecting different miRNAs synthesized or even extracted from real samples.

In additon to solid-gas interface-based SERS, other interfaces including solid-solid, solid-liquid and solid-vacuum have been observed to have SERS effect. Wang *et al.* developed the miRNA detection method based on SERS effect on solid-liquid interface using plasmonic coupling phenomenon combined with the LNA-based nucleic acid hybridization process, in which plasmonic coupling enhancment in the gap regions between nanoparticles contributes to the high SERS enhancement [62]. As seen in Fig. 8, silver nanoparticles (AgNPs) functionalized with the thiolated oligonucleotides with the same sequences as the miRNA targets and labled with Cy3 Raman dye worked as reporter-NP, while AgNPs functionalized with the complementary LNAs worked as capture-NP. In the absence of the miRNA targets, the two probes assembled into a three dimension (3D) nanonetwork through hybridization, inducing plasmonic coupling between adjacent NPs to produce increased SERS signal of the Raman lebels upon laser excitation. In the presence of the miRNA targets, the plasmonic coupling is interfered by the formation of LNA-miRNA duplex between capture-NPs and miRNA, and thus leading to the reduction in SERS intensity. The method was successfully applied to 100 nM miR-21 detection. However, the initial experiments should be further validated by detecting a series of miRNAs with different concentrations and sequences. Therefore, there is still a long way to go for multiplexed simultaneous assays of miRANs in a single sample solution.

In conclusion, the SERS technique for miRNA detection is in its fledging period, which facing the problem on sophisticated read-out system, complicated analysis skill, and convoluted data interpretation and verification [58].

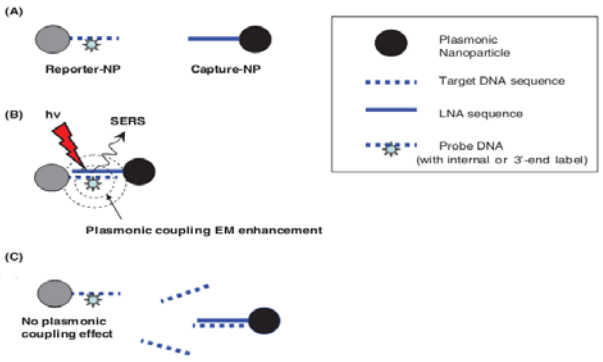

Fig. 8 Scheme of DNA/AgCNs probe for miRNA detection. Copyright permission from ref. [54].

However, as for label-free and multiplexed detection of miRNA, SERS is undoubtedly a primary selection and has potential for development of novel miRNA detection tools.

4.2 Other Scattering

Besides SERS, other light scattering signals including Rayleigh scattering are also capable of miRNA detection with the help of advanced optical detection technology.

Exploiting the Brownian motion and the stronger resonance Rayleigh scattering of silver nanoparticles (AgNPs) [63], the single AgNPs counting system (SSNPC) by photon-burst counts with ultra-high sensitivity, high spatial resolution (0.5 fL), and good reproducibility was established to detect miRNA in homogeneous solution. The principle of the method is shown in Fig. 9, in which two kinds of AgNPs conjugated with oligonuleotides respectively complementary to the two ends of the target sequence worked as probes, producing high signals without miRNA targets while low signals with miRNA targets in SSNPC detection. The method was able to detect synthetic miRNA as low as 0.5 fM with high reproducibility. Though with higher sensitivity by further reducing the sample volume using microfluidic droplet technique, the method is hard to simultaneously detect multiplexed miRNAs.

Based on the enhancement of light scattering by gold nanoparticles (AuNPs), a high density scanometric miRNA microarray (Scano-miR) was constructed [64]. As seen in Fig. 10, miRNA targets in a sample mixture was firstly attached with universal DNA linker by T4 RNA ligase, and then hybridized with the DNA complementary to the miRNA target on the microarray. After washing the unbound miRNA, spherical nucleic acid-AuNPs (SNA-AuNPs) conjugated with oglionucleotides complementary to the universal linker sequences were added and hybridized onto the microarray followed by a second washing. The final microarray after further gold enhancement was detected by scanometric system. The Scano-miR system was first applied to detection of miR-16 with detection limit at 1 fM (~600 copies/μL serum), as well as human serum miRNAs with good performances

Fig. 9 Scheme of homogenous detection of miRNA based on SSNPC system. Copyright permission from ref. [63].

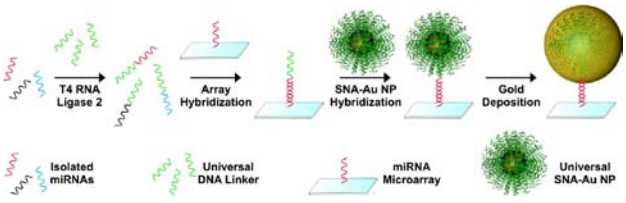

Fig. 10 Scheme of scanometric array-based multiplexed detection of miRNA species. Copyright permission from ref. [64].

in specificity, reliability and reproducibility. More importantly, the array was useful for miRNA profiling in cell lines and even tissue samples, thus showing as robust high throughput tools for screening miRNA biomarkers.

5. Colorimetry-based microRNA detection

Colorimetric method for miRNA detection is generally superior to other optical detection methods in terms of low-cost equipment. And the widely used nanomaterial in this method is microarray-based AuNPs combined with silver enhancement to realize the high throughput detection [23].

With the help of AuNPs and silver enhancement, a simple, sensitive, and specific colorimetric method for miRNA detection was developed [65]. The principle of the method is shown in Fig. 11, in which the biotinylated oligonucleotides as capture probes complementary to the 3' end of the target miRNA sequence and AuNPs as reporter probes conjugated with oligonucleotides complementary to the 5' end sequences were hybridized with the miRNA target, followed by immobilizing onto the streptavidin-coated microplate, and the final colorimetric detection by a microplate reader after washing and silver enhancement steps. The present method was able to detect miR-122a/128 in isolated RNA from only 2 ng sample of mouse brain or liver tissue, and detect synthesized miR-122a ranging from 10 fM to 10 pM with the capability to discriminate one single oligonucleotide mismatch. However, it is limited for multiplexed miRNA detection due to complex probes design. Anyway, the colorimetric method does not require expensive equipment and an advanced read-out system, and can be performed in any standard laboratory [66].

6. Conclusions and Outlook

In this review article, the current nanoparticle-based miRNA detection by optical technologies were summarized from the following aspects about fluorescence, scattering and colorimetry (shown in Table

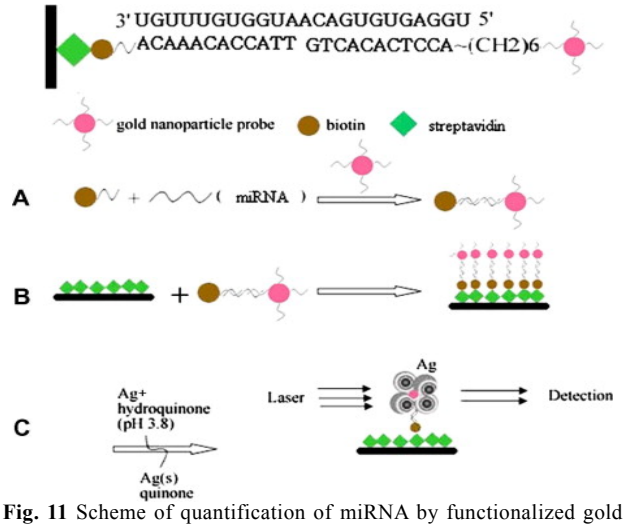

Fig. 11 Scheme of quantification of miRNA by functionalized gold nanoparticles. Copyright permission from ref. [65].

1). Among three of them, more attention has been drawn by the former two-based miRNA detection in the last few years.

Fluorescence-based miRNA detection is peculiar to in vivo and in situ imaging, as well as high sensitivity in single miRNA quantification in solution. And a myriad of nanomaterials are able to be applied to fluorescence detection, including self-fluorescent structures such as QDs, AgNCs, and conjugated fluorophores such as fluorescent magnetic nanoparticles and metal nanoparticle fluorophores, thus providing sufficient material elements for selecting to develop the optimal miRNA detection method with high sensitivity or imaging method with identified safety. Moreover, the development of multimodal probes for fluorescent imaging emerges as a promising tool for miRNA-based cancer diagnosis and therapy.

Scattering-based methods to detect miRNA have great advantages in label-free multiplexed detection. The widely used nanomaterials for this method are AgNPs- or AuNPs-based substrate or solution. The limited scattering substrates create bottle-neck for SERS-based miRNA detection with high sensitivity. However, in recent study, it is prospective to construct miRNA microarray with high throughput based on scattering detection.

Compared to the above methods, colorimetric miRNA detection with simple and low-cost instrument or even naked eyes makes it a potential portable sensor for public use out of the laboratories.

In a word, the emerging nanoparticle-basd optical technologies pave the way for detection of miRNA by developing novel nanosensors or probes. Based on the current studies, there is still a lot of work to do to put it into clinical practice in the next few years. Firstly, environment-friendly and bio-safe nanomaterial should be further synthesized to meet the application to in vivo imaging or detection of miRNA in homogeneous solution or on microarray. For example, AgCNs produced by DNA templates showing good biocompatibility have attracted great attention for direct miRNA detection. On the other hand, we should pay attention to the novel properties of the nanomaterials when applied to miRNA detection. Secondly, sufficient experiments should be performed to validate the robustness of the designed method including sensitivity, specificity, reliability, reproducibility and so on. The combination of conventional miRNA detection methods with the emerging nanotechnologies is a promising way to obtain ultrahigh sensitivity, such as the isothermal amplification of miRNA combined with QD-based nano-sensor [47]. Last but not the least, based on the above elements, the advanced optical detection technology such as the microfluidic setup or surface plasmon resonance imaging (SPRi) [67] is becoming the hot study for miRNA detection with ultrahigh sensitivity.

Anyway, employing nanoparticle-based optical technology, multiplexed miRNA detection or in vivo imaging will be widely used in biomarker identification for cancer diagnosis, prognosis and even therapy in the near future.

Table 1 Summarization and comparation of the current nanoparticle-based optical miRNA detection methods.

Solution/surface-based or FISH	miRNA modification (amplification,labeling,ligation,etc.)	Probes	Detection signal	Samples	Performance (sensitivity, specificity, dynamic range, etc.)	Ref.
Surface-based	No modification	Capture probes; QDs-modified reporter probes	Fluorescence intensity	Synthetic dual short RNAs	LOD at 10 pM in 100 μL with dynamic range 10pM-15nM.	[42]
Solution- based	Isothermal amplification	605QD/reporter oligonucleotide Y/Cy5 complexes	Burst counts in FRET	Let-7a,let-7b,and let-7c miR-21	Near-zero background noise; LOD at 0.1 aM; Clearly discriminate let-7a, b, c.	[47]
Solution-based	No modification	DNA/AgNCs probe	Fluorescence dropping value	MiR-160	Picomole level detection of miRNA.	[54]
-[a]	No modification	MF-miR124a beacon	Fluorescence intensity	MiR-124a	Capable of in vitro intracellular imaging and in vivo imaging	[44]
Solution-based	No modification	Capture probe: molecular becon; Detection probe: aptamer with NCs	Fluorescence intensity in CXFluo Amp	Synthetic miR-21; RNA extracts from tissues	LOD at 35 fM with dynamic range of 7 orders of magnitude.	[55]
FISH	No modification	Metal shell/Si core bound with the single-strand oligonucleotide probe	Fluorescence intensity and lifetime in FISH	MiR-486 in cell lines positive to lung cancer	Capable of detecting and locating low levels of miR-486 in cells.	[43]
Surface-based	No modification	thiolated ssDNA	SERS	Let-7f	LOD at 28nM.	[61]
Solution-based	No modification	Capture NPs: AgNPs with thio-LNAs; Reporter NPs:AgNPs with Cy3-labeled DNA	SERS in PCI	MiR-21	100nM.	[62]
Solution-based	No modification	AgNPs conjugated with oligonucleotides	Photon counts in SSNPC system	MiR-21, 23a, P	LOD at 0.5 fM.	[63]
Surface-based	MiRNA ligation by T4 RNA ligase	Universal DNA linker, miRNA microarray and universal SNA-Au NPs	Light scattering on scano miR arrays	Synthetic miR-16; RNA extracts from cell line and serum	LOD at 1 fM with single nucleotide polymorphism (SNP) selectivity.	[64]
Surface-based	No modification	Biotinylated probe and gold nanoparticle probe	Colorimetric absorbance	Synthetic miR-122a; RNA extracts from tissues	LOD at 10 fM with specificity to discriminate one single base mismatch in 0.01 to 10 pM.	[65]

[a]. denotes no classification as solution or surface-based detection.

Acknowledgements

This work was supported by Chinese 973 Project (2010CB933901 and 2011CB933100), 863 Key Project (No. 2007AA022004), the National Natural Science Foundation of China (No. 81225010, 81101169 and 31100717), New Century Excellent Talent of Ministry of Education of China (NCET-08-0350), Special Infection Diseases Key Project of China (2009ZX10004-311) and Doctorial Position Budget (20070248050).

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