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REVIEW

Recent progress of SERS optical nanosensors for miRNA analysis

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This review focus on emerging applications of surface-enhanced Raman spectroscopy (SERS) optical nanosensors for miRNA analysis, in which the key enhancement factors of SERS signal, i.e. SERS-active substrates, SERS nanoprobes and nano-assembly strategy, are emphasized. This article includes many nanomaterials for miRNA analysis by SERS techniques. We summarize these reported nanomaterials mainly according to their function in the miRNA assay biosensor. We also briefly summarized the research progress of these nanomaterials in SERS detection of intracellular miRNA. In the end, we discussed the prospect and limitations of SERS nanosensors for analyzing miRNA.

1. Introduction

MicroRNA(miRNAs) are a subset of 18–25 base single-stranded RNA molecules that are expressed in cells of both plants and animals to control cellular key events.[1-5] MiRNAs have emerged as a novel kind of biomarkers, since they regulate gene expression and closely related to many diseases, such as diverse cancers, diabetes and myocardial infarction.[6-10] Analyzing miRNAs is an urgent problem, many optical nanosensors based on a variety of optical techniques, including fluorescence,[11-13] chemiluminescence,[14-16] electrochemistry,[17-19] and so on, have been designed to analyze miRNA in disease biology. However, most of these optical nanosensors can't take all factors under biological conditions into account.[20-23] Low detection limits, good selectivity and ideal performance are still desired in the biological environment. Therefore, some new nanomaterials combining advanced spectrum have emerged to construct new optical nanosensors for miRNA detection. Among these new nanosensors, sensors based on surface-enhanced Raman spectroscopy (SERS) is considered to be promising in the field of biology since SERS is an ultra-sensitive and non-destructive technique.[24-27] Meanwhile, some novel or multifunctional nanoprobes are applied for miRNA analysis with different optical technology.

SERS, as a newly found spectroscopy method firstly reported at roughened silver electrodes in 1974, has become a fascinating tool in a variety of research fields.[28-30] Since SERS technology greatly improves Raman signal and can even provide unique fingerprint peaks of single-molecule with high specificity, SERS has been widely used for organic pollutant analysis, heavy metal ion detection, protein measurements and so on.[31-35] With the rapid development of Raman spectroscopy and the urgent need for disease diagnosis, SERS has been extensively applied for analyzing disease-related miRNAs.[20, 36-40]

SERS nanosensors have many unique benefits for miRNA detection.[41, 42] Firstly, ultra-sensitive miRNA detection has been realized owing to the high sensitivity of SERS. Secondly, the rapid detection speed of SERS would promote miRNA as a practical indicator of clinical diagnosis. Thirdly, the unique fingerprint peaks of SERS can ensure simultaneous detection of multiple miRNAs. Besides, the length of miRNAs is appropriate for generating plasmonic “hot spots”, which can greatly improve the signal of SERS. With the development of SERS and nanotechnology, more unique benefits for miRNA detection by SERS nanosensors will be found. Since miRNAs usually exist in complex biological environments, the analytic process is usually interfered by a variety of substances.[43] Besides, miRNAs are easily degraded and sometimes multiple homologous miRNAs are present simultaneously. To meet the requirements of simultaneous detection of miRNAs, high throughput miRNA detection, ultra-sensitive miRNA detection and so on, different detection means have been developed. Novel nanomaterials have been synthesized and used as probes, plasmonic materials have been prepared as SERS-active substrates, multiple nucleic acid amplification strategies have been reported, and different magnifying elements are often combined together.[44-46]

Many comprehensive reviews on miRNA analysis from different insights have been published, including different detection means, different amplification technologies, multifunctional nanomaterials and so on.[47-51] However, some emerging biosensors for miRNA analysis are not timely reviewed and research prospects also desired to inspire others. This review focus on emerging applications of SERS nanosensors for miRNA analysis, the key enhancement factors of SERS signal, i.e. SERS-active substrates, SERS nanoprobes and nano-assembly strategy, are emphasized.

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Other reviews published on a similar topic often give a general overview of SERS application in biological detection. Our reviews will focus on the emerging applications of SERS sensors, especially in miRNA analysis. In general, we separated the miRNA analyzing platform according to the nanomaterials used for nanosensors. Active substrates like Ag/Au nanomaterials, layered materials, metal arrays, metal oxides, etc. usually generate strong electromagnetic field enhancement under laser irradiation, which play an important role for SERS enhancement.[52-54] Besides, nanoprobes are also a key component for optical nanosensors, as they not only act as a signal readout probe but also as a signal amplifier carrier.[55, 56] With the development of nanotechnology, nano-assembly with traditional or advanced materials is an advanced technology which opens a new way for miRNA analysis. Besides, to meet the requirements of disease diagnosis and biological analysis many optical nanosensors are applied in intracellular detection and imaging.[57, 58]

2. MiRNA detection with SERS-active substrates

For many biological analysis platforms that based on SERS, the active substrates can undergo charge transfer with the signal molecules to produce SESR signals. SERS-active substrates are an important component for SERS biosensors. A large number of studies show that the metallic composition of nanomaterial, the size and shape of nanostructures has a great effect on the activity of substrates.[59] Therefore, nanostructures with strong plasmonic effects are studied to construct efficient SERS biological analysis platforms. This section includes different types of SERS-active substrates in miRNA detection.

2.1 MiRNA detection with Ag/Au nanomaterials as SERS-active substrates

AgNPs and AuNPs are the most used nanomaterials for constructing SERS-active substrates. A pure substrate facilitates the use of complex nucleic acid amplification techniques. Yang et. al designed a sensitive miRNA detection platform by combining miRNA-responsive DNA microcapsule with Si@Nafion@Ag SERS active substrate.[60] With the help of duplex-specific nuclease, Raman dye can be released from TB@$CaCO_3$ composite, then strong Raman signal can be obtained since there is a large amount of AgNPs on Si@Nafion@Ag substrate.

2.2 MiRNA detection with magnetic nanoparticle substrates

Magnetic nanoparticle substrates based SERS platform for the assay of disease-related miRNA biomarkers are widely reported. Silica, gold, and silver-coated magnetic nanoparticle are reported as capture for miRNA assay by SERS.[61-63] He et. al has constructed an ultrasensitive miRNA detection platform by combing synthesized magnetic Co@C/PEI/Ag SERS substrate with rolling circle amplification strategy, which can achieve a wide measuring range from 100 aM to 100 pM and the detection limit of miRNA-155 is 70.2 aM (Fig. 1A).[20] As well, they constructed another novel miRNA-155 detection platform by combing synthesized leaf@nafion@Ag SERS substrate with 3D DNA hydrogel.[36] In their work, 3D DNA hydrogels can quickly respond to miRNA-155, then releasing Raman reporters on leaf@nafion@Ag SERS substrate. As a result, this platform also displays high sensitivity and selectively of target miRNA with a wide detection range (0.1 fM-100 pM) and ideal LOD (0.083 fM) (Fig. 1B).

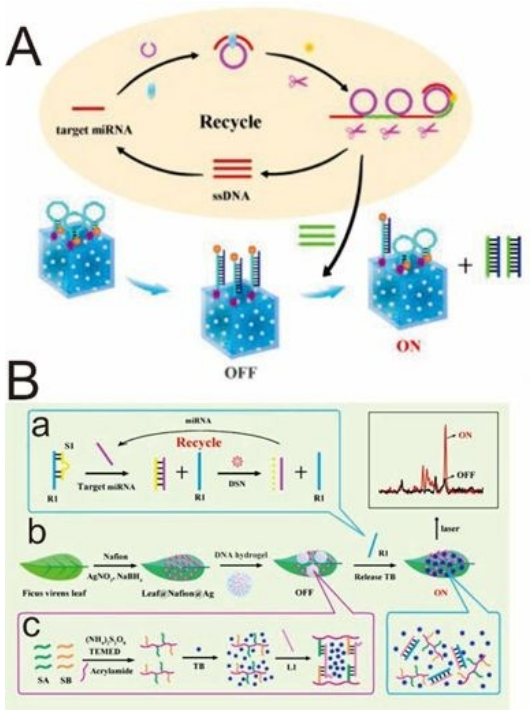

Fig. 1 (A) Schematic illustration of miRNA detection platform by combing synthesized magnetic Co@C/PEI/Ag SERS substrate with rolling circle amplification strategy (reprinted with permission from Y. He, X. Yang, R. Yuan and Y. Chai, Anal. Chem., 2017, 89, 2866-2872, American Chemical Society). (B) Schematic illustration of miRNA-155 detection platform by combing synthesized leaf@nafion@Ag SERS substrate with 3D DNA hydrogel. (reprinted with permission from Y. He, X. Yang, R. Yuan and Y. Chai, Anal. Chem., 2017, 89, 8538-8544, American Chemical Society).

2.3 MiRNA detection with two-dimensional layered nanomaterials substrates

Traditional metal nanostructures exhibit strong plasmonic enhancement effects, while there are still many shortcomings, making SERS application limited.[23, 64] Then, the plasmonic effects of some two-dimensional layered nanomaterials are studied to explore new SERS substrates.[65] Nanoarray structures are often used as SERS substrates since they can produce strong plasmon resonance effects. Zhu's group constructed a "signal-off" miRNA-21 detection platform by integrating T7-Exo assisted recycling amplification with gold decorated silicon nanowire arrays. In their work, they further enhanced the sensitivity of SERS detection by introducing Au nanostar probes, since they can produce a coupled electromagnetic field between the nanoarray substrate.[66] In 2019, Tian's group constructed an ultrasensitive miRNA assay platform with

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composite two-dimensional nanomaterial CuPc@HG@BN, which was assembled by boron nitride nanosheets and copper(II) phthalocyanine. This nanostructure not only played as SERS substrate but also as photodynamic therapy source.[22]

3. MiRNA detection with SERS nanoprobes

Efficient SERS nanoprobes open up the avenue to analyze disease markers by SERS. Therefore, nanomaterials with different components, shape and size have been extensively studied as SERS nanoprobes. A large number of probes are used to construct sandwich-type detection platforms. Biosensors with different SERS nanoprobes for miRNA analysis are summarized.

3.1 MiRNA detection with pure Au/Ag nanoprobes

Liu's group improved the enhancement effect of pure gold and silver nanoparticles by decorating AuNPs on Ag@SiO_2 nanocomposite.[67] This composite nanomaterial was used for developing a plasmonic sandwich miRNA assay approach, which exhibited ultra-high sensitivity with a determination of 10 fM miRNA in human serum. Ye et. al synthesized AuNPs modified biobarcode SERS probes for miRNA and ATP detection. They proposed an asymmetric SERS signal amplification method by combing the bifunctional probe with the isothermal amplification method.[21] Ye's group also proposed a simultaneous miRNA detection strategy with gold nanobridged nanogap particles as SERS nanoprobes.[37] In this sandwich hybridization assay, hollow silver microspheres were used as capture substrates, miRNAs in cancer cells are successfully determined and the LOD is 10 fM. Ag@4-mercaptobenzoic acid@biotin-modified DNA probes was reported for miRNA-21 detection and realize cascade amplification. The LOD was significantly improved by three orders of magnitude with cascade amplification.[38]

3.2 MiRNA detection with Ag/Au composite nanoprobes

Su et. al report a sandwich-type SERS detection platform for multiplex and simultaneous detection of nucleic acids with gold−silver nanosnowmen as SERS nanoprobes.[68] Our group modified thiol-containing Raman dyes on Au@Ag nanosnowmen nanoprobes, which were used for sandwich-type detection platform and realizing simultaneous miRNA detections (Fig. 2A).[69] We also develop a sandwich-type SERS biosensor for analyzing myocardial infarction-related miRNAs in serum by combing hollow Ag/Au nanoprobes with the target-catalyzed hairpin assembly strategy. Importantly, the stability and SPR effect of this hollow Ag/Au nanoprobes are controllable by adjusting the ratio of gold and silver.[70]

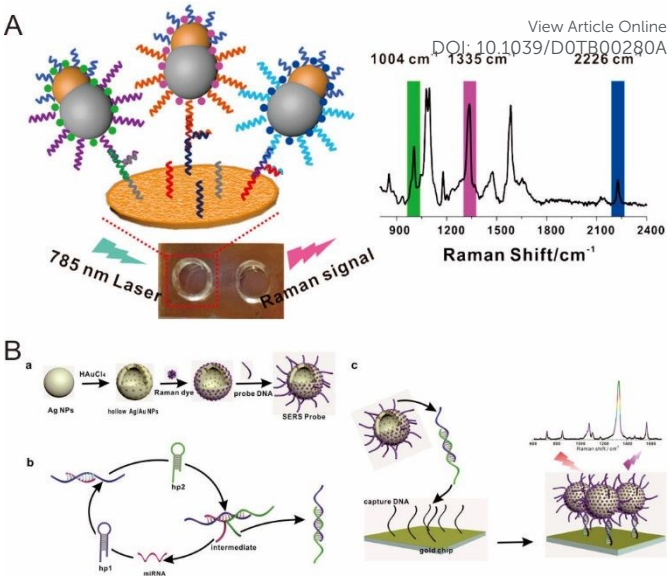

Fig. 2 (A) Schematic illustration of the multiplex miRNA detection with the Au@Ag nanosnowmen nanoprobes (reprinted with permission from R. Guo, F. Yin, Y. Sun, L. Mi, L. Shi, Z. Tian and T. Li, American Chemical Society). (B) Illustration of sandwich-type SERS biosensor for analyzing myocardial infarction-related miRNAs in serum by combing hollow Ag/Au nanoprobes with the target-catalyzed hairpin assembly strategy (reprinted with permission from Y. Sun and T. Li, Anal. Chem., 2018, 90, 11614-11621, American Chemical Society).

4. Application of nano-assembly in SERS detection of miRNA

4.1 MiRNA detection with DNA-driven assembly strategy

Metallic nanoparticle aggregates with abundant hot spots can further improve electromagnetic and realize low abundant detection by SERS. Nano-assembly is an advanced technology which opens a new way for miRNA analysis. Xu's group developed a DNA-driven assembly strategy for simultaneous detection of miRNAs and other biomarkers in cells.[71] In the presence of targets, Raman dye modified Au NPs were separated from graphene oxide, leading to the change of SERS signal and achieve a LODs of 0.03 amol/ng miRNA. Besides, Xu's group also chose a DNA-driven assembly strategy for simultaneous detection of nucleic acids and other biomarkers.[72] In the presence of target biomarkers, gap length change in AgNPs-pyramids will cause Raman signal enhancement.

4.2 MiRNA detection with miRNA-trigged nano-assembly strategy

MiRNA-trigged nano-assembly strategy is a rapidly developing technology, which can meet the needs of in-situ intracellular monitoring. Liu's group realized in-situ miRNA imaging in living cells through a miRNA-mediated AuNPs dimerization strategy, which enables enough electromagnetic hot spots for SERS detection.[37, 73] They also developed a general SERS strategy for miRNA detection by in situ hot-spot assembly based on hybridization chain reaction.[74] Zhang's group reported a miRNA-trigged nano-assembly strategy, which achieved a LOD

down to 0.85 aM(Fig. 3A).[75]In the presence of target miRNA, AuNPs are assembled onto Au nanodumbell cores through catalytic hairpin assembly, resulting in plasmonic satellite structure with multiple SERS hot-spots, which can produce strong SERS signal. In 2017, Xu's group developed a novel strategy for simultaneous quantification of intracellular miRNA and other biomolecules with Au nanorod Dimer-UCNP Core–Satellite Nanostructures. In the presence of targets, Au nanorod dimers will be dissolved and cause a decrease of SERS signal. This two-signal approach is unique and universal for the quantitative detection of biomolecules in cellular systems(Fig. 3B)[76]. In 2018, Xu et. al reported a miRNA driven in situ side-by-side self-assembly of Au-nanorod strategy, realising real-time SERS monitoring of miRNAs in living cells. Their project provides insights into the understanding of the physiologic function of miRNA.[77]

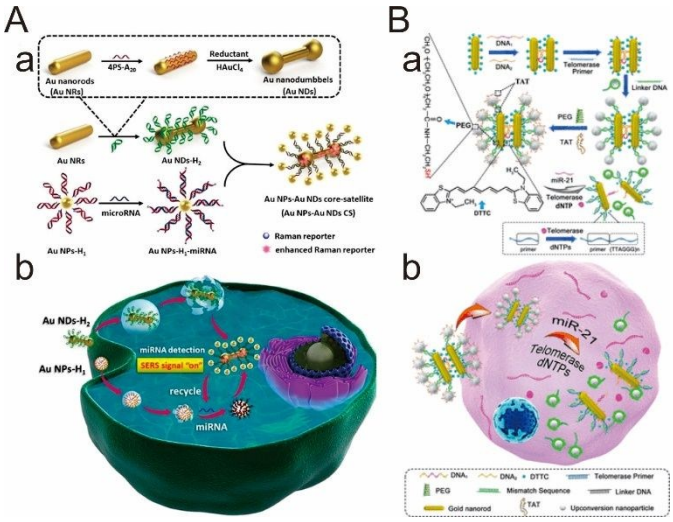

Fig. 3 (A) miRNA-trigged nano-assembly strategy. (reprinted with permission from C. Liu, C. Chen, S. Li, H. Dong, W. Dai, T. Xu, Y. Liu, F. Yang and X. Zhang, Anal. Chem., 2018, 90, 10591-10599. American Chemical Society). (B) simultaneous miRNA and telomerase detection strategy with Au nanorod Dimer-UCNP Core–Satellite Nanostructures (reprinted with permission from W. Ma, P. Fu, M. Sun, L. Xu, H. Kuang and C. Xu, J Am Chem Soc, 2017, 139, 11752-11759., American Chemical Society).

5. Intracellular miRNA analysis with SERS

SERS has a great application prospect in intracellular detection and imaging. It is reported that gold nanostars were used as nanoprobes for in vivo miRNA imaging within whole leaves by integrating SERS techniques with other techniques.[78] Tang's group developed a simultaneous miRNA imaging platform by designing a fluorescence-Raman dual-signal switchable nanoprobes.[79] The method combined the advantages of two techniques and achieved intracellular images of miRNAs. Zhang et. al also realized fluorescence and SERS dual spectrum imaging of miRNA in cells by designing a dual-signal twinkling with AuNPs as core.[80]

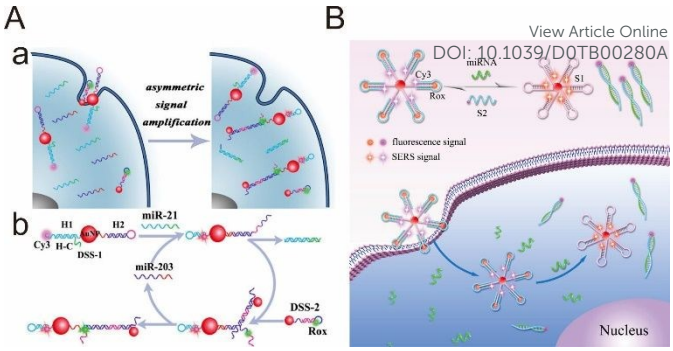

Fig. 4 (A) Illustration of the simultaneous miRNA imaging platform by designing a fluorescence-Raman dual-signal switchable nanoprobe. (reprinted with permission from S. Ye, X. Li, M. Wang and B. Tang, Anal Chem, 2017, 89, 5124-5130, American Chemical Society) (B) Schematic illustration of the fluorescence and SERS dual spectrum imaging of miRNA in cells by designing a dual-signal twinkling with AuNPs as core. (reprinted with permission from N. Zhang, S. Ye, Z. Wang, R. Li and M. Wang, ACS Sens., 2019, 4, 924-930, American Chemical Society).

6. Conclusion and outlook

Research progress of optical nanosensors for miRNA analysis in the last five years are summarized. Since active SERS substrates, efficient SERS nanoprobes are important for developing sensitive nanosensors, the component, shape and size of nanomaterials have been extensively studied to improve their SERS performance. SERS biosensors with novel nanomaterials or functional nanostructures open up new avenues for miRNA assay and imaging. However, efforts are still needed for constructing optical biosensors for in vivo miRNA assay, since the complexity, time-consuming and low sensitivity. SERS provides new insight for applications in miRNA detection and intracellular imaging by combining suitable nanostructures with high efficient amplification strategy. In the future, more nanomaterials will be developed to meet the demand of biomedicine. Nucleic acid aptamers and noble metal composites, noble metals and transition metal composites, organic functionalized nanoparticles are all potential SERS probes or SERS active substrates. With the development of nanotechnology, SERS technology will bring new opportunities for precision medicine.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

The acknowledgments come at the end of an article after the conclusions and before the notes and references.

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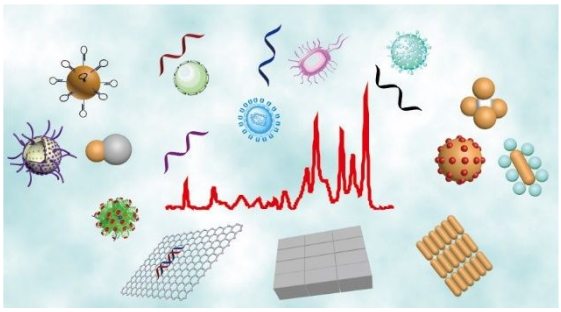

SERS-active substrates, SERS nanoprobes and nano-assembly strategy have inspired emerging applications of SERS nanosensors for miRNA analysis.