Dimensional Surface-Enhanced Raman Scattering Nanostructures for MicroRNA Profiling

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Surface-enhanced Raman scattering (SERS) spectrum is an ultrasensitive quantitative tool for molecular fingerprinting and is very suitable for the analyzing and detecting trace substances, including low-abundance miRNAs. Researchers have already investigated the effects of nanomaterial-based SERS profiling on miRNA. As review herein, these SERS-active nanostructures can be generally classified into 0D, 1D, 2D, and 3D nanostructures according to their distinct structure. These SERS-active structures with differential structural dimensions exhibit differences in terms of detection performance which can be attributed to the unique nanomaterials involved and the overall design of the SERS nanostructures. In this review the authors focus on the design of SERS nanostructures in previous research, the advantages and disadvantages in miRNA profiling of the different dimensional nanostructures are analyzed, and some design principles to develop guidelines, to fabricate more effective SERS nano-sensors with ultra-high sensitivity and high levels accuracy are summarized, thereby promoting the practical application of miRNA profiling by SERS technology in complex life systems.

1. Introduction

As a major class of biomarkers, microRNA (miRNA)s are endogenous and highly conservative small RNAs. These miRNAs are usually 19–23 nucleotides in length and play a critical role in cell differentiation, biological development, and disease progression. Generally, miRNAs can be complementary paired with their target mRNAs to affect the transcription process by inhibiting gene expression, thus causing biological dysfunction, including many human cancers, diabetes, cardiovascular and autoimmune diseases, as well as mental and neurological diseases. There are more than 1000 miRNAs in human cells. The most commonly studied miRNAs are miR-21, miR-155, miR-103b, and let-7; these miRNAs have been associated with cancer and can be used clinically to predict the risk of developing disease. Therefore, the quantification of single of multiple miRNA levels is of great significance for the assessment of human health, and can be applied to evaluate the different staging of cancer, the physical conditions of patients preoperatively, and prognosis.

Many profiling technologies have already been applied to quantify miRNA levels; this work has made significant contributions in a range of molecular diagnostic tests in biomedicine. Typically, quantitative real-time polymerase chain reaction (qRT-PCR) technology, microRNA microarrays, and northern blotting represent the gold standard tools for miRNAs profiling. However, these techniques are associated with certain limitations. For example, qRT-PCR is associated with high costs while microRNA microarrays and northern blotting are associated with low sensitivity. Consequently, it is very difficult to perform efficient and economical miRNA analysis with such techniques. Consequently, there is a critical need to develop other analytical methods to allow us to rapidly and accurately quantify miRNA levels.

Surface-enhanced Raman scattering (SERS) refers to the fact that the Raman scattering signal of molecules adsorbed in the surface of some metal plasmonic conductors can be significantly enhanced by substrate materials, such as gold (Au), silver (Ag), and copper (Cu) plasmonic nanomaterials, and some rare multielement composite materials, and act as a typical signal amplifier. The main mechanisms underlying the Raman phenomenon can be attributed to an electromagnetic field enhancement mechanism (EM) and a chemical enhancement mechanism (CM). According to the EM, these SERS-active plasmonic nanomaterials can usually generate a strong local surface plasmon resonance (LSPR) effect when interacting with incident light. This can be effectively coupled to metal nanoparticles to further enhance the local surface electromagnetic field of nanoparticles; via this process, the SERS intensity can undergo a significant increase, by 10^3–10^6 times. In contrast with the EM, the CM is mainly responsible for the electron density deformation of surface molecules and the transfer of electrons between target molecules and substrates. This process occurs in the presence of strong interactions from the optical electric field and the atoms in the metal surface; these processes can also influence the SERS signal intensity. Generally, the EM, as a typical physical enhancement, shows no selectivity with regards to the enhancement of molecules adsorbed near the substrate; this usually dominates the enhancement effect. In contrast,
the CM only contributes 1–2 orders of magnitude while closely related to the surface property. Based on these two mechanisms, SERS spectroscopy gradually superseded the shortcomings of traditional Raman spectroscopy and acquired structural information from exiguous molecules adsorbed on the surfaces of the plasmonic nanoparticles with high resolution and selectivity. The superior performance of SERS renders this technique as one of the most powerful tools for supersensitive detection.

The miRNAs are degradable and are found in low-abundance; consequently, it has proved to be very difficult to accurately quantify the levels of miRNAs. There is an urgent need to develop rapid and sensitive methods to identify and quantify the levels of miRNAs for biomedical research and clinical diagnosis. By relying on high spectral resolution, SERS has become a powerful technology for profiling miRNAs. The assembly of nanomaterials as SERS nanostructures, based on plasmonic nanomaterials and the nucleic acid aptamers, can produce an enhanced electromagnetic field and improve the signal-to-noise ratio of the Raman signal. Collectively, these features render SERS as an excellent tool for the highly sensitive monitoring of miRNAs.

2. The Fabrication of SERS Nanostructures for MicroRNA Detection

Many fast and sensitive SERS methods have been developed for the detection of miRNAs; these are mostly based on a range of nanomaterials as a substrate. The fabrication of superior SERS nanostructures usually depends on the combination of high-performance SERS-active nanomaterials and the creative design of nanostructures.

2.1. SERS-Active Nanomaterials

It is already evident that SERS-active nanomaterials can play a critical role in the high performance of ultratrace miRNA detections. Various types of nanomaterials have been used as SERS substrates to enhance Raman scattering and amplify the SERS signal intensity, including plasmonic metals (gold, silver, and copper), semiconductor materials (metal oxides and metal sulfides), carbon-based nanomaterials (graphene and carbon nanotubes), and some composite nanomaterials (metal-organic frameworks [MOFs] and core–shell nanostructures). The current mainstream nanomaterials used as SERS substrates are still noble plasmonic metals. This is because the plasmons of these nanomaterials can resonate with the selected incident excitation light in Raman testing, thus forming a local electromagnetic field on the surface, thus contributing to high levels of Raman activity. Typically, the physical EM is considered to produce the strongest impact on the SERS signal with a Raman-enhanced factor (EF) of up to $10^4$–$10^6$. This Raman enhancement by the noble metal nanomaterial substrates is closely associated with the plasmonic properties of the nanomaterials themselves. Consequently, the enhancement involves dielectric function in combination with the shape, size, composition, and the local dielectric environment of the nanostructures. Therefore, from a practical point of view, it is necessary for us to focus significant efforts on engineering nanostructures, if we are to take full advantage of the SERS EMs.

Other non-noble metal types of nanomaterials usually depend on the CM instead of the EM. In such cases, the CM can only contribute to a $\approx 1000$-fold enhancement of the Raman signal. This is due to its near-infrared or infrared plasmonic resonance band that does not match the laser wavelength region used in Raman testing. From this point of view, different materials can contribute differently to SERS. However, this does not mean that we must choose high SERS-active noble metals as a substrate material to obtain high-quality SERS signals. In fact, the development of SERS technology has been hindered by noble metals, to some extent; this is due to limited choice, high costs, and the susceptibility to environmental factors, such as Au, Ag, and Cu.

2.2. The Design of SERS Nanostructures

To achieve an ultrasensitive, highly specific, and reliable detection method, we also need to consider how to effectively and innovatively design SERS nanostructures once the substrate nanomaterial is also determined.

In terms of the nanostructure design, there are many factors that can further regulate the SERS signal. First, the most typical factor is by regulating the hotspot, a high intensity local electric field that exists in the gaps of noble metal nanoparticles. These hotspots are usually produced by adjusting the distance between nanomaterials through the assembly, like the metal dimers and core-satellite nanostructures, or by creating tiny gaps between nanoparticles, like core–shell nanoparticles, or by improving the surface roughness of nanomaterials, like the spiky metal nanoparticles. Hotspots are also closely related to the morphology of nanomaterials, as well as their size, and valuable area. For instance, monodisperse nanoparticles with a small end curvature radius as SERS substrate have a high EF value, a strong local electromagnetic field enhancement effect above $10^10$ could be produced when these nanoparticles approach each other; therefore, the shape of nanomaterials is an important factor to influence the signal intensity, and as the curvature of nanomaterials get higher, the SERS intensity generally would be enhanced. As for the size, the EF value of noble metal nanoparticles with the size range of 20–100 nm can reach $10^4$–$10^5$, and can also be increased by 1–2 orders of magnitude when the molecules are in direct contact with nanoparticles; the decreasing nanoparticle diameter can have the LSPR effect getting weaker, whereas the too large particle diameter could also weaken the LSPR activity, both of which will affect the SERS enhancement factor.

In addition, there are also other factors that can make a significant contribution to the performance of SERS nanosensors, including the design of the molecular switch and the miRNA aptamers, the position of the Raman labels molecules on the surface of metal nanoparticles, and the binding form of nanomaterials and biomolecules.

Worthy mentioned, the substrate nanomaterials and the target molecule are two indispensable factors in miRNA SERS detection, and both together can produce characteristic fingerprint SERS peak to qualitative and quantitative target profiling; this is the unlabeled SERS technology, but the complex biological systems in miRNA detection are an unwanted external environment that would significantly influence the SERS spectrum peaks, and
the lack of standard peaks would also limit the label-free Raman miRNA profiling. To solve these problems, specific organic Raman label molecules with strong and stable signal, and characteristic Raman peak, are often modified on the nanomaterials to provide ultrasensitivity for miRNAs trace analysis of biological samples. Common Raman labels molecules are 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), 2-bromo-4-mercaptobenzoic acid (BMA) and 4-mercaptobenzoic acid (MBA), 4-aminothiophenol (4-ATP), Cy3, Cy5, Rhodamine 6G (R6G), tetramethyl rhodamine, and Texas red, etc., and these molecules were labeled on the surface of metal nanomaterials through the physical adsorption interaction or covalent bonding (Au–S bond, Ag–S bond, and Cu–S bond), or modified on the target aptamers molecules as Raman labels. The characteristic fingerprint of Raman labels molecules generated under the laser excitation would be used to target miRNAs detection.

Generally, both SERS active nanomaterials and the nanostructure design can finally determine the SERS performance, finally forming into diverse structures in different dimensions. Different dimensional SERS nanostructures have different structural complexity, and these SERS nanosensors featuring nanostructures of different dimensions could yield unique performance with regards to the detection of miRNAs. The lower the dimension, the simpler the structure. For example, low-dimensional structures usually consist of single nanoparticle (spherical or rod-shaped, etc.) or simple dimers, whereas high-dimensional nanostructures tend to higher complexity in nanostructure fabrication, helping realize multiple targets or multiple signal pathways SERS detection. Moreover, given that researchers have systematically distinguished the dimensions of matter according to their size characteristics in length, width, and height, SERS nanostructures can usually be divided into 0D nanostructures, 1D nanostructures, 2D nanostructures, and 3D nanostructures, as shown in Figure 1.

3. Nanostructures in the SERS Detection of MicroRNAs

3.1. 0D Nanostructures

If all three dimensions of the nanostructure are at the nanometer scale, and the structure is approximately spherical, then we would consider thus as a 0D nanostructure. Examples of 0D structures include nanoclusters, single metal nanoparticles, and hybrid metal nanoparticles, as shown in Figure 1. These are easy to prepare and use in the creation of simple SERS nanostructures. These structures do not require complex assembly or tedious synthesis steps.

First, in regard to the SERS-active nanomaterials in 0D nanostructures, gold or silver nanoparticles are probably the best known anisotropic noble metal plasmonic nanomaterials and have already became the most ideal SERS substrate. This is due to the fact that these materials generate electronic oscillations in response to incident light, thus causing the polarization of surface molecules, thus creating molecular vibration and rotation and enhancing the Raman signal intensity. Previous researchers have accurately quantified miRNAs based on gold nanoparticles (Au NPs) or silver nanoparticles (Ag NPs). As shown in Figure 2A, Lee et al. modified Au NPs, the Raman labels, and the sulphydryl at the end of three different DNA chains, to create special triangular SERS nanostructures that could be broken down by its target miR-155 via complementary base pairing interactions with DNA molecules. The authors explained that as two individual Au NPs approach each other; a closer distance will allow the oscillating electrons on the surface of the two adjacent nanoparticles to become coupled to form a stronger electric field, thus creating a “hot spot” this resulted in a sevenfold increase in the Raman signal produced and a low detection limit for miR-155 (60 aM). Although gold nanomaterials exhibit excellent SERS activity and chemical stability, it has been demonstrated that silver nanoparticles usually show a superior performance with regards to SERS enhancement than gold nanoparticles because of the strong near-field coupling effect between silver particles and the sharp plasmonic peak. Attracted by the performance of silver in plasmonic materials, Qi et al. used silver nanomaterials as a SERS substrate to simultaneously quantification of the levels of three different miRNAs: miR-21, miR-31, and miR-98 (Figure 2B). In addition, considering that the morphology of metal nanostructures can exert significant influence over the signal enhancement amplitude and detection sensitivity, various silver nanoparticles have been synthesized, in many different patterns, including a truncated stellar octahedron shape, a truncated tetrahedron shape, and a cubic morphology. Silver nanoparticles that modify DNA probes would approach each other and form a dimer structure due to complementary base pairing with the target miRNAs; the local electromagnetic field would then be further enhanced, and the intensity of the Raman signal would be greatly improved. In general, just as the structure determines the function,
nanoparticles with a sharp corner, or distinct edges, can usually produce highly localized charge polarization at the edges and corners. This would significantly affect the electromagnetic field, via the creation of a nanotriangle or nanocube, thus creating differing performances in terms of SERS enhancement.

Single metal nanomaterials are usually associated with limited application. Typically, the practical application of silver nanomaterials is heavily limited by chemical degradation and cytotoxicity. Therefore, silver is usually integrated with gold to form bimetallic nanoparticles; this combines the best chemical and plasmonic properties from each of these materials. Many researchers have reported the use of Au@Ag hybrid nanoparticles as SERS substrate;[67,68] these can significantly contribute to a better Raman performance.[69] As shown in Figure 2C, Wang and et al.[70] designed OFF-to-ON SERS nanostructures to quantify the levels of miR-21 and miR-34a using silver-coated gold nanostars (AuNS@Ag). Raman labels were linked at the ends of a single-strand nucleic acid sequence (ssDNA). The miRNA aptamer paired with the placeholder ssDNA, although the aptamer would detach into solution once bound with the target miRNAs. The ssDNA existed in a more stable hairpin structure and then brought the AuNS@Ag and the Raman label close to each other; under laser excitation, this produced a strong Raman enhancement effect. In contrast with Wang’s research, in preparing gold and silver hybrid SERS nanostructures, Su and et al.[71] modified DNA probes and Raman labels on the surface of Au NPs and then grew silver caps to form a mushroom-like bimetallic nanostructure (Au–Ag NMs)(Figure 2D). In this case, the Raman labels and the DNA molecules were squeezed into a tiny gap with abundant “hotspots” between the gold and the silver; this brought about an enhanced electronic field and a strong SERS signal. The target miRNAs triggered the Au–Ag NMs to form a sandwich structure with micromagnetic nanoparticles (MMPs) via base pairing interaction, thus quantifying the miRNAs.

Thus far, research involving 0D SERS nanostructures and nanostructures that are based on noble metals have achieved good performance with regards to the analysis of miRNAs. However, a SERS detection system created with these 0D nanostructures is relatively simple. The hotspots contributed by 0D SERS nanostructures, composed of simple morphological nanoparticles or simple assembly structures, are less and weak, making it not easy to have the Raman enhancement effect significantly strong, thus limiting their potential applications as ultrasensitive biomarkers.

3.2. 1D Nanostructures

1D nanostructures have three dimensions or two dimensions on the nanoscale and one dimension that is significantly larger than the other two dimensions. Common SERS-active 1D nanomaterials include nanowires, nanorods, and nanotubes; some of these
can simultaneously possess the characteristics of nanomaterials and macromaterials, thus connecting the microscopic and macroscopic worlds. In fact, due to the superior optical responding property of 1D nanomaterial, it was more commonly seen in SERS detection to use 1D nanomaterials than 0D nanomaterials as substrates. Meanwhile, the significantly structural differences of several typical 1D nanomaterials influenced their applications in SERS detection. Therefore, it would be more appropriate to introduce the practical performance of these nanomaterials separately according to the typical morphological features.

Nanorods, as a typical 1D nanomaterial, are widely used in the life sciences, and represent nanomaterials with rod-like shapes. Commonly, gold nanorods (Au NRs)\(^{[74]}\) can exhibit huge electric field enhancement under resonance excitation, mainly located in two ends of the nanorods. At the same time, these anisotropic Au NRs can form into diverse structural assemblies with different morphologies, thus exhibiting distinct plasmonic properties. These characteristics together make nanorods a very common SERS substrate nanomaterial, and it would be more challenge to regulate the nanorods into high-performance SERS nanostructure by the creative molecular switch. As shown in Figure 3A, Xu et al.\(^{[75]}\) used Au NRs as SERS substrates to detect miR-21 in living cells. To fabricate superior 1D SERS nanostructure, these authors decorated two different ssDNA aptamers on the side surfaces of Au NRs by selectively modifying HS-PEG molecules on the two ends of the nanorods. The target miRNAs in living cells could assemble these two types of nanorods with different aptamers into the side-by-side nanorod dimer structures by complementary base pairing between the target miRNAs and the ssDNAs. The formation of the Au NRs dimer structure in the cell could result in strong local plasmonic resonance and an increased electromagnetic field, thus causing enhancing the Raman signal intensity. Considering that the electromagnetic field intensity of nanorods near the surface can be regulated by changing the shape of nanorods, nanorods with arrow-like high curve tips, referred to as arrowhead gold nanorods (AH-NRs),\(^{[76]}\) could also be chosen as SERS nanosubstrate. These AH-NRs have been shown to exhibit stronger electromagnetic coupling\(^{[77]}\) and can significantly alter the polarity of the Raman label molecules in between. Our laboratory\(^{[78]}\) recently fabricated AH-NRs as an SERS substrate to detect and image miR-21 in living cells (Figure 3B). By selectively modifying the DNA aptamers and PEG molecules on the surface of AH-NRs, we successively constructed arrowhead end-to-end nanorod (AH-ETE-NRs) dimers and arrowhead side-by-side (AH-SBS-NRs) dimers. An intense electromagnetic field was generated after the high curvature tips approached each other; this can be used for the ultrasensitive detection of miRNAs in accordance with intensity of the SERS signal.

Except for nanorods, nanotubes are a new option for 1D SERS nanomaterials, which usually relate to carbon nanotubes. As a typical layered carbon-based 1D nanomaterial, carbon nanotubes exhibit stable and excellent optical properties, as well as distinctive Raman scattering features that are induced from the chemical enhancement. In a previous study, Zheng et al.\(^{[79]}\) found that single-walled carbon nanotubes (SWNTs) can be bundled with ssDNA molecules to form a SERS-active SWNTs/DNA/Ag complex after reducing silver ions to silver nanoparticles on the surface (Figure 3C). The SERS signal intensity, jointly attributed to chemical and plasmonic enhancement, showed a positive correlation with the number of ssDNA aptamers for miR-21; the limit of detection (LOD) was 10 pM.

In addition, nanowires, representing another 1D structure, have many unique merits; for example, they are adjustable and easy-to-prepare. In particular, nanowires usually possess excellent transmittance and electrical conductivity.\(^{[80]}\) The surface plasmon propagation length of nanowires is often much higher than other nanomaterials, and can effectively couple far-field light to harvest more light as an “antenna.” Light energy can also be further transferred into the vibrational energy of molecules.\(^{[81]}\) Consequently, nanowires are regarded as an ideal SERS substrate and superior SERS detection systems can be achieved by regulating other factors together. Based on a single-crystal plasmonic gold nanowire (Au NWs), Lee et al.\(^{[82]}\) designed a protein probe-based miRNA sensor by surface plasmon resonance (SPR) analysis. Therein, a protein probe (PAZ) was a target miRNA binding protein that was used to distinguish between specific double-stranded RNA (dsRNA) and nonspecific single-stranded RNA (ssRNA); this practice would avoid false-positive signals. Here, Au NWs were used as nanoplatform to immobilize ssRNAs and enhance the Raman signal. However, this technique yielded an LOD of only 10 pM. It is possible that the highly conservative and common mutation sequences of miRNAs may cause false-positive results. Considering these issues, Kang et al.\(^{[83]}\) modified the thiolated locked nucleic acids (LNAs) as an aptamer for miRNAs on the surface of the Au NWs (Figure 3D). By raising the temperature from 42 to 64 °C, the imperfect base-pairing between the miRNAs and the LNAs sequences was not stable enough to protect against such a high temperature. Consequently, there was no contribution to the Raman signal, thus avoiding false-positive signals and achieving an LOD of 100 aM.

### 3.3. 2D Nanostructures

2D nanostructures exhibit at least one dimension on the nanoscale, whereas the size of the other two dimensions is significantly larger than that of the third dimension. These are usually fabricated by direct synthesis, assembly, etching, or chemical deposition. Typical 2D nanomaterials usually exhibit an ultrathin structure, a highly specific surface area, as well as unique optical properties. Some of these properties are present simultaneously on nano and macroscopic scales. These structures represent a promising SERS matrix that is associated with a lower cost, higher selectivity, and a superior throughput (Figure 4).

Worthy mentioned, the single-atom-layer graphene oxide (GO) is a typical 2D nanomaterial and features high-frequency Dirac fermions conduction and high carrier transmission speed.\(^{[84]}\) Collectively, these contribute to an ultrafast and efficient optical response to further enhance Raman scattering,\(^{[85]}\) and have GO a potential SERS substrate to replace the noble metal nanomaterials. Previous study by Ma et al.\(^{[86]}\) have successfully fabricated an unique GO and Au NPs assembly nanostructure with a strong plasmonic coupling effect, thus resulting in an enhanced local electromagnetic field (Figure 4A). Moreover, the DNA aptamer modified with the Raman label FAM, acted as a
linker between GO and the Au NPs, these then bound to the target miR-21, thus disintegrating the local assembly structure and reducing the Raman intensity, to effectively quantify the miRNA.

For another 2D SERS-active nanomaterials, boron nitride is a single atom honeycomb layered structure with a graphite-like structure. This is a wide bandgap semiconductor with excellent electrical and magnetic properties; this materials is also chemically inert but another potential SERS substrate. For instance, Liu et al. combined hexagonal boron nitride nanosheets (h-BNNS) with insoluble copper phthalocyanine (CuPc) and a
hairpin-structure DNA aptamer (HGDNA) to form CuPc@HG@BN complexes under π–π stacking interaction (Figure 4B). The highly polar B–N bond can cause perturbation in the symmetry of the CuPc structure, thus resulting in a high EF value of $7.42 \times 10^4$. When the target miR-21 appeared, the CuPc would escaped from the surface of h-BNNS, with the Raman signal intensity correspondingly weakened.

Unlike the nonmetallic single-layer nanomaterials mentioned earlier, most 2D SERS substrates are inorganic metal nanomaterials in the form of large area films or nanomaterial arrays.
However, these substrates can also represent a macroscopic structure with a height on the nanometer scale and are usually fabricated by top-down methods, such as electrochemical deposition and etching rather than the bottom-up method in solution. It should be also noted that GO-like 2D materials are too thin and cannot absorb a sufficient amount of light; this inevitably limits their effective applications. Therefore, the plasmonic metal used as nanomaterials in 2D structures can enhance light absorption via the plasmonic effect when used for important optical applications.

By depositing a layer of branched nanodendritic gold substrates on the surface of the ITO/Ti/Au substrate, Song et al. \[89\] constructed a superwettable SERS active substrate with a superhydrophilic array and surrounding superhydrophobic area. This was then used to anchor multiple microdroplets and enrich analytes to effectively prevent the coffee ring effect (Figure 4C). Furthermore, the gold nanodendritic structure provided multiple hotspots to enhance the SERS intensity of the Raman label which featured modified ssDNA so as to achieve an OFF-to-ON SERS signal that was regulated by the target miR-141. As shown in Figure 4D, Lee et al. \[89\] used nanoimprint lithography and oblique deposition techniques to fabricate a highly robust, uniform, and periodically arranged gold nanobowls (Au NBs) structure on a polymer substrate. We then deposited silver nanostructures by high overpotential reduction inside the Au NBs with a EF value of 4.8 x 10^3. This achieved an excellent SERS signal and achieved an LOD for miR-34a of 50 aM. In another work, Lee et al. \[91\] combined the mask-less reactive ion etching (RIE) method and electrochemical deposition methods to construct a plasmonic gold nanopillar SERS substrate (Figure 4E). The analyte solution or air drying could change the solvent conditions of the substrate surface. The gold nanopillars were able to lean toward each other, thus creating a “head-flocking” effect. This occurred under capillary force and the elastic deformation of the plasmonic gold nanoparticles. The coupling effect of the surface local plasmon was significantly enhanced with regards to the detection of the target miR-21, miR-222, and miR-200c at 1 aM.

### 3.4. 3D Nanostructures

3D nanostructures represent stereoscopic nanostructures with comparable sizes in three dimensions. Typical 3D nanomaterials include core-satellite (CS) nanostructures, the pyramidal nanostructure, and some composite bulk nanostructures. These 3D nanostructures can often form a restricted 3D plasmonic field and lead to enhanced electromagnetic fields with the 3D space. At the same time, the coupling of LSPR fields can occur due to the tiny gaps among these nanostructures. This creates high density “hotspots,” thus resulting in a significant Raman signal amplification and improved sensitivity of the SERS nanostructures (Figure 5), which is a common strategy to fabricate SERS nanostructures.

CS nanostructures are one of the most well-known 3D nanostructures and can be prepared from a range of from different nanomaterials, including gold, silver, up-conversion nanoparticles (UCNPs) and some magnetic nanoparticles. The close distance among the satellites and core could help the local electromagnetic field enhancement. This would have a positive influence on the SERS performance of the entire nanostructures. A typical OFF-to-ON gold nanoparticle CS assembly was fabricated and used as a SERS substrate by He et al. \[92\] (Figure 5A). These authors modified ssDNA on the surface of gold nanoparticles in a frozen environment. This effectively improved the modification efficiency and precisely regulated the number of DNA molecules. Based on this, these authors created a CS assembly of gold nanoparticles in a shorter time by targeting the miR-21 sequence; this was associated with an LOD of 0.12 pM. Ma et al. \[93\] used gold nanorod side-by-side dimers (Au-SBS dimers) as a core and UCNPs as satellites to form a dual-functional CS SERS nanostructure that was able to achieve dual-signal and dual-target quantification (Figure 5B). The plasmonic coupled “hotspots” among the Au NRs and UCNPs formed in large quantities and enhanced the SERS signal. In addition, the intensity of the SERS signal can respond to the target miR-21, thus initiating the collapse of the assembly structure. In another study, Liu et al. \[94\] used gold nanodumbbells as a core, and gold nanoparticles as satellites, to fabricate a CS assembly to detect miR-1246 (Figure 5C). Finite difference time domain (FDTD) simulation of the CS nanostructure proved that the electric field intensity at the spherical ends of the nanodumbbells was significantly higher than other regions. At the same time, this method also combined target miRNAs-mediated catalytic hairpin assembly (CHA) as a signal amplification strategy; thus achieved a minimum detection limit of 0.85 aM. In addition to using the common CS nanostructure as a SERS substrate, Xu et al. fabricated pyramid-shaped stereoscopic assembly nanostructures driven by DNA molecules; these significantly enhanced the electromagnetic field and allowed the detection of cancer biomarkers; these structures included Au NPs pyramids, \[95\] Ag NPs pyramids, \[96\] and Au-UCNP pyramids. \[97\] DNA molecules could be used to determine the distance between these inorganic nanoparticles in the pyramid structure, and the distance could be adjusted by the target biomarkers to achieve effective SERS detection. In short, for the SERS nanosensors based on 3D CS nanostructures, the nucleic acid molecules usually are a common strategy to regulate the hot spots and the SERS signal.

Aside from using monotonous novel metal nanostructures as SERS substrates, there has been the combination of metals with other types of nanomaterials, such as MOFs and magnetic nanomaterials. These are new strategies that are used to regulate hot spots and enhance the SERS signal. MOFs are stable porous structures that contain a uniform distribution of metal nanoparticles within their pores. These structures have abundant hot spots that enrich the target analytes simultaneously. The magnetic nanoparticles can contribute to the formation of a magnetic-field-induced hotspot. Drawing on the features of MOFs and magnetic nanomaterials, He et al. \[98\] carbonized ZIF-67 to synthesize Co@C nanoparticles and reduced the incorporation of silver nitrate into silver nanoparticles on the surface of Co@C, thus forming a complex SERS substrate of magnetic and porous Co@C/PEI/Ag. This helped to generate multiple hot spots, enrich the target miR-155 and Raman label molecules, and produce a stronger SERS signal intensity (Figure 5D).

Furthermore, the rolling circle amplification (RCA) strategy was used to amplify the target sequence. This SERS
nanostructure achieved a sensitive and effective low detection limit for miR-155 at 70.2 aM. It is worth noting that when used as SERS substrates, these composite materials can usually avoid a large number of noble metal nanomaterials and reduce the cost of SERS analysis.

Although 3D SERS nanostructures create relatively larger amounts of hot spots, the complex multistep assembly process will not only increase the difficulty of fabrication but also limit the yield of 3D SERS nanostructures.

4. Conclusion

Our research showed that the fabrication methods for SERS nanostructures could be mainly categorized into two strategies: regulation of the electromagnetic field of the nanomaterials as a substrate, and the design of molecular switch to create OFF-to-ON or ON-to-OFF SERS signals switched by the target miRNAs. Both of these two factors were indispensable for a successful SERS nanostructure and jointly determined the performance.
of the SERS nanostructures when detecting miRNAs. SERS nanostructures, when used in the four different dimensions (0D, 1D, 2D, and 3D nanomaterials) have achieved superior performance for the detection of miRNAs and are commonly applied in living cells and real blood samples.

However, although many SERS nanostructures are based on nanomaterials and show excellent performance for the profiling of miRNAs, there are still many barriers to be overcome. 1) The binding stability of Raman active molecules and nanomaterials makes it difficult to achieve high-sensitivity SERS nanosensors with good signal reproducibility and long-term stability. 2) The lack of uniform and reproducible SERS substrates, the insufficient selectivity for miRNA detection, and the fluctuation of SERS signals hinder the reliable quantification of miRNAs using SERS. 3) The highly sensitive, easy-to-operate, and low-cost Raman spectrometer is crucial to transform the technology into clinical practical application. Although, existing studies have shown that SERS overcome the shortcomings of traditional methods for high sample requirements and time-consuming in miRNA detection applications, realize sensitive and rapid detection and analysis, and provide effective analysis tools for real-time rapid detection of nucleic acid molecules and real-time accurate diagnosis of clinical diseases. There are many future avenues to be developed, such as single-molecule-level miRNA detection in real samples by supersensitive SERS technology. The use of SERS nanostructures for the analysis of miRNAs are likely to be of significant value for clinical point-of-care human health monitoring and accelerate the process of modern digital medicine.

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Conflict of Interest

The authors declare no conflict of interest.

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