Trends in Food Science & Technology 138 (2023) 615–627

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Advances in CRISPR-based SERS detection of food contaminants: A review

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ARTICLE INFO

Keywords:
SERS
CRISPR-Cas
Nucleic acid
Disease diagnosis
Detection

ABSTRACT

Background: The integration of CRISPR-Cas systems and surface-enhanced Raman spectroscopy (SERS) has emerged as a powerful approach for developing highly sensitive biosensors. This review explores recent studies that demonstrate the potential of CRISPR-Cas12a and Cas13a systems combined with SERS technology in detecting nucleic acids with exceptional sensitivity and specificity.

Scope and approach: This review examines studies that showcase the application of CRISPR-Cas and SERS technology in various fields, including healthcare, food safety, environmental monitoring, and biodefense. The reviewed studies highlight the capability of CRISPR-Cas systems to target specific DNA or RNA sequences, while SERS serves as a highly sensitive detection method. The combination of these technologies enables the development of biosensors for the detection of pathogens, genetic markers, drug resistance genes, chemical compounds, and adulterants in food.

Key findings and conclusions: The reviewed studies demonstrate the significant findings and potential of integrating CRISPR-Cas and SERS technologies for biosensing applications. The studies showcase the ability of CRISPR-Cas12a and Cas13a systems to detect nucleic acids with high sensitivity and specificity, even at trace levels. The use of SERS as a detection method offers advantages such as enhanced sensitivity, selectivity, and reliability. The combination of CRISPR-Cas and SERS technology has the potential to revolutionize diagnostic testing in various sectors. Although challenges remain, including improving detection limits and reducing interference from complex matrices, the promise of these biosensors in early disease diagnosis, pathogen detection, and environmental monitoring is evident. Further research and development are warranted to harness the full potential of CRISPR-Cas and SERS technology for future biosensing applications.

1. Introduction

Food safety is a major global concern due to the potential health risks associated with consuming contaminated foods (Ehuwa, Jaiswal, & Jaiswal, 2021). Foodborne diseases, caused by ingesting food contaminated with pathogenic microorganisms and toxic chemicals, pose a significant threat to human health, with an estimated 600 million people falling ill and 420,000 deaths occurring worldwide each year (Lee & Yoon, 2021). Thus, detecting foodborne pathogens and contaminants plays a crucial role in ensuring food safety. The accurate and rapid detection of foodborne pathogens is of paramount importance to prevent foodborne illnesses (Yan et al., 2021). Traditional detection methods for foodborne pathogens rely on microbial isolation, culture, and biochemical identification techniques. However, these conventional methods have limitations in terms of accuracy, sensitivity, and turnaround time, which hinder their practical application (Panwar et al., 2022). To address these challenges, numerous advanced detection methods have been developed, including molecular-based assays such as PCR (Vinayaka et al., 2019), surface plasmon resonance (Zhou et al., 2019), and CRISPR-Cas-based nucleic acid detection systems (Wu et al., 2022). These methods have demonstrated great potential for detecting foodborne pathogens with high sensitivity, specificity, and rapid turnaround time. Therefore, there is an urgent need to study advanced food detection techniques that can improve the detection of foodborne pathogens and ensure food safety (Sun, Li, Zhu, & Jiang, 2022).

The field of molecular diagnostics has been revolutionized by the development of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems (Bonini et al., 2021). The CRISPR-Cas system, originally discovered as a bacterial immune system, has been harnessed for gene editing, gene regulation,

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https://doi.org/10.1016/j.tifs.2023.07.001
Received 16 May 2023; Received in revised form 30 June 2023; Accepted 1 July 2023
Available online 3 July 2023

and nucleic acid detection (Li, Li, Wang, & Liu, 2019). The development of CRISPR-based nucleic acid detection systems has provided a powerful tool for rapid, sensitive, and specific detection of pathogenic microorganisms in clinical (Wang, Zhang, & Li, 2020; Zhang et al., 2022), environmental (Liu, Hussain, et al., 2022), and food samples (Zhuang et al., 2022). The CRISPR-based detection systems utilize the highly specific and programmable nature of the CRISPR-Cas system for nucleic acid detection, allowing for the detection of pathogens with high sensitivity and specificity (Liu, Hussain, et al., 2022).

Several CRISPR-based nucleic acid detection systems have been developed, including specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) (Kellner, Koob, Gootenberg, Abudayyeh, & Zhang, 2019) and DNA endonuclease targeted CRISPR trans reporter (DETECTR) (Broughton et al., 2020). These systems utilize the CRISPR-Cas system for the detection of target nucleic acids in a sample, and the detection of the resulting signals is typically achieved through the use of fluorescent or colorimetric reporters. These CRISPR-based detection systems have been successfully applied for the detection of viral and bacterial pathogens, including SARS-CoV-2 (Palaz, Kalkan, Tozluyurt, & Ozsoz, 2021), Zika virus (Kellner et al., 2019), and *Staphylococcus aureus* (Zhou, Yin, et al., 2020). Several studies have been conducted on the use of CRISPR in detection methods for food samples. For instance, Wang et al. (2020) developed a CRISPR/Cas12a-based lateral flow biosensor for the detection of *Salmonella enterica* (Zhu et al., 2020). Similarly, Li et al. (2021) developed a CRISPR/Cas12a-based biosensor for the detection of *Listeria monocytogenes* in food samples (Li et al., 2021). Liu, Hussain, et al. (2022) developed an RPA-CRISPR-Cas12a detection method for *S. aureus* (Liu, Hussain, et al., 2022). Another study by Wang et al. (2021) used CRISPR-Cas12a to detect *Escherichia coli* O157:H7 in milk samples (Wang, Fan, et al., 2021). These studies indicate the potential of CRISPR-based methods for the rapid and accurate detection of foodborne pathogens, which is crucial for ensuring food safety.

Surface-enhanced Raman spectroscopy (SERS) is a sensitive and selective analytical technique used for detecting and identifying trace amounts of molecules (Gillibert, Huang, Zhang, Fu, & Lamy de la Chapelle, 2018). SERS involves the interaction of light with a metal surface coated with a layer of molecules, enhancing the Raman scattering signals of the molecules for improved detection. The high sensitivity and selectivity of SERS make it a valuable tool for sensor identification and design, particularly in medical diagnostics (Nima et al., 2014), environmental monitoring (Sivaprakasam & Hart, 2021), and food safety (Nilghaz et al., 2022). Its ability to detect low concentrations of substances, versatility in analyzing various sample types, and specificity in identifying unique molecular vibrations of a molecule make SERS a powerful technique in sensor design. Integrating SERS and CRISPR methods holds immense potential for designing a new generation of sensors to identify different samples with high accuracy and specificity (Li, Man, Ye, Liu, & Ma, 2022). By leveraging CRISPR's specificity in targeting and binding to the analyte of interest and SERS's sensitivity and selectivity in detecting and identifying analytes, it is possible to create sensors that can accurately and rapidly identify various samples such as biomolecules, chemicals, bacteria, and other contaminants in different fields, including medical diagnostics, food safety, and environmental monitoring (Zhuang et al., 2022). The integration of these two techniques could revolutionize the sensor design, creating highly sensitive and selective devices for various analytical applications.

This review article aims to provide an overview of the latest studies on the use of CRISPR and SERS technologies in sensor design, with a focus on identifying target analytes (Fig. 1). Specifically, the applications of this combined technology in the identification of nucleic acids, viruses, microorganisms, and various factors affecting the quality of food products are elaborated. Through a comprehensive review of the literature, the potential of CRISPR and SERS technologies in designing a new generation of diagnostic sensors with high sensitivity and specificity is explored. This review will provide valuable insights into the future development of biosensors and their applications in various

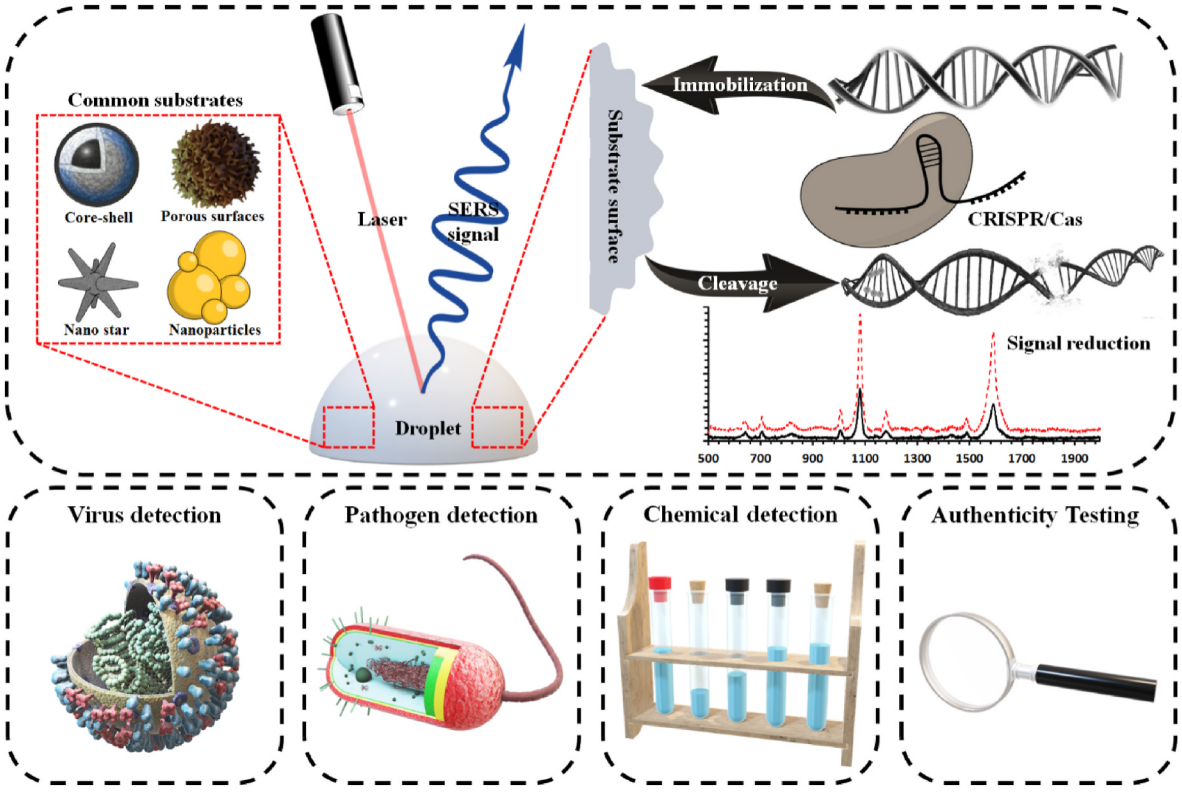

Fig. 1. An overview of the working mechanism of sensors designed using CRISPR and SERS technologies.

fields, including food safety, medical diagnostics, and environmental monitoring.

1.1. SERS

Surface-enhanced Raman scattering (SERS) is a powerful analytical technique that has revolutionized the field of surface science and molecular spectroscopy. SERS involves the use of a metallic substrate, such as gold or silver, to amplify the Raman signals of molecules adsorbed onto the substrate surface. This results in an enormous enhancement of the Raman scattering signals, enabling the detection of very low concentrations of analytes (Pilot et al., 2019). Compared to normal Raman scattering, SERS can enhance the Raman signals by more than 10^6 times (Shahine, Mevellec, Richard-Plouet, Humbert, & Tessier, 2022), making it a highly sensitive analytical tool.

The superiority of SERS over other methods used in making diagnostic sensors lies in its ability to detect and identify analytes at very low concentrations, often in the femtomolar to attomolar range (Bai, Serien, Ma, Obata, & Sugioka, 2020). This makes it ideal for use in various diagnostic applications, including disease diagnosis, environmental monitoring, and food safety testing. Unlike other analytical techniques, SERS can be used to identify a wide range of analytes, including biological molecules, such as proteins (Feliu et al., 2017), peptides (Altuntas & Buyukserin, 2018), and nucleic acids (Sitjar et al., 2021), as well as inorganic molecules, such as heavy metals (Guo et al., 2021) and pesticides (Wang, Wang, et al., 2021).

Some useful applications of SERS include the detection and identification of various analytes, including bacteria, viruses, and cancer cells. For example, SERS has been used to detect the presence of bacteria in food samples, such as *E. coli* (Asgari et al., 2022) and *Salmonella* (Ma, Lin, Xu, & Wang, 2021). In addition, SERS has been used to identify cancer cells in biological fluids, such as blood (Gao et al., 2021) and urine (Chamuah, Saikia, Joseph, & Nath, 2019), enabling early diagnosis and treatment of cancer. Other applications of SERS include the detection of environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs) (Zhou, Yin, et al., 2020) and heavy metals (Guo et al., 2021), as well as the identification of counterfeit drugs (Huo, Yang, Wilson, & Jiang, 2022) and explosives (Gao et al., 2020).

The working mechanism of SERS involves the interaction of incident light with a metallic substrate, such as gold or silver, that has been roughened or coated with nanoparticles. When a sample containing analytes is introduced onto the substrate, the incident light causes localized surface plasmons on the metallic surface, creating hot spots where the Raman signals are greatly enhanced (Sharma, Frontiera, Henry, Ringe, & Van Duyne, 2012). This amplification is due to both the electromagnetic enhancement, which arises from the interaction between the incident light and the surface plasmons, and the chemical enhancement, which results from charge transfer between the analyte molecules and the metallic surface (Hajikhani & Lin, 2022; Sharma et al., 2012).

There are two main modes of SERS: in the first mode, known as the "substrate mode", analytes are adsorbed directly onto the roughened or nanoparticle-coated substrate. In the second mode, known as the "colloidal mode", analytes are adsorbed onto nanoparticles that have been dispersed in solution (Israelsen, Hanson, & Vargis, 2015). In the colloidal mode, the nanoparticles act as both the substrate and the enhancer, providing a highly sensitive platform for detecting analytes in solution (Israelsen et al., 2015). Another mode of SERS is the "tip-enhanced mode", which involves the use of a scanning probe microscope (SPM) to enhance the Raman signals of analytes (Shao & Zenobi, 2019). In this mode, a sharp metallic tip is brought into contact with the sample, creating a nanoscale "hot spot" where the Raman signals are greatly enhanced. This mode is particularly useful for imaging biological samples at high resolution, as it allows for the identification of specific molecular components in a complex mixture (Shao & Zenobi, 2019).

1.2. Substrate

The choice of substrate is an important consideration in the SERS method, as it can significantly affect the sensitivity and reproducibility of the Raman signals. Commonly used substrates in SERS include roughened metal surfaces, such as silver or gold, as well as metallic nanoparticles and nanorods (Krajczewski, Ambroziak, & Kudelski, 2021). Roughened metal surfaces are typically created by depositing a thin layer of metal onto a substrate, such as glass or silicon, and then subjecting it to various treatments, such as annealing or etching, to create a surface with a high density of nanoscale features (Han, Huang, & Lee, 2014). These features act as "hot spots" for the amplification of the Raman signals, enabling highly sensitive detection of analytes (Han et al., 2014).

Metallic nanoparticles, such as gold or silver nanoparticles, are also commonly used as substrates in SERS. These nanoparticles can be synthesized with a range of sizes and shapes, which can influence the enhancement of the Raman signals (Pérez-Jiménez, Lyu, Lu, Liu, & Ren, 2020). For example, nanorods can provide stronger SERS signals than spherical nanoparticles due to their high aspect ratio and strong plasmonic resonances (Pérez-Jiménez et al., 2020). In addition to metals, other materials have been explored as substrates for SERS, including semiconductors, carbon-based materials, and dielectrics. These materials can provide unique advantages, including greater stability, lower cost, or specific surface chemistry for binding specific analytes (Cong, Liu, Jiang, Zhang, & Zhao, 2020).

Overall, the choice of the substrate in the SERS method depends on the specific application and the desired sensitivity and reproducibility of the Raman signals. Advances in substrate fabrication and optimization continue to drive the development of more sensitive and robust SERS substrates for various applications.

1.3. CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a prokaryotic immune system that provides adaptive immunity against foreign nucleic acids, including bacteriophages and plasmids. It is a complex system that involves multiple genes, including Cas (CRISPR-associated) genes and CRISPR arrays, which consist of repeated sequences separated by unique sequences called spacers. These spacers are derived from viral or plasmid DNA that the bacterium has encountered and integrated into its genome as a defense mechanism against future invasions (Hille & Charpentier, 2016). The Cas component, on the other hand, encodes for proteins that are responsible for identifying and destroying foreign genetic materials, using the information stored in the CRISPR spacer sequences to guide the targeting and cutting of specific DNA sequences (Zhang, Zhang, Unver, & Zhang, 2021).

The CRISPR system consists of two main components: the CRISPR-associated (Cas) protein and a guide RNA molecule. Cas proteins are responsible for cleaving the DNA at specific locations, while the guide RNA provides the specificity for targeting the desired DNA sequence (Asmamaw & Zawdie, 2021). The classification of CRISPR-Cas systems is based on the organization and composition of the Cas genes, as well as the structure of the CRISPR array. The current classification system di-vides the CRISPR-Cas systems into two classes, six types, and multiple subtypes (Chaudhuri, Halder, & Datta, 2022):

Class 1: This class is characterized by multi-subunit effector complexes, which are composed of multiple Cas proteins. The Class 1 systems are further divided into three types:

- Type I: Type I Cas proteins are characterized by the presence of a large, multi-subunit complex called Cascade (CRISPR-associated complex for antiviral defense). The Cascade complex includes several Cas proteins that work together to bind to target DNA, cleave it, and degrade the resulting products. Type I Cas systems are further

divided into seven subtypes (I-A to I–F, and I–U), which differ in the composition of the Cascade complex and the mechanism of DNA cleavage. The key Cas proteins in Type I systems are Cas3, Cas5, Cas7, and Cas8. Type I systems are commonly found in bacteria and are used for both DNA and RNA interference (Xue & Sashital, 2019).

- Type III: Type III Cas proteins are characterized by the presence of a large, multi-subunit complex called Csm (CRISPR-associated complex for the targeting of mobile genetic elements). The Csm complex includes several Cas proteins that work together to bind to target RNA, cleave it, and degrade the resulting products. Type III Cas systems are further divided into four subtypes (III-A to III-D), which differ in the composition of the Csm complex and the mechanism of RNA cleavage. The key Cas proteins in Type III systems are Cas10, Cas7, and Cas5. Type III systems are used for both DNA and RNA interference and have been implicated in regulating bacterial gene expression (Chaudhuri et al., 2022).
- Type IV: This putative type is characterized by the presence of the Cas12 and Cas12b proteins, which are single-subunit effector proteins. Cas12 is guided to the target DNA by a single crRNA molecule. Type IV systems have been used in genome editing and detection applications (Zhou et al., 2021).

Class 2: This class is characterized by single-subunit effector complexes, which are composed of a single Cas protein. The Class 2 systems are further divided into three types:

- Type II: Type II Cas proteins are represented by the CRISPR-Cas9 system, which is widely used in genome editing. Cas9 is a single, large protein that is guided to specific target DNA sequences by a short RNA molecule called a guide RNA (gRNA). Cas9 then cleaves the target DNA, resulting in gene disruption or replacement. Type II Cas systems are further divided into three subtypes (II-A to II-C), which differ in the composition of the Cas9 protein and the specificity of the gRNA (Chylinski, Makarova, Charpentier, & Koonin, 2014).
- Type V: Type V Cas proteins are represented by the CRISPR-Cas12 system, also known as Cpf1. This system is characterized by the presence of a single Cas12-like protein called Cas12e, which is a single, large protein that is responsible for the targeting and cleavage of the foreign DNA. Cas12 then cleaves the target DNA, resulting in gene disruption or replacement. Type V Cas systems are further divided into three subtypes (V-A to V–C), which differ in the composition of the Cas12 protein and the specificity of the gRNA (Tong et al., 2021).
- Type VI: Type VI Cas proteins are characterized by the presence of a large, multi-subunit complex called C2c2. The C2c2 complex includes several Cas proteins that work together to bind to target RNA, cleave it, and degrade the resulting products. Type VI Cas systems are further divided into four subtypes (VI-A to VI-D), which differ in the composition of the C2c2 complex and the mechanism of RNA cleavage. The key Cas proteins in Type VI systems are Cas13 and Cas10. Type VI systems are used for RNA interference and have been adapted for use in various molecular biology techniques (Chaudhuri et al., 2022).

The different types of Cas proteins and CRISPR-Cas systems have distinct advantages and disadvantages in different applications. For example, the Cas9 system is widely used for genome editing because of its ease of use and specificity, but it requires a relatively large gRNA and has limitations in targeting certain sequences. The Cas12 system, on the other hand, has a smaller gRNA and can target a broader range of sequences, but is less well-studied than Cas9. The type III and type VI Cas systems have potential applications in RNA targeting and RNA interference, respectively (Makarova et al., 2015).

In addition to genome editing, CRISPR-Cas systems have been used for a variety of identification methods, such as CRISPR-based diagnostics (Table 1). In this approach, a specific gRNA is designed to target a specific pathogen or genetic variant, and the resulting cleavage products are detected by a variety of methods such as fluorescence or colorimetry (Kim, Ji, & Koh, 2021). This approach has been utilized for the identification of various RNA viruses, such as the Ebola virus and Zika virus, as well as DNA viruses, including human papillomavirus (HPV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and BK polyomavirus (BKV) (Najafi et al., 2022).

1.4. SERS and CRISPR integration approach

The combination of CRISPR and SERS allows for the development of highly specific and sensitive detection sensors. In this approach, CRISPR-Cas enzymes are engineered to recognize and bind to a specific DNA or RNA sequence of interest. Once the CRISPR-Cas complex binds to the target, the Cas enzyme can cleave the DNA or RNA, resulting in a change in the SERS signals (Zhuang et al., 2022). To achieve this, a SERS-active substrate is functionalized with capture probes that are complementary to the target sequence. When the target binds to the capture probe, the CRISPR-Cas complex is recruited to the substrate, leading to the cleavage of the target and a change in the SERS signals. By measuring the change in the SERS signals, it is possible to detect and quantify the target sequence (Ma et al., 2023). This CRISPR-SERS approach has several advantages over traditional detection methods. It is highly specific, meaning that it can distinguish between closely related sequences, which is important for detecting disease-causing pathogens or genetic mutations (Dubey et al., 2022). It is also highly sensitive, allowing for the detection of low concentrations of analytes.

Overall, the combination of CRISPR and SERS has enormous potential for various applications, including disease diagnosis, environmental monitoring, and food safety testing. With further development and refinement, this approach could become a valuable tool for improving our ability to detect and monitor a wide range of biological and environmental targets.

1.5. Virus detection

The identification and detection of viruses are crucial for understanding their pathogenicity and developing appropriate therapies. One approach that has shown significant potential for virus detection is the combination of SERS with CRISPR technology. The use of CRISPR allows for highly specific targeting of viral RNA sequences, while SERS provides a highly sensitive detection platform (Liang et al., 2021). Together, these techniques offer a powerful tool for identifying viruses with high specificity and sensitivity, surpassing the limitations of traditional detection methods, such as ELISA or PCR, and potentially allowing for rapid, field-deployable diagnostic systems.

In recent years, several studies have been conducted to explore the potential of using SERS in combination with CRISPR for virus detection. In this section, the latest studies in this field are reviewed and discussed. These studies have demonstrated the feasibility and effectiveness of using these techniques for detecting a wide range of viruses, including influenza, Zika, and SARS-CoV-2, among others. The ability to rapidly and accurately detect viruses using this approach has significant implications for public health, especially in the context of emerging infectious diseases. In the following paragraphs, the methodologies and results of these studies are discussed, highlighting the advantages and potential applications of this technology.

A recent study conducted by Jinan University presents a new diagnostic platform for SARS-CoV-2 detection, using SERS-based CRISPR technology. The SERS-based CRISPR diagnostic platform is an amplification-free assay that utilizes the high sensitivity of SERS to detect specific DNA sequences. The assay employs the CRISPR-Cas12a system to specifically cleave the target DNA and release Raman-labeled oligonucleotide probes that generate SERS signals (Liang et al., 2021).

Table 1
An overview of the studies conducted in the field of detection through the implementation of CRISPR and SERS methods.

Detection group	Capture probes	Raman probe	Analyte/Organism	Sample matrices	Limit of detection	Reference
Virus	Cas12a-crRNA	MBs[a]-ssDNA-AgNPs	SARS-CoV-2	Nasopharyngeal swab extract	1 fM	Liang et al. (2021)
	CRISPR/Cas12a activated by crRNA[b]	SH-DNA@AuNPs	HPV 16 and HPV 18	Human serum	pM level	Su et al. (2022)
	CRISPR/Cas12a-based viral DNA	AgNPs@4ATP	HPV	Infected body fluids	0.1 pM	Du et al. (2023)
	Activated CRISPR-Cas12a by viral DNA	Au nanoparticles on the graphene oxide/triangle Au nanoflower array	HBV and HPV	Plasma	1.25 fM for HBV 2.5 fM for HPV	Choi et al. (2021)
Pathogens	RPA-Cas12a-μPAD[c]	AuNS@4-MBA@Au@DNA[d]	Salmonella typhimurium	Spiked milk and meat samples	3–4 CFU/mL	Zhuang et al. (2022)
	dCas9/gRNA	Au coated magnetic nanoparticles (Fe3O4@SiO2@Au NPs)	Multidrug-resistant bacteria, species Staphylococcus aureus, Acinetobacter baumannii, and Klebsiella pneumoniae	Plasma	14.1 fM for MDR S. aureus, 9.7 fM for MDR A. baumannii, and 8.1 fM for MDR K. pneumoniae	Kim et al. (2020)
	dCas9/sgRNA RNP[e]	Au-NTA-Ni2+	drug resistance gene macB	Simulated in vitro buffer	22 ng for macB gene in milk 1.9 fM for a total of 2.82 Mbp of the S. aureus genome	Du et al. (2022)
Authenticity Testing	Biotinylated ssDNA-PB[f] NP	Au@Ag core–shell	Identifying and detecting milk adulteration	Milk	224 aM	Pan et al. (2022)
	CRISPR/Cas12a with *trans*-cleavage activity on ssDNA linkers utilized to capture liposomes	Gold nanoparticle	Identification of adulterated beef samples with different proportions of duck meat	Beef and duck meat	100 aM	Liu et al. (2021)
Chemical compounds	Cas12a/crRNA complex with fluorescence reporter	MXenes nanosheets	Aflatoxin B1	Peanut	0.92 pg/mL	Wu et al. (2023)
	Cas12a/crRNA	Core–shell Au@PB[f]@Au NPs	Aflatoxin B1	Milk and soy sauce	3.55 pg/mL	Zhang et al. (2023)
	Cas12a/crRNA and biotin-DNA-digoxin	Au@BDT@Au	17β-estradiol	Water, milk, serum, and urine	180 fM	Li, Li, et al. (2022)
	CRISPR-Cas12a/crRNA	AuNPs, Fe@Au40 NPs, and modified Fe@Au40 NPs	B.1.1.529/Omicron variant of SARS-CoV-2 viral RNA sequence (Orf1ab gene)	PBS buffer (7.4), Tris-HCL (7.4), blood plasma and cfBALF[g]	1 aM	Yin et al. (2022)

[a] : Magnetic beads.
[b] : CRISPR ribonucleic acid.
[c] : Recombinase polymerase amplification (RPA)-integrated microfluidic paper-based analytical device (μPAD).
[d] : Thiolated ssDNA conjugated gold nanostar@4-mercaptobenzoic acid@goldnanoshell structures.
[e] : ribonucleoprotein (RNP).
[f] : Prussian blue.
[g] : Cell-free Bronchoalveolar lavage fluid.

The authors also developed a portable Raman plate reader that provides accurate and stable measurements of Raman signals at a low cost. The platform was validated using 112 nasopharyngeal swab samples collected from patients in a hospital, and the results showed 87.5% sensitivity and 100% specificity for SARS-CoV-2 detection compared to RT-qPCR, with 30–40 min assay time (Liang et al., 2021). The authors suggest that this technology can be further developed to detect other genes of the SARS-CoV-2 genome, increase sensitivity, and automate the portable Raman plate reader for more reliable and efficient diagnostic testing. The potential of S-CRISPR for nucleic acid detection and its advantages of being rapid, economical, and easy-to-use highlight its importance in disease-control strategies and the management of patient treatments (Liang et al., 2021).

A similar study conducted by Jilin University described a new method for detecting viral genes using a combination of SERS and CRISPR/Cas12a technology (Fig. 2). The system uses a Cas12a/crRNA complex to recognize and capture target dsDNA, which activates the *cis*-cleavage function of the complex on the target dsDNA and nearby linker ssDNA, leading to aggregation of the designed SERS probes loss. The method is fast, simple, and sensitive, and exhibits superior specificity for the target dsDNA. The system has been shown to be valid for HPV 16 and HPV 18 in pseudovirus and serum samples and can measure pM levels of target DNA without target gene amplification. The authors conclude that this novel detection system is a universal gene detection platform that can be modified simply by changing the crRNA sequences and has potential applications in point-of-care tests and virus infection diagnosis in clinics (Su et al., 2022).

Another similar study for HBV detection described the development of a CRISPR/Cas12a-based viral DNA biosensor that uses SERS to amplify the signals without amplifying the target DNA. The biosensor combines magnetic spheres with AuNPs modified for Raman report analysis to form SERS probes that enhance the Raman signals by forming hot spots with AuNPs. The use of CRISPR/Cas12a improves the sensitivity of viral DNA detection through the *trans*-cleavage properties of this protein. The method allows simpler and more sensitive detection with no subsequent operations required for cleaning the target DNA, and the CRISPR-mediated SERS technology for DNA detection offers low detection limits and short response times. The biosensor can be used to identify other targets by altering crRNA sequences and can also detect other biomarkers. In conclusion, the CRISPR/Cas12a-based biosensor

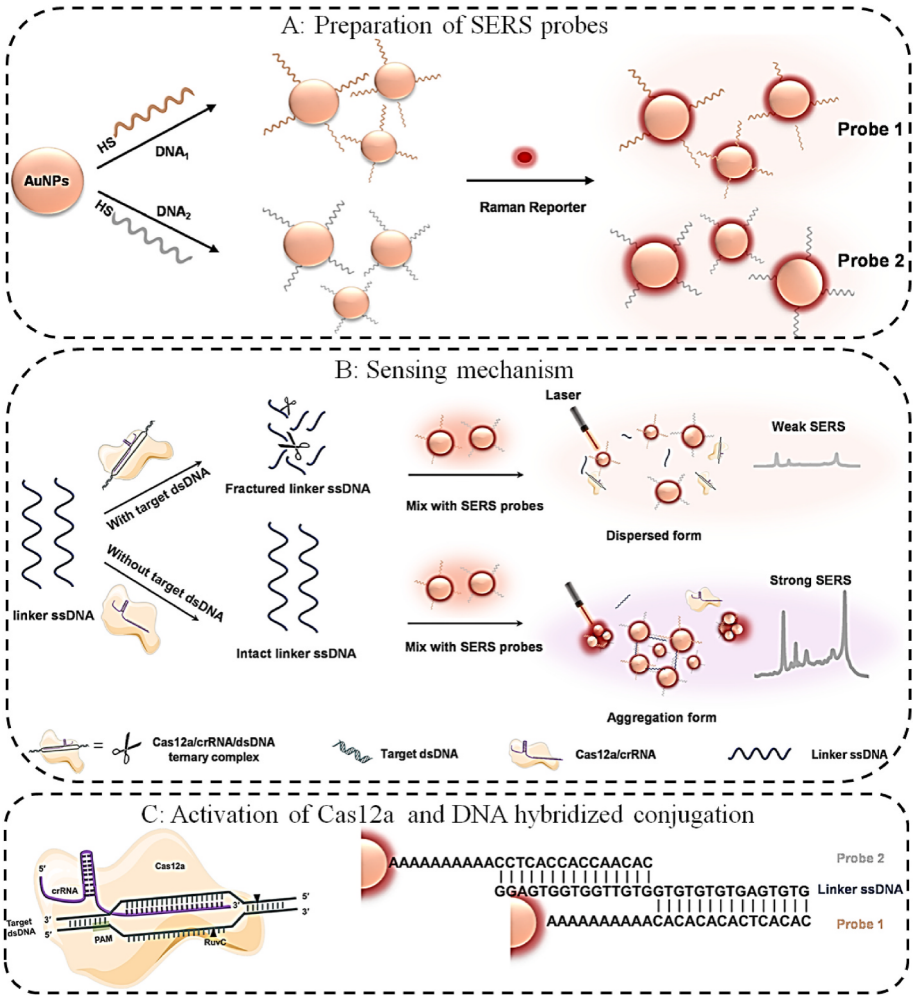

Fig. 2. The design of the CRISPR/Cas12a-based SERS gene detection assay involves the preparation of two probes, Probe 1 (AuNPs@DNA1@MBN) and Probe 2 (AuNPs@DNA2@MBN), which will be used to detect the target dsDNA. The sensing mechanism of the assay relies on the activation of Cas12a protein by the crRNA, which will then bind with the target dsDNA and initiate *trans*-cleavage. The SERS signal output elements comprise Probe 1, Probe 2, and Linker ssDNA, and their monodispersion or aggregation form will depend on the presence or absence of the target dsDNA. The distance-dependent optical and SERS properties of the AuNPs@DNA@MBN pairs will then determine the intensity of the SERS signal output. The recognition of the target by Cas12a activates nonspecific ssDNA cleavage, resulting in a staggered cut using a single RuvC nuclease. The relationship between Probe 1, Probe 2, and linker ssDNA is illustrated in the diagram (Su et al., 2022). Copyright 2022, Elsevier.

enables rapid, simple, and sensitive detection of HBV DNA and has the advantage of nucleic acid-free amplification. The biosensor can also be used to identify the target site specifically by altering the sequence of crRNA, and the system can detect RNA and proteins in addition to viral DNA, thereby improving the sensitivity of detecting multiple biomarkers (Du et al., 2023).

A recent study conducted by Sogang University describes the development of a new biosensing platform that utilizes SERS to detect DNA targets with high sensitivity and specificity (Fig. 3). The biosensor is based on a triangle Au microstructure with an overlaid equilateral triangle consisting of Au–Ag co-deposited electrodeposition. The platform is further modified with graphene oxide (GO) to enhance the SERS signals. To detect specific DNA targets, Raman-active nanoprobes (RAuNPs) functionalized with single-stranded DNA (ssDNA) are immobilized on the GO-TANF array through NH_2-GO interactions (Choi et al., 2021). The biosensor is tested for the detection of hepatitis B virus (HBV) and human papillomavirus (HPV) DNA targets, using the CRISPR-Cas12a system for DNA cleavage and release of ssDNA from RAuNPs. The SERS signals are measured using a Raman spectrometer, and the results show high specificity and sensitivity in detecting the target DNA with a limit of detection (LOD) of 1.25 fM for HBV DNA and 2.5 fM for HPV DNA (Choi et al., 2021).

The study also includes finite-difference time-domain (FDTD) simulations to investigate the electromagnetic and chemical enhancement on the RAuNP-functionalized GO-TANF array. The simulations show that the GO-TANF array enhances the electromagnetic field and provides a chemical enhancement for SERS signals (Choi et al., 2021). Finally, the biosensor platform described in the article offers a promising approach for sensitive and specific detection of DNA targets and has potential applications in clinical diagnostics and environmental monitoring.

A recent study conducted at Hong Kong Polytechnic University describes the development of a biosensing system that combines CRISPR-Cas12a and SERS for the detection of a specific DNA sequence. The system consists of gold nanoparticles (AuNPs) functionalized with DNA probes that can hybridize with the target DNA sequence. The hybridization triggers the Cas12a enzyme to cleave a reporter DNA sequence, which releases a quenched SERS probe that generates Raman signals upon binding to a nearby AuNP. The Raman signals can be measured and used to detect the presence of the target DNA sequence (Yin et al., 2022). The authors first optimized the CRISPR-Cas12a reaction conditions and tested the biosensor with synthetic DNA samples in buffer solutions, as well as in cell-free bronchoalveolar lavage fluid (BALF) and blood plasma from mice. They then demonstrated the detection of a viral RNA sequence (Orf1ab gene) in cultured cells infected with the B.1.1.529/Omicron variant of SARS-CoV-2, the virus responsible for the COVID-19 pandemic (Yin et al., 2022).

To validate the biosensor's sensitivity and specificity, the authors conducted simulations and statistical analyses. They used finite-difference time-domain (FDTD) simulations to model the Raman scattering properties of the AuNPs and SERS probes and showed that the biosensor could detect as few as 10 copies of the target DNA sequence in a sample. They also performed statistical analyses of the Raman spectra from multiple samples and showed that the biosensor could discriminate between the target DNA sequence and non-target sequences with high accuracy (Yin et al., 2022). Overall, the study demonstrates the potential of combining CRISPR-Cas12a and SERS for sensitive and specific

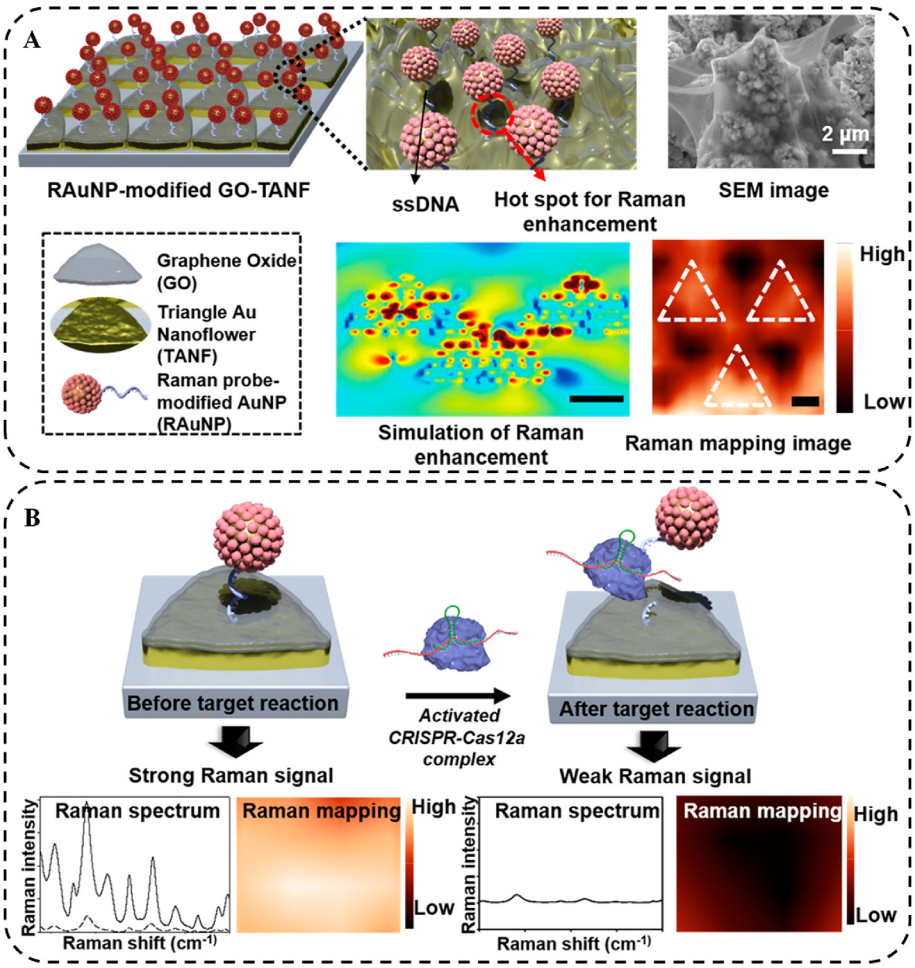

Fig. 3. The schematic diagram illustrates the approach for detecting viral DNA using SERS-active graphene oxide (GO)/triangle Au nanoflower (GO-TANF). (A) To increase the SERS signal, a Raman probe-modified Au nanoparticle (RAuNP) was added to GO-TANF to generate a hot spot between RAuNP and GO-TANF. (B) The ssDNA degradation caused the separation of Pro-immobilized RAuNPs from the surface, leading to a decrease in the maximized SERS intensity (Choi et al., 2021). Copyright 2021, American Chemical Society.

detection of DNA and RNA sequences, including viral sequences. The biosensor has potential applications in point-of-care diagnostics and environmental monitoring, among others.

Overall, the use of CRISPR and SERS techniques together shows promise as a highly effective method for virus detection. Further research and testing are needed to validate the approach for the detection of other viruses and in real-world settings.

1.6. Pathogenic bacteria

CRISPR and SERS are two advanced technologies that can be used to design a sensor for identifying foodborne pathogens. Scientists can use CRISPR to target specific genes in pathogens that are responsible for causing foodborne illnesses. SERS, on the other hand, is a spectroscopic technique that can be used to detect and identify molecules based on their unique vibrational properties. To design a sensor for identifying foodborne pathogens, scientists can use CRISPR to target specific genes in the pathogen that are known to cause illnesses. They can then amplify the DNA using polymerase chain reaction (PCR) and use SERS to detect the presence of the pathogen based on its unique DNA sequence. This approach has several advantages over traditional methods for identifying foodborne pathogens, such as culture-based methods. It is faster, more sensitive, and can identify multiple pathogens at once. It also has the potential to be integrated into a portable device that can be used in the field, allowing for rapid and accurate detection of foodborne pathogens (Zhuang et al., 2022).

In a similar study conducted by Tianjin University of Science & Technology, researchers described the development of a novel biosensor for bacterial detection based on a microfluidic paper-based analytical device (μPAD) combined with SERS and CRISPR-Cas12a. The biosensor takes advantage of the sensitivity and specificity of the CRISPR-Cas system, specifically the *trans*-cleavage activity of Cas12a, which acts as a bio-catalytic signal amplifier. The SERS signal transducer and amplifier enable the conversion of the DNA of interest into SERS signals, allowing for fast and sensitive detection of bacterial DNA (Zhuang et al., 2022). The biosensor is designed to be portable and cost-effective, making it ideal for use in point-of-need (PON) detection and resource-limited areas. The biosensor can achieve excellent detection sensitivity and dynamic range and could be applied as a universal DNA detection platform in a variety of fields, including medical diagnostics, forensic sciences, and food safety inspection (Zhuang et al., 2022). The article also highlights the limitations of the current biosensor, specifically the need for separate steps in operation. Future work could focus on merging DNA extraction, amplification, and Cas12a *trans*-cleavage into a one-pot fashion or making it more automated to promote field use (Zhuang et al., 2022).

Another similar study describes a novel method for detecting multidrug-resistant (MDR) bacteria using a CRISPR-mediated SERS assay (Fig. 4). The assay involves using an Au MNP-dCas9/gRNA probe to bind to the target bacteria's genomic DNA, which is then amplified and detected using SERS. The assay was tested on MDR *A. baumannii*-infected mice and was found to be effective in detecting bacteria in lung, spleen, liver, and blood samples (Kim et al., 2020). The article also describes the fabrication of a 3D complex nanopillar array swab for on-site capture and detection of MDR bacteria. The swab was designed using a CAD program and fabricated using a 3D printer. The swab was coated with a Ti/Au layer and treated with a solution of $HClO_4$, aniline, ammonium persulfate, and DI water to form a 3D complex nanopillar

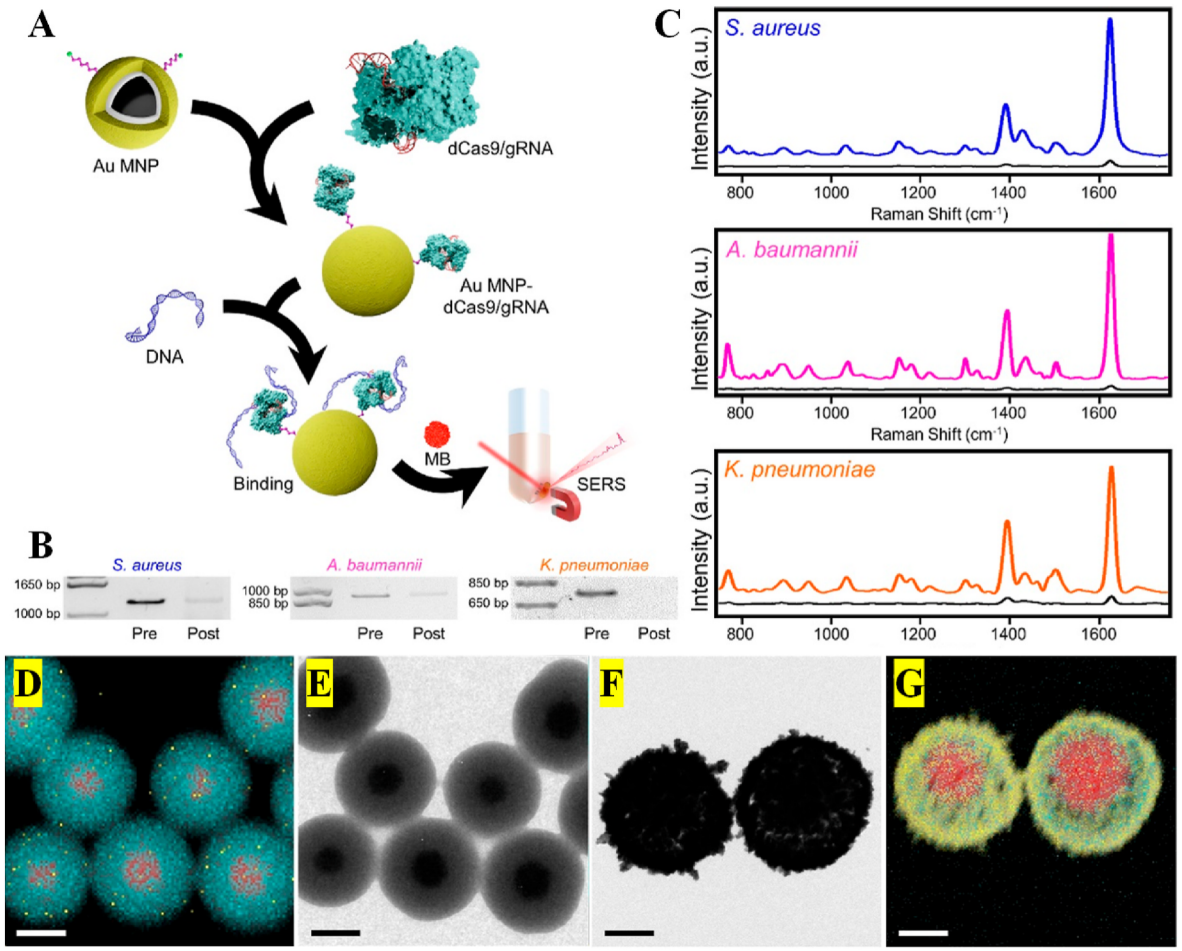

Fig. 4. (A) The evaluation of the CRISPR-mediated SERS assay involved the preparation of Au MNP-dCas9/gRNA and SERS-based DNA detection procedures, as shown in the schematic representation. (B) The PCR amplicons were subjected to gel electrophoresis before and after the reaction with Au MNP-dCas9/gRNA probes, as depicted in the images. (C) The results of the CRISPR-mediated SERS assay for PCR amplicons are presented. TEM and EDX-TEM mapping images of (D, E) Fe3O4@SiO2, and (F, G) Fe3O4@SiO2@Au NPs (Kim et al., 2020). Copyright 2020, American Chemical Society.

array on the surface. To test the swab's efficacy, MDR *A. baumannii* was dropped onto various surfaces, including mouse skin, tail, foot, and surgical instruments, and then swabbed using the 3D complex nanopillar array swab. The swab was found to effectively capture and detect the bacteria on all surfaces tested (Kim et al., 2020). Overall, the CRISPR-mediated SERS assay and the 3D complex nanopillar array swab show promise as rapid and sensitive methods for detecting MDR bacteria in clinical and on-site settings.

Researchers have developed a new method to detect drug resistance genes using a combination of CRISPR-Cas and SERS technologies. The CRISPR-Cas system was used to target the macB gene, a drug-resistance gene found in bacteria. The dCas9/sgRNA complex in the CRISPR system was used as a targeting material, and Raman spectroscopic information was acquired through a Raman reporter molecule MB. The results showed that the proposed CRISPR-SERS technology could detect the macB gene at levels as low as fM, without the need for complex steps such as amplification or the use of gene fragments. This technology has the potential to revolutionize gene detection and might have significant impacts in the biomedical field (Du, Han, An, Wang, & Gao, 2022).

In a recent study, a novel method was described for detecting non-pathogenic *E. coli* and HPV-16 using CRISPR-mediated graphene field-effect transistors (gFETs). The researchers used a single-stranded HPV-16 target sequence to demonstrate the versatility of the CRISPR-mediated gFET for both ssDNA and dsDNA detection. They prepared the CRISPR-Cas12a crRNA and the non-pathogenic *E. coli* target plasmid, and an amplification-free assay consisting of LbaCas12a, crRNA, synthetic DNA target, nuclease-free water, HEPES buffer, and magnesium chloride solution was applied to the probe-functionalized gFET array for on-chip CRISPR reaction (Weng et al., 2023).

To measure the transfer characteristics, the gFET array was gated by 2 mM $MgCl_2$ solution or 1 × phosphate-buffered saline (PBS) with a silver/silver chloride (Ag/AgCl) gate electrode. The transfer characteristics were recorded by sweeping the gate voltage from −1 to 1 V with a step size of 1 mV under a 100 mV source–drain bias voltage. The probe-functionalized gFET chip was incubated in a test solution (2 mM $MgCl_2$ or 1 × PBS) for 15 min to soak the graphene channels with the test solution for stable signal responses, and then rinsed with the test solution to remove loosely bound blocking agents (pre-cleavage) or residual cleaved probes (post-cleavage). The CRISPR-Cas12a assay was applied to the chip followed by incubation in a dark room at room temperature in a humid chamber for 30 min. Finally, the chip was washed with nuclease-free water to remove the assay, and post-cleavage transfer characteristics were measured after the incubation and rinsing procedure. The results showed that the CRISPR-mediated gFET was able to detect nonpathogenic *E. coli* and HPV-16 with high sensitivity and specificity. The method could potentially be used for rapid, portable, and cost-effective diagnostic applications (Weng et al., 2023).

1.7. Authenticity testing

CRISPR and SERS are two powerful technologies that can be combined to design a sensor for authenticity testing. In the context of

authenticity testing, CRISPR can be used to target specific DNA sequences that are unique to a particular species, strain, or variety. By designing a CRISPR-based assay, one can detect the presence or absence of a specific DNA sequence in a sample, which can be used to determine the authenticity of the sample.

The SERS technique, on the other hand, is highly sensitive and can be used to detect trace amounts of molecules, making it an ideal tool for detecting small molecules or biomolecules in complex samples. By combining CRISPR with SERS, one can design a biosensor that can detect the presence or absence of a specific DNA sequence in a complex sample with high sensitivity and specificity. This technology could be applied to a wide range of industries, including food and beverage, pharmaceuticals, and forensics.

A recent study introduces a biosensing strategy for detecting trace-level DNA using a combination of CRISPR/Cas12a signal amplification and SERS spectroscopy (Fig. 5). The strategy involves a nanolabel probe consisting of polydopamine-coated silver nanoparticles (PB NP) conjugated with single-stranded DNA (ssDNA) that specifically binds to the target DNA sequence. The CRISPR/Cas12a system amplifies the signals of target DNA by triggering a *trans*-cleavage reaction, which leads to the production of many copies of the signal amplification molecule. The PB NP-ssDNA probe is target-responsive, ensuring a high signal-to-noise ratio for SERS detection. The detection limit of the proposed method is as low as 224 aM, and it can be detected using a portable Raman spectrometer. The authors applied their method to 12 commercially available milk products and demonstrated that it can detect target milk adulteration in real samples. Recovery tests were performed by spiking cow milk in the samples, and the recoveries of adulterated cow milk were calculated to be 94.20–106.16%. The authors suggest that this sensing method could be a universal platform applied to various fields ranging from clinical diagnosis to food safety monitoring (Pan et al., 2022).

Another similar study described a novel biosensor based on the CRISPR/Cas12a system that can detect trace amounts of target nucleic acid in complex matrices. The biosensor uses liposomes to amplify the signals and enhance the sensitivity of detection. The biosensor can detect both single-stranded and double-stranded DNA with high specificity and sensitivity, with a detection limit as low as 10 aM. The detection can be performed both by SERS and naked-eye analysis. The biosensor was also successfully applied to detect meat adulteration, demonstrating its practicality and reliability. Recovery tests were performed on different meat samples, showing high accuracy and low relative standard deviation. The authors conclude that the CRISPR/Cas12a-based biosensor has the potential to be a universal platform for nucleic acid detection in various applications, such as environmental monitoring and clinical diagnosis (Liu et al., 2021).

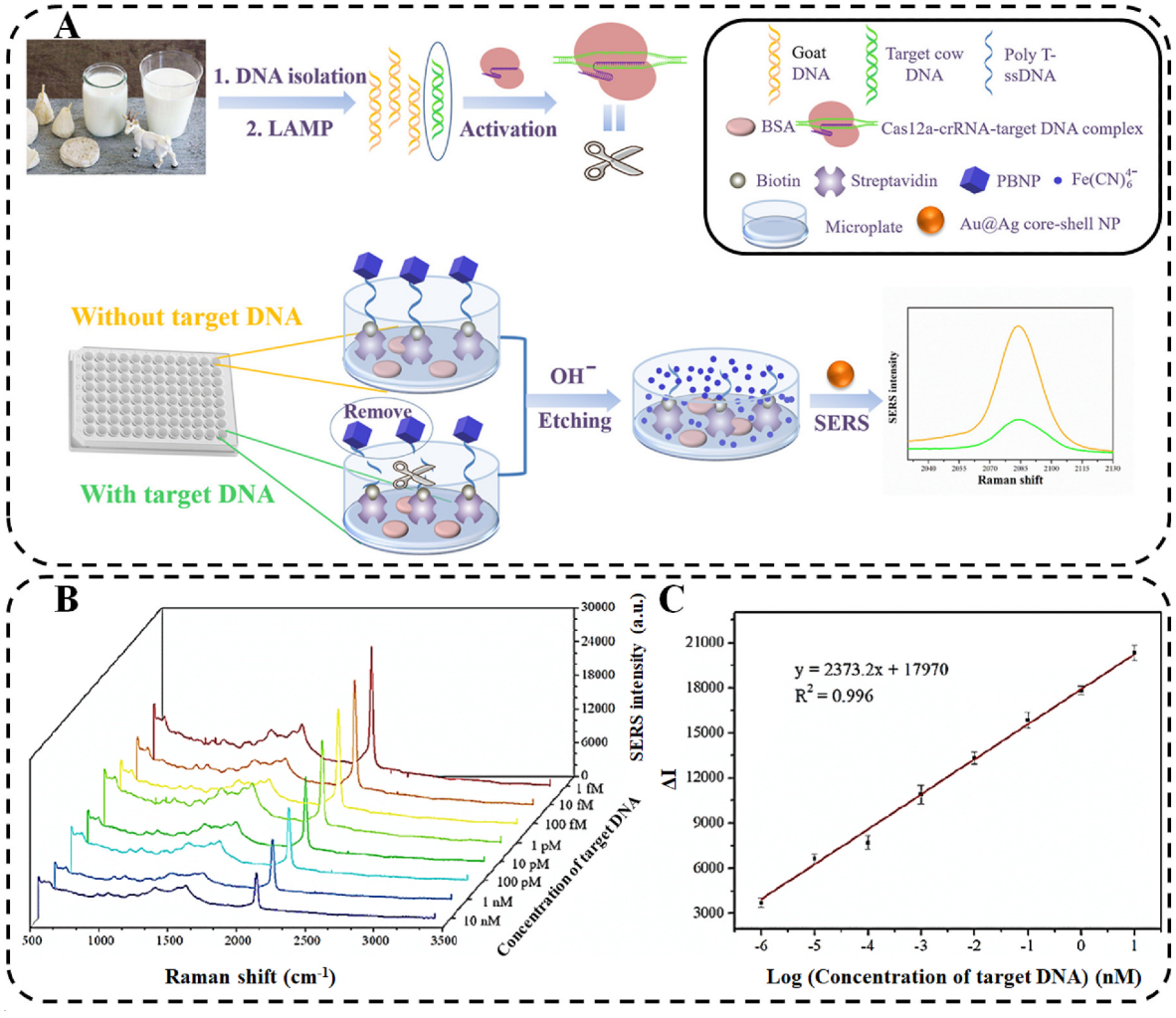

Fig. 5. (A) A schematic illustration depicting the CRISPR/Cas12a-driven SERS biosensor for detecting adulteration in goat milk. (B) SERS spectra were obtained for the detection of target DNA at varying concentrations ranging from 1 fM to 10 nM. (C) A linear calibration curve was generated by plotting the ΔI at 2083 cm^{-1} against the concentration of target DNA. (Pan et al., 2022). Copyright 2022, American Chemical Society.

1.8. Identification of chemical compounds

The CRISPR-SERS biosensor has the potential to revolutionize chemical compound identification by providing a rapid, sensitive, and specific method for detecting specific DNA sequences that are associated with particular compounds. This technology could be applied in various industries, including pharmaceuticals, food and beverage, environmental monitoring, and forensic science.

In a similar study conducted at the South China University of Technology, scientists developed a biosensor for detecting Aflatoxin B1 (AFB1) using the CRISPR/Cas12a complex and SERS-based MXenes/ssDNA-FAM reporter. The biosensor demonstrated a linear relationship in the range of 0.001–80 ng/mL of AFB1 with a detection limit of 0.92 pg/mL. The fluorescent biosensor was also capable of detecting AFB1 in spiked peanut samples, with recoveries ranging from 87.60% to 92.10% and relative standard deviation (RSD)values of 4.32%–7.47%. The entire workflow of the CRISPR/Cas12a-based fluorescent biosensor was completed in approximately 80 min (Wu, Sun, Pu, & Wei, 2023). The study's achievement was developing a novel biosensor based on CRISPR/Cas12a and SERS for the detection of AFB1, providing a new idea for the detection of non-nucleic acid targets. The biosensor exhibited specificity and anti-interference abilities, making it promising for practical applications, and demonstrated excellent potential in the detection of AFB1 in spiked peanut samples. The use of CRISPR/Cas12a and SERS in biosensor design could provide a prospective alternative strategy for rapid quantification of various targets (Wu et al., 2023).

Researchers in another similar study described the development of a biosensor for the detection of aflatoxin B1 (AFB1) using a combination of CRISPR-cas12a and nucleic acid amplification strategy. The biosensor utilizes core-shell nanoparticles embedded with Prussian blue (PB) to reduce background interference and calibrate the SERS signal. The biosensor has a detection limit of 3.55 pg/mL and is found to be specific to AFB1 and not to other substances such as AFG1, AFB2, ATP, and GSH. The biosensor is tested on milk and soy sauce samples and is found to have good sensitivity and reproducibility with an RSD of 6.04%. The biosensor is compared to other detection methods and is found to have a lower detection limit, making it a promising tool for practical detection of AFB1. The study concludes that the biosensor has the potential to provide a new approach for the detection of non-nucleic acid targets in the future (Zhang, Jiang, Li, Yuan, & Yang, 2023).

The researchers of Capital Medical University, in a similar study, developed a new biosensor for the detection of the hormone 17β-estradiol (E2) using a combination of split aptamers, CRISPR/Cas12a, and SERS technology (Fig. 6). The biosensor was designed to be a lateral flow assay (LFA), which is a simple and rapid method for detecting various analytes in a sample. The authors aimed to develop a more sensitive and specific method for E2 detection compared to existing methods, which have limitations in terms of sensitivity and accuracy (Li, Li, et al., 2022). The biosensor works by using split aptamers, which are short segments of DNA that can bind to a target molecule, to regulate the *trans*-cleavage ability of Cas12a/crRNA. In this way, the biosensor can detect E2 with high sensitivity and specificity. The authors also used SERS technology to enhance the sensitivity of the biosensor. The biosensor was tested using spiked samples of milk, tap water, urine, serum, and NEBuffer2.1, and the results showed that it had high reliability and accuracy for real samples detection (Li, Li, et al., 2022). The results of the study showed that the biosensor could detect E2 as low as 10 pM, with a detection limit of 180 fM, which is at least 103-fold lower than the recently reported lateral flow immunoassays. The biosensor was also found to be simple and convenient to operate compared with previous aptamer-activator DNA-aptamer sandwich lock-structure-based CRISPR/Cas12a biosensors. The authors suggest that their biosensor could be used to detect other small molecules or proteins by using split aptamers for different targets (Li, Li, et al., 2022).

1.9. Recent studies

Researchers at Tianjin University of Science & Technology have developed a novel method for ultrasensitive detection of SARS-CoV-2 in food and environmental samples (Ma et al., 2023). The method eliminates the need for nucleic acid pre-amplification and enables on-site detection, particularly in food samples. The study involved several steps. Initially, SERS nanoprobes were created by modifying gold nanoparticles with 4-mercaptobenzoic acid (4-MBA), resulting in enhanced SERS signals. These nanoprobes were then mixed with different concentrations of linker single-stranded DNA (ssDNA) to assess

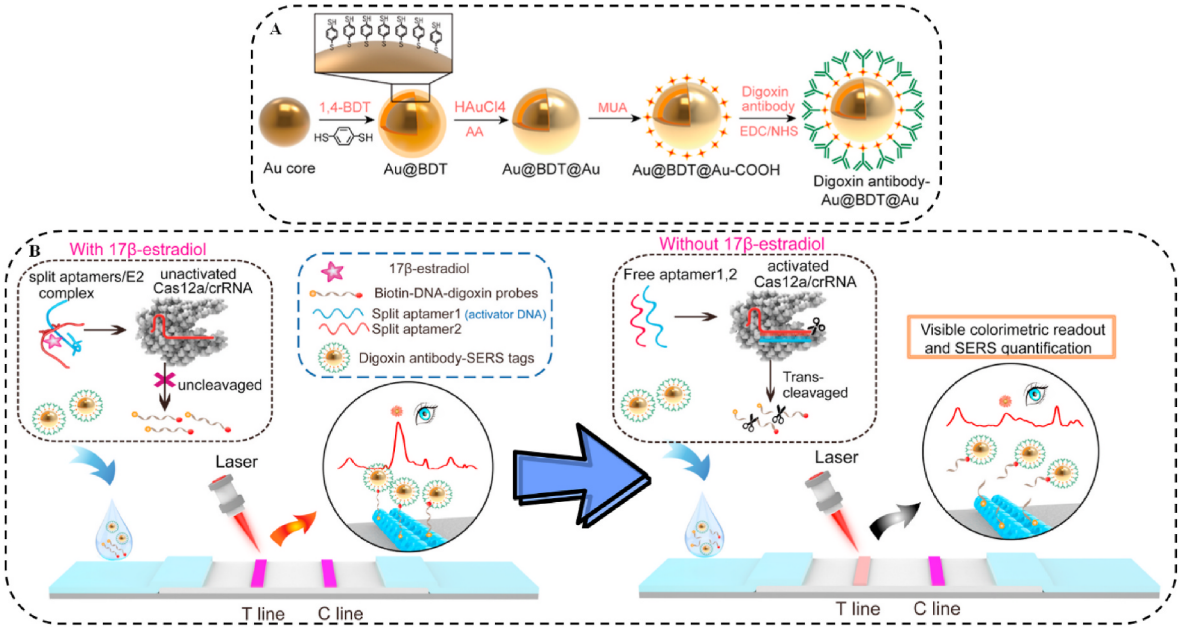

Fig. 6. (A) The preparation of the anti-digoxin antibody modified Au@BDT@Au SERS tags is illustrated in the schematic diagram. (B) The principle of detecting 17β-estradiol using split aptamers regulated CRISPRSERS LFA is shown, where the test line and control line of the strip are represented by T line and C line, respectively (Li, Li, et al., 2022). Copyright 2022, Elsevier.

their crosslinking behavior. The aggregation of the nanoprobes in the presence of linker ssDNA confirmed successful hybridization. To test the feasibility of the nanobioassay, plasmids containing the SARS-CoV-2 nucleocapsid (N) gene were used. The activated Cas12a enzyme degraded the linker ssDNA non-specifically upon recognizing the SARS-CoV-2 N gene, leading to a significant increase in Raman signals. The performance of the nanobioassay was evaluated for SARS-CoV-2 detection, achieving a sensitivity of 200 copies/mL, with a linear correlation between Raman intensity and SARS-CoV-2 concentration. The repeatability and reproducibility of the assay were excellent, with all RSD values below 10%. The nanobioassay was further tested on clinical samples, including nasopharyngeal swabs, exhibiting 100% specificity and sensitivity. Even samples with ultralow viral loads in the "grey zone" of RT-qPCR were accurately identified. A correlation between Raman intensity and RT-qPCR Ct values confirmed the assay's accuracy (Ma et al., 2023). Additionally, the nanobioassay successfully detected SARS-CoV-2 in environmental water, cold-chain food, and food packaging samples, demonstrating its robustness and ultrasensitive detection capabilities. The methodology integrated CRISPR/Cas12a's nucleic acid targeting and programmability with SERS-based biosensing, providing high sensitivity and specificity. The assay's simplicity, time-saving nature, and reduced instrument dependency were achieved through a one-vessel reaction system and the elimination of pre-amplification steps (Ma et al., 2023).

Another similar study presents the development of a rapid and sensitive aptasensor based on the CRISPR/Cas12a system for the detection of ampicillin, with a focus on its application in food analysis. The aptasensor utilizes specific aptamers that bind to ampicillin, triggering the activation of the Cas12a enzyme and generating fluorescent signals. The study investigates various factors influencing the aptasensor's performance, such as optimal ratios of Cas12a to gRNA and concentrations of gRNA and fluorescent probe. The results demonstrate that the aptasensor exhibits high specificity for ampicillin, showing low fluorescence signals in the presence of other antibiotics. The LOD for ampicillin is determined to be 0.01 nM, which is well below the recommended threshold limit set by the European Union for ampicillin in food. Comparisons with other detection assays highlight that although the aptasensor has relatively lower sensitivity, it offers advantages in terms of simplicity, speed, and minimal equipment requirements, making it suitable for food analysis applications (Yee, Shafiqah, Mohd-Naim, & Ahmed, 2023). The aptasensor is successfully applied in real sample analysis, showing accurate quantification and good recovery rates in different food matrices. The study emphasizes the importance of considering factors such as aptamer selection, assay optimization, and detection methods in the development of biosensors for food analysis (Yee et al., 2023).

2. Conclusion

The integration of CRISPR-Cas systems with SERS sensing technology offers new possibilities for highly sensitive and specific biosensors. The article reviews studies that demonstrate the potential of CRISPR-Cas12a and Cas13a systems in detecting nucleic acids, including viral RNA sequences and disease-related microRNAs. SERS technology shows excellent performance in terms of sensitivity, selectivity, and reliability. This combination has the potential to revolutionize diagnostic testing in healthcare, food safety, environmental monitoring, and biodefense.

However, there are challenges to implementing CRISPR-based SERS detection in food contamination. One challenge is the complexity of food matrices, which can interfere with detection sensitivity and specificity. Sample preparation methods need to be developed to effectively remove or minimize interfering substances while preserving the target analytes. Another challenge is improving the biosensors' detection limits to reliably detect trace amounts of contaminants in food samples. This may involve optimizing biosensor design, enhancing signal amplification strategies, or exploring new SERS-active materials for improved sensitivity. Ensuring specificity in food analysis is crucial. Selecting and designing specific recognition elements, such as aptamers or CRISPR guide RNAs, with high affinity and selectivity for target analytes can address this challenge. To overcome these challenges, future research should focus on developing advanced sample preparation techniques, exploring new SERS-active materials, and expanding the range of detectable analytes. With further research and development, CRISPR-based SERS biosensors have the potential to become essential tools for early disease diagnosis, pathogen detection, and environmental monitoring. By addressing challenges and pursuing innovative strategies, these biosensors can enhance food safety and public health in the future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

This research was financially supported by the USDA National Institute of Food and Agriculture (2018-67017-27880, 2019-67021-29859, 2020-67030-31336, 2023-67017-40165), the National Science Foundation (CBET-2103025 & CBET-2031242), and the Robert T. Marshall Scholarship.

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