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Bacterial detection with electrochemical, SERS, and electrochemical SERS sensors

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Bacteria are responsible for a plethora of infectious diseases, with potentially serious complications, including sepsis, especially in young, elderly, and immunocompromised populations. Quick and accurate bacterial detection is becoming critically important, in many areas, e.g. food safety, medical diagnostics, and public health. However, the currently available bacterial detection methods, such as plate culture, flow cytometry, enzyme-linked immunosorbent assay, and polymerase chain reaction, face limitations, being either too time consuming, too costly, or not diagnostically accurate. Alternative approaches seek to provide rapid and accurate diagnostics; electrochemical sensors and optical assays based on surface enhanced Raman scattering (SERS) aim to minimize cost and processing time while improving diagnostic accuracy. Here, we provide a review of recent reports utilizing these techniques for bacterial detection in various settings, as well as their combination, namely, electrochemical surface enhanced Raman spectroscopy (EC-SERS).

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1. Introduction

Pathogenic bacteria are a major threat to human health. Between 2025 and 2050, it is estimated that 92 million deaths will be attributed to bacteria;¹ by 2050, bacterial infections will account for 10 million annual deaths, surpassing the current cancer-related death toll.² Moreover, the rise of antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), causes a concerning increase in mortality, with an estimated 700 000 individuals killed worldwide by antibiotic resistant strains every year.² Furthermore, bacteria are implicated in food safety, as even small numbers of bacteria can compromise food quality and lead to spoilage or contamination.³ Bacterial biofilms in infrastructure further compound the threat, as they can become a persistent cause of nosocomial infections, especially when caused by antibiotic-resistant strains; biofilms also contribute to the corrosion of equipment in other settings, for example pipes used in water distribution and oil and gas production.⁴

Given the impact of bacteria on human health, as well as in materials and the food industry, there is a growing need for the development of straightforward, quick, and sensitive biosensors that allow bacterial identification and monitoring. The “gold standard” in bacterial detection is bacterial culture, which requires a laboratory setting and several hours. Other well-established methods include the performance of polymerase chain reaction (PCR), flow cytometry, or mass spectrometry

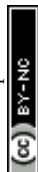
techniques, all of which are resource intensive, costly, and can have long turnaround times, making them unsuitable for routine bacterial detection and monitoring.⁵ New diagnostic techniques based on electrical and optical sensors, are emerging, aiming to complement existing assays, and provide specific bacterial detection quickly and at low costs.

Electrochemical (EC) sensors have become an established technology, with many applications over the last 50 years;⁶ they offer respectable sensitivity and selectivity, ease of fabrication, quick detection times, and potential for miniaturization.⁷ A successful paradigm, the commonly-used glucometer, utilizes screen-printed electrodes, and is commonly used at home by patients with diabetes.⁸ Electrochemical sensors monitor changes in electrical signals at the electrode surface to infer the presence of the analyte of interest. Combined with bio-recognition elements, such as antibodies, aptamers, enzymes, and phages, EC sensors can become a powerful tool for the rapid and specific detection of pathogenic bacteria.⁹

Surface enhanced Raman spectroscopy (SERS) is another emerging alternative for bacterial detection.¹⁰ It is an optical spectroscopic technique based on metallic nanostructures, that produces spectral “fingerprints” of molecules under laser illumination, identifying many analytes, including ones related to bacteria, with unparalleled sensitivity.¹¹ SERS is extremely versatile, but has, thus far, only been reported in the laboratory setting. SERS can be combined with electrochemistry, in a hybrid electro-optical method, EC-SERS, merging the strengths of both techniques.¹²

Furthermore, the increased demand for automated point-of-care detection and identification of bacteria has led to the

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development of lab-on-chip devices that incorporate these sensing technologies.^{12–15} Microfluidic integration is increasingly applied to pathogen detection, due to multiple advantages offered by the miniaturisation of the analytical processes. High sensitivity and selectivity of detection can be achieved on-chip, while simultaneously decreasing the sample volume needed for detection and accelerating the workflow. This results in decreased operating costs, as well as less generated waste.

In this mini review we present recent reports of EC, SERS, and EC-SERS sensors with applications related to bacterial detection, in healthcare and other settings. The three methods are illustrated in Fig. 1.

As one of the major focal points in this review is the detection of bacteria, it is important to distinguish between the two major bacterial types, Gram positive (+ve) and Gram negative (–ve) bacteria. This categorization originates from crystal violet staining, commonly used in microbiology, which penetrates and stains the thicker outer layer of Gram +ve bacteria.¹⁶ Gram –ve bacteria, such as *E. coli*, *Salmonella* spp. (species within the *Salmonella* genus) and *Klebsiella* spp. are often associated with food spoilage and the pathogenesis of sepsis and more severe infections.^{17,18} Gram +ve bacteria, such as *Staphylococcus* spp. and *Listeria* spp. are commonly associated with respiratory and skin infections and gastrointestinal issues.¹⁹ Moreover, the antibiotic resistance of *S. aureus* is becoming increasingly more threatening, highlighting the need for rapid bacterial identification.¹⁹

When discussing sensors, the term Limit of Detection (LOD) is used extensively. LOD is defined as “the lowest analyte concentration likely to be reliably distinguished from the blank and at which detection is feasible”.²⁰ The suitability of LOD varies between applications and sample matrices. Whereas chemical analytes are reported in units of molar, for bacteria, LOD is given in Colony Forming Units (CFU) per millilitre or gram, *i.e.* how many bacterial colonies develop after applying the nominal quantity of sample to a culture plate. For example, according to Public Health

Laboratory Services guidelines in Europe, the limit of Gram –ve bacteria in food is <100 CFU g^{–1}.¹⁷ In contrast, clinical samples have varying diagnostic bacterial cutoff limits – for the diagnosis of urinary-tract infections, a bacterial count higher than 10⁴ CFU mL^{–1} is usually indicative of infection;²¹ whereas, during sepsis, the concentration of bacteria found in blood is much lower (between 1 and 100 CFU mL^{–1} in adult patients).²²

2. Electrochemical bacterial sensors

An electrochemical biosensor functions by generating an electrical signal that correlates with the concentration of an analyte of interest, often a biomarker. It consists of three primary electrodes: the sensing electrode, which provides the signal for biomarker detection; a reference electrode, which provides a stable potential; and a counter electrode, which facilitates current flow to complete the circuit. These components are immersed in the liquid sample, typically an aqueous solution with electrolytes to provide electrical conductivity.²³ EC measurements can be performed in various modes, such as cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). Reviews covering electrochemical sensors extensively are available.^{23–26}

EC sensors can be categorized as either labelled or label-free. Label-free sensors rely on the analyte of interest to induce the change in electron flow, whereas labelled sensors use a reporter molecule to facilitate current flow. In both cases, a receptor molecule needs to be tethered to the sensing electrode, to provide affinity for the target. Label-free detection relies on the specific binding between the receptor and the target and is often simpler and more cost-effective, reduces contamination from additional labelling steps, has a shorter readout time, and is typically preferred for point-of-care systems.²⁷ However, compared to labelled approaches, this technique is limited by lower sensitivity, non-specific binding, and the generation of false-positive responses, hence care must be taken to ensure diagnostic reliability.^{27,28}

On the other hand, labelled detection employs additional components, other than the receptor, such as redox reporters (ferrocene, methylene blue), enzymes, nanoparticles, and other species. These moieties are either attached to the receptor or the target (*via* a “sandwich assay”) and provide a strong signal upon target binding. A major benefit of labelled sensors is increased sensitivity and specificity, especially in complex matrices.²⁹ For example, the widely used blood glucose meter detects glucose through enzymatic oxidation.³⁰ However, labelled sensors are often held back by increased costs, time-consuming reactions, and the risk of contamination through the introduction of additional steps.^{28,29} The sections below present recent label-free and labelled electrochemical approaches for sensing bacteria.

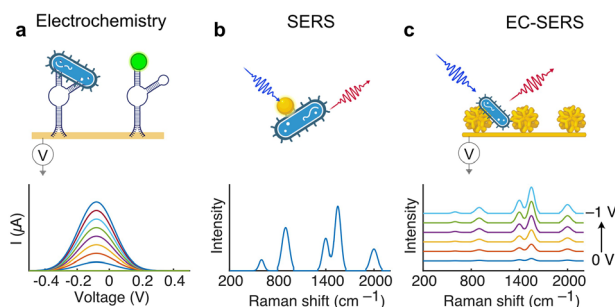


Fig. 1 (a) Electrochemistry-based approach utilizing aptamers for bacterial detection. An electrochemical reporter molecule can be used to provide indirect signal. (b) SERS nanoparticles provide spectral signals for label-free bacterial detection. (c) EC-SERS uses electrodes and metal nanoparticles for label-free SERS-based bacterial detection. Different Raman peaks are preferentially enhanced by the applied voltage, facilitating detection.



2.1 Label free EC sensing

There is an increasing demand for the development of label-free sensors, as their low fabrication costs and quick turn-around times make them attractive for fieldwork and point-of-care testing.²⁷ Notably, the global market for label-free detection has grown by about 9% over the past five years and is expected to reach \$3 billion in value by the end of 2025, while the point-of-care industry is expected to reach a value of \$72 billion by 2027.^{31,32}

Label-free EC sensors feature a working electrode functionalized with receptor molecules that directly bind the target analyte, such as aptamers, antibodies, or other moieties. Target binding is detected *via* changes in the electrical characteristics of the circuit without the need of an additional reporter.

Fig. 2 shows examples of three different label-free EC sensors for the detection of bacteria using aptamers (a), antibodies (b) and imprinted polymers (c).

2.1.1. Aptamer-based sensors. Aptasensors are sensors utilizing aptamers for target selectivity. Aptamers make a popular receptor for label-free sensors, due to their low production cost, high sensitivity, and ease of integration. Readers are referred to existing reviews covering the mechanisms of aptamer detection and conventional aptamer applications in sensing, including bacterial detection.^{36–39}

Recently, aptamers have been included in more advanced applications, typically as parts of nanocomposites. For example, a sensor utilizing a graphene oxide/poly deep eutectic

solvent/nickel oxide was combined with aptamers specific to *S. aureus*, reporting a LOD of 10 CFU mL⁻¹.⁴⁰ Nanoparticles are often coupled with aptamers and used in EC sensors as a means of increasing surface area and sensitivity. For example, carbon nanofibers, gold nanoparticles and an aptamer specific to *Acinetobacter baumannii*, a pathogen associated with meningitis and sepsis, were used for detection of the pathogen in water and urine with a LOD of 0.6 CFU mL⁻¹.⁴¹ The rapid detection of *E. coli* was investigated, using aptamer-conjugated silver nanoparticles on screen printed carbon electrodes, reporting a LOD of 150 CFU mL⁻¹.³³ In a different study, a glassy carbon electrode was modified by silicon-methylene blue nanoparticles and gold nanoparticles, followed by aptamer attachment *via* Au–N bond. The sensor reported sensitive detection of *Listeria monocytogenes* in fresh produce samples.⁴²

2.1.2. Immunosensors. Single-antibody immunosensors (without the need of a secondary antibody) are commonly used as receptors in label-free sensors. For example, anti-*S. aureus* antibodies were immobilized on lipoic acid structured on screen printed electrodes made of gold nanoparticles coated with iron oxide, reporting the sensitive (0.10 CFU mL⁻¹) detection in milk and meat samples.³⁴ Similarly, antibodies have been used in conjunction with a nanoporous platinum layer, which increased the surface area, reporting rapid detection of *S. typhimurium* down to 10 CFU mL⁻¹ concentrations.⁴³ A fluidic integrated electrochemical-cell-chip has also been constructed, where antibodies against *Aeromonas salmonicida* were immobilized on microfabricated gold electrodes, enabling bacterial detection in seawater.⁴⁴

Similarly to aptasensors, nanoparticles and nanocomposites are often used in combination with antibodies to provide superior detection. One study described the use of zinc oxide nanorods on a sensor board, combined with anti-*S. aureus* antibodies, reporting sensitive and rapid detection on human hand skin.⁴⁵ Another study reports the use of ferric oxide ionic liquid nanocomposite on gold electrodes, followed by polyglutamic acid deposition, on which anti-*Salmonella* antibodies were deposited, resulting in bacterial detection in milk.⁴⁶ A similar study on the same pathogen and sample matrix reported the use of ferric oxide on graphene as a nanocomposite combined with antibodies, reporting similar levels of detection.⁴⁷

Finally, label-free immunosensors can be used for the simultaneous detection of multiple bacterial species. A recent example describes the use of a graphene oxide-copper (GO-Cu) metal-organic framework (MOF) nanocomposite on dual screen-printed electrodes, followed by functionalization by pyrene and a different antibody immobilized on each electrode.⁴⁸ The sensor reported simultaneous detection of *Mycoplasma pneumoniae* and *Legionella pneumophila* in tap water samples.

2.1.3. Polymers. Polymer coatings on sensors have been explored as a versatile tool. Polymer films can provide affinity/specificity for the target molecule, facilitate the attachment of detection moieties such as antibodies, or provide a secondary signal, such as fluorescence.

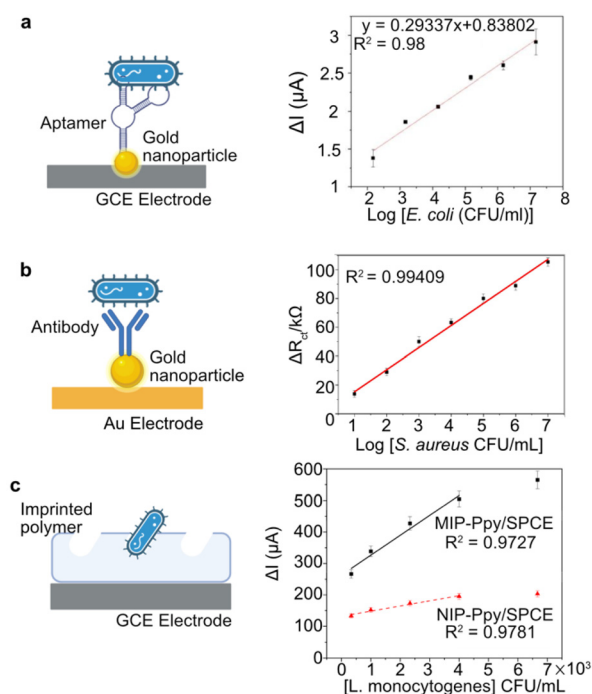


Fig. 2 Bacteria captured by receptors provide direct EC signal. (a) Aptamers,³³ (b) antibodies,³⁴ and (c) imprinted polymers³⁵ have been reported for label free EC bacterial detection.



For example, an EC immunosensor based on a polydopamine film on the surface of a copper-iron (Cu-Fe) MOF nanocomposite was developed. The anti-*Salmonella* antibodies were immobilized onto the polydopamine film, enabling *Salmonella* detection in water and milk samples.⁴⁹ Similarly, another sensor based on an indium tin oxide (ITO) electrode used electropolymerized poly melamine and poly glutamic acid film, on which anti-*Shigella* antibodies were immobilized, reporting sensitive detection in food samples.⁵⁰ Another example reports the use of fluorescent nanofibers obtained by the electrospinning of quantum dot mixture in polyacrylonitrile solution, before being deposited on the surface of gold electrodes, followed by the attachment of anti-*Streptococcus agalactiae* antibodies.⁵¹ The system reported real-time sensitive detection in diluted blood plasma.

Imprinted polymers offer selectivity for various analytes without the use of other targeting elements and are becoming increasingly popular in label-free sensors. One study reports the detection of *L. monocytogenes* in aqueous samples through the use of an imprinted polymer sensor. To produce the imprinted polymer, bacteria and pyrrole were mixed, and following electropolymerization, the bacteria were removed, leaving behind imprinted cavities in their shape. The study reported a LOD of 70 CFU mL⁻¹.³⁵ Imprinted polymers can also be combined with aptamers, creating hybrid imprinted polymers. The hybrid approach has gained popularity, as it allows for more sensitive detection of molecules, as the incorporation of recognition elements in molecularly-imprinted polymers (MIPs) is hypothesized to result in more homogenous binding sites that are readily accessible by the target.⁵² One recent example describes the development of a hybrid imprinted sensor, combining aptamers, magnetic nanoparticles and polymers. Using a magnetic nanoparticle-modified glassy carbon electrode as base, aptamer-modified gold nanoparticles were deposited on the sensor's surface. Following attachment of *S. aureus*, *o*-phenylenediamine was electro-polymerized, and the bacteria were subsequently washed away, revealing aptamer-lined cavities. The sensor was reported to sensitively detect *S. aureus* in milk, conduit water, and apple juice samples.⁵³

Additionally, polymers can be combined with aptamers or antibodies in non-imprinted sensors. One such example describes the use of self-polymerized polydopamine on glassy-carbon electrodes, combined with amino-functionalized vertically-ordered mesoporous silica film, on which aptamers were bound, reporting *V. parahaemolyticus* detection in shrimp samples.⁵⁴ Moreover, polydopamine has been used as a cross-linker on an MXene platform to immobilize antibodies against *Listeria monocytogenes*.⁵⁵

Label-free EC sensing of bacteria has been integrated with microfluidic devices of various configurations. Microfluidic devices featuring electrodes functionalised with antibodies^{56,57} or aptamers⁵⁸ have been used for selective bacteria detection, demonstrating fast detection times, and depending on the device configuration, achieving low LODs. For example, a microfluidic device was developed which used fluidic focusing

to direct bacteria to interdigitated electrode arrays (IDEAs) functionalized with antibodies for impedance sensing. This device demonstrated a LOD as low as 39 CFU mL⁻¹ for *E. coli* 0157:H7,⁵⁶ while a similar device was reported to have achieved a LOD of 7 CFU mL⁻¹ for *Salmonella* sp.⁵⁷

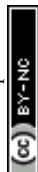
Beyond simple detection, however, an advantage that microfluidic devices offer is the ability to trap bacteria on-chip in order to monitor cell behaviour and cell growth over time. This can be further combined with electrochemical sensing for cell growth analysis and antibiotic susceptibility testing.^{59,60}

2.2 Labelled EC sensing

The majority of published electrochemical work for bacterial detection describes labelled approaches. It is important to clarify that the term “labelled”, when used in the context of electrochemical sensors, refers to the use of secondary reporters (*e.g.*, aptamers/antibodies, electrochemical labels, molecular markers) in addition to a primary detection moiety. These secondary reporters serve to provide a measurable electrochemical signal in the presence of the target. Fig. 3 shows examples of labelled EC sensing utilizing (a) aptamers, (b) antibodies, (c) peptides, and (d) imprinted polymers, all in conjunction with secondary labelled molecules.

2.2.1. DNA amplification. Recently, there has been an increase in the number of scientific works utilizing amplification techniques to improve the EC signal, and enzymatic DNA amplification methods are a popular choice. It is important to note that in this context whole bacteria are the detection target – DNA serves solely as a convenient means of signal amplification, due to the well-established nature of DNA amplification methods.

One recent example, illustrated in Fig. 3a, used enzymatic tyramide signal amplification technology to detect *S. aureus*. Specifically, an aptamer was used to immobilize the bacteria on the sensors, followed by a secondary aptamer labelled with Horseradish Peroxidase (HRP), which catalyzed the amplification technique, achieving a low LOD of 3 CFU mL⁻¹.⁶¹ Another amplification approach is described in a study which utilized saltatory rolling circle amplification combined with the enzyme Cas12a to detect *S. aureus*. The binding of the bacteria onto thiolated, methylene blue-labelled DNA strands activated the cleavage activity of the enzyme, releasing methylene blue into the solution and resulting in a drop of the electrochemical signal, allowing detection with LOD of 3 CFU mL⁻¹.⁶⁵ Another amplification approach is described in a study, where the detection of *S. aureus* by aptamers caused the release of DNA by the aptamer, triggering a cascade which resulted in the binding of ferrocene-labelled DNA, achieving increased detection sensitivity, with a LOD of 0.3 CFU mL⁻¹ in both sterile water and seawater samples.⁶⁶ CRISPR/Cas12a amplification has also been used to detect *E. coli* in conjunction with aptamers in both water and milk, with LOD of 5 CFU mL⁻¹ in both samples, as well as for the detection of *Vibrio parahaemolyticus*, in both aqueous and mussel samples, with a LOD of 1.5 CFU mL⁻¹.^{67,68} Hybrid approaches have also been developed, through the use of aptamers for the capture of *Salmonella enteritidis* combined with Cas12a and MOF-based electro-



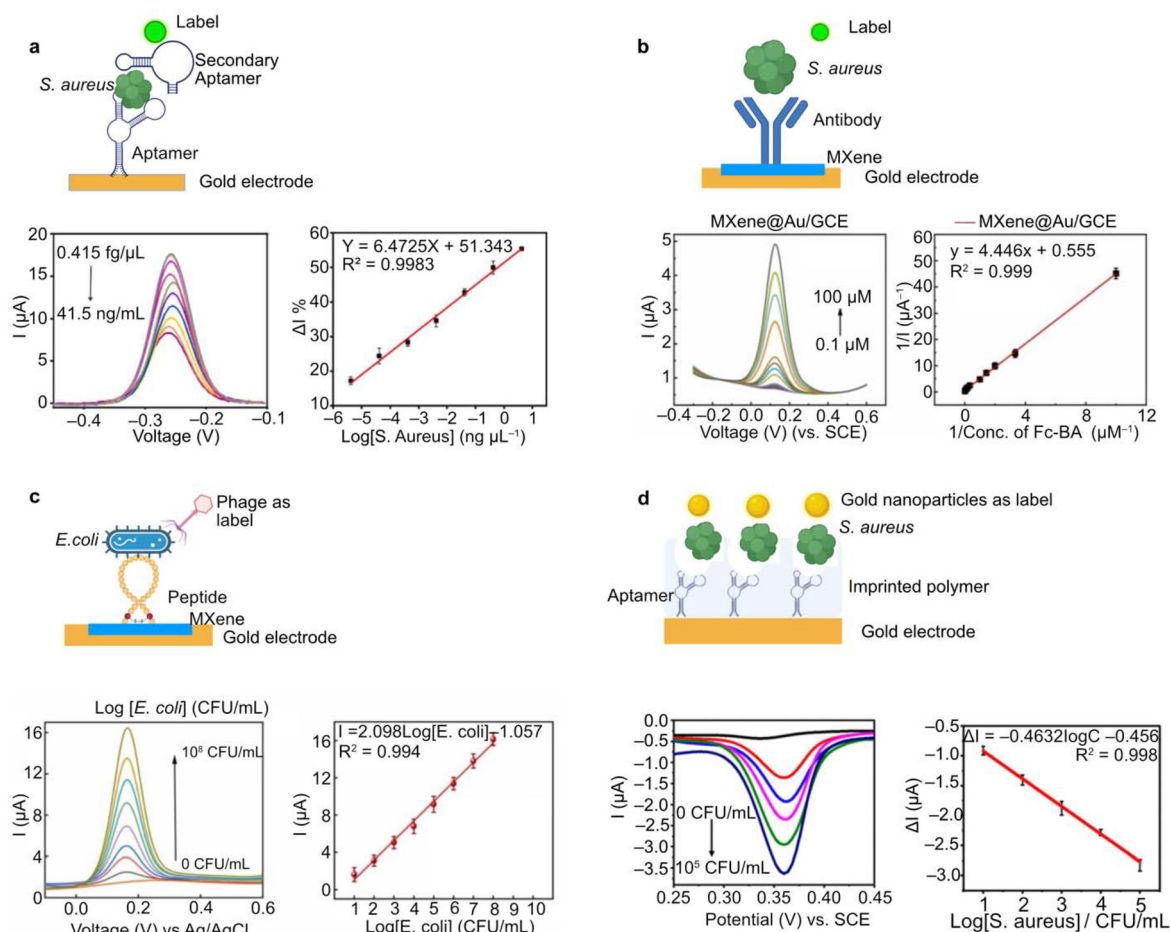


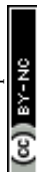
Fig. 3 Examples of labelled electrochemical sensor for bacterial detection. (a) Using *S. aureus* aptamers in conjunction with DNA cycling amplification based on CRISPR-Cas12a.⁶¹ (b) MXene-based sensor utilizing antibodies specific for *S. aureus*.⁶² (c) MXene-based sensor utilizing antimicrobial peptides for *E. coli* detection.⁶³ (d) Glassy-carbon based sensor utilizing an imprinted polymer film for the detection of *S. aureus*.⁶⁴

chemiluminescence emitters, with a LOD of 37 CFU mL⁻¹, followed by successful detection of the bacteria in waste water, milk, and whole blood.⁶⁹

There are also examples of sensors utilizing an enzymatic, non-CRISPR amplification cascade. One such example describes the use of lubricant-infused surface DNazymes, which bind onto species-specific proteins present on the cell wall of bacteria, resulting in successful detection in both water and milk samples.⁷¹ Another similar sensor used photoactive DNazymes modified with semiconductor structures between titanium dioxide (TiO₂) nanomaterials, enabling the sensitive detection of *E. coli* in water, even in the presence of other bacteria.⁷² Finally, a dual-mode electrochemical-electrochemiluminescence sensor has been described, where hybridized aptamer-silver nanocomposites bound and killed *S. aureus*, resulting in the release of hybridized DNA into the solution, initiating an endonuclease cascade which resulted in the hybridization of ruthenium-labelled sequences, thus enabling sensitive detection (1 CFU mL⁻¹).⁷³

Non-enzymatic amplification. Non-enzymatic signal amplification sensors utilize nanostructures as amplification labels,

complementary DNA and RNA sequences and recycled hybridization reactions, instead of enzymes. Such examples have been reported, one of which integrated gold nanoparticles with a MOF and aptamers specific to *S. aureus*. This study used a dual DNA amplification approach which resulted in a low LOD (1 CFU mL⁻¹) in aqueous samples, as well as in orange juice and commercial milk.⁷⁴ Similarly, another dual amplification approach has been described, where aptamers specific to *S. aureus* were used in combination with polymethylene blue nano-dumbbells and a hybridization chain reaction, resulting in LOD of 1 CFU mL⁻¹.⁷⁵ An interesting, non-enzymatic approach, which combines MIPs with MOF-assisted redox recycling amplification, has been reported, which utilized methylene blue as a label and recorded the redox recycling of iron facilitated by methylene blue, resulting in LOD of 1 CFU mL⁻¹.⁷⁶ A novel approach utilizing quantum dots and mesoporous silica nanospheres as amplification labels was able to detect *S. aureus* in both aqueous and milk samples with LOD of 2 CFU mL⁻¹.⁷⁷ A dual amplification approach using aptamers specific to *Pseudomonas aeruginosa*, MIL-101(Cr)/multi-walled carbon nanotubes, and a carbon



nitride complex with silver nanoparticles as a label, achieved LOD of 1 CFU mL⁻¹ in both aqueous solutions and diluted blood serum.⁷⁸

2.2.2. MXenes. MXenes are 2D materials composed of carbides and nitrides of transition metals commonly used for electrochemical bacterial detection. Thanks to their conductivity, extensive surface area, hydrophilic nature and facile functionalization, they have attracted the interest of researchers.⁷⁹

Antibodies have been combined with MXenes to produce novel and sensitive sensors. One example describes the use of MXene@Au on a glassy carbon electrode combined with antibodies specific for Methicillin-resistant *S. aureus* (MRSA), employing DPV and ferroceneboronic acid as a label, and detecting bacteria with an LOD of 3.8 CFU mL⁻¹ in cerebrospinal fluid samples, as shown in Fig. 3b.⁶² Antibodies were also used for the development of a sensor capable of detecting *Vibrio parahaemolyticus*, using antibodies to immobilize the bacteria followed by a MXene/gold nanobipyramid/antimicrobial peptide signal probe and a ferrocene-labelled DNzyme, resulting in an LOD of 6 CFU mL⁻¹ in aqueous samples.⁸⁰

Aptamers have similarly been used with MXenes. For example, an aptasensor specific for *S. aureus*, was reported based on recycling of DNzyme activation on gold nanoparticles-functionalized MXene nanomaterials, using methylene blue-labelled DNA probes, achieving LOD of 5 CFU mL⁻¹ in aqueous samples and retaining its activity in food samples.⁸¹ Aptamers can also be paired with imprinted polymers, as was the case in a study which developed a hybrid polymer specific for *S. aureus*, paired with *in situ* growth of a MOF on MXene tagged with methylene blue and vancomycin as a label, resulting in LOD of 10 CFU mL⁻¹ in aqueous solutions and spiked food samples.⁸² Moreover, aptamers can be combined with signal tags, such as two-dimensional Zn-MOF on MXene sheets, allowing detection of *E. coli*, *S. aureus* and *S. typhimurium* by using the suitable aptamer.⁸³ An interesting example of an MXene aptasensor describes the use of 2D titanium carbide (Ti₃C₂T_x) MXene, on which aptamers were bound. Following that, a ferrocene-based MOF containing gold nanoparticles and 4-mercapto-phenylboronic acid, which binds to endotoxin, was used as a signal label, which also functioned as a Faraday cage.⁸⁴

MXene nanocomposites can also be combined with enzymatic amplification strategies, such as with the CRISPR/Cas12a enzymatic amplification cascade. One such sensor utilized ss-DNA sequences to capture *S. Typhimurium*, enabling detection in aqueous and chicken meat samples, with a LOD of 160 CFU mL⁻¹.⁸⁵ Non-CRISPR enzymatic amplification is also possible; aptamers specific to *S. aureus* were attached to gold nanoparticles-functionalized MXene nanomaterials, and subsequently hybridized with DNA sequences containing the blocked DNzyme.⁸¹ Upon bacterial binding, the DNzyme was released, initiating a DNA recycling cascade, resulting in bacterial quantification down to 5 CFU mL⁻¹.

Additionally, MXene sensors can be developed without any biorecognition molecules. One such example is an impedi-

metric imprinted sensor for the detection of *Salmonella*, where mixture of pyrrole, MXene and *Salmonella* was electrochemically polymerized onto a glassy carbon electrode, resulting on a MIP with LOD of 23 CFU mL⁻¹ in aqueous and food samples.⁸⁶

Other labelled sensors. Antimicrobial peptides are also used in sensors, as illustrated in Fig. 3c. One example describes the development of an antimicrobial peptide/MXene platform specific to *E. coli*, followed by a phage-apoferritin@CuO₂ label, which resulted in a LOD of 30 CFU mL⁻¹ for live bacteria and 6 CFU mL⁻¹ for all bacteria tested, in aqueous and food samples.⁶³ Furthermore, aptamers have also been used in conjunction with antibiotics. One such example describes the use of vancomycin as a receptor, and the use of an aptamer as a labelling molecule, reporting successful detection of *S. aureus* and *B. cereus* in water, milk and bovine serum.⁸⁷

There have been reports of other sensors utilizing aptamers. For example, an aptasensor specific for *E. coli* has been developed, where methylene blue-labelled aptamers were used on gold screen-printed electrodes.⁸⁸

There is interest in the development of dual-mode sensors combining electrochemistry and a secondary detection method. One such sensor combined electrochemistry and electroluminescence and used dual-bipolar electrodes, capture-DNA and luminol filled anodes and ferrocene-labelled aptamers, resulting in the sensitive detection of *E. coli* (LOD 1 CFU mL⁻¹).⁸⁹ Aptamers have also been combined with glucose oxidase labelled probe-DNA, releasing the enzyme upon recognition of *S. aureus*, allowing it to react with glucose, causing a decrease in signal and the sensitive quantification of bacteria.⁹⁰ Another example describes the use of functionalized gold nanoparticles utilizing tetrahedral DNA nanostructures to bind *Vibrio parahaemolyticus*, combined with CuO₂ nanodot-mediated MOF nanozymes, releasing copper ions upon bacterial recognition, which can be subsequently detected by both colorimetry and square wave voltammetry.⁹¹

Aptamers can also be combined with imprinted polymers. One such example, shown in Fig. 3d, describes the combination of aptamers with bacterial imprinted polymer film, plus 6-(ferrocenyl)-hexanethiol functionalized gold nanoparticles, capable of detecting *E. coli* at the single cell level, while also demonstrating satisfactory detection in milk.⁶⁴

In addition, sensitive aptasensors can be developed through the inclusion of nanomaterials. For example, a *S. aureus* specific aptamer was immobilized on a 3D reduced graphene oxide and chitosan film, followed by hybridization with a DNA-zeolitic imidazolate framework-8-ferrocene labelled sequence, the displacement of which allowed for bacterial quantification.⁹²

Numerous sensors utilizing antibodies have been reported in literature, either in combination with a secondary antibody or nanoparticles. In particular, “sandwich” approaches are common with antibodies, providing superior detection and selectivity compared to single antibodies. One example used laser-scribed, graphene oxide electrodes, combined with anti-*E. coli* antibodies plus HRP-labelled secondary antibodies,



allowing bacterial detection in water and artificial urine samples with a LOD of 283 CFU mL⁻¹.⁹³ Similarly, a 3D printed sensor containing electroplated bismuth film on the sensor surface, which utilizes anti-*S. typhimurium* antibodies followed by streptavidin labelled with quantum dots, achieved sensitive detection in chicken broth (1 CFU per 25 mL).⁹⁴ Electrodeposition has also been used to deposit chitosan on glassy carbon electrodes, on which anti-*S. Typhimurium* antibodies were bound, in combination with HRP-labelled antibodies.⁹⁵

3. SERS for bacterial detection

Surface enhanced Raman spectroscopy (SERS) was first observed in 1974 when an electrochemical apparatus with roughened silver electrodes was illuminated using a Raman laser; Fleischmann *et al.* noted exceptionally high quality Raman spectra for pyridine adsorbed on the electrodes.⁹⁶ This effect was identified as a distinct phenomenon shortly thereafter and coined as SERS. Its spectral signal offers excellent molecular specificity, typically referred to as a molecular “fingerprint”, while the enhancement afforded by nano-engineered substrates allows even single-molecule sensitivity.⁹⁷ SERS has found many applications in molecular imaging, medical diagnostics, chemical analysis, food safety and environmental monitoring, including in clinical and food samples.^{10,11,98,99}

By tuning the plasmonic resonances of metallic nanostructures with the excitation laser, the unique “fingerprint” signal of the target can be enhanced by 10¹⁴–10¹⁵, which makes SERS a powerful analytical technique, including for bacterial detection.¹⁰⁰ The first reported use for bacterial classification using silver nanocolloids was in 2004 by Roger M. Jarvis;¹⁰¹ more recently, novel nanomaterials have emerged for this application, including nanowires, nanoislands and nanopancakes.^{101–104} Additionally, SERS can be paired with microfluidic chips, which can improve sample processing and assay accuracy, as well as lateral-flow assays capable of detecting multiple pathogens.^{105,106}

It is important to distinguish between direct SERS and SERS tags (labelled or indirect SERS). Direct SERS refers to the simplest layout, where intrinsic molecular vibrational modes of the target analyte are enhanced by plasmonic nanostructures and provide its unique spectrum identifying the molecule. In contrast, indirect SERS involves using tags with Raman reporters with affinity to the target analyte, that rely on the reporter's spectrum – not the analyte's – for detection. Additionally, molecular chemosensors can serve for indirect SERS sensing, as they are subjected to changes in their spectral profile and/or intensity due to recognition events involving the target analyte.¹⁰⁷

3.1 Direct SERS sensing

Label-free SERS approaches are preferred for bacterial detection, as evidenced in the literature. This is due to the method's

accessibility and low cost. The employed nanomaterials can be divided in two categories: colloidal suspensions and nanostructures on a substrate. For direct SERS sensing, extensive spectral processing and analysis is required for target identification, particularly in complex media. Traditionally this is performed with multivariate analysis methods such as principal component analysis (PCA), least-squares regression (CLS, PLS, *etc.*), often followed by discriminant analysis (DA); more recently methods based on machine learning have been reported. It is worth noting that most studies report detection of the presence and identification of the bacterial species, while quantitative detection (along with LOD) is typically not provided.

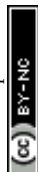
3.1.1 Nanoparticles in solution

Silver nanoparticles. Silver-based nanomaterials are commonly used in SERS, as silver produces high enhancement to the Raman scattering signal, low toxicity to non-pathogenic cells, has notable thermal stability, near ideal dielectric properties, and lower cost, compared to gold.¹⁰² An important consideration for the detection of bacteria is their peptide structure, which exhibits an affinity for silver, facilitating the attachment of silver-based structures for SERS.¹⁰²

A simple approach is the deposition of silver nanoparticles (AgNPs) mixed with the sample on silicon wafers or a glass slide, to form the “coffee-ring” stain. Once dried, the deposited nanoparticles are interrogated with a Raman laser to provide the spectral “fingerprint” of the sample.¹⁰⁸ This method has been used along with a multiscale deep-learning method, reporting the discrimination of four different strains of *Shigella*.¹⁰⁹ A similar approach reported the use of a machine learning model for differentiation between microorganisms (*Lactobacillus* spp., *Candida* spp.) and detection of bacterial vaginosis.¹¹⁰ Alternatively, Raman interrogation can be performed in liquid droplets. Droplet optical microcavities on aluminized hydrophobic glass slides were used for the detection of lactic acid bacteria (*Lactocaseibacillus paracasei* subsp. *tolerans*) reporting a high detection rate (>95%).¹¹¹

Silver coated gold nanoparticles have been successfully employed for spectral analysis of *E. coli*, *S. aureus* and *S. typhimurium*. Spectral analysis was performed to characterize bacteria in mixed-species samples.¹¹² Silver nanoparticles have been used for the identification and classification of multiple pathogens in beef.¹¹³ The colloid was mixed with the bacterial solution to optimize the SERS signal, and, following signal acquisition, the bacteria were differentiated. The sensor was capable of classifying *S. typhimurium*, *E. coli*, *S. aureus*, *L. monocytogenes*, *L. innocua* and *L. welshimeri* with reported average accuracy of 95.65%.

The rise of antibiotic resistance causes widespread concern, as the emergence of drug-resistant bacteria is becoming one of the leading causes of illness and mortality, and rapid detection of antibiotic-resistant bacteria and proper antibiotic guidance can be lifesaving.⁷⁰ SERS has been used for the discrimination between antibiotic resistant and susceptible bacteria. Silver nanoparticles have been used as SERS substrates to successfully differentiate between carbapenem antibiotic-resistant and



antibiotic-sensitive *Klebsiella pneumoniae* bacteria through spectral analysis using a convolutional neural network (CNN) model.¹¹⁴

Similarly, silver and gold nanoparticles were mixed with both antibiotic resistant and susceptible *K. pneumoniae* strains, and following spectral acquisition and analysis, antibiotic resistance was reportedly predicted with 99.46% accuracy.¹¹⁵ A recent study developed a residual neural network model, capable of not only identifying carbapenem antibiotic resistant bacteria but also discriminating them by species and enzyme type, as well as successfully predicting corresponding sensitive antibiotics.¹¹⁶ Similar methods have been employed for the detection and characterization of methicillin-resistant and susceptible *Staphylococcus aureus*, through spectral analysis using deep-learning models.¹¹⁷

Bacteria infected with bacteriophages can also be characterized using SERS. Silver nanoparticles were used to monitor the changes in *S. aureus* after interaction with bacteriophages. Specifically, after infection, SERS was performed to monitor temporal changes in pellets of infected bacteria.¹¹⁸ A similar study differentiated between phage-resistant and phage-susceptible methicillin resistant *S. aureus*, reporting 100% accuracy and specificity, in bacterial pellets and supernatant.¹¹⁹

AgNPs have also been used in microfluidic devices, where the sensitive SERS detection can be combined with the precise cell handling that microfluidics facilitates, to further enhance bacterial detection. As a result, on-chip enrichment of bacterial samples using a nanoporous membrane has been applied to improve detection sensitivity¹²⁰ and optical tweezers have even been used to achieve single cell immobilisation for SERS interrogation, significantly enhancing the stability of the SERS signal recorded.¹²¹

In a separate instance, an optofluidic device was used to achieve parallel quantification and identification of pathogens on-chip at concentrations as low as 4 CFU mL⁻¹.¹²² Quantitative detection was achieved by automated counting of the spectral events recorded as the bacterial cells flowed through the photonic fibre. Microfluidic devices also enable the *in situ* synthesis of the nanoparticles. AgNPs were synthesized *in situ* in a microfluidic device for the detection of *E. coli* in food samples. The AgNP synthesis was guided by aptamers that had been previously bound to the bacteria cells and which enabled uniform distribution of the AgNPs on the cell surface, enhancing the specificity and sensitivity of SERS detection.¹²³

Gold and other materials. Gold nanoparticles have also been used for the successful monitoring of the vaginal microbiome.¹²⁴ AuNPs were mixed into aqueous bacterial solutions and synthetic vaginal fluid solution, before being deposited on a glass slide covered with aluminum foil and being allowed to dry. The study reported the successful detection of *Lactobacillus* species, *L. crispatus* and *L. iners* in complex solutions. *E. coli*, *S. aureus*, and *P. aeruginosa* have been detected through the use of aluminum foil integrated pegylated gold nanoparticles as a SERS substrate.¹²⁵ Notably, the bacteria in this study were used directly from the culture without any pretreatment, and the

gold nanoparticles were drop-casted on the aluminum slide, reporting sensitive detection with the least bacterial concentration necessary for clinical diagnosis of urinary tract infections. Non-pathogenic bacteria have also been investigated with SERS. Bacteria capable of removing sulfur from fossil fuels though the metabolism of dibenzothiophene can also be detected using SERS combined with gold nanoparticles. One such example reports the use of gold nanoparticles for the identification and characterization of sulfur-metabolizing bacteria (*Chryseobacterium* sp.*IS*, *Gordonia* sp.*4N*, *Mycobacterium* sp.*J2*, and *Rhodococcus* sp.*J16*) leading to bacterial differentiation with high accuracy in soil and sludge samples.¹²⁶

Over the past ten years, molybdenum disulphide (MoS₂) has attracted a lot of interest in the field of materials science and in SERS because of its layered structure, which resembles graphite, and because it exhibits unique anisotropic electrochemical, electrical, and optical properties.¹²⁷ It can often be combined with tin sulfide (SnS₂), forming a nanocomposite that is more economical than gold or silver. A study used one such nanocomposite, functionalized with L-cysteine, reporting accurate and specific detection of *E. coli*.¹²⁷

Silicon-based SERS substrates have also been reported in published literature. Aptamer-coated magnetic beads were used to bind to and isolate target bacteria (*E. coli*, *S. Typhimurium*, *S. aureus*).¹²⁸ Following that, the bacteria were isolated and pipetted onto a silicon-based SERS substrate, which, combined with machine learning and spectral analysis, reported identification and classification of bacteria.

3.1.2 Non-colloidal nanostructures. Metal nanostructures can be grown on a surface to provide the plasmonic enhancement necessary for SERS. Although this approach is in general more complex and costlier than the use of colloidal aggregates, it can be considerably more controllable and reproducible. A more versatile approach is to tether pre-synthesized nanoparticles on a larger fixed structure such as a polymer, producing a more stable nanoparticle arrangement without the need of extensive nanofabrication.

Silver-based sensors. A simple nanostructured SERS substrate was reported with silver nanoparticles combined with the elastomer polydimethylsiloxane (PDMS), forming a sponge-like structure through the “mold transfer-surface embedding” method.¹⁰⁰ This substrate also provided the benefit of reduced risk of shedding and oxidation of silver nanoparticles, prolonging their usage. The sponge structure successfully and rapidly entrapped *E. coli* in close proximity to the nanoparticles and reported sensitive detection (LOD 1 CFU mL⁻¹) in water and milk samples and differentiation between six bacterial strains using an ML model. Silver nanocubes embedded in PDMS have been also employed for the detection of *E. coli* and *S. typhimurium* on eggshells. The nanocubes were prepared by a sulfide-mediated polyol method, followed by arrangement on PDMS membranes. Following spectra acquisition, analysis was performed to differentiate between bacterial spectra with high accuracy reported.¹²⁹

Nanospheres, nanowires, and nanoseeds have been successfully combined on an amine-functionalized PDMS surface.¹⁰²



The amine groups displayed high affinity for silver, allowing the orderly arrangement of nanospheres and nanowires on the PDMS surface, creating ideal hotspots for bacterial detection with high density. The sensor was capable of detecting *Pectobacterium carotovorum* subsp. *Carotovorum*, a plant pathogen, in kimchi cabbage juice, at single cell level.

Paper has also been used as a support for plasmonic nanostructures. Two-arm zigzag nanorods have been fabricated on a porous and fibrous cellulose substrate using glancing angle deposition method, which reported a tenfold increase in signal compared to nanorods deposited on a glass substrate, owing to the wicking ability of the paper substrate.¹³⁰ Detection of *P. aeruginosa*, *E. coli*, and *S. aureus* was reported, as well as distinction between Gram +ve and Gram -ve bacteria.

Nanowires are also widely used as SERS substrates. One example described the use of silver coated nanowire SERS chips, which formed a 3D matrix containing tightly placed metal nanoparticles, providing ideal hotspots for bacterial attachment.¹³¹ Combined with a neural network model, the sensor was reported to differentiate between twelve bacterial strains and two strains of antibiotic-resistant *E. coli* in real time. Silver nanowires have been used in a three-layer arrangement, reporting the sensitive detection of *Enterobacter*, *Staphylococcus* and *S. albus*. The composite was constructed with nanowires at the center, ZIF-8 as a shell, and AgNPs on the exterior, and was capable of entrapping and inactivating bacteria, as well as enhancing localized surface plasmon resonance via ZIF-8 control. The sensor was capable of semiquantitative analysis as well as characterization and differentiation of the bacteria in water samples.¹³²

A microfluidic device utilised a membrane filter to trap and enrich *E. coli* samples and then detect the SERS produced via a silver film substrate incorporated in the same device. The enrichment resulted in a 4-order of magnitude improvement in detection sensitivity over what could be achieved off-chip.¹³³ An example of a SERS-active substrate used for microfluidic interrogation after the microfluidic device fabrication is found in a different study, where a microfluidic microwell device was used to trap bacteria. This was combined with SERS detection to monitor the growth of trapped bacteria and perform antibiotic susceptibility tests.¹³⁴ A silver island-film coated glass substrate was placed on the microwell device to provide the SERS enhancement.^{134,135}

Gold-based sensors. One example of gold nanoparticle application for bacterial detection describes the use of an inverted pyramid microcavity array, a microchannel cover plate and a multilayer gold nanoparticle substrate at the bottom of the microcavities, using only a few picoliters of sample to identify live bacteria.¹³⁶ Each microcavity only contained one cell or no bacteria at all, and the substrate provided sensitive, single-cell bacterial detection.

E. coli and *S. aureus* were detected using gold nanocubes on two-dimensional (2D) delaminated nano mica platelets, forming a hybrid substrate.¹³⁷ Through self-assembly, the nanocubes and the mica platelets were able to form hotspots,

enhancing the Raman signal. The combined hydrophilicity and hydrophobicity of the substrate attracted both *S. aureus* and *E. coli*, reporting sensitive detection for both bacteria.

An example microfluidic device incorporating a SERS active substrate to detect *E. coli* contained a uniform layer of upconversion nanoparticles with silica coating and gold nanoparticles formed through a self-assembly and chemical plating process.¹³⁸

MXene has gained popularity in SERS sensors, in addition to electrochemical ones, as they have demonstrated antibacterial properties, which can potentially make them an attractive target for an antibacterial strategy.¹³⁹ In a recent study, an MXene-gold nanoparticle nanocomposite was synthesized through a self-assembly method before being deposited on a cellulose substrate by freeze-drying. The paper sensor reported sensitive detection of *E. coli* and MRSA in porcine skin and blood serum, in addition to bactericidal effects.¹³⁹ A similar study, utilizing MXene and gold nanoparticle nanostructures on electrostatic self-assembly, reported rapid and label-free detection of *E. coli* without tedious pre-processing.¹⁴⁰ Additionally, laser irradiation increased the substrate temperature to 50 °C within 200 seconds, resulting in the release of reactive oxygen species, efficiently killing the bacteria.

Gold nanostars were used as a solid-state substrate on etched stainless steel by chemical replacement reaction, reporting detection of *E. coli*, *S. epidermidis*, *S. enteritidis* and *S. aureus* in beverage samples.¹⁴¹ Following Raman acquisition, spectral analysis allowed the identification and classification of the pathogens.

Vertically-aligned gold nanowires have been successfully used for the detection of *S. aureus*, *E. coli*, *P. aeruginosa* and *S. epidermidis* in tear samples.¹⁴² Coupled with a microfluidic chamber producing turbulent flow, which promoted contact of the bacteria with the nanowires, the sensor allowed the sensitive detection and discrimination of bacteria.

3.2 SERS tags

Unlike direct SERS sensing, which seeks the spectral signature of the analyte itself, indirect sensing uses SERS nanoparticles with a pre-encoded spectrum as reporters to infer the presence of the analyte. These nanoparticles are typically bright, chemically stable, and rely on targeting molecules (e.g. antibodies) to bind to their target. They can serve for molecular imaging in living organisms or incorporated in point-of-care diagnostic assays.¹⁴³ In contrast to label-free sensors, the majority of published work on labelled SERS sensors utilizes gold substrates, due to the ease of functionalization with thiolated receptors and its chemical inertness.

Silver coated, gold core-shell nanorods have been combined with aptamers for the sensitive detection of *S. Typhimurium*.¹⁴⁴ In particular, 4-mercaptobenzoic acid (4-MBA) was used to provide the Raman spectrum and also to bind aptamers onto Au@Ag nanorods. Upon sample addition, the aptamers preferentially bound to the bacteria, reducing 4-MBA concentration and resulting in a quantifiable decrease in SERS intensity.



Gold nanoparticles are often paired with aptamers. One such example describes the use of gold colloid nanoparticles, coupled with a blocked aptamer with high affinity for *E. coli*.¹⁴⁵ In the presence of *E. coli*, the blocked aptamer released the blocker DNA, hybridizing with the capture DNA on the SERS probe and kickstarting a hybridization chain reaction, resulting in double-stranded DNA formation and reduction of the SERS signal intensity of 4-mercaptobenzonitrile. The sensor reported high specificity and selectivity for *E. coli* in tap water and milk, using a portable Raman spectrometer.

SERS tags can be combined with magnetic elements to help separate them from within complex media. Gold-core, silver outer shell magnetic round nanoparticles, functionalized with concanavalin A, which specifically binds terminal α -D-mannosyl and α -D-glucosyl on bacteria, formed a sandwich SERS sensor which was used for the sensitive (LOD 1 CFU mL⁻¹) detection of *E. coli*, *S. aureus* and *P. aeruginosa*, following exposure to a magnetic field, in water and milk samples.¹⁴⁶ Raspberry-like, wheat germ agglutinin-modified gold based magnetic nanoparticles have been reported as a bacterial entrapment method, followed by aptamer-functionalized gold nanoparticles.¹⁴⁷ This sensor was also combined with co-freezing aptamer/5,5'-dithiobis-(2-nitrobenzoic acid) gold nanoparticles as signal tags. The sensor reported sensitive and rapid (>20 minutes) detection of *E. coli* in different sample types, including human urine, serum, and skimmed milk, as well as DNA from *Mycobacterium tuberculosis*. Superparamagnetic iron oxide nanocomposites, combined with a layer of self-assembled gold nanoparticles have been applied for the detection of *K. pneumoniae* and *A. baumannii*.¹⁴⁸ In particular, the gold nanoparticles were functionalized with vancomycin, which functioned as a universal capture probe for both bacteria strains. Following bacterial capture, gold nanoparticles functionalized with SERS tags (5,5'-dithiobis-(2-nitrobenzoic acid), 4-mercaptobenzoic acid) and aptamers were used as signal target probes. The dual-recognition strategy resulted in sensitive (10 CFU mL⁻¹), simultaneous detection of bacteria in milk, orange juice, chicken, and sputum samples. Gold nanoparticles were also combined with aptamer functionalized-macroporous magnetic silica photonic microsphere.¹⁴⁹ This 3D substrate reported sensitive detection of *E. coli*, owing to the aptamer's specific capture and the formation of uniform hot-spots caused by the 3D substrate. Gold nanoparticles functionalized with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and a secondary aptamer were used as signal tags. Importantly, the sensor integrated sample pretreatment and sample separation, further shortening the turnaround time. Successful detection was reported for water, milk and minced pork samples.¹⁴⁹

Antibodies and nanogapped, self-assembled SERS tags (Si@Au/Au) have been reported in literature for the simultaneous detection of *S. pneumoniae* and FluA H1N1 virus in clinical samples.¹⁵⁰ In particular, following the formation of a silicon dioxide nanoparticle layer, continuous assembly of two layers of dense gold nanoparticles resulted in an ultranarrow

intraparticle gap. The nanoparticles were functionalized *via* DTNB and 4-MBA probes, providing sites for anti *S. pneumoniae* and anti-H1N1 antibodies. The study reported bacterial detection (LOD 16 CFU mL⁻¹) in aqueous and saliva samples.

An interesting study reported the use of silver-etched silver-coated gold 'nanopancakes' which were made as a dual-function SERS substrate and as a reservoir for silver ions.¹⁰⁴ The nanopancakes were decorated with a Prussian blue analogue, capable of binding with bacteria through a cyano group. Bacteria were first tagged with 4-mercaptophenyl boronic acid (4-MPBA). Upon mixing, the nanopancakes provided intrinsic SERS signal from the bacteria as well as from the 4-MPBA (which served as an internal standard). The study reported detection and antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*, with a low LOD in aqueous samples, blood, and biofilms.

Magnetic sample enrichment in microfluidic devices has also been reported. Antibody-modified MNPs were used to selectively capture *E. coli* from complex milk samples in a capillary-driven microfluidic device. SERS was subsequently detected using 4-aminothiophenol (4-ATP)-labelled gold nanorods as SERS probes.¹⁵¹ A microfluidic platform was reported for the simultaneous detection of *S. aureus* and *E. coli* O157:H7 which utilized MNPs functionalized with antimicrobial peptides to selectively capture the bacteria, followed by SERS interrogation using bacteria-specific aptamer-functionalized SERS nanoparticles with peaks in the spectral silent region (around 2100 cm⁻¹). In this case, performing both the magnetic enrichment and the SERS detection in a microfluidic chip provided flexibility in rapidly switching between magnetic enrichment and elution of the samples.¹⁵²

Urchin-shaped, platinum layered nanoparticles based on a core of gold and silver nanoparticles have been reportedly used for the multimodal detection of *E. coli*, *S. aureus*, and *P. aeruginosa* in aqueous and human blood samples, using a lateral flow setup.¹⁰⁶ 4-MBPA was used as an antibody analogue, reporting high affinity for the bacteria used in the study. Interestingly, this study used a triple colorimetric-SERS-photothermal-catalytic method, reporting simultaneous bacterial detection.

A variety of SERS nanoparticles have been used in microfluidic devices to enable the sensitive and selective detection of bacteria. SERS-tagged gold nanostars have been functionalized with a monoclonal antibody to rapidly detect *Listeria monocytogenes* bacteria on-chip with high specificity.¹⁵³ Gold nanoparticles with different Raman reporter molecules were used to selectively detect *E. coli* O157:H7 and *Salmonella enterica*. The nanostars were functionalised with either fluorescein or rhodamine 6G (R6G) and streptavidin (SA), followed by tagging with species-specific antibodies for targeting, resulting in two distinct and specific SERS-nanotags.¹⁵⁴ The system was successfully demonstrated for *E. coli* detection in food samples.¹⁵⁵ Finally, SERS detection can be further combined on-chip with tools such as CRISPR/Cas12a genome editing, broadening the scope of detection that is currently achievable. Gold nano-



star@4-mercaptobenzoic acid@goldnanoshell structures (AuNS@4-MBA@Au@DNA) conjugated with thiolated ssDNA were used in one such microfluidic platform for the SERS detection of *Salmonella typhimurium* in food samples.¹⁵⁶

4. Electrochemical SERS

Recently, there has been increasing interest in the combination of electrochemistry with SERS. EC sensors are often held back by their lack of specificity and SERS often faces challenges in the acquisition of reproducible signals. EC-SERS aims to enhance the Raman signal by applying controlled electrode polarization on the SERS active electrode.¹⁵⁷ This approach can allow the detection of the target molecule at a desired applied voltage as well as the formation of a biologically relevant electric field. This can provide insights on the rotational configuration of molecules under certain voltages, as well as the electrochemical stability of analytes.¹⁵⁸

4.1 Introduction to EC-SERS

Electrochemistry and SERS have always been intertwined, as the first SERS spectra were obtained from an electrochemical cell in the 1970s.⁹⁶ The signal enhancement of SERS is attributed to two mechanisms: electromagnetic (plasmonic) and chemical enhancement; both can be modulated by the application of electric potential.^{159,160} The aim of EC-SERS is to measure Raman (SERS) spectra of adsorbed species on the surface of the electrode, while also measuring any changes caused by varying the applied voltage. Changes in the voltage can lead to species adsorption/desorption, reorientation and composition of the electrochemical double layer.^{12,161} The combination of these effects leads to improved analytical performance, which has found applications in molecular identification and characterization as well as detection of low-affinity species, including drugs, metabolites and other chemicals.¹⁶² Published literature has reported that the combination of electrochemistry and SERS leads to an average 10-fold improvement in signal enhancement compared to SERS alone; the varying voltage can promote analyte adsorption to the SERS substrate boosting the distance-dependent electromagnetic enhancement, but also facilitate charge transfer mechanisms related to the chemical enhancement.¹⁶³

An EC-SERS setup consists of a Raman spectrometer, a potentiostat, and an electrochemical cell with the working electrode used as a SERS substrate.¹⁵⁷ Working electrode materials are similar to those used in electrochemical applications and most commonly include gold, silver, platinum and glassy carbon, as their plasmonic properties allow generation of SERS signal.¹⁵⁷ Screen-printed electrodes have gained popularity as EC-SERS substrates given their low cost, reliability, efficiency and simplicity, and are utilized by the majority of published work on EC-SERS.¹⁶⁴ Furthermore, advances in nanomaterials have led to the development of novel and improved SERS substrates, such as bimetal complexes and nanowires, nanoislands, and nanopancakes.^{87,93–95,148}

In the past three years, there has been an increasing number of published work describing the use of EC-SERS for the detection of plasticizers (phthalate esters and bisphenol A),^{165,166} insecticides and pesticides,^{6,164,167–172} drugs,^{173–183} pigments,¹⁸⁴ nucleic acids,¹⁸⁵ viral lysates,¹⁸⁶ endotoxin,¹⁸⁷ protein markers,¹⁸⁸ and even whole bacteria.¹⁸⁹

Fig. 4 shows examples of EC-SERS applications for bacterial detection, categorized as direct bacterial detection (a), detection of bacterial metabolites (b), and detection of bacterial-derived DNA (c).

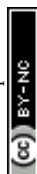
4.2 EC-SERS for bacterial detection

A relatively recent EC-SERS application is in the more sensitive detection of bacteria and their metabolites. Since 2018, when the first EC-SERS application for a bacterial detection was described by Lynk *et al.*, numerous other published works have described EC-SERS applications for various bacteria species and metabolites, using various EC techniques. Commonly used EC techniques applied with SERS include voltage stepping, cyclic voltammetry (CV) and differential pulse voltammetry (DPV), all of which can be used in electrochemical sensors by themselves.^{163,190} Table 1 lists examples of detection of bacteria and their metabolites using EC-SERS.

Lynk *et al.*, in their 2018 publication, reported the use of screen-printed electrodes, on which three layers of silver nanoparticle paste were deposited by drop-coating.¹⁶³ Following a rinse with 0.5 M KCl, the electrode was incubated with a bacterial suspension for 16 hours, before being rinsed and dried prior to use. Firstly, spectra for bacterial metabolites and nucleotide breakdown products (adenine, hypoxanthine, xanthine, guanine, uric acid, AMP, and guanosine) were obtained, by applying voltage in -0.1 V steps, until a final potential of -1.0 V was reached. Next, *E. coli* and *B. megaterium* were assessed, using the same method and voltage application. In both cases (metabolites and bacteria), the application of voltage resulted in clearer spectra with more well-defined and more intense peaks compared to the control spectra, highlighting the enhancement provided by electrochemistry.

The same group, in a subsequent study published in 2019, further examined the effect of EC-SERS on bacterial detection.¹⁹⁰ Using the same protocol (screen printed electrodes (SPEs) and silver nanoparticles), the study further investigated the initial findings and reported that the electrochemical enhancement was caused by the release of bacterial metabolites from both species (*E. coli* and *B. megaterium*), such as adenine, xanthine, hypoxanthine, guanine, guanosine, adenosine monophosphate and uric acid, all of which dominate the SERS features of bacteria. Such metabolites are released by bacteria upon exposure to environmental stressors, and due to their affinity for the SERS substrate, result in spectral enhancement.

The same year, EC-SERS was employed for the detection of pyocyanin, a blue-coloured characteristic metabolite produced by *P. aeruginosa*, which is heavily involved in pathogenesis.¹⁹¹ As pyocyanin is known for being both redox-active and Raman-



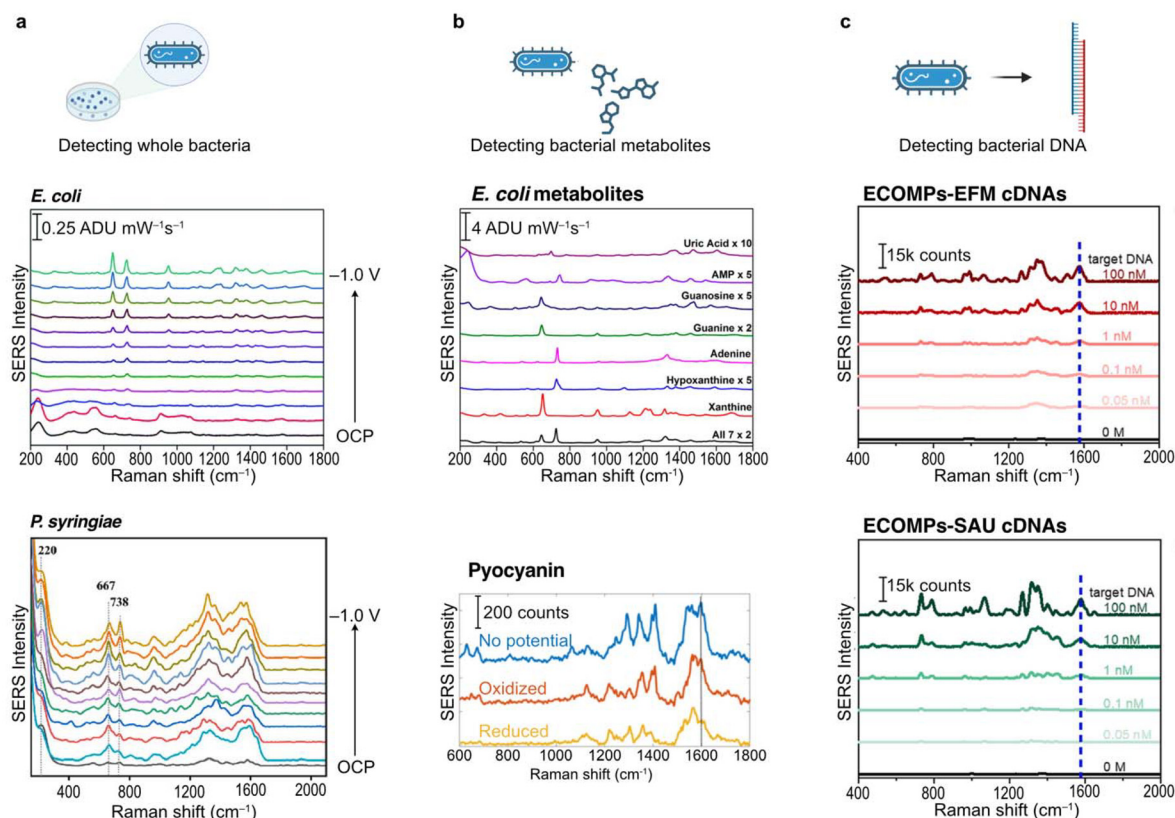


Fig. 4 Examples of EC-SERS applications for bacterial detection. (a) EC-SERS label-free detection of *E. coli* and *P. syringiae*.^{189,190} (b) EC-SERS detection of bacterial metabolites.^{163,191} (c) EC-SERS detection of bacteria-derived cDNA, from *E. coli* and *E. faecium*.¹⁹²

active, it was anticipated that EC-SERS would provide valuable information. Overall, it was reported that following application of external potential, reduced pyocyanin generated weaker spectra compared to its oxidized form. In addition, the sensor reported the early detection of pyocyanin in low concentrations, which could enable rapid *Pseudomonas* detection.

The combination of electric field and SERS as an EC-SERS system was explored using gold nano-islands (20 nm) which were deposited on a 3D laser-scribed graphene (LSG) substrate to increase the sensitivity and reproducibility of SERS detection.¹⁹³ The authors amplified the SERS signal (633 nm laser; 10 seconds integration time) by applying an electric-field stimulus to the Au-LSG SERS substrate and measured the electric-field-induced chemical enhancement (CE) SERS signals of uremic toxins (urea and creatine) for the determination of chronic kidney diseases. The detected molecules displayed a 4-fold increase in Raman signal at low voltage potentials (−0.2 V), demonstrating the capability of enhancing the SERS signal and its sensitivity.

A recent paper describes the use of applied voltage, DPV and CV for the label-free detection of *Pseudomonas syringae* pv. *actinidiae* (Psa), a kiwifruit pathogen responsible for economic losses worldwide.¹⁸⁹ In this work, silver nanoparticles with iodide and calcium ions on a nanostructured silver electrode were used as a SERS substrate. Following preparation of the

bacterial culture and resuspension in ultrapure water, the modified electrode was immersed into the culture for 16 hours, followed by air drying and spectral acquisition. Following a screening process, it was reported that application of −0.8 V led to a significant enhancement in SERS spectra, possible caused by potential-induced reorientation of Psa molecules on the electrode surface.

Additionally, EC-SERS can also be used for the detection of bacterial spores. The use of silver nanoparticles in a cuttlebone-derived organic matrix was reported. Following electrochemical enhancement through the application of 2 V, the sensor reported sensitive detection of *Bacillus cereus* spores in water and milk samples, with LOD of 0.3 nM.¹⁹⁴

Novel SERS composites can be exploited for the detection of bacterial metabolites, with one such example describing the use of a pH-responsive block copolymer membrane with nanopore electrode arrays filled with gold nanoparticles, capable of isolating and detecting phenazine-1-carboxylic acid, an important *Pseudomonas* metabolite, in bacterial cultures.¹⁹⁵ The pH-responsive membrane ensured the transfer of the negatively-charged analyte in acidic culture medium, across its membrane and into the gold-nanoparticle filled nanopores. Square-wave voltammetry performed from −0.8 to +0.2 V on different *Pseudomonas* phenazines revealed that each species generated distinct spectra, aiding their characterization.

Table 1 Bacteria and bacterial metabolites detection using EC-SERS

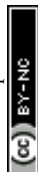
Bacterial species/ analyte	Label	Substrate	Wavelength	EC	Sample type	Ref.
Whole bacteria	Label free					
<i>E. coli</i> , <i>B. megaterium</i> , Bacterial metabolites		AgNPs and SPEs	780 nm	Applied voltage	Aqueous solutions	163
<i>B. megaterium</i> , <i>E. coli</i>		AgNPs and SPEs	780 nm	Applied voltage	Aqueous solutions (pbs)	190
<i>Pseudomonas syringae</i> <i>pv. actinidiae</i>		AgNPs with calcium and iodide ions	633 nm	Enhancement at -0.8 V, DPV, CV	Aqueous solutions	189
Whole bacteria	Labeled					
<i>V. vulnificus</i>	Detection antibodies	Mxene, rhodamine 6, Au nanorods	780 nm	CV	Aqueous solutions	203
<i>Mycobacterium</i> <i>tuberculosis</i>	Antibodies	AgNPs, carbon SPE	785 nm	Voltage stepping	Aqueous, urine, sputum	201
Bacterial metabolites	Label free					
Uric acid		Au/Ag substrate layers	532 nm	Applied voltage	Aqueous solutions	198
Pyocyanin from <i>Pseudomonas</i>		AgNPs and SPEs	532 nm	CV	Aqueous solutions	191
Uric acid		Ag SPEs	785 nm	CV	Synthetic urine	199
Uric acid		Polycarbonate, Au nanospheres	632 nm	Applied voltage	Aqueous solutions, simulated urine	200
<i>Pseudomonas</i> metabolites		AuNPS block copolymer (BCP) membrane, nanopore electrode arrays (NEAs)	785 nm	CV, SWV, DPV	Aqueous solutions	195
DPA from <i>Bacillus</i> spores		AgNPs/cuttlebone-derived organic matrix	785 nm	Applied voltage	Water, milk	194
Bacterial metabolites & DNA	Labeled					
Guanine, adenine as bacterial metabolites	Aptamer	SPEs, AgNPs	785 nm	CV	Aqueous solutions	158
DNA from <i>E. faecium</i> and <i>S. aureus</i>	Capture DNA with cyanine 5	Ag nanopillars	785 nm	CV EC deposition of Au on metallic nanopillars	H ₂ O, 100× diluted blood	192
Endotoxin	Aptamer	Au@Ag nanocubes	785 nm	CV, EIS	Aqueous solutions	187

A common bacterial metabolite is uric acid, often produced by aerobic bacteria as part of the classical metabolic pathway of purines.¹⁹⁶ Some bacteria can break down uric acid, such as *Bacillus* spp., *P. aeruginosa*, *K. pneumoniae* and *Lactobacillus* spp.¹⁹⁷ There have been reports of label-free EC-SERS sensors in literature designed for the detection of uric acid. One such sensor describes the use of a multilayered gold and silver substrate, constructed by depositing monodisperse gold and silver nanoparticles on the carbon working electrode surface of screen printed electrodes.¹⁹⁸ By applying voltage in a stepwise approach, it was realized that application of -0.9 V resulted in the best Raman spectral enhancement in aqueous samples. Another similar study described the use of silver, screen-printed electrodes, combined with cyclic voltammetry between -0.40 V and $+0.40$ V, reporting uric acid detection even in complex samples, such as synthetic urine.¹⁹⁹ Finally, a more recent study reported the use of a nanostructured, polycarbonate nanocone array, decorated with a tightly packed array of gold nanoparticles.²⁰⁰ Voltage application at -1.0 V was reported to assist uric acid detection, in both aqueous and synthetic urine samples.

4.2.1. Labelled EC-SERS applications. Labelled EC-SERS is considered an indirect detection approach. Commonly used EC-SERS labels can include antibodies, aptamers and peptides.

A point of care sensor utilizing disposable carbon screen printed electrodes combined with silver nanoparticles with a polyethylene coating has been developed for the detection of *Mycobacterium tuberculosis*. *M. tuberculosis* is the causative pathogen of tuberculosis, infects around one third of the global population and is a leading bacterium-based infectious disease-causing pathogen worldwide.²⁰¹ Disposable carbon screen printed electrodes were combined with silver nanoparticles with a polyethylene coating, which attracted bacteria, anti-*M. tuberculosis* antibodies and a customized voltage-stepping sequence-based protocol. The application of both anodic and cathodic voltage in sequence was able to specifically detect and identify mycobacteria, including three clinically important MTB strains. Additionally, the sensor differentiated between mycobacteria and Gram +ve and -ve bacteria (*E. coli* and *S. aureus*). The study reported *M. tuberculosis* detection in aqueous samples, as well as in urine and sputum samples, with little to no sample interference.

Bacterial DNA can be sensitively detected using a combination of electrochemistry and SERS. Electrochemical deposition was performed to deposit gold onto nanopillars, containing a plethora of hot-spots, on which DNA strands complementary to the target DNA were attached.¹⁹² The complementary DNA strands were labelled with Cyanine 5, a Raman active



4.3.1. Electrochemiluminescence. ECL is closely related to electrochemistry; the applied voltage drives a redox reaction between a luminophore and a sacrificial co-reactant resulting in the emission of light, which in turn serves as the sensor signal.²⁰² ECL has been used along with SERS for bacterial detection. Specifically, MXene and antibodies were combined, reporting detection of *V. vulnificus*, an especially high-risk *Vibrio* strain, responsible for gastroenteritis, sepsis, necrotizing wound infection and death.²⁰³ The sensor achieved sensitive bacterial detection in physiological and seawater samples. Although the ECL and SERS measurements were performed separately, this work demonstrates that the two techniques are closely intertwined, allowing cross-verification of results between the two methods. A recent, novel labelled approach reported the use of dual-mode ECL and SERS sensing of bacterial endotoxin, one of main components of Gram -ve bacterial cell walls, which functions as a major mediator for sepsis in the human body.¹⁸⁷ In particular, the study reported a complex, adjustable cascade where the binding of endotoxin to the sensor initiated rolling-cycle amplification, causing the release of large amounts of poly-C DNA, which gradually etched the silver shell of silver-coated, gold core nanocubes, through removal of Ag⁺ ions. Subsequently, the poly-C DNA was reduced to silver nanoclusters. This reaction resulted in the loss of dye-signal and the disappearance of hot-spots, resulting in a noticeable, quantifiable reduction in SERS signal. The study reported sensitive detection of endotoxin in aqueous samples, as well as dilute milk and serum samples.

4.3.2. Field-effect transistors. As electric potentials can modulate SERS on EC electrodes, a similar approach can be pursued by using field-effect transistors (FETs). The combination of an electric field and SERS provides a unique approach to modulate the electronic state of the SERS substrate and enhance the Raman effect by controlling the electric field around the hot-spot nanostructures and any adjoining molecules. Tuning the applied potential of the metallic electrode contacts in the electric field-stimulated Raman device increases plasmonic enhancement and facilitates electron

transfer to the molecule. The combination of electric field-stimulation and SERS has not been fully explored concurrently, with most studies employing FET structures as sensing platforms for SERS, serving as a supplemental detection technique for analytes.^{204–206}

A recent study reported an electrically tunable back-gate FET on silica/silicon substrates with pre-patterned Au/Ti electrodes comprising a tungsten/molybdenum oxide hybrid as the SERS active layer deposited *via* spin-coating (Fig. 5a).²⁰⁷ The study reported that the applied gate bias (from 0 V to +15 V) tunes the SERS enhancement factor and Raman intensity (Fig. 5b). The SERS performance was evaluated using rhodamine 6G as the Raman reporter, excited by 532 nm and 785 nm lasers, exhibiting a LOD as low as 10^{-8} M. According to the authors, when a positive voltage is applied to the gate electrode, the band bends downwards, and electrons from the source/drain electrodes accumulate near the interface between the hybrid semiconductor and the gate dielectric. This enhances the electron density near the Fermi level, ensuring that more electrons can be transferred to the molecular orbital. The reported study provides a substantial contribution to the understanding of the fundamental properties of FET-SERS mechanism using metal oxide semiconductors.

The limited number of studies highlights the research gap in electric field-stimulated SERS and its potential for analyte detection. Moreover, the FET-SERS platform shows potential for bacterial detection, presenting a flexible method for quickly and accurately identifying harmful microorganisms.

4.3.3. Electrokinetics. Following a different approach, electric fields shaped by microelectrodes can be used alongside SERS. For example, dielectrophoresis (DEP) has been reported to concentrate or isolate bacteria, facilitating detection. Specifically, negative DEP has been combined with SERS, reporting rapid concentration, isolation and label-free identification of *E. coli*.²⁰⁸ The SERS substrate was composed of poly (ethylene terephthalate) polymer foil, a layer of indium tin oxide subjected to dielectric barrier discharge and a layer of silver. DEP assisted the separation, concentration and controlled deposition of bacteria on the SERS-active area of the substrate. Longer DEP times (180 seconds) resulted in improved *E. coli* deposition and clearer SERS spectra. The

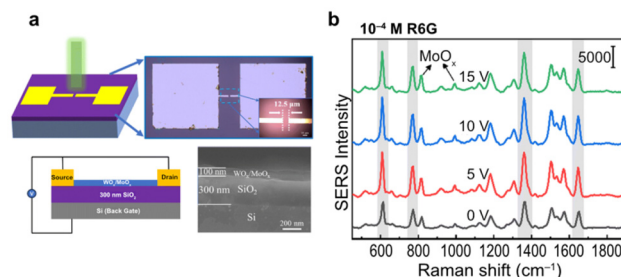


Fig. 5 (a) Optical image, 3D schematic structure, and cross-sectional SEM image of a WO_x/MoO_x-based FET-SERS substrate. (b) SERS spectra of 10⁻⁴ M R6G adsorbed on the WO_x/MoO_x-based SERS substrate with applied voltages from 0 to +15 V.²⁰⁷

study reported bacterial detection in aqueous solution, apple juice and urine samples.

5. Discussion

It is evident that bacterial detection remains a hot area of research. Miniaturized and rapid sensors are necessary to supplant the currently used lab-based protocols. Electrochemistry, SERS, and their combination, all exhibit potential towards this goal. These methods have been employed for bacterial detection, identification, and quantitation, in a variety of different settings.

To produce useful and relevant sensors, however, a few key parameters must be kept in mind: timeliness, ease of use, sensitivity and quantitation.

5.1. Time is of the essence when dealing with bacterial infections in a healthcare setting

A major limitation of bacterial detection is the reliance on bacterial cultures. Most detection methods require bacterial cultures either to amplify the numbers of bacteria above the detection threshold, or to ensure the bacteria are in a medium suitable for the sensor. Cultures take time to grow (typically 24 hours or more) taking a toll on the benefits these new technologies provide. For the deployment of a useful sensor, reliance on cultures should be eliminated. Methods such as SERS may be able to detect low abundance of bacteria, obviating the need for time consuming cultures.

5.2. Any commercial application of sensors requires ease of use

Electrochemistry is an established technique that may be conducive to miniaturization and automation. The equipment needed is cost-effective and lightweight. However, most EC sensors found in the literature require several steps of sample handling and reagents, making their field deployment cumbersome. SERS may be simpler to perform compared to EC, especially when using non-colloidal substrates, but data analysis is more complex, and the equipment needed a little more costly. One key technology that can greatly expedite and support the integration of EC-SERS and its related methodologies into commercial applications is microfluidics. Microfluidics can miniaturize and parallelize the analysis, performing several assays with a small volume sample. Tedious steps such as sample washes, serial reagent additions, and nanoparticle mixing can be automated. Additionally, microfluidic devices can integrate other mechanisms to facilitate detection, such as FETs, or electrokinetic traps for sample enrichment. Magnetic nanoparticles can be used, offering yet another modality for detection, complementing the electrical and optical signals. For example, a platform based on droplet microfluidics and magnetoresistive sensing has been employed for the selective detection of *K. pneumoniae* in urine.²⁰⁹

5.3. Sensitivity and quantitation are crucial for most applications

New sensor materials have allowed EC to push down its LOD, especially when secondary labels are used. EC sensors for bacteria have been reported in different contexts, demonstrating that EC detection is a robust strategy. Also, EC sensors are – by design – specific to only a single target, meaning that while they may be appropriate for dedicated applications, they will not detect other pathogens that may be present in the sample.

On the other hand, SERS boasts extremely low limits of detection, revealing low abundance targets. In the case of direct SERS, it offers the possibility of exploratory analysis, where multiple bacterial species may be detected with the same platform, making it more versatile and cost effective. However, making quantitative measurements using SERS is challenging; most studies provide qualitative results, such as yes/no detection or classification of bacterial species. This may be sufficient for applications such as sepsis or detection of *Salmonella*, where the presence of a single pathogen is indicative of infection, but it is limiting in applications such as UTI diagnosis or detection of *Campylobacter* spp in raw poultry, where quantitation is necessary.

The combination of the two methods as EC-SERS aims to leverage their strengths synergistically. The two techniques supplement each other, SERS providing the low limits of detection while EC ensures specificity.

One limitation in the field lies in the inconsistency of reporting of LODs. Most studies, regardless of the sensing methodology, report a theoretical LOD, based on extrapolation of measurements in a linear sensing region. Few studies report the last measurable concentration different than the blank.

Additionally, low target concentrations become increasingly harder to detect as the sample volume is decreased *via* miniaturization. *I.e.*, low abundance targets will not be sufficiently represented in small sample volumes. There may be a compromise to be reached between miniaturization, sensitivity, and sample enrichment (through culture).

6. Conclusion

The combination of EC, SERS, and other related methods has the potential to produce reliable sensors, with application-relevant sensitivities, democratizing bacterial detection and saving lives and operational costs. To achieve this, it is necessary for researchers to have usability in mind, as the final product will need to be deployed very widely to make a meaningful impact.

While EC sensors are more mature, they are limited by the use of specific recognition moieties, limiting their generalizability. SERS, on the other hand, can be used to identify many bacterial species, but struggles to provide quantitative information. EC-SERS may be able to harness the strength of both, providing sufficient sensitivity, quantitation, and identification of bacterial species.

To date, the number of reports on EC-SERS in the literature remains limited. This may be due to the distinct skillsets



required to employ the two techniques. The few examples demonstrated, however, seem to be more effective than ones using the parent techniques alone. Specifically, compared to SERS alone, EC-SERS is more likely to provide quantitative information; whereas the spectra acquired at different voltages contain a wealth of information that can be used to identify bacterial species with higher specificity than EC alone.

Moving forward, we recommend that the research community working in the bacterial diagnostics field be aware of these limitations. While the existing literature showcases the capabilities of the detection methodologies, there is limited discussion of application specific requirements. For example, regulatory requirements are different for different fields (*e.g.*, health care and food safety) but also within the same fields (*e.g.*, diagnostics of sepsis *vs.* UTIs). Conforming to such requirements is essential for any product's approval by the relevant regulatory authorities. This may be an important reason for the limited commercial success these methods have shown to date. Additionally, few studies report applications such as antimicrobial resistance, indicating a major gap in knowledge and potential area of research. One thing, however, remains constant: the need for rapid and cost-effective bacterial detection will only increase in the future.

Author contributions

Conceptualization: CA, KK; funding acquisition: CA; writing – original draft: KK, TL, MC; writing – review & editing: CA.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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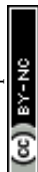
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