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# Nano/Micro and Spectroscopic Approaches to Food Pathogen Detection

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## Abstract

Despite continuing research efforts, timely and simple pathogen detection with a high degree of sensitivity and specificity remains an elusive goal. Given the recent explosion of sensor technologies, significant strides have been made in addressing the various nuances of this important global challenge that affects not only the food industry but also human health. In this review, we provide a summary of the various ongoing efforts in pathogen detection and sample preparation in areas related to Fourier transform infrared and Raman spectroscopy, light scattering, phage display, micro/nanodevices, and nanoparticle biosensors. We also discuss the advantages and potential limitations of the detection methods and suggest next steps for further consideration.

## 1. INTRODUCTION

Detection of pathogens is fundamental for identifying and minimizing foodborne disease outbreaks and preventing foodborne disease. There is a universal need among regulators, food producers and/or processors, and researchers for rapid, precise, and accurate detection methods of foodborne pathogens. Traditional detection methods (e.g., culturing and plating) have been sustained as the gold standard for foodborne pathogen detection primarily because they can detect cells in initially low numbers. Selective enrichment of food or environmental samples (food and nonfood contact surfaces) propagates initially low numbers of cells to levels detectable on selective media or downstream assays such as polymerase chain reaction (PCR)-based methods. Other advantages to traditional detection methods include recovery of live cells for further analyses (e.g., genome sequencing), ease of interpretation, and limited need for specialized equipment. However, the major limitation of traditional detection methods is time to results. In many instances, traditional sample processing and analysis may require more than seven days to final results. This is problematic for both industry and regulatory agencies because products with short shelf lives may spoil before test results are available and time is critical when identifying and controlling foodborne disease outbreaks. An ideal pathogen-detection platform would have utility across diverse food matrices, deliver fast results, contain an internal positive control, have high sensitivity and specificity, be easy to perform and interpret, have the capacity to multiplex, have the ability to recover or identify stressed and/or injured cells, and be quantitative and cost effective. To this end, the technologies detailed in the following sections work to overcome the current challenges of traditional methods through innovation and engineering as steps toward the development of faster, accurate assays to enhance public health and the economic well-being of the food industry. **Figure 1** gives a general overview of the conventional and current methodologies for the detection of pathogens that are introduced in this review. This review primarily focuses on the recent trends in technology development for food pathogen detection.

## 2. SAMPLE PREPARATION

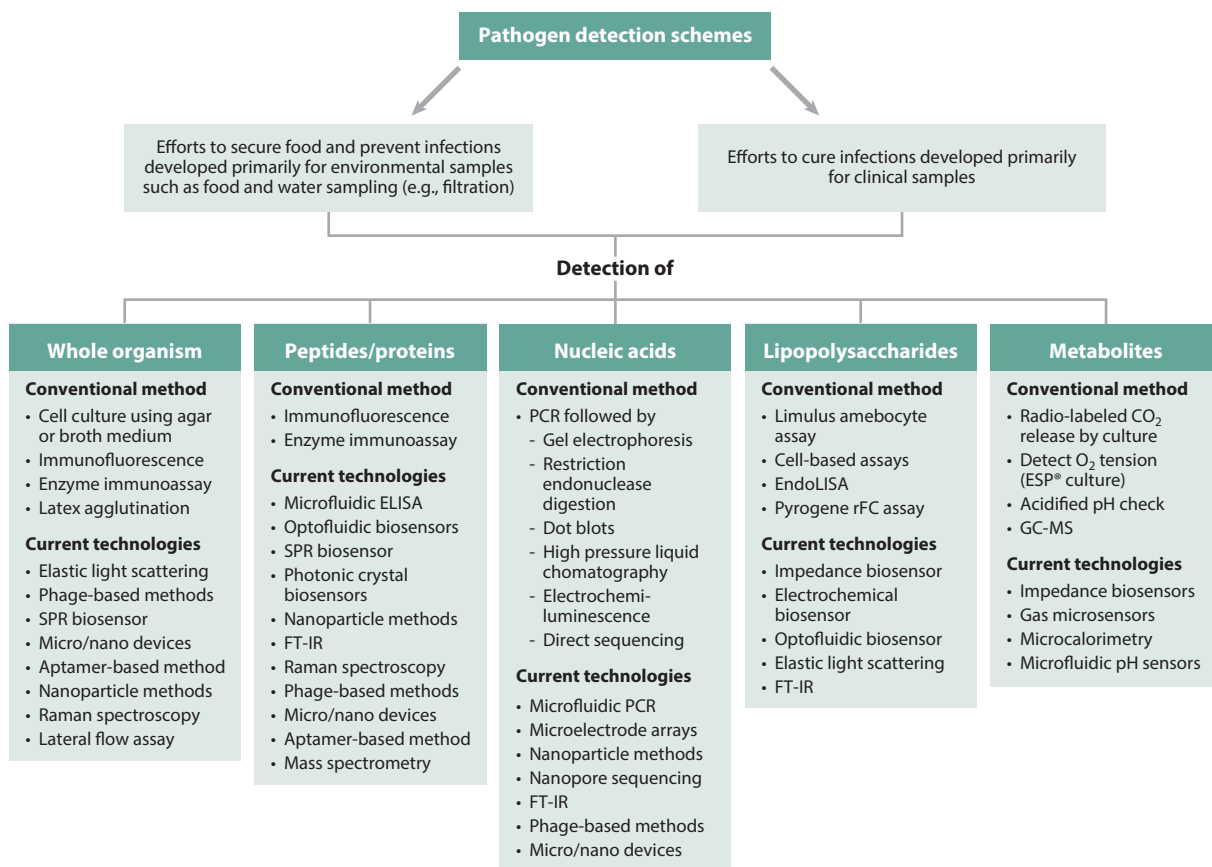
Rapid detection of food pathogens is challenging because of the need to recover microorganisms from food matrices (ground meat, vegetable matter, and milk), either by separating and/or propagating them so that they are differentiated from nonpathogenic microorganisms and then detecting and identifying the pathogenic organisms, if they are present. Although technology has been developed for detecting pathogenic microbes that are grown or suspended in buffer and/or growth media so that the target cells approach a monoculture, direct detection in foods is much more difficult owing to the presence of overwhelming numbers of nonpathogenic microorganisms as well as food materials and molecules (lipids, fats, nucleic acids, polysaccharides) that interfere with the assay. Although traditional sample preparation methods overcome these limitations, they require culturing, enrichment, and, in some cases, resuscitation steps. Several days may elapse between the sampling and propagation of a culture that is suitable for probing for the presence of pathogens.

### 2.1. Microfiltration

Microfiltration techniques represent a conceptually simple way to reduce large samples to a small volume and effectively increase cell concentration without lengthy culturing and enrichment steps. Dead-end filtration techniques using a flat-sheet membrane are effective for concentrating cells for microbiological analysis of water, dairy, and food products (1–5). They have also been used to

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**Figure 1**

Overview of pathogen-detection techniques. Abbreviations: ELISA, enzyme-linked immunosorbent assay; FT-IR, Fourier transform infrared spectroscopy; GC-MS, gas chromatography–mass spectrometry; PCR, polymerase chain reaction; rFC, recombinant Factor C; SPR, surface plasmon resonance.

show how membrane filtration using a syringe filter concentrates *Listeria monocytogenes* by 95-fold with up to 95% recovery of living microorganisms via microfiltration of 50 mL of a food sample into a volume of 500  $\mu$ L. These techniques, coupled with a microfluidic biosensor, provided an initial indication that a 4-h detection assay is possible.

Microfiltration through a flat-sheet membrane is effective for concentrating cells using a syringe pump with an attached filter holder. However, the subsequent actions needed to recover the microbial cells from a flat-sheet membrane surface into a small volume and then deliver this volume into a microfluidic biosensor platform remain problematic. Manual intervention is required to remove the membrane, harvest the sample, and recover a small volume of concentrated microorganisms from the surface of the membrane. Fouling, caused by the accumulation of fats, oils, particles, and proteins on the surface of the membrane, occurs in a manner that causes the formation of a layer that blocks fluid transmission through the membrane. This ultimately limits the volume of fluid that may be processed. Hence, the configuration of this approach has been transitioned from a flat-sheet membrane to hollow fibers with flow-through.

## 2.2. Hollow-Fiber Filtration

Hollow-fiber filtration offers several advantages over traditional flat-sheet membranes: a high surface area to volume ratio, a higher flux per unit volume of the membrane module, and continuous operation that avoids manual handling of the membrane and sample. The membrane may be back-flushed to recover concentrated cells in an aqueous buffer. Cross flow across the membrane reduces material accumulation at the surface of the membrane and improves flux (filterability). A hollow-fiber module has a lower transmembrane pressure compared with that of a flat-sheet membrane, and fouling at the membrane surface is reduced owing to a tangential flow of the liquid across the membrane. Cross-flow microfiltration using a hollow fiber may effectively reduce clogging of the filtration membrane by maintaining a high velocity and tangential flow of the feed across the membrane's surface. The hollow fiber's operation allows it to be coupled with a microfluidic biosensor where liquid sample is first reduced in volume through hollow-fiber microfiltration and is then transported onto a microfluidic detection device (i.e., "chip") where further concentration, capture, and analysis occur in an automated manner (6).

Both the flux and tangential convection is typically proportional to the transmembrane pressure. As fluid flows along the length of the membrane, there will be a pressure loss per unit length of the membrane, causing axial convection to start with a high transmembrane flux and then decrease along the length of the membrane. Concurrent with this effect, the magnitude of the shear-induced diffusion will remain constant. Ideally, the shear gradient is high and the tangential convection remains low. Under normal operation, however, this is not possible because the tangential convection and shear-induced diffusion are coupled. This may be resolved by pumping the permeate across the permeate side of the membrane such that the transmembrane pressure remains constant along the length of the membrane. This uncouples the forward and backward transport mechanisms and allows for increased rates of flux and sample concentration. These concepts have recently been assembled into a prototype instrument that couples high flux, concentration in less than 30 min, reusable membranes, and self-cleaning features in an automated system.

In addition to these filtration methods, other types of sample preparation have also been introduced for maximum recovery of foodborne pathogens from food matrices. Kim et al. (7) compared different sample preparation methods such as pummeling, pulsifying, sonication, and shaking by hand from iceberg lettuce, perilla leaves, cucumbers, green pepper, and cherry tomato that were contaminated with *Escherichia coli* O157:H7, *Salmonella typhimurium*, *L. monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. They reported that pummeling and pulsifying were optimal sample preparation methods for detecting microorganisms under 90% relative humidity. Detaching microorganisms without preprocessing using a spindle apparatus also yields high recovery from various types of food samples. This spindle method causes less damage to sample matrices and shows better reproducibility than hand massaging and stomacher methods (8). The following sections discuss recent attempts that use key technologies for pathogen detection.

## 3. NANOPARTICLE BIOSENSORS

Nanoparticles play a major role in biosensor development. Owing to their excellent physical properties, e.g., high surface to volume ratio, fast diffusion rate (9), nanosensors have been considered as promising signal tracers for detecting biomolecule target analytes. For decades, various types of detection methods, such as optical, electrochemical, and magnetic, in combination with nanoparticle technology, have been introduced to detect harmful bacteria that contaminate food and cause foodborne illness. Compared with other detection methodologies, nanoparticle-based techniques enable rapid and sensitive detection of target pathogens without complex sample handling. Moreover, this can be widely measured by not only optical and electrochemical means, but

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also by spectroscopic approaches such as infrared (IR), fluorescence, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), and mass and nuclear magnetic resonance (NMR), thus making nanoparticles versatile entities in diagnostics. Three types of metal nanoparticles, gold nanoparticle (AuNP), silver nanoparticle, and magnetic nanoparticle (MNP), have been primarily used to detect pathogens, and these are usually incorporated with the several detection techniques described above to attain signal measurements. To enable the capture of a specific target pathogen, biomolecules such as antibodies, antibiotics, carbohydrates, and other binding proteins are conjugated with the nanoparticles. Furthermore, the nanoparticle-based methods can be combined with microfluidic and membrane-based lateral flow technologies to enable the use of point-of-care measurement devices that are practical and ready for onsite testing. Here we describe recent reports on nanoparticle-based pathogen detection.

### 3.1. Colorimetric Methods

Pathogen detection based on colorimetric assay using nanoparticles allows signals to be perceived by the naked eye or a cheap colorimetric detector, enabling simple procedures without expensive instruments. Some nanoparticles such as CeO<sub>2</sub>, Pt, and Fe<sub>3</sub>O<sub>4</sub> possess intrinsic peroxidase-like activity, which can be used similarly to horseradish peroxidase, a commonly used enzyme in immunoassays. Au@Pt bimetallic alloy nanoparticles have been adopted in immunoassays (10) and glucose assays (11). By employing the biomimetic catalytic activity of the Au@Pt nanoparticle, researchers detected *E. coli* O157:H7 in the range from 7 to  $6 \times 10^6$  cfu/mL (12). Owing to their ionic property, nanoparticle surfaces can be modified into either a cationic or an anionic state such that the protein can be electrostatically coupled.  $\beta$ -galactosidase coupled to cationic AuNPs functionalized with amine head groups was used as an enzyme-nanoparticle biosensor for bacteria detection (13). When *E. coli* XL1, whose surface has a negative charge, binds to the cationic nanoparticle,  $\beta$ -galactosidase can be released from the particle to restore its enzymatic activity. Using this concept, *E. coli* XL1 has been detected at the level of 100 cfu/mL in solution. Assembly of nanoparticles yields another excellent approach for visualizing as well as enhancing colorimetric signals. The network approach using AuNPs conjugated with antibody and peroxidase has demonstrated extremely high sensitivity (3 cfu/mL for *E. coli* O157:H7 and 15 cfu/mL for *S. typhimurium*) in liquid food samples (14). Controlling the aggregation of AuNPs with switchable linkers to bridge nanoparticles in proportion to the concentration of target analyte has also revealed high analytical performance by detecting less than 100 cfu/mL of *E. coli* cells (15).

### 3.2. Optical Methods

Fluorescence analysis using organic dyes have played a key role in pathogen sensor development. Recent methods combined with nanoparticles have been introduced as novel optical detection methods not only to enhance signals but also to circumvent the disadvantages of fluorophores such as their low photostability (16). Although quantum dots may serve as an alternative fluorescence tracer, they must be labeled with binding molecules such as antibodies and require multiple washing steps. SPR is a charge-density oscillation at the interface of two media (for instance, between metal and dielectric) (17), measuring the refractive index change upon analyte binding. As such, it enables rapid, label-free, and real-time monitoring of target pathogens without multiple steps. However, low analytical sensitivity due to a small refractive index, slow diffusion-driven mass transfer, and insufficient depth of layer influenced by SPR (18) are intrinsic problems with conventional SPR methods, even though antibody-nanoparticle conjugates can be adopted in sandwich assays as signal enhancers. Long-range SPRs that propagate along thin metal film embedded in a refractive index symmetrical layer architecture combined with MNPs have been

reported and used to detect pathogens (18). MNPs have the potential to concentrate pathogens at the sensor surface to detect *E. coli* O157:H7 at a limit of detection (LOD) of 50 cfu/mL. Dark-field imaging associated with AuNPs showed good performance, with an LOD for *E. coli* of  $10^4$  cfu/mL, within only 30 min (19). By employing fluorescent polymers such as poly(*para*-phenylene ethylene) that electrostatically interact with AuNPs, various types of bacteria can be easily detected with simple and fast handling steps (20).

### 3.3. Magnetic Detection Methods

For decades, MNPs have been utilized to separate and concentrate target analytes from complex media. The use of MNPs is considered a powerful technique for sample purification without complex instrumentation. Owing to their excellent superparamagnetic property, iron oxide nanoparticles have been utilized as a promising tracer for pathogen detection. Magnetic glycol nanoparticles where MNPs were functionalized with D-mannose captured *E. coli* with outstanding efficiency (21). When labeled with antibiotics such as gentamicin and vancomycin, MNPs also bind to the receptors on the cell walls of bacteria. Such MNPs have also been used for rapid and highly efficient capture of target pathogens (22, 23). Furthermore, NMR spectroscopy has been considered as a powerful measurement tool for magnetic particles. This technique has been used to measure the spin-spin relaxation time ( $T_2$ ) of water photons on MNP-tagged bacteria samples, which can be further enhanced by synthesizing iron-based MNP with high transverse relaxivity (24, 25).

### 3.4. Electrochemical Methods

Metallic nanoparticles that intrinsically possess electronic properties have also been used to generate electrochemical signals via their interaction with pathogens. Impedance biosensors based on interdigitated array microelectrodes incorporated with antibody-coated MNPs have been successfully used to detect bacteria in ground beef (26). Via the principle of impedance,  $7.4 \times 10^4$  cfu/mL of *E. coli* O157:H7 can be detected within 35 min from sampling to measurement (26). Cyclic voltammetry measurements on screen-printed carbon electrodes combined with immunomagnetic separation processes yield high performance sensors for assessing *B. cereus* and *E. coli* O157:H7, with LODs of 40 cfu/mL and 6 cfu/mL, respectively (27). Amperometric sensors in combination with AuNPs and MNPs are also highly efficient detectors of *Salmonella* at an LOD of 143 cells/mL (28).

### 3.5. Surface-Enhanced Raman Spectroscopy

SERS amplifies the Raman scattering signal of molecules adsorbed in the vicinity of metal surfaces to yield a  $10^6$ – $10^{12}$ -fold enhancement of the signal. SERS has also been used to detect pathogens (29). Silver nanospheres formed by assembling silver nanocrystals have been successfully used as SERS substrates to detect three types of bacteria (*E. coli* O157:H7, *S. typhimurium*, and *S. aureus*) at an LOD of 10 cfu/mL (18). AuNPs associated with silica-coated MNPs have been successfully used to separate and detect bacteria via simple reaction steps. When Raman reporters comprising of thiolated compounds such as mercaptobenzoic acid and mercaptopyridine were coupled to AuNPs, detection of multiple target analyses was possible. In the study by Wang et al. (18),  $10^4$  cfu/mL of *S. typhimurium* and *S. aureus* were simultaneously detected via the multiplex SERS measurement. SERS was further refined with a filter membrane (0.45- $\mu$ m pore size) that can trap only nanoprobe specifically bound to pathogens. This membrane-associated SERS method enables multiplex, fast, and sensitive detection of bacteria via a series of simple steps (30).

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### 3.6. Mass Spectrometry

Bacterial pathogen detection based on matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has been reported. Owing to its excellent proteome profiling property, this technique has enabled rapid identification as well as analysis of target bacteria (31). However, analytical sensitivity has been a major problem in mass-based measurements. To increase sensitivity, various nanoparticles (Au, Pt, TiO<sub>2</sub>, Se, CdTe, Fe<sub>3</sub>O<sub>4</sub>, and Pt) have been investigated (32). Once numerous nanoparticles are covered on the bacterial cell, ionization can be facilitated. Recently, antibody-functionalized Pt nanosensors have been used to identify *Bacillus subtilis*, and signals from protein markers of bacteria have been successfully enhanced using the MALDI assay (33).

### 3.7. Lateral Flow Immunochromatography

Nanoparticles have been used as tracers in immunoassays based on lateral flow chromatography that utilizes the capillary action as the driving force to move sample and reactions on membrane strips for detection (34). In this approach, the antibody-antigen interaction can be performed as a one-step reaction. Thus, it may be used in point-of-care diagnostics because it is rapid and easy to use. With an AuNP coupled to an antibody specific to the target bacteria, *Vibrio cholerae* O139 was detected at an LOD of 10<sup>4</sup> cfu/mL (34). To address its lack of sensitivity, this assay was further expanded by using MNPs to concentrate target pathogen from a complex media. As a result, the assay realized a signal enhancement of up to 60-fold. In addition, signals from the magnetic concentration can be further enhanced by combining it with enzymes such as horseradish peroxidase and *L. monocytogenes* to detect 100 cfu/mL in milk (35).

## 4. MICRO/NANODEVICES

The advent of micro/nanofabrication to build micro total analysis systems ( $\mu$ TAS) has made it possible to conceptualize automated handling of food or water samples as well as detection of pathogens therein. The development of  $\mu$ TAS started with the use of standard semiconductor processes on silicon and glass substrates such as photolithography, bulk etching, thin-film deposition, thin-film patterning, and semiconductor packaging. Gradually, polymeric substrates such as polydimethyl siloxane, polymethyl methacrylate, polycarbonate, SU-8, and dry-film resists have been introduced into the development of  $\mu$ TAS. Efforts to detect pathogens using  $\mu$ TAS have been successful in automating routine microbiology practices using microfluidic channels, microvalves, and micropumps. Most importantly, the advantages of  $\mu$ TAS have been leveraged by interfacing with novel sensors of sizes similar to those of pathogens, thus providing a higher sensitivity of detection. Additional advantages of  $\mu$ TAS include the drastic reduction in required reagent volumes and associated costs, significant reduction in detection times owing to novel pre-concentration schemes, and higher portability owing to their miniaturized nature. Construction of these micro/nanodevices, often called biomicroelectromechanical systems or microfabricated biosensors, can be simplified to essentially two layers: the receptor layer and the transducer layer.

### 4.1. Receptors and Anchoring of Receptors to Surfaces of Micro/Nanodevices

The receptor layer typically consists of biological elements such as antibodies, antimicrobial peptides, mammalian pathogen interaction receptors, bacteriophages, aptamers, or single-stranded DNA that impart selectivity and specificity to these micro/nanodevices (6, 36). Antimicrobial peptides including sarcotoxin IA, melittin, polymyxin B, attacin, omiganan pentahydrochloride (MBI-27 and -28), and magainin-2 form another class of molecules that are often used to capture

pathogens or membrane fragments such as lipopolysaccharides. These molecules are not as specific as antibodies; however, they are applicable to the capture of all pathogens in general. The function of antibodies and antimicrobial peptides is essentially due to their structure, which is susceptible to denaturation upon immobilization to rigid surfaces of the transducer. This has led to the development of a novel class of molecules called aptamers, which are usually a short chain of oligonucleic acids that have been engineered completely in a test tube through repeated rounds of in vitro selection to bind to small-molecule targets with specificity that rivals that of the antibodies (37).

Micro/nanostructures used in microfabricated devices have high surface area to volume ratios. This makes it possible to sample loss through nonspecific adsorption. Although the detection mechanism may be highly sensitive, the increased nonspecific adsorption may lead to an overall lower sensitivity of the device. Huang et al. (38) showed that nonspecific binding of proteins or organisms such as *E. coli*, *Listeria innocua*, and *L. monocytogenes* on the SiO<sub>2</sub> surfaces of a biochip can be prevented by adsorbing bovine serum albumin (BSA) or biotinylated BSA after modification with an octadecane (C18) coating.

## 4.2. Transducer Technologies for Micro/Nanodevices

**4.2.1. Mechanical detection.** Microfabricated cantilevers, initially developed for atomic force microscopy, are now the microstructures most widely used for mechanical sensing. A cantilever is a diving board-shaped, single-clamped, suspended beam. A microcantilever system has two main sensing methods: (a) stress detection and (b) mass detection (**Figure 2**). In the stress-sensing mode, a biochemical reaction is performed selectively on one side of the cantilever, thus changing the surface free energy and surface stress. This results in a measurable bending of the cantilever and a label-free detection of the bioanalyte. The stress sensitivity is increased by reducing the spring constant of the cantilever. In the mass-detection mode, the cantilever vibrates at its resonant frequency in response to an external excitation or ambient thermal noises.

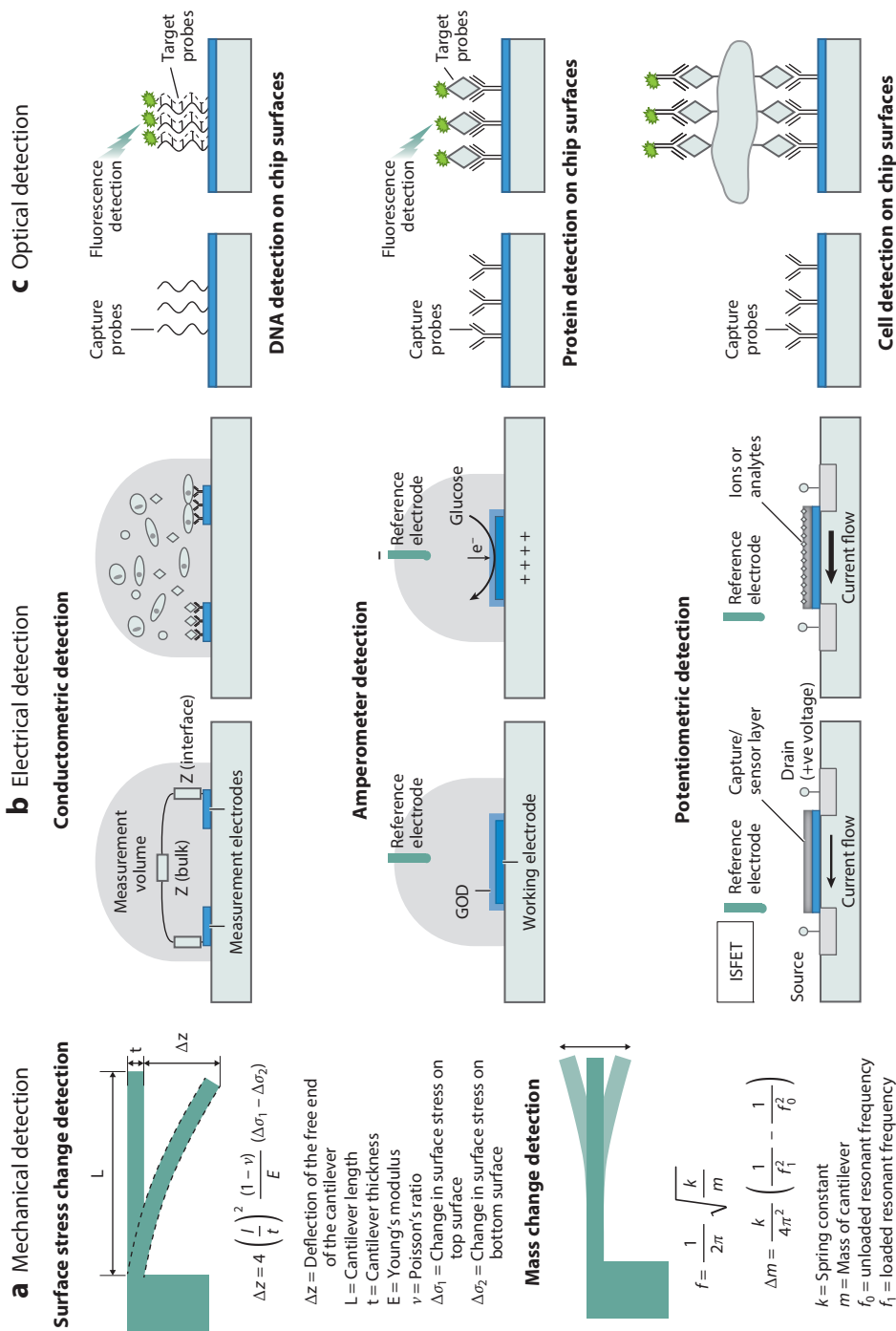
Bashir and colleagues have developed a microcantilever-based ultrasensitive pH sensor with a dynamic range of 5.9 to 6.5 and a sensitivity of  $5 \times 10^{-5}$  pH for a 1-nm deflection of the microcantilever tip (40, 41). Such pH sensors can accurately monitor acidification of culture medium with growth of pathogens in microbioreactors. Bashir et al. (41) surface micromachined low-stress silicon microcantilevers and decorated their surface with anti-*Listeria* antibodies and BSA. They then captured *L. innocua* on the microcantilever surface. Resonant frequency shifts showed adsorption of 59 pg of antibody and 166 pg of BSA as well as capture of 180 bacterial cells, each averaging  $\sim 85$  fg. Recently, Park et al. (42) used an array of microelectromechanical systems to develop resonant mass sensors resembling an oscillating pedestal that can be used to directly measure the biophysical properties, mass, and growth rate of single adherent cells. Unlike conventional cantilever mass sensors, these sensors retain a uniform mass sensitivity over the mammalian cell attachment surface. With these resonant sensors, researchers can develop mammalian cell-based pathogen sensors to monitor the health of the cell after exposure to a pathogen-containing sample. For a broad overview of microcantilever biosensors, we refer readers to a few reviews (43–45).

**4.2.2. Electrical detection.** Compared with bulky optical detection setups, electrical or electrochemical transduction strategies are more amenable to miniaturization concepts. Many reports have used enzymes such as horseradish peroxidase that produce electroactive compounds such as hydrogen peroxide during the conversion of their substrates. Brewster & Mazenko (46) showed rapid detection of *E. coli* O157:H7 by incubating pathogen with an enzyme-labeled antibody, capturing the enzyme-antibody-pathogen complex on a filter membrane, pressing the filter against an electrode surface, and then amperometrically detecting conversion of the substrate

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**Figure 2** Some commonly used transduction schemes implemented in micro/nanodevices for direct pathogen detection. Figure reprinted with permission from *Advanced Drug Delivery Reviews* (39).

(*para*-aminophenyl phosphate) to an electroactive product (*para*-aminophenol) via the enzymes attached to the pathogens. The sensor has an LOD of 5,000 cells/mL in an assay time of 25 min. Detection of lipopolysaccharides with concentrations as low as 50 ng/mL from *E. coli* O127:B8 was demonstrated amperometrically using immobilized diaphorase layer and ferrocenylboronic acid derivatives.

Potentiometric detection of microorganisms, similar to amperometry, involves the use of a permselective outer layer and a bioactive material, usually an enzyme. The enzyme-catalyzed reaction generates or consumes a species that is detected by an ion-selective electrode. Another approach is to use semiconductor-based ion-sensitive field-effect transistors. In contrast to the linear concentration dependence of amperometry, potentiometry provides logarithmic concentration dependence. Use of nanomaterials such as carbon nanotubes has allowed better ion-to-electron transduction in potentiometry, thereby linearizing the response to concentration. Zelada-Guillén et al. (47) showed detection down to 6 cells/mL of *E. coli* CECT-675 in milk samples using aptamer-immobilized carbon nanotube electrodes. Inclusion of electroactive polymers such as polyaniline also improved detection capability.

Impedance spectroscopy-based detection relies on attachment of the receptor layer between two electrodes, typically as interdigitated electrode arrays or in a point-and-lid arrangement, and application of a small AC excitation  $V(t)$ . The resulting current  $I(t)$  is measured. The ratio  $V(t)/I(t)$  at a particular frequency is defined as the impedance ( $Z$ ) of the cell. This measurement is repeated at different frequencies ( $\omega$ ), yielding  $Z(\omega)$ . The electrode-cell system behavior is then investigated by fitting experimental impedance data to an equivalent circuit model. Gómez et al. (48) provided the first demonstration of microscale impedance-based detection of bacterial metabolism using live *L. innocua*. They demonstrated the viability of a few bacterial cells in a 5.27-nL volume of fluid and detected ionic strength modification of a low conductivity medium due to bacterial metabolism. Gómez et al. (49) later used the same microdevice to show that it is possible to differentiate between live and heat-killed cells for as few as 100 *L. innocua*, 200 *L. monocytogenes*, and 40 *E. coli* cells. They also integrated a dielectrophoretic preconcentrator to concentrate bacterial cells from a dilute sample, by factors on the order of  $10^4$  to  $10^5$ , and detected their metabolic activity via impedance spectroscopy.

**4.2.3. Optics embedded fluidics.** Real time polymerase chain reaction using a fluorescent DNA intercalating dye is a classical example of pathogen DNA detection demonstrated in micro/nanodevices. Bhattacharya et al. (50) presented a novel, glass-silicon chip platform for the dielectrophoretic preconcentrator capture of bacterial cells and their identification using PCR. Micro/nanodevices also offer the advantage of carrying out fluorescence-based DNA, protein, or cell detection (**Figure 2**) directly on a light-sensitive charge-coupled device (CCD), thereby increasing the sensitivity of the assay (51, 52). Alternatively, fiber optics can be integrated into microfluidic channels to carry out macroscale assays including agglutination, fluorescence, Förster resonance energy transfer, and Raman spectroscopy in a confined low volume. The attachment of target molecules changes the local index of refraction, thus changing the resonance conditions of the surface plasmon waves. Taylor et al. (53) demonstrated the first immunoassay using SPR, and such work has been widely replicated for pathogen detection.

## 5. SCATTERING AND SPECTROSCOPY

### 5.1. Light Scattering

Biosensors that do not require a labeling reagent or probe for interrogation of target analyte are highly desirable because they can allow real-time high-throughput screening and are amenable

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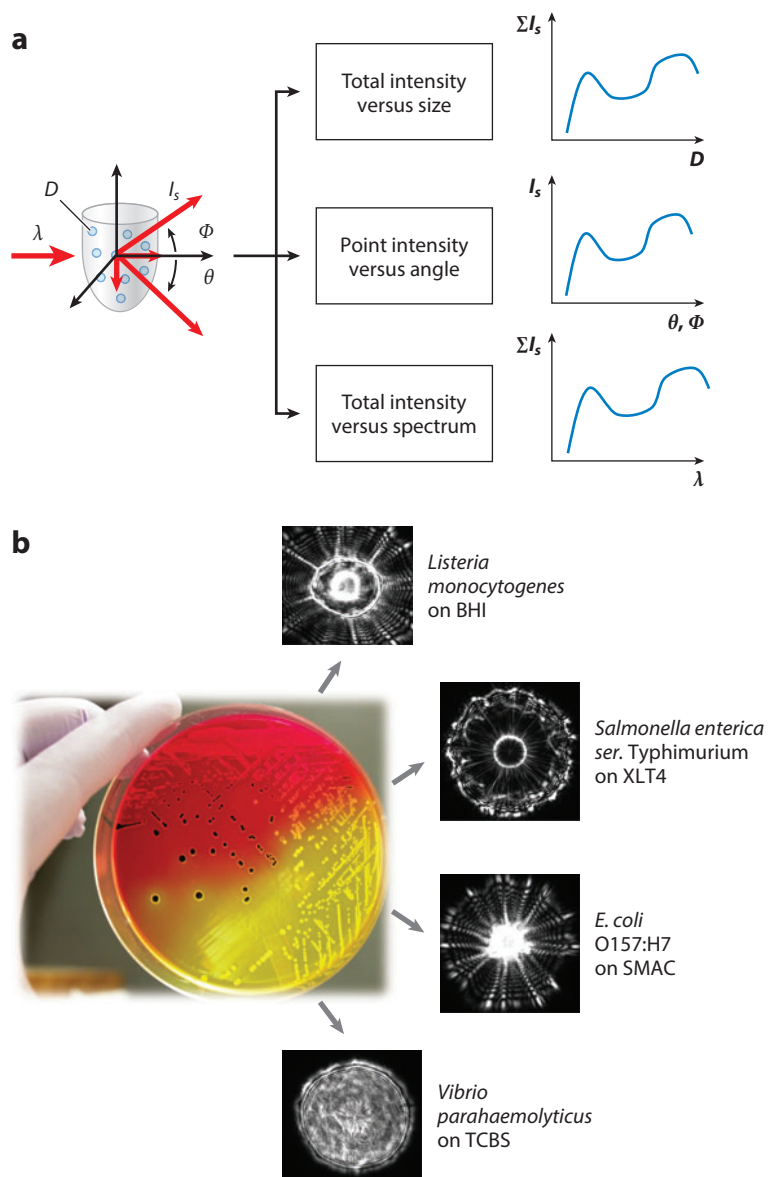


to automation (54). Such platforms include spectral-based elastic light scattering (ELS) sensors and inelastic sensors such as Fourier transform infrared (FT-IR) and Raman spectroscopy. FT-IR spectroscopy is discussed in the next section while a detailed account of Raman spectroscopy and its applications in food is provided by Irudayaraj and coworkers (55). Additionally, hyperspectral imaging (imaging spectrometry) is also receiving increasing attention due to its ability to monitor abnormality or contaminants on food surface in real-time (56, 57). Hyperspectral imaging spectrometry has the ability to generate spatio-spectral map of an object by collecting the spatial intensity information across many electromagnetic spectra. These label-free optical sensors use a spectral library to identify the analyte; thus, it is possible to generate a comprehensive library to cover a broad range of pathogens and toxins of concern. ELS measures the spatial distribution of the scattered light from an object and is captured by a photodetector, PMT (photomultiplier tube), or CCD. A PMT has high gain amplification capacity and thus performs well as a point detector, whereas a CCD collects the two-dimensional spatial distribution of scattered light. The signal strength of ELS sensors is very high compared with other spectroscopic or inelastic techniques and allows instantaneous signal measurement. They have been used widely in the semiconductor industry for wafer inspection and in biology to investigate microbial and eukaryotic cellular properties. **Figure 3a** illustrates the principles of an ELS system. Depending on the instrumental design, ELS can be used to detect a single cell in aqueous suspension or bacterial colonies, which consist of millions to billions of single cells on a solid agar surface.

A novel light scattering device called BARDOT (bacterial rapid detection using optical scattering technology) that utilizes a 635-nm laser and a CCD detector was developed to capture two-dimensional scattering patterns from bacterial colonies. In BARDOT, the wavefront modification of the incoming wave by bacterial colony exerts individual and accumulative effects resulting from the morphological (shape, size, thickness) and composition (refractive indices) properties to produce a unique scattering pattern, which is then stored in an image library. Zernike polynomials and a more advanced support vector machine are used to classify and discriminate scatter patterns for automated identification (58). A Bayesian approach based on Wishart priors was also employed to identify previously unclassified bacteria (59).

BARDOT is discriminatory for bacteria belonging to different genera such as *Listeria*, *Salmonella*, *Escherichia*, *Vibrio*, and *Staphylococcus* (60). It can also differentiate bacteria at the species level (see **Figure 3b**). For example, *L. monocytogenes* scatter patterns are distinct from those of other species within the genus, thus providing a unique nonredundant scatter signature for each species (61). Similarly, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *V. cholerae* scatter patterns can be separated from each other or other species within the genera (62). More recently, BARDOT's ability to discriminate different serovars belonging to a species has also been explored. BARDOT-based discrimination of bacteria is possible because of the distinct genetic and phenotypic makeup of each bacterial species and of serovars (defined by different antigenic properties) and their utilization of growth substrates in solid agar media. Nutrient utilization and the resulting metabolic by-products coupled with their cellular morphology contribute to their scatter signatures. Extracellular polysaccharide appears to have a greater influence on scatter signatures than do the proteins (60).

Bacterial growth on solid surfaces results in the formation of colonies, and thus growth rates may vary depending on the bacterial genera/species. Because a colony diameter of  $1.0 \pm 0.2$  mm is needed for analysis, fast-growing bacteria such as *Bacillus* can be detected in fewer than 8 h when a desired colony size is achieved; by contrast, *E. coli*, *Salmonella*, *Vibrio*, and other gram-negative bacteria can be detected in fewer than 16 h and gram-positive *Listeria* in 24–48 h. Designed for use with culture-based methods, BARDOT is considered the gold standard for pathogen detection: Detection is possible in a much shorter time (within 12–24 h in most cases). It has been used for



**Figure 3**

(a) Diagram depicting principles of elastic light scattering sensor. When incident light ( $\lambda$ ) impinges on a particle with diameter  $D$ , scattered light ( $I_s$ ) will spread out in a hemispherical direction ( $\theta$  and  $\Phi$ ). Scattered light intensity can be measured and plotted against  $D$ ,  $\theta$  and  $\Phi$ , or  $\lambda$  to retrieve sample information.

(b) Scatter signature patterns of select bacterial pathogens. Abbreviations: BHI, brain-heart infusion agar; SMAC, sorbitol MacConkey agar; TCBS, thiosulphate citrate bile salts sucrose agar; XLT4, xylose lysine tergitol-4 agar.

pathogen testing from inoculated or real-world food samples and is highly sensitive with an LOD of 1 cfu/g/mL of food products. BARDOT could also be useful for screening clinical specimens for pathogens to aid in clinical medicine and in studies of bacterial communities in food products or in the environment. Scattering is one of several optical methods. Unlike scattering, spectroscopy produces unique signatures based on the vibrational modes of the chemical groups of the organism. In this context, IR spectroscopy and Raman spectroscopy are discussed.

## 5.2. Fourier Transform Infrared Spectroscopy

IR spectroscopy techniques generate spectra (absorbance or transmission) of molecules excited by IR radiation at specific wavelengths. The IR region ( $10\text{--}12,500\text{ cm}^{-1}$ ) of the electromagnetic spectrum is divided into three regions: near, mid, and far. Of these, the mid-IR region ( $400\text{--}4,000\text{ cm}^{-1}$ ) is the most commonly used for bacterial analysis because all molecules possess characteristic absorbance frequencies and primary molecular vibrations in this wavelength range. Therefore, mid-IR spectra of bacterial cells can theoretically be used to analyze their total composition (including proteins, fatty acids, carbohydrates, nucleic acids, and lipopolysaccharides) and to identify or classify bacteria. With the advent of FT-IR spectroscopy and computer-assisted computational analysis in the late 1980s and 1990s, Naumann and coworkers (63, 64) reintroduced FT-IR methods for in-situ analysis of bacterial cells and complex spectral analysis to identify, differentiate, and classify bacteria. Since then, the use of FT-IR techniques coupled with different chemometric analyses have been expanded and successfully applied to detect, discriminate, identify, and classify bacteria belonging to different species (65, 66), including foodborne pathogens such as *Listeria* (67), *E. coli* (68–72), and *Salmonella* (73–75). Near-IR spectroscopy ( $4,000\text{--}12,500\text{ cm}^{-1}$ ) has also been applied to the compositional analysis and detection of bacteria (76, 77), although to a lesser extent than mid-IR spectroscopy owing to the inherent limitations of the small absorptivity and broad molecular overtone and combination vibrations that occur in the near-IR region.

Functional groups present in a molecule tend to absorb IR radiation in the same wave number range regardless of other structures in the molecule, and spectral peaks are derived from the absorption of bond vibrational energy changes in the mid-IR region. Thus, there is a correlation between IR band positions and chemical structures in the sample. A summary of the wave number regions in which common functional groups in bacteria absorb IR radiation is presented in **Table 1**. The molecular composition of bacteria varies from species to species and even at strain levels; therefore, each bacterium can be identified by its unique and characteristic spectrum in which both peak positions and peak intensities provide useful information (63).

Detecting pathogenic bacteria in food using FT-IR has been done using direct and indirect methods, although the indirect approach is most common. In a direct method, IR spectra of the contaminated food are collected directly from the sample and compared with spectra of an uncontaminated sample. If successful, direct analyses could enable near real-time food analysis because spectral acquisition requires at most 5 min. The presence of a new absorbance peak and/or a change in peak intensity from the baseline spectra of an uncontaminated sample may indicate the presence of a pathogen. For example, studies have differentiated and quantified various species of bacteria from an apple juice matrix using an FT-IR attenuated total reflectance method (78). In this study, eight bacteria were inoculated into the juice, the juice was pipetted directly onto an attenuated total reflectance crystal, and the spectrum of each sample was collected after 2 min. Raw spectra were processed using a vector algebra-based algorithm to remove the juice background contribution to the spectra and further processed using chemometrics to differentiate and quantify the microorganisms in the juice. For seven of the eight bacteria, excluding *Proteus vulgaris*, quantification of the bacteria ( $10^3\text{--}10^8$  cfu/mL) was consistent with standard plate counts (78).

**Table 1 The major vibration bands in the mid-infrared spectra of bacteria and their related functional groups**

Wave number (cm <sup>-1</sup> )	Molecular vibrations of functional groups and the biomolecule contributor
3,200	N–H stretching of amide A in proteins
2,955	C–H asymmetric stretching of –CH <sub>3</sub> in fatty acids
2,930	C–H asymmetric stretching of >CH <sub>2</sub> in fatty acids
2,898	C–H stretching of ≥C–H of amino acids
2,870	C–H symmetric stretching of –CH <sub>3</sub> in fatty acids
2,850	C–H symmetric stretching of >CH <sub>2</sub> in fatty acids
1,740	>C=O stretching of lipid esters
1,715	>C=O stretching of ester, in nucleic acids and carbonic acids
1,695–1,675	Amide I band components of proteins
1,655	Amide I of α-helical structures of proteins
1,637	Amide I of β-pleated sheet structures of proteins
1,550–1,520	Amide II band of proteins
1,515	Tyrosine band
1,468	C–H deformation of >CH <sub>2</sub> in lipids and proteins
1,415	C–O–H in-plane bending in carbohydrates, DNA/RNA backbone, and proteins
1,400	C=O symmetric stretching of COO– group in amino acids and fatty acids
1,310–1,240	Amide III band components of proteins
1,240	P=O asymmetric stretching of phosphodiester in phospholipids
1,200–900	C–O–C, C–O dominated by ring vibrations in various polysaccharides
1,085	P=O symmetric stretching in DNA, RNA, and phospholipids
720	C–H rocking of >CH <sub>2</sub> in fatty acids and proteins
900–600	“Fingerprint region”

However, in many cases the spectra collected in a direct method contain a complex spectral background owing to the presence of food that interferes with spectral interpretation for pathogen identification. Indirect detection reduces or eliminates this spectral background from food by using a bacterial separation step such as filtration or immunomagnetic separation prior to spectral acquisition (73, 79, 80). Filtration of growth media or fruit juices through a filter with 0.45-μm pore size has enabled the capture of inoculated *E. coli* or *Salmonella* bacteria on a surface suitable for further analysis using FT-IR (79). Solid foods require additional sample handling steps prior to the final bacteria capture filtration: For example, liquid media may need to be added and a solid surface may need to be washed, as occurs in a chicken rinse water from chicken inoculated with *Salmonella enterica* (73). Alternatively, liquid media may need to be added, a step followed by stomaching with, perhaps, a multistep filtration with decreasing pore sizes to remove food particulates prior to capturing the target bacteria, as was done with ground beef inoculated with *E. coli* O157:H7 (80). Subsequent analysis of spectra collected from filter surfaces proved useful for identifying contamination if the bacteria were present at sufficient levels. In these approaches, the LOD for pathogens varied between 10<sup>3</sup> and 10<sup>6</sup> cfu/mL, depending on the type of food and method used to analyze the spectra.

Bacterial viability determination is a major concern in the food industry because injured bacteria pose a significant health threat if they revive during food distribution and storage. Thus, it is also important to examine the efficacy of various intervention treatments. The majority of conventional microbiology methods cannot give an accurate measure of both live and dead cells. Because FT-IR methods are based on an analysis of the biochemical composition of cells, they may be used to



differentiate live and dead cells. To that end, FT-IR methods have been used to differentiate live and heat-killed *E. coli* O157:H7 in ground beef (68). Differences in the spectra of live and dead cells are very small and occur mainly in the amide and nucleic acid regions, likely owing to heat-induced denaturation of these biomolecules in the killing step. For discrimination studies, second derivative spectra were generated to increase the number of discriminative spectra features, and PCA (perchloric acid) was used to successfully classify live and dead *E. coli* O157:H7 (68). Another study used FT-IR spectroscopy to differentiate not only live and dead *E. coli* O157:H7, but also cells subjected to various inactivation treatments including heat, salt, ultraviolet light, antibiotics, and alcohol (69). Partial least-squares analysis of the spectra quantified live *E. coli* O157:H7 in the presence of dead cells with an  $R^2 > 0.996$ , and canonical variate analysis differentiated between spectra of 1% live cells: 99% dead and 100% dead cells. Treatment-induced changes in the 1,800–900  $\text{cm}^{-1}$  spectral region enabled clear separation in Cooman plots between the clusters of spectra of bacteria exposed to the different inactivation treatments, which may be used to assess the degree of cell damage caused by each treatment (69).

Owing to overlapping absorbance bands, FT-IR spectra of intact bacterial cells often cannot provide complete information of specific cellular components. The spectra of bacteria may also change, depending on the growth medium and culture conditions. FT-IR techniques were used to gain insight into bacterial cell structures and surface components before the pioneering study of FT-IR characterization of bacteria (81), and later studies reported the discrimination of bacteria on the basis of various cellular components. For example, spectra of fatty acid methyl esters isolated from 14 gram-positive and gram-negative foodborne pathogens were used for species- and strain-level identification. FT-IR spectra of outer membrane proteins of *Salmonella* were used to differentiate between six *S. enterica* serovars (74). Spectra of crude lipopolysaccharide extracts from *E. coli* and *S. enterica* were used for serotype-level differentiation with 95–100% correct classification (70).

Given the importance of tracking outbreaks of foodborne illness and the emergence of new virulent subtypes of foodborne pathogens, rapid and reliable subtyping methods are needed. FT-IR spectra represent phenotypic fingerprints of bacteria that may enable the differentiation of bacteria at different taxonomic levels. FT-IR spectroscopy has been used successfully to identify and classify different microorganisms at the species, subspecies, and strain levels on the basis of IR absorbance patterns of whole bacteria (63) or cell components such as proteins and lipopolysaccharides (70, 74). FT-IR spectroscopy has been applied to the taxonomic study of *Listeria* genera for species identification (82) as well as for serotype and haplotype identification (67, 83). The use of FT-IR techniques to subtype and identify *E. coli* O157:H7 strains with varying virulence and genetic relatedness has had comparable accuracy to that of multilocus variable number tandem repeat analysis and requires <16 h to obtain results (72).

## 6. PHAGE-BASED METHODS

Viruses that infect bacteria are called bacteriophages (phages). Phage surface proteins are responsible for the selective attachment to the host, thereby determining which bacteria are susceptible to that phage (host range) (84). A significant advantage of using phage-based detection methods over most molecular techniques such as PCR and ELISA (enzyme-linked immunosorbent assay) is that they can propagate only in live bacteria, thus reducing the chance of false positives (85). Phage proteins also have similar binding properties to those of antibodies but are inexpensive and easy to produce and have greater stability. Phages have two types of life cycles: lytic (virulent) or lysogenic (temperate) (86). Lytic phages lyse the host cell after using the host cell's machinery to multiply. Lysogenic phages can insert a copy of their DNA into the host genome (prophage) to

be replicated but will not produce phage particles unless induced. Lysogenic phages can be used to transfer exogenous genes to the host genome. The strength and specificity of phage binding to the host cell has been exploited in several detection methods. After attachment to the host bacteria surface and injection of the phage genetic material into the host, phages take over the host cellular machinery to amplify their proteins and genome. Exogenous genes that produce detectable traits can be added to phages, which are produced in the host along with the phage genes. These modified phages are called reporter phages (87).

### 6.1. Direct Use of Phages

Phages can be labeled in different ways to simplify the detection of the host cell once the phage is bound. Various fluorescent dyes have been used to label phage DNA, which can be detected via flow cytometry or visualized via fluorescent microscopy. Goodridge et al. (88) detected 2 cfu/g of *E. coli* O157:H7 in ground beef and 10 cfu/mL in raw milk using the fluorescent dye YOYO-1 to stain the DNA of phage LG1. Phages can also be labeled using fluorescent proteins (89). Quantum dots can be used to enhance the detectability of labeled phages (90, 91). Edgar et al. (90) detected 10 cfu/mL *E. coli* using phage T7 expressing biotin peptides in conjunction with quantum dots functionalized with streptavidin. Injection of the phage genome into the host results in the release of  $\sim 10^8$  ions per cell into the surrounding environment. The small change in voltage from these ions can be detected with the SEPTIC (sensing of phage-triggered ion cascade) assay and correlated to the number of bacteria present. This approach exploits the initial stages of infection by any phage (lytic, lysogenic, wild type, or engineered) and does not require incubation. An LOD of  $10^7$  cfu/mL of *E. coli* has been demonstrated, but the authors hypothesized that with further improvements an LOD of 1 cfu/mL is possible (92, 93).

Phages can be used in place of antibodies in SPR-based detection. SPR detects changes in the refractive index of a probe when a ligand binds to its surface. Balasubramanian et al. (94) used phage 12600 to detect  $10^4$  cfu/mL of *S. aureus* with the SPR-based SPREETA sensor. SPR-based bioassays using a phage as the biorecognition element have also been used to detect other bacteria, e.g., *E. coli*, *L. monocytogenes*, and methicillin-resistant *S. aureus* (95–97). These studies have demonstrated LODs ranging from  $7 \times 10^8$  to  $7 \times 10^2$  cfu/mL. Magnetoelastic biosensors detect a change in resonance caused by a change in mass that can arise from the binding of a target pathogen to a phage on the sensor surface. Different filamentous phages engineered to selectively bind specific pathogens have been used on magnetoelastic biosensors in various studies to detect *S. typhimurium* (98–102) and *Bacillus anthracis* spores (98, 103), reaching LODs as low as  $5 \times 10^2$  cfu/mL.

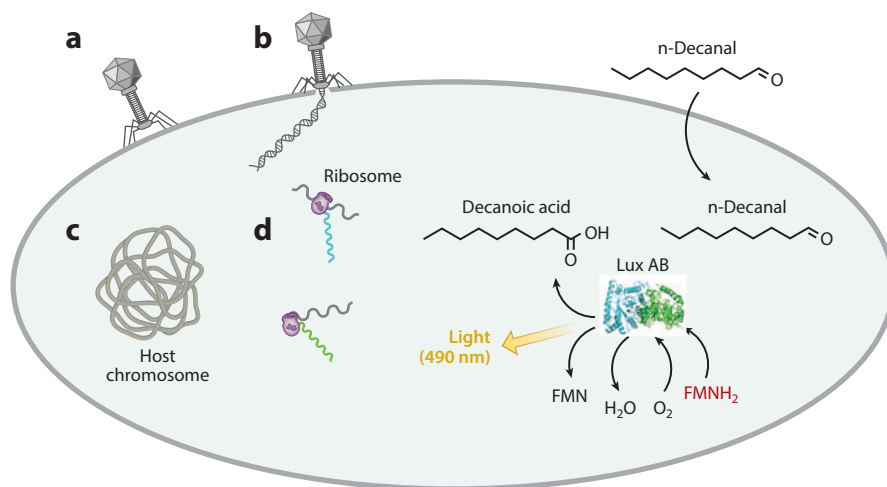
The binding of *E. coli* K-12 to T4 phages immobilized on screen-printed carbon electrode microarrays causes a change in impedance in the chip, thereby allowing the detection of  $10^4$  cfu/mL (104). The cell-binding domain of a *Listeria*-specific phage endolysin has also been used to functionalize a screen-printed carbon electrode, thus enabling the detection of  $10^5$  cfu/mL in 2% milk and  $1.1 \times 10^4$  cfu/mL in culture media (105). Acoustic wave biosensors have also detected changes in resonance frequency when a target binds to the biosensor surface. Olsen et al. (106) used filamentous phages specific for *S. typhimurium* on the probe of an acoustic wave biosensor to detect  $10^2$  cfu/mL *S. typhimurium*.

### 6.2. Phage Replication and Metabolism-Based Methods

As noted above, genetically modified phages that produce a detectable marker (e.g., a specific analyte) are called reporter phages.  $\beta$ -galactosidase encoded by the *lacZ* gene can catabolize a colorimetric, bioluminescent, chemiluminescent, or fluorescent reaction, depending on the

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**Figure 4**

Infection of a bacterium by a reporter phage results in the production of an easily detectable product. A widely used reporter is bioluminescence. Once a reporter phage that is engineered to carry the *luxAB* genes infects the target host pathogen, it will use cellular resources to produce the heterodimeric Luciferase (LuxAB; PDB:1LUC). The oxidation of an externally added long-chain aldehyde (e.g., n-decanal) results in the production of light at 490 nm. The specificity of the phage ensures that light is produced only in the presence of the target pathogen.

exogenous substrate used. Small gene size and easy visual detection are the main advantages of *lacZ*, but the natural occurrence of *lacZ* in some bacteria can cause false positives. Goodridge & Griffiths (107) a *lacZ* T4 reporter phage to achieve an LOD of 100 cfu/mL of *E. coli*. Green fluorescent protein (GFP) is another small reporter gene with many variants that can be added to phage proteins. GFP will autofluoresce when excited by ultraviolet light. Funatsu et al. (108) were the first to use a GFP reporter phage to detect *E. coli* by microscopy. Some bioluminescent reporter genes can also produce light. The 2.3 kbp *luc* gene from fireflies (*Photinus pyralis*) encodes luciferase that can catalyze the conversion of D-luciferin to oxyluciferin to produce light at 560 nm. Light production is usually triggered by the exogenous addition of D-luciferin. Luc reporter phages have been used for *Mycobacterium tuberculosis* detection, thus reducing the detection time to a few days instead of weeks (87). In addition, *luxAB* genes from bacteria (e.g., *Photobacterium luminescens* and *Vibrio fischeri*) can produce light at 490 nm (Figure 4) (109, 110). The use of very sensitive light detectors, e.g., CCD or PMT, and the lack of any natural bioluminescence make this a valuable reporter with LODs as low as 0.1 cfu/g (109, 111). *luxAB* reporter phages have been used to detect many bacteria including enteric bacteria (112), *E. coli* O157:H7, *L. monocytogenes* (109, 111), *S. enterica* (113), *S. typhimurium* (114), *Yersinia pestis* (115), and *B. anthracis* (116).

### 6.3. Phage Amplification–Based Assays

Once phages infect the host, they replicate in 1–2 h and produce a large number of progeny phages that can be detected and measured directly or indirectly. Phage amplification indicating the presence of the host bacteria can be detected using high-performance liquid chromatography, as demonstrated by Hirsh & Martin (117, 118). They detected the amplification of the *Salmonella*-specific phage Felix-O1 (117, 118). Detecting phage DNA using quantitative PCR circumvents the possibility of false positives from dead bacteria DNA. PCR amplification of phage DNA has

been used to detect several pathogens such as *B. anthracis* (119–121) and *Y. pestis* (119, 122, 123). MALDI-TOF mass spectrometry can also be used to detect the amplification of phage components in the presence of host bacteria in a sample. This is a sensitive method that has been used to detect various pathogens, e.g., *E. coli* and *S. enterica* via phages MS2 and MPSS-1, respectively (124).

#### 6.4. Phage Lysis–Based Methods

Each bacterial cell contains a consistent amount of adenosine-5'-triphosphate (ATP), which along with cellular enzymes (e.g., adenylate kinase) and other molecules gets released into the environment when phages lyse the cell. Using luciferase to detect light produced correlates to the ATP present and number of bacteria specifically lysed by the phage (125, 126). Addition of external adenosine diphosphate to be converted to ATP by the released adenylate kinase also enhances the assay sensitivity. Wu et al. (127) used AT20 and SJ2 phages to detect  $10^3$  cfu/mL of *E. coli* and *S. enteritidis*, respectively, in 2 h. The lysis of host cells by phages releases into the environment enzymes and other cytoplasmic components that can cause detectable electrochemical changes. Various approaches based on electrochemical changes have been used to detect bacteria including *E. coli* O157:H7 (128), *Listeria* (105), *B. cereus*, and *Mycobacterium smegmatis* (129).

### 7. CONCLUSIONS

In this review, we attempt to provide background on sample preparation, which is critical to sensor development. We also expound on five key sensing modalities for pathogen detection: spectroscopy (FT-IR and Raman, including SERS), light scattering, phage display, micro/nanodevices, and nanoparticle biosensors. Each method has its own intrinsic advantages that improve analytical sensitivity and proposed potential for onsite sensing. The universal challenge among all detection systems remains the complexity and importance of sample preparations that currently rate limit the undeniable potential of sensor-based systems. Although significant improvements in instrumentation have occurred owing to advances in the design and development of the components, the design of field-deployable biosensors has not been integrated with sample preparation. However, this presents exciting opportunities for the development of instrumentation with multimodal sensing compartments that can report on the presence of pathogens. These units can also be integrated with a sample preparation module to realize a hybrid instrument that not only reports but also cross-validates findings. Given the infusion of smart devices and a range of detector options, products or product-specific detection devices are more likely and easily adopted than a sensor that functions equally well in all samples. Together with advancements in sample preparation, developments in sensor platforms based on spectroscopy, molecular biology, and nanotechnology will offer sensitive, specific, cost-effective, and robust pathogen-detection platforms that perform in the lab, in the field, and across food matrices. These systems are paramount to achieving the common goal of industry and regulators to prevent foodborne disease through detection.

### DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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