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Review

Optical Biosensors for the Detection of Pathogenic Microorganisms

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Pathogenic microorganisms are causative agents of various infectious diseases that are becoming increasingly serious worldwide. For the successful treatment of pathogenic infection, the rapid and accurate detection of multiple pathogenic microorganisms is of great importance in all areas related to health and safety. Among various sensor systems, optical biosensors allow easy-to-use, rapid, portable, multiplexed, and cost-effective diagnosis. Here, we review current trends and advances in pathogen-diagnostic optical biosensors. The technological and methodological approaches underlying diverse optical-sensing platforms and methods for detecting pathogenic microorganisms are reviewed, together with the strengths and drawbacks of each technique. Finally, challenges in developing efficient optical biosensor systems and future perspectives are discussed.

Trends

Rapid and accurate pathogen diagnosis is important for saving lives from infection.

Various optical sensors have been developed for the detection of pathogens.

Microfluidic-integrated optical sensors are useful for point-of-care diagnostics.

Smartphone-based optical sensors provide a simple user interface for rapid sensing at reduced cost with ubiquitous capabilities.

Improvement is needed for more rapid, accurate, and multiplex sensing of pathogens.

Pathogenic Microorganisms as Causative Agents of Infectious Diseases

Pathogenic microorganisms cause various infectious diseases and even death. Despite early triumphs over infectious diseases with the development of vaccines and antibiotics, new and multidrug-resistant pathogens are continuously emerging [1]. Furthermore, current pathogen-diagnostic methods are inefficient and slow, especially in resource-limited regions, which remain hard pressed for appropriate solutions. Thus, there is a greater need for developing faster, more accurate, and multiplex diagnostic methods that do not require complicated and expensive assay steps.

Conventional culture-based assays, the gold standard method, are inherently time consuming and labor intensive. This problem is compounded by the expanding spectrum of pathogens, which markedly reduces the sensitivity of culture-based systems for detecting pathogens, causing serious healthcare problems throughout the world [2–4]. Thus, successful pathogen-diagnostic systems with enhanced multiplex capacity, sensitivity, selectivity, speed, and cost-effectiveness need to be developed to overcoming these problems.

Biosensors integrated with nanobiotechnology offer several advantages over conventional methods, including high-throughput screening, low limit of detection (LOD), real-time analysis, label-free detection, and the small sample volume required, among others. As nanobiotechnology advances rapidly, various types of binding receptors and ligands, physicochemical methods, and nanoplatforms have been exploited, creating novel strategies for enhancing detection performance. These biosensors hold great promise for addressing the analytical needs in practical pathogen diagnostics, as described below.

Various pathogen-detecting biosensors have been developed by using electrical [5], electrochemical [6], mechanical [7], nuclear magnetic resonance (NMR) [8], and optical-sensing [9]

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methods. Among these, optical sensors, especially colorimetric sensors, allow easy-to-use, rapid (within 15 min), portable, and cost-effective diagnosis. Additionally, plasmonic biosensors, which are also optical biosensors, offer superior sensitivity and multiplexing capability. These distinct advantages have led to the development of several ingenious optical sensors, some of which are currently available on the market (Table 1). Furthermore, optical sensors integrated with microfluidic system are under active commercial development, and some are ready to be launched on the market by several companies, such as LAMDAGEN. Furthermore, much effort has been exerted to develop smartphone-based system that have both a light source and a light detector, providing a simple user interface for rapid sensing at reduced cost. Thus, it can be envisioned that optical sensors will have increasingly important roles in pathogen diagnostics and point-of-care (POC) monitoring under various clinical and environmental settings.

Here, we review recent advances in optical biosensors for pathogen diagnosis, especially colorimetric and plasmonic sensors, and introduce technologies and strategies that constitute those optical biosensors with such sensing performances. Recent examples of optical biosensors are reviewed and their advantages and limitations are discussed. Finally, future perspectives on enabling strategies for the development of desirable biosensors with rapid, accurate, and multiplexing capability are provided. All the examples of optical biosensors, including those described here together with others that have been developed and used for the detection of pathogenic microorganisms are summarized in Table 2. Their advantages and limitations, together with strategies to overcome the latter, are summarized in Table 3.

Optical Biosensor: A Trend Toward POC Pathogen Detection

An ongoing trend in the area of pathogen diagnostics is the development of biosensor for POC testing. Some of the driving forces for developing POC systems include the increasing need for a patient-centered medical system and for more convenient, inexpensive, and efficient diagnostic systems applicable in underdeveloped and developing countries, and to eliminate the need for sample transportation for medical and environmental monitoring. Also, developing POC systems has become more feasible thanks to recent advances in technologies related to the integration of microfluidics and optics, the miniaturization of devices and communication, and the advent of simplified fabrication technologies, among others. The key advantages of POC biosensors include reduced test and therapeutic turnaround times resulting from immediate diagnostic test results, the reduction and/or elimination of sample transport, and reduced sample volume and data management, and easy integration with information processing [10]. Thus, the successful establishment of POC biosensors would enhance patient survival and treatment outcomes through rapid decision-making at the hospital bedside, in ambulatory care settings (including clinics and physician offices), and for acute care in emergency rooms. Also, applications can be easily extended to food, beverage, and drinking water safety testing [10–12]. It is desirable to develop POC sensors that allow rapid, label-free, multiplexed detection with high sensitivity and specificity, thereby improving healthcare through real-time and remote monitoring [10–12]. Various components for the development of advanced optical biosensors are illustrated in Figure 1. The following sections highlight some of the important optical biosensors that have been applied to pathogen detection. Also, recent trends in the integration of optical biosensors with microfluidics are described.

Current Optical Biosensors for Pathogen Diagnostics

Colorimetric biosensors are an attractive optical biosensor system because one can easily and instantly observe with the naked eye the presence of pathogenic microorganisms in the sample through a color change without the need for any analytical instrument. Colorimetric biosensor can be divided into two system formats: flat substrate based and solution based. Flat substrate-based sensors that generally use paper and glass are favored for their simple use and small volume of sample analyzed. As a representative example, some lateral flow assay (LFA)-based

Table 1. Examples of Commercially Available Optical Sensors (Systems) for the Detection of Pathogenic Microorganisms[a]

Manufacturer	Assay	Detection Limit	Target	Assay Time	Sample Type	Note	Website
Creative Diagnostics	Colorimetry	NI	*Campylobacter*, *Escherichia coli* O157: H7, *Legionella pneumophila*, *Salmonella*, *Treponema pallidum*, *Streptococci* Group B	10 min	Feces, stool, urine, vaginal swab	LFA-based sensor; Ag-Ab interaction; cassette or card type	http://www.creative-diagnostics.org/
bioMérieux	Colorimetry	NI	*Streptococcus* sp.	5 min	Throat swab	LFA-based sensor; Ag-Ab interaction; cassette or dipstick type	http://www.biomerieux-diagnostics.com
			Legionella pneumophila	15 min	Urine	LFA-based sensor; Ag-Ab interaction; cassette type	http://www.biomerieux-diagnostics.com
DuPont	Colorimetry	1 CFU/assay	*E. coli* O157, *Salmonella*, *Listeria*	15 min	NI	LFA-based sensor; Ab-whole cell interaction; (optional) enrichment step (+8 h assay time).	http://www.dupont.com
Abaxis	Colorimetry	NI	*Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*	10 min	Whole blood, serum, plasma (canine)	LFA-based sensor	http://www.abaxis.com
Micro Identification Technologies	Light scattering	10–50 CFU/assay	*E. coli*, *Cryptosporidium*, *Giardia*	10 min	Cells from colony	Analysis of reflection pattern of incident laser light from the outer surface of bacterium and penetrates body of bacterium	http://www.micro-imaging.com
rap.ID	Raman Spectroscopy	1 CFU/assay	Bacteria, yeast	3–10 min	Cells from colony, liquid medium, product/raw material, surfaces	Analysis of fingerprint spectra of vibrations of pathogenic cell	http://www.rap-id.com
Battelle	Raman spectroscopy	1 CFU/assay	*Alcaligenes*, *Pseudomonas*, *Brevundimonas*, *Candida*, *E. coli*, *Bacillus*, *Ralstonia*	3 min	Cells from colony, liquid medium, product/raw material, surfaces	Analysis of spectral signature of bacterial cells.	http://www.battelle.org

[a]Abbreviations: Ag, antigen; Ab, antibody; CFU, colony forming unit; LFA, lateral flow assay; NI, not identified.

Table 2. Examples of Optical Biosensors for the Detection of Pathogenic Microorganisms[a]

Substrate	Receptor	Analyte	Target Pathogen(s)	Signal Amplification method	Sensing Approach	Assay Time	Detection Limit	Evaluation of Practical Samples	Remarks	Refs
Paper	DNA	RNA	*E. coli*	NA	Colorimetry	<25 min	5.0×10^4 CFU/ml	NA	LFA	[13]
	Bacteria-specific enzyme (i.e., β-galactosidase for *Escherichia coli*; PI-PLC for *Listeria monocytogenes*; esterase for *Salmonella enterica*)	Lysed cell	*E. coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*	NA	Colorimetry	8–12 h	10 CFU/cm^2	Bacteria spiked into ready-to-eat meat samples	PAD; requirement for enrichment step	[17]
	Antibody	Whole cell	*E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*	NA	Colorimetry	NA	5.0×10^2~5.0×10^3 CFU/mL	NA	LFA; multiplex mode with 3-test strips	[82]
	Antibody	Whole cell	*E. coli*	Pre-concentration step using magnetic separation	Colorimetry	30 min	5 CFU/mL	NA	PAD	[14]
	Aptamer	Whole cell	*E. coli*, *L. monocytogenes*, *S. enterica*	NA	Colorimetry	NA	3.0×10^2 CFU/assay	NA	LFA; use of quantum dot instead of Au-NP	[83]
Glass slide	DNA	DNA	57 pathogenic bacteria and fungi[b]	NA	Fluorescence	10 h	10~1.0×10^3 CFU/assay	Clinical isolates from blood culture specimen	Identification of largest number ($n = 57$) of microbial pathogens, including bacteria and fungi	[3]
	DNA	DNA	50 pathogenic bacteria[c]	NA	Fluorescence	18 h–19 min	NA	Blood samples from patients with clinically	Application of largest number ($n = 3318$) of clinical samples	[4]

Table 2. (continued)

Substrate	Receptor	Analyte	Target Pathogen(s)	Signal Amplification method	Sensing Approach	Assay Time	Detection Limit	Evaluation of Practical Samples	Remarks	Refs
								suspected sepsis		
	Ag NP	Whole cell	*E. coli*	NA	SERS	NA	2.5×10^2 CFU/mL	NA	Use of reducing agent for synthesis of Ag-NP on cell wall of bacteria	[34]
Polymer	Chemically responsive dye	Whole cell	*S. aureus*, MRSA, *Staphylococcus epidermidis*, *Staphylococcus sciuri*, *P. aeruginosa*, *Enterococcus faecium*, *E. faecalis* (VRE), *E. coli*	NA	Colorimetry	10 h	NA	NA	Monitoring volatile compounds produced by cell	[62]
Multilayer on glass substrate (2nd Au layer-floropolyer-1st Au layer)	Antibody	Whole cell	*E. coli* O157:H7	Magnetic bead-based signal amplification	Long-range SPR	30 min	50 CFU/ml	NA	Capturing bacteria with antibody-attached magnetic NP	[24]
Polystyrene microparticle	Antibody	Whole cell	*S. typhimurium*	NA	Light scattering	NA	10 CFU/ml	Liquid from processed raw chicken	Immunoagglutination assay	[84]
Au NP	DNA	DNA	*Neisseria gonorrhoeae*, *Treponema pallidum*	Multiple turnovers by MNAzyme	Colorimetry	NA	500 pM pathogenic DNA	NA	See Figure 1, main text	[85]
	Aptamer	Whole cell	*S. aureus*	Magnetic bead amplification method	Light scattering	1.5 h	~10 CFU/assay	NA	Selection of aptamer through SELEX; see Figure 1 (main text)	[86]
Au NW	DNA	DNA	*Aspergillus fumigatus*, *Candida glabrata*, *Candida krusei*,	Exonuclease III-aided target recycling reaction	SERS	NA	100 fM fungal DNA	Clinical isolates	Multiplexed mode; see Figure 1 (main text)	[32]

Table 2. (continued)

Substrate	Receptor	Analyte	Target Pathogen(s)	Signal Amplification method	Sensing Approach	Assay Time	Detection Limit	Evaluation of Practical Samples	Remarks	Refs
			Cryptococcus neoformans							
	DNA	DNA	*E. faecium*, *S. aureus*, *Vibrio vulnificus*, *Stenotrophomonas maltophilia*	NA	SERS	NA	10 fM pathogenic DNA	Clinical isolates from cerebrospinal fluid, stool, pus, and sputum	Multiplexed mode; see Figure 2D (main text)	[31]
Magnetic bead	Aptazyme	Whole cell	*E. coli*	Magneto signal amplification	Colorimetry	1-2 h	5×10^2–5×10^3 CFU/assay	NA	Enable detection of single bacterium after 7 h of culturing; see Figure 2B, main text	[20]
Polymerdot (Pdot)	Antibiotics	Whole cell	*S. aureus*, *P. aeruginosa*	NA	Fluorescence	NA	1.0~3.1×10^4 CFU/mL	NA	Antibiotic-conjugated Pdots that have microbe-killing as well as microbe-targeting properties	[87]
Copper shell/silica nanoparticle core structure on glass slide	DNA	DNA	*S. aureus*, *Vibrio vulnificus*, *Salmonella*, *S. epidermidis*, *E. faecalis*, *Klebsiella oxytoca*, *N. gonorrhoeae*	NA	LSPR	NA	10 fM (50 zmol) of pathogenic DNA	Clinical isolates from blood, pus, sputum, and urine	Multiplexed mode; see Figure 2C (Figure 1)	[26]
Au-silica NP dielectric layer on glass slide	Aptamer	Whole cell	*P. aeruginosa*, *S. typhimurium*,	NA	LSPR	NA	30 CFU/assay	NA	Multiplexed mode	[25]
Carbon nanotube	Aptazyme	Whole cell	*Salmonella paratyphi*	Luminol oxidation-based signal amplification method	Chemiluminescence	NA	1.0×10^3 CFU/mL	Bacteria spiked into city water samples	Selection of aptamer through SELEX	[88]
Au plate	Bacteriophage	Whole cell	MRSA, *E. coli* O157: H7	NA	SPR	15 min	1.0×10^3 CFU/mL	NA		[58]

Table 2. (continued)

Substrate	Receptor	Analyte	Target Pathogen(s)	Signal Amplification method	Sensing Approach	Assay Time	Detection Limit	Evaluation of Practical Samples	Remarks	Refs
	Antibody	Whole cell	MRSA	NA	SPR	>20 min	1.0 × 10 CFU/mL	Clinical isolates	Use of PBP2a-specific antibody; detection of MRSA, MSSA, BORSA	[61]
	Antibody	Whole cell	*E. coli*	Magnetic signal amplification	SPR	>70 min	3 CFU/mL	Bacteria spiked into water	Capturing bacteria with biotin-antibody/avidin/ MUA/Au-coated magnetic NP	[23]
Au plate/magnetic bead	Bacteriophage	Whole cell	*Campylobacter jejuni*	NA	SPR	25 min	1.0×10^2 CFU/ml	NA	Use of receptor binding protein of phage NCTC 12673; immobilization of GST-Gp48 fusion protein on to magnetic bead and Au plate	[60]

[a]Abbreviations: BORSA, borderline oxacillin-resistant *S. aureus*; CFU, colony forming unit; LFA, lateral flow assay; LSPR, localized surface plasmon resonance; MNA, multicomponent nucleic acid enzyme; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; MUA, mercaptoundecanoic acid; NA, not applicable; NP, nanoparticle; NW, nanowire; PAD, paper-based analytical device; PBP2a, penicillin binding protein 2a; PDMS, polydimethylsiloxane; PI-PLC, phosphatidylinositol-specific phospholipase C; SELEX, Systematic evolution of ligands by exponential enrichment; SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance; VRE, vancomycin-resistant Enterococci.

[b]*Acinetobacter baumannii*, *Anaerobiospirillum succiniproducens*, *Burkholderia cepacia*, *Brahamella catarrhalis*, *Bacteroides ovatus*, *Bacteroides vulgates*, *Bacteroides fragilis*, *Cardiobacterium hominis*, *Elizabethkingia meningoseptica*, *Comamonas acidovorans*, *Enterobactercloacae*, *Klebsiella oxytoca*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Morganella morganii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella paratyphimurium*, *Kingella kingae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Ochrobactrum anthropi*, *Neisseria meningitides*, *Neisseria gonorrhoeae*, *Fusobacterium necrophorum*, *Aggregatibacter actinomycetemcomitans*, *Listeria monocytogenes*, *Streptococcus mutans*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Peptostreptococcus anaerobius*, *Proteus mirabilis*, *Proteus vulgaris*, *Stentrophomonas maltophila*, *Serratia marcesence*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus bovis*, *Streptococcus sanguis*, *Streptococcus anginosus*, *Streptococcus pneumoniae*, *Vibrio cholera*, *Vibrio vulnificus*, *Cryptococcus neoformans*, *Candida glabrata*, *Candida krusei*, *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*.

[c]*Neisseria meningitides*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella enterica subspecies enterica*, *Serratia marcescens*, *Enterobacteriaceae family*, *Achinetobacter baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Haemophilus influenzae*, *Campylobacter jejuni*, *Campylobacter coli*, *Bacteriodes fragilis group*, *Staphylococcus aureus*, *MRSA*, *Staphylococcus epidermidis*, *Coagulase-negative staphylococci*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae subspecies equisimilis*, *Streptococcus pneumonia*, *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria monocytogenes*, and *Clostridium perfringens*.

Table 3. Advantages and Limitations, and Strategies to Overcome the Limitations of Various Optical Sensors for the Detection of Pathogenic Microorganisms[a]

Sensor type		Advantages	Limitations	Strategies to Overcome Limitations	Refs
Colorimetric	Flat based (e.g., LFA)/solution based (e.g., Au NP aggregation)	Simple detection; rapid detection; portability; cost-effective system; analytical equipment not required	Low sensitivity	Use magnetic beads for pre-concentrating cells	[14]
				Use quantum dots instead of Au NPs	[83]
				Use chemiluminescent substrate	[89]
				Use multiwell plate	
			Limited multiplexing capability	Substrate patterning by photolithography, inkjet printing plasma etching, and wax printing	[15–17]
			Limited quantification capability (commonly on-off detection)	Semiquantification using mobile phone	[19,90]
Plasmonic	SPR	Superior sensitivity; multiplexing capability; label-free detection; quantification capability	Requirement of a relatively large equipment	Portable plasmonic equipment developed by several companies such as LAMDAGEN and GenOptics	
			Limited detection of whole cell	Increase reflective index sensitivity	[27]
				Optimization of decay length	[28]
			Limited LOD (>1.0 × 10^3 CFU/ml)	Use long-range SPR	[24]
				Use LSPR on Au NP-capped nanostructure	[25]
				Use LSPR on Cu NP-capped nanostructure	[26]
			Complex system	Integration of microfluidic system	[46,47]
	SERS	Superior sensitivity; multiplexing capability; ability to characterize analyte in more detail; label-free detection	Limited quantification capability	Addition of cucurbit [n]urils to Au colloids to create uniform gap distances	[30]
				Use stable SERS active substrate, such as atomically smooth NW	[31,32]
				Use Ag nanorod array as SERS active substrate	[33]
				Use Au-coated silicon substrate with regular inverse	

Table 3. (continued)

Sensor type		Advantages	Limitations	Strategies to Overcome Limitations	Refs
				pyramidal pattern (commercialized by Renishaw Diagnostics Co.)	
			Limited LOD	Combination with exonuclease III-aided target-recycling reaction	[32]
			Difficulty of analyzing spectra	Use analysis software	[34]
				Use bacterial SERS database	[35]
			Complex system	Integration of microfluidic system	[91]

[a]Abbreviations: LFA, lateral flow assay; LOD, limit of detection; LSPR, localized surface plasmon resonance; NP, nanoparticle; NW, nanowire; SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance.

biosensors are currently available in the marketplace (Table 1). A LFA-based biosensor produced by DuPont Co. can detect *Escherichia coli* O157, *Salmonella*, and *Listeria* within 10 min using antibodies. BioMérieux's product was designed to detect *Streptococcus* and *Legionella pneumophila* within 15 min. These products take advantages of the capillary flow of sample in the membrane and color change by the aggregation of gold nanoparticles (Au NPs). The injected sample in LFA-based biosensor diffuses toward the Au NPs attached to receptors capturing desired pathogens. Then, the Au NP–receptor–pathogen complex flows Toward a membrane region that also has pathogen-specific receptors attached. Upon sandwich binding of pathogens by receptors, a red line is formed on the device due to the aggregation of pathogen–receptor–Au NPs, yielding the readout that is detectable by naked eyes within minutes (Figure 2A).

However, the LFA-based biosensors have the major drawback of low sensitivity [13]. This can be enhanced by using various signal amplification methods, for example, using magnetic beads for pre-concentrating cells. In this method, receptors are attached to magnetic beads and target cells are isolated from samples with a magnet, enabling isolated cells to be easily concentrated by resuspending them in any desired assay volume [14]. This approach can concentrate cells by 10–100-fold and, thus, enabled the detection of 5 colony-forming units (CFU)/mL of *E. coli* O157:H7 within 30 min [14]. LFA-based sensors also have limited potential for the multiplex detection of target analytes, which is another major disadvantage. The need for multiplex detection on a single substrate has motivated efforts to develop additional fabrication processes for the specific immobilization of receptors. In addition, a variety of fabrication methods, including photolithography, inkjet printing [15], plasma etching, and wax printing [16,17], can be used to create physical or hydrophobic channels and barriers in hydrophilic and porous paper, yielding a 2D or even 3D device. Combinations of these fabrication and origami-based methods can form multiple spots and layers with different patterns, enabling the semiquantitative detection of analytes because of the different wicking rates of fluids in each layer, as well as multiplex detection [18].

The solution-based sensors that use nanomaterials can be advantageous for rapid and simple detection with large volume of samples to be analyzed. Solution-based biosensors generally use

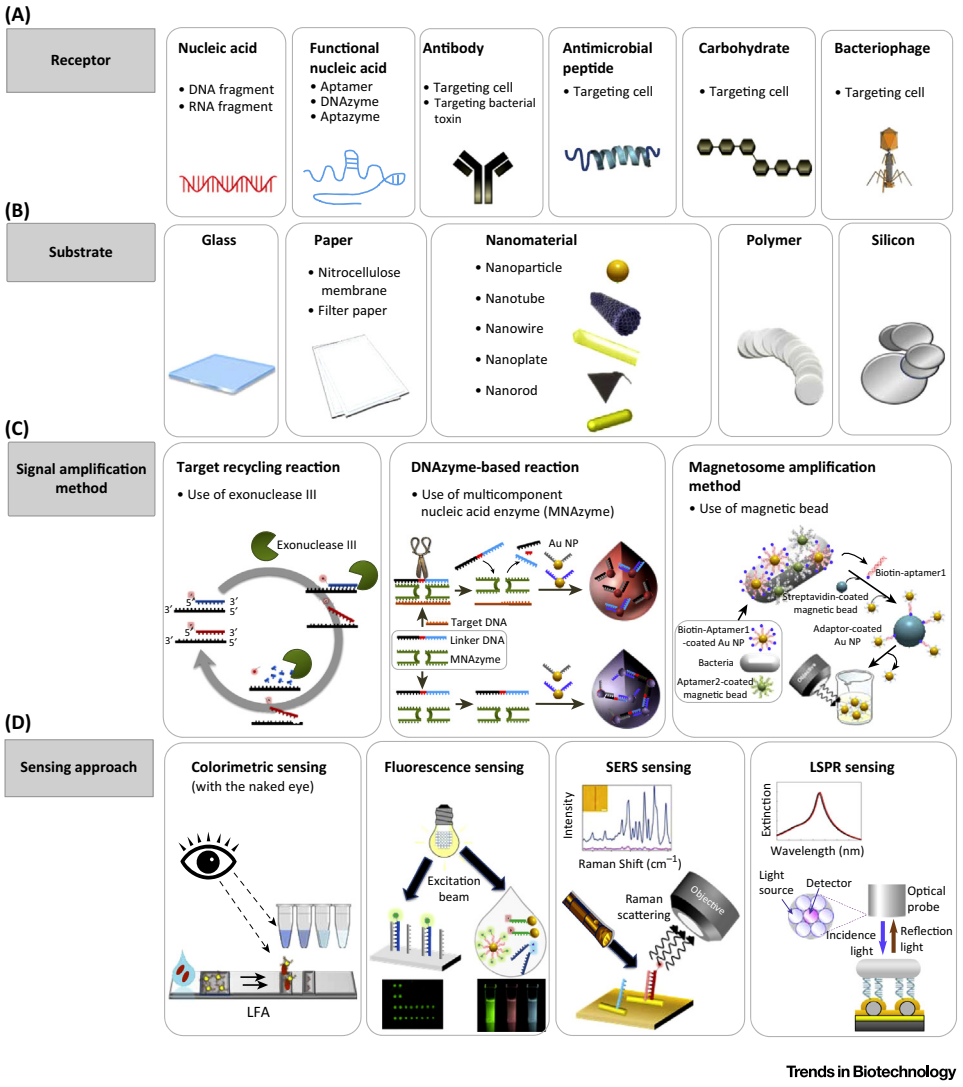

Figure 1. Operating Steps in Developing a Pathogenic Microbe-Detecting Sensor. Schematic illustration of representative components and techniques applied to date that can be integrated into a biosensor for the detection of pathogenic microbes: (A) substrate, (B) receptor, (C) signal-amplification method, and (D) sensing approach. Abbreviations: LSPR, localized surface plasmon resonance; SERS, surface-enhanced Raman scattering.

colloidal Au NPs and the color change can be detected via Au-NP aggregation. Receptor-attached Au NPs react with the corresponding pathogens, and the receptor–pathogen complex then leads to NP aggregation, causing a visible change in color from red to purple. Recently, an NP aggregation-based sensor linked to a mobile phone camera was developed. Using this sensor system, *E. coli* cells present in a sample could be not only detected with the naked eye, but also quantified by using a mobile phone camera and an imaging analysis tool on a computer; the LOD was as low as 8 CFU/ml [19]. A new type of solution-based colorimetric method has also been developed [20]. In this method, one end of an RNA-containing aptazyme binds to magnetic beads through biotin–streptavidin interactions and the other end binds to a urease attached to a pH-sensitive dye via DNA–DNA hybridization (Figure 2B). Upon aptazyme-mediated capture of bacterial cells, the RNA region of the aptazyme is cleaved, splitting the aptazyme into two. The part of the aptazyme conjugated to the magnetic beads is separated

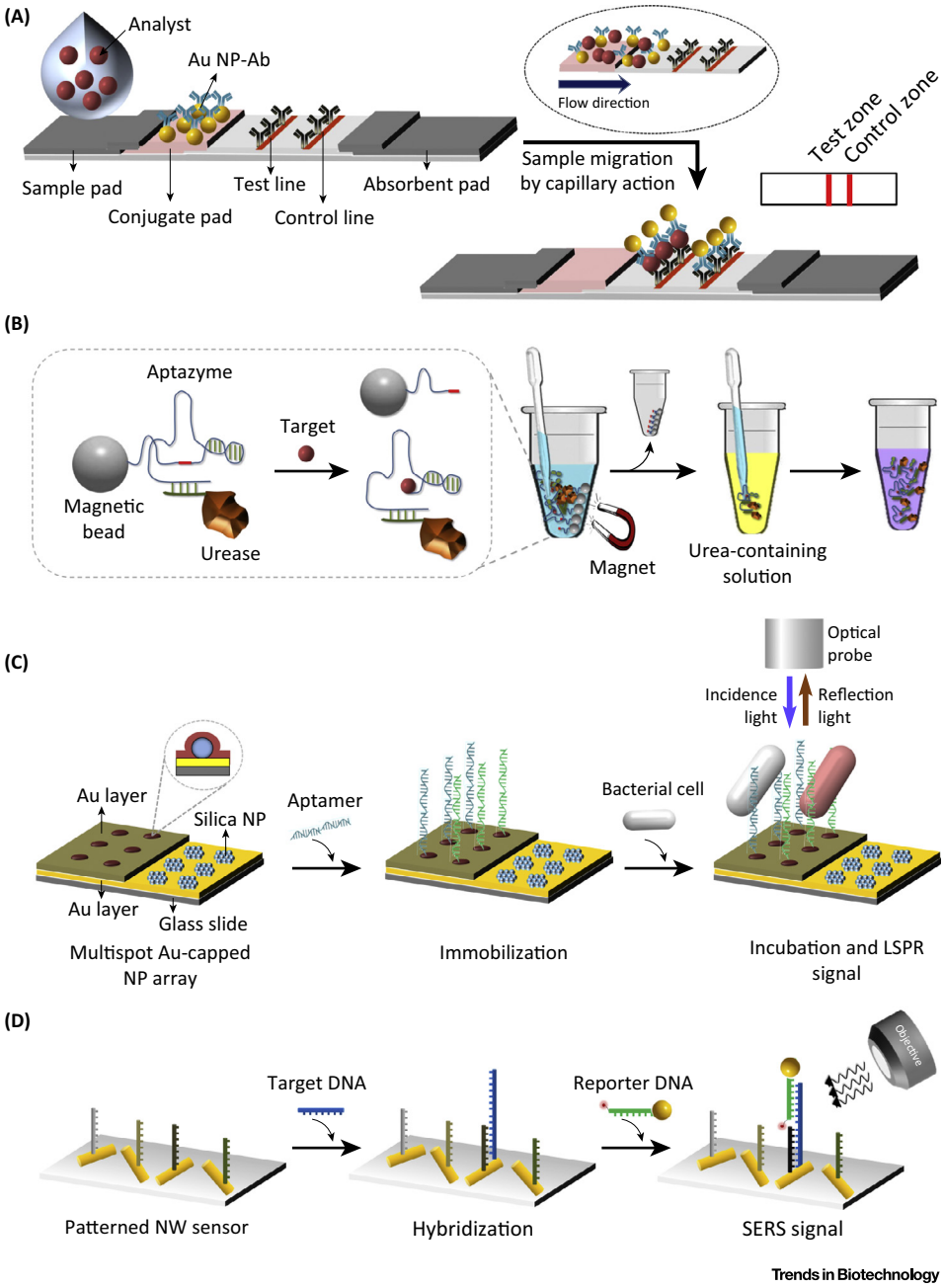

Figure 2. Representative Optical Biosensors Integrating Various Sensing Approaches in the Field of Pathogen Diagnostics. The representative types of biosensor are: (A) lateral flow assay (LFA)-based biosensor [82]; (B) solution-based colorimetric biosensor [20]; (C) localized surface plasmon resonance (LSPR)-based biosensor [25]; and (D) surface-enhanced Raman scattering (SERS)-based biosensor [31]. Abbreviation: NP, nanoparticle.

from samples using a magnet and the urease-containing part is transferred to a urea-containing solution. The associated urease hydrolyzes urea in solution, and the resulting increase in pH induces a color change mediated by the attached pH-sensitive dye, phenol red. Although this method is simple and inexpensive, its sensitivity is relatively low (5×10^2 CFU/assay at 1 h and 5×10^3 CFU/assay at 2 h), suggesting that further improvements are required.

One of the notable recent trends in biosensors is the development and application of plasmonic biosensors for pathogen detection. Two major plasmonic biosensors use surface plasmon resonance (SPR) and surface enhanced Raman spectroscopy (SERS) [21,22]. The general SPR sensor has low sensitivity (LOD, 1.0×10^3 CFU/ml) because the limited penetration depth of SPR into a metal surface and the similarity in refractive index between the bacterial cytoplasm and the aqueous medium [9,21], limit the detection of relatively large objects, such as whole microbial cells. Recently, a SPR sensor combined with a magnetic separation method using Au-coated magnetic NPs resulted in detection with an LOD of 3 CFU/ml [23]. Also, long-range SPR that exhibits a low loss of surface plasmon, a high surface electric field strength, and narrow resonance resulted in an LOD of 50 CFU/ml [24]. The use of localized SPR (LSPR) on the Au NP-capped nanostructure allowed simultaneous identification of three different bacterial cells with an LOD of 30 CFU/assay using a single sensor (Figure 2C) [25]. An LSPR sensor that uses Cu instead of Au as a shell material on a silica NP core structure orderly arranged on an Au surface was also developed for the ultrasensitive detection of pathogenic DNAs with an LOD of 10 fM [26]. The sensitivity of LSPR sensors can be maximized by increasing their reflective index sensitivity and optimizing the decay length so that the binding reaction between the analyte and receptor occurs in the appropriate range of the electromagnetic field [27,28]. In practical applications, even animal cells approximately 10 μm long could be detected using 500-nm diameter NPs, which have a long electromagnetic field decay length and enhanced near-field electric field intensity [29]. Given that SPR sensors still require relatively large equipment for a laboratory-based test, much effort has recently been exerted on reducing the equipment size and complexity of sensors by integrating microfluidics.

SERS has also been used in various biosensors for the detection of pathogens because of its single molecule-level sensitivity, molecular specificity, and insensitivity to quenching [22]. Most of the currently available SERS biosensors require bulky optical components, such as optical microscopes, lasers, monochromators, and detectors. Thus, simple and miniaturized instrumentation is consistently needed for SERS biosensors. Another challenge when using SERS biosensors is the quantitative detection of pathogens in the sample. This originates from the nature of SERS, whereby enhancement in the Raman signal depends on the nanoscale roughness feature of the metal surface. Recent studies have focused on obtaining highly controlled SERS signals by (i) adding cucurbit[n]urils to Au colloids for immediate bridging of adjacent NPs to create uniform gap distances [30]; (ii) using atomically smooth nanowires (NW) with Å roughness [31,32] as a SERS active substrate; (iii) using Au-coated silicon substrates with regular inverse pyramidal pattern (commercialized by Renishaw Diagnostics Co.), and (iv) by using a Ag nanorod array as a SERS active substrate [33]. For example, combining SERS with stable SERS active NW enabled the sensitive detection of bacterial DNAs with an LOD of 10 pM (Figure 2D) [31]. A SERS sensor combined with an exonuclease III-aided target-recycling reaction could increase the sensitivity for detecting multiple fungal DNAs of as low as 100 fM [32]. Furthermore, there is a growing trend toward the development of SERS-based, whole-bacteria detection methods [34,35]. For example, newer bacterial SERS detection methods use electrostatic interactions between cationic metal ions and negatively charged walls originating from teichoic acid (in Gram-positive bacteria) and outer membrane lipopolysaccharides (in Gram-negative bacteria) as the driving force to uniformly deposit NPs on the cell wall [34]. This method allowed the generation of a homogenous SERS signal from cells in bulk solution within 10 min [34]. Bacterial species can be identified by a hierarchical cluster analysis that exploits the fact that the components of the cell wall (polysaccharide, protein, lipids, nucleic acids, etc.) exhibit different SERS spectra; thus, the SERS spectral 'signature' for each microbe is distinct since pathogenic microbes have different cell wall components. The requirement for analysis software together with a database on the standard SERS spectra of the pathogens are still preventing this system being more widely accessible.

Optical Biosensors Integrated with Microfluidics

Similar to many other healthcare-related systems, diagnostics systems, including that for pathogen detection, are evolving toward to a more patient-centered system [10,11]. Such systems aim to collect samples, test them, and obtain results rapidly at or near the location of the patient so that the treatment plan can be immediately implemented or adjusted as required. In response to such demand, the integration of optical sensors with microfluidics is receiving great interest and has become feasible. Such biosensor system integration with microfluidics allows the delivery, separation, preparation, and analysis of samples on a single device and, thus, provides many benefits, including low cost, short reaction time required for analysis, multiple sample detection, parallel detection, automation, portability, versatility in design, and minimal handling of hazardous materials [36]. Some of the key factors in developing such an integrated biosensor include the separation and concentration of pathogens from a complex sample, integration of optical and fluidic components into single substrate, establishment of a system with network connectivity for data acquisition and transmission, and compatibility with personal electronics.

Efficient isolation of pathogens from a complex sample is crucial for the precise detection of pathogens using an integrated biosensor. To achieve this, several new microfluidic separation methods using a propagating surface acoustic wave [37], inertial focusing using spiral microchannels [38], deterministic lateral displacement [39], and dielectrophoresis-based separation [40] have been developed. The most recent example for the detection of pathogens is the use of helical microchannels with a trapezoid cross-section [41]. The separation efficiency was enhanced by injecting the sheath flow through an additional inlet of the microfluidic device and inducing strong Dean vortex cores in the trapezoidal cross-section of the channel. Only bacterial cells captured by antibody-functionalized magnetic NPs are trapped in the inner wall of the channel [41]. Using this method, the ultraviolet (UV)-vis spectroscopy-integrated biosensor showed a LOD of 100 CFU/ml [41].

Much effort has also been exerted to develop automated and miniaturized systems [10,36]. For example, a fluorescence sensor that couples a PCR assay in a microfluidic chip enabled the real-time detection of multiple pathogens [42]. To integrate a thermal cycling PCR machine, microfluidic devices have recently been developed that use isothermal cycling PCRs, such as nucleic acid sequence-based amplification (NASBA) and loop-mediated isothermal amplification (LAMP) [43,44]. In some cases, the microfluidic device is designed as multiwall plate, as used for the multiplex detection of waterborne pathogens [43].

Current fluorescence sensors still require a long assay time, external lenses, and large and complex detector, which limit their practical use as a POC device. Plasmonic biosensors, which enable the fast, real-time, label-free detection of pathogens, are potential alternatives to fluorescence-based biosensors. Conventional SPR-based biosensors require complex instruments that incorporate a prism coated with a thin metal layer to excite propagating plasmons on the metal surface and free space optics, limiting its miniaturization. To overcome this limitation, various SPR-based integrated biosensors have been developed for the detection of pathogens. For example, the SPRi-Lab+ instrument (GenOptics) equipped with an light-emitting diode (LED) source, a charge-coupled device (CCD) camera, and a microfluidic cell, could detect 0.45 fM *L. pneumonia* 16S rRNA [45]. More recently, a portable lightweight (850 g and even as light as 40 g) microfluidic SPR device that contains a complementary metal–oxide–semiconductor (CMOS) sensor instead of a CCD camera has been developed [46,47]. Also, Liu and colleagues developed a smartphone-based portable SPR detection instrument whereby the smartphone provides a light source and serves as a detector, while fiber-optic SPR is used as the sensing element [48]. As all optical components are connected by optical fibers, this SPR sensor can be easily installed and removed from a phone case [48].

Biosensors integrated with localized (L)PSR that use a white light source [49–51] are also being actively developed for rapid diagnostic assays using 96-well plates, a lateral flow system, microtiter plates, and microfluidic systems by LAMDAGEN. Such integrated biosensors have a multifunctional microfluidic chamber that comprises a LSPR-sensing region and the connected flow and control channels. However, sample delivery and isolation are controlled by external micromechanical valves [49]. Recently, a multifunctional device capable of isolating cells by an on-chip trapping method was developed [50]. Cells are mixed with antibody-attached microbeads and, after injection of a mixed sample into the chamber, only microbead-bound cells become trapped in the micropillar arrays due to the mechanical rigidity of the beads [50]. This device has patterned multiple-sensing regions and each sensing region has its own inlet where the samples are analyzed, which provides individual control and multiple detection of analytes [50]. Although these two methods have been used for detecting cancer cells [49,50], they might also be applicable for the detection of microbial pathogens.

Biosensors with wireless communication capability can be more powerful pathogen-diagnostic systems because they enable remote monitoring and provide real-time feedback information in various practical settings [10]. For example, a recently developed remote biosensor capable of identifying bacterial cells was fabricated in a two-step process: integration of a graphene transducer on a silk film using a printing process, followed by incorporation of an Au-inductive, coil-based electrode pattern on the graphene-silk hybrid for wireless transmission. The capacity for wireless remote sensing of the device allowed real-time and continuous monitoring of bacteria from various samples from teeth, muscle, and an intravenous bag [52].

Furthermore, all the results recorded and analyzed by biosensors could be sent to the doctors or pharmacists via personal electronic devices when the users approve and agree to transmit such information. For example, a wireless adhesive device with a free-floating strip-line network assembled between structured electronic substrates could monitor the results from the biosensor and transmit data real-time to a cell phone or computer [53]. In the near future, such connected biosensor devices will have increasingly important roles in all patient care environments.

Concluding Remarks and Future Perspectives

It is expected that optical biosensors for pathogen diagnosis will become increasingly important, impacting the fields of clinical research, forensics, biodefense, food safety, animal healthcare, pathology, and drug discovery. Several methods have been developed, and the advantages of each can be combined to develop more advanced diagnostic devices. Recent advances in nanotechnology are facilitating the development of smaller, inexpensive, multiplex, easy-to-use, and fully integrated biosensors for diagnostics, environmental monitoring, and food and water safety [54–57]. However, challenges remain for the practical usefulness of optical biosensors (see Outstanding Questions). For example, few pathogen-detecting biosensors have been applied to a large number of clinical and environmental samples in a high-throughput mode to evaluate their practical usefulness. This might be attributable to difficulties in obtaining clinical samples from pathogen-infected patients. Further direct tests on pathogens in samples without a prior cultivation step are needed for applications in a clinical setting. Large-scale clinical trials should also be performed to evaluate the practical utility of these pathogen-diagnostic sensors.

One important research topic on pathogen diagnostics is developing a system for the detection of antimicrobial-resistant pathogens. Despite its importance, most pathogen-diagnostic biosensors focus on the detection and identification of the species or subtypes of pathogens in a sample. The optical biosensors developed so far for the detection of antimicrobial-resistant pathogens use only limited types of receptor: bacteriophage (e.g., BP14) [58–60], antibodies specific to antimicrobial resistance-related protein [e.g., anti- penicillin binding protein 2A

Outstanding Questions

How can we apply an optical sensor to clinical and environmental samples in a high-throughput manner?

How can we improve the sensitivity of optical sensors for the successful detection of pathogens in clinical samples, for example in blood, without additional cultivation, given that the number of microbial pathogens is very small?

How can we more efficiently detect antimicrobial resistance together with microbial pathogen identification in a single assay?

What new and more efficient receptors could be used to rapidly and specifically detect microbial pathogens?

(PBP2a) [61], and volatile organic compounds [62] (Table 2). Thus, much research effort needs to be exerted to discover new biomarkers for the development of more diverse receptors specific to resistance-relevant molecules. New insights into the design and screening of synthetic receptors by using powerful selection methods, such as systematic evolution of ligands by exponential enrichment (SELEX) and its alternatives [63–65], as well as click chemistry [66], will enable more sensitive and specific sensing of diverse pathogens. Also, a recent study on the use of antimicrobial resistance detection for theranostic applications is notable. In this system, the change in cantilever fluctuation returning to baseline levels after injection of antibiotics was used to indicate that all bacteria were dead and confirmed the antibiotic sensitivity of the test strain [67].

As emphasized throughout this review, the design and use of a receptor that specifically binds to the pathogen of interest is critical for developing optical sensors with high sensitivity and specificity. Such receptors can be designed and selected by using the various methods described above. One more important method for developing novel receptors is phage display [68,69] or cell surface display technology [70–72]. Phages (e.g., M13 or fd) or microorganisms (e.g., *E. coli* or yeast) that are designed to display a library of various receptors, such as peptides (magainin I [69] or leucocin-A [73]) or proteins (including antibodies) can be screened in a high-throughput manner to identify the most suitable receptor capable of binding the pathogen of interest. For example, the library of candidate receptor variants can be constructed and displayed on the cell surface. The cell can be designed to emit fluorescence by the operation

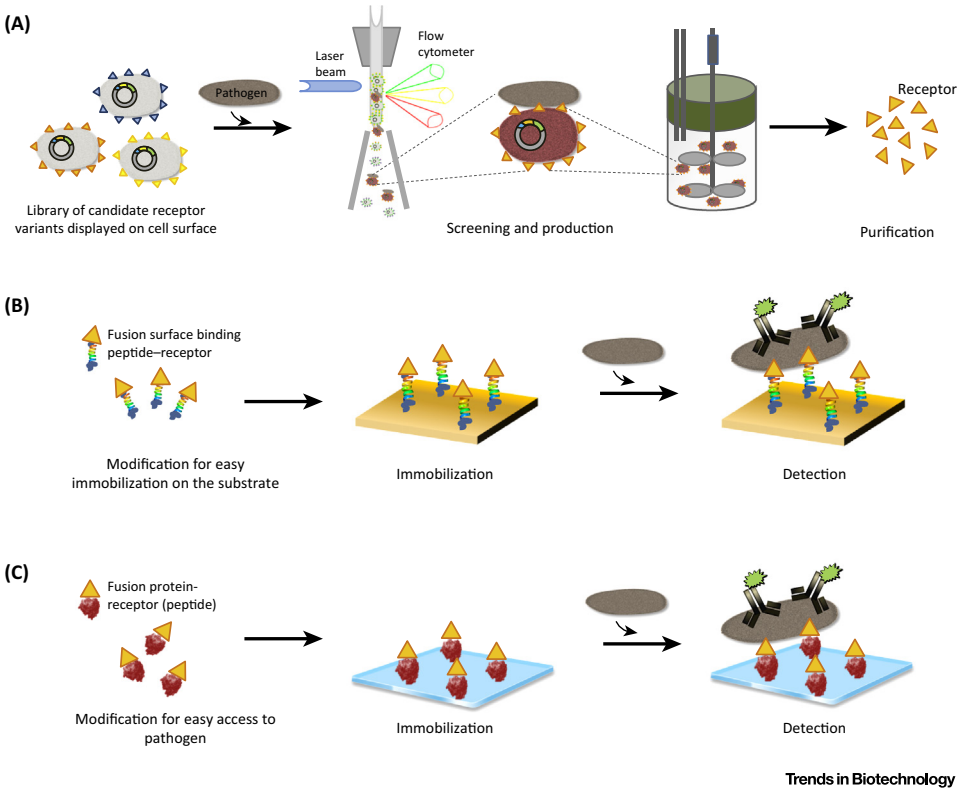

Figure 3. Schematic Illustration of Developing Pathogen-Specific Receptors. (A) A pathogen-specific receptor can be efficiently developed by cell surface display of a receptor library followed by high-throughput fluorescence-activated cell sorting (FACS) screening. The screened receptor can be produced and used for optical biosensors. In addition, the receptor can be modified by a gene fusion system using various fusion partners, including surface binding peptides (B) and fusion proteins (C) for its easy immobilization on the substrate surface and better access to target molecule, respectively.

of the cellular fluorescence circuit through the bound pathogen-induced signaling cascade when the pathogen binds to the receptors, thereby allowing easy screening on multi-well plates or more rapidly by fluorescence-activated cell sorting (FACS). The best receptor identified by screening can then be overproduced by an engineered microorganism for its use in various optical-sensing platforms (Figure 3A). A little tweak of this method can result in easy immobilization of receptors and easy access for the target molecules to the active sites of receptors; for example, surface-binding peptide or proteins that are capable of binding Au, Ag, silica, or Cu [74–76] can also be fused to the receptors for their selective immobilization on the sensor surface (Figure 3B). A fusion protein–peptide strategy can be used to efficiently screen peptide receptor libraries. Since it is difficult for peptides directly immobilized on the surface to interact with the target protein due to steric hindrance, elevating the peptide on the layer of protein allows enhanced peptide–protein interactions on the solid surface [77] (Figure 3C). Furthermore, it is not necessary to chemically synthesize a library of peptides, which can be rather costly, because a

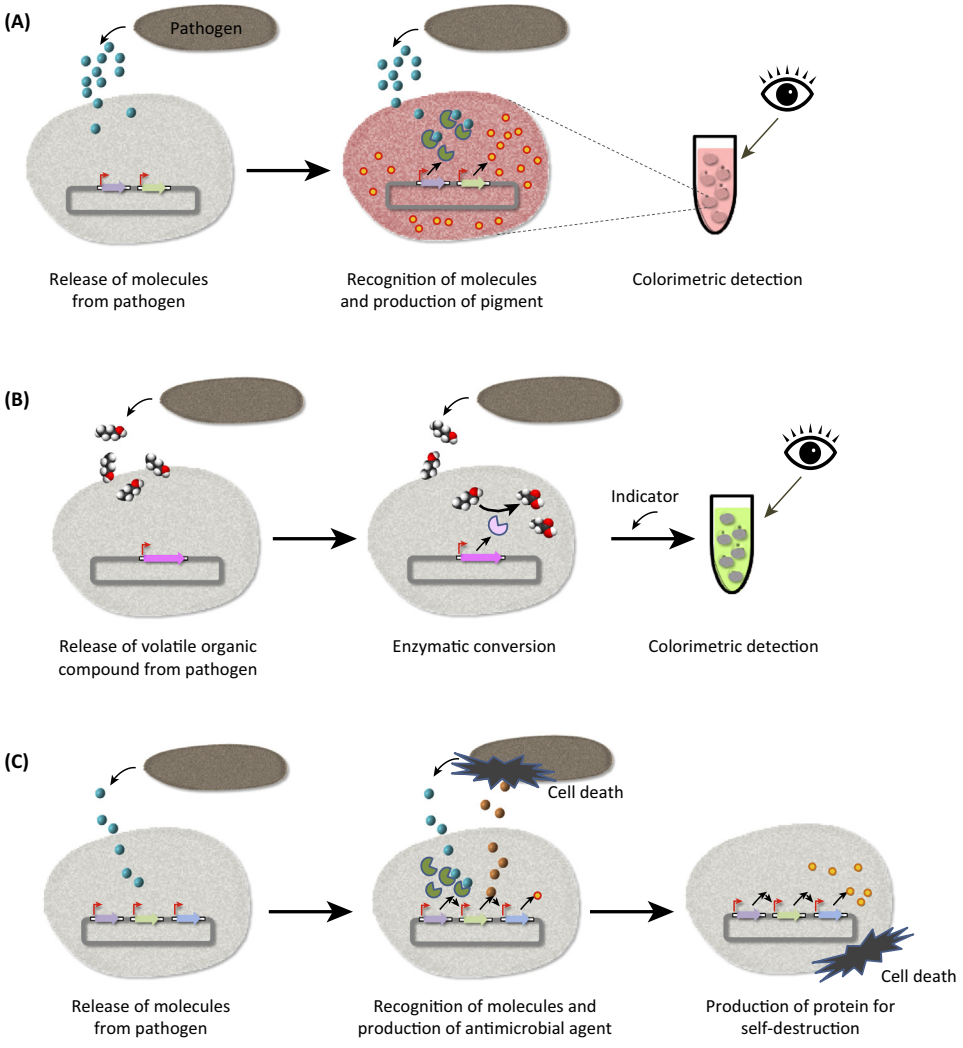

Figure 4. Schematic Illustration of Pathogen-Detecting Strategies Using Programmed Cell-Based Optical Sensors. Whole cells can be designed to recognize pathogen-releasing molecules to detect pathogens by monitoring the color change caused by pigment production (A) or enzymatic conversion of released molecules (B). In addition, further circuits can be introduced to induce the death of both the pathogen and the programmed cell (C).

fusion protein–peptide library can be made simply by fermentation of engineered microorganisms expressing the fusion gene library; only the DNA region encoding the peptide receptor can be mutated. All such technologies will enable the cost-effective development of more specific receptors for optical biosensors (Figure 3).

Metabolic engineering and synthetic biology will also contribute to developing more advanced optical biosensors. Whole cells equipped with reconstructed genetic circuits and/or pigment-producing pathways can be used as optical sensors (Figure 4A). For example, one can construct programmable cells that recognize molecules released by a pathogen (e.g., acyl-homoserine lactones) [78,79] and produce the pigment as a response. This will allow colorimetric detection (or even with the naked eye) of pathogens. Along the same line, engineered cells that can amplify enzymes for desired pathway reactions can also act as a whole-cell optical sensor (Figure 4B). For example, to detect pathogens (e.g., *Staphylococcus aureus* and *Salmonella typhimurium*) that produce butanol as a volatile organic compound (VOC) [80], one can make bacterial cells that constitutively produce enzymes converting butanol to butyric acid. Butyric acid thus produced lowers the pH of the reaction sample that also contains a pH-dependent indicator, which can easily be detected by the naked eye by monitoring the color change. Such color change can be further enhanced by using a matrix, such as agarose gel, for VOC trapping [81].

The application of engineered cells as a whole biosensor can be expanded for their use in the human body or in polluted areas by endowing them with additional functions, such as pathogen killing upon detection [78,79] and self-destruction after use, to address biosafety issue [79] (Figure 4C). Such engineered cells with preprogrammed multifunctions commonly comprise feed-forward genetic activating interactions. For example, upon detecting quorum-sensing molecules, the circuits turn into an ON state in consecutive order and then express mobility-associated proteins for seeking (e.g., CheZ), secretion-tagged antimicrobial peptides (or proteins) for killing (e.g., MccS or Pyocin), and proteins for autodestruction (e.g., E7 Lysis protein) [78,79].

Thus, diverse ideas relating to biosensor systems are being generated continuously. These are likely to lead to the successful development and commercialization of sensitive, accurate, inexpensive, convenient, multiplexed, fully integrated, and wireless communicable biosensor systems for pathogen diagnosis for a range of applications.

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References

1. Gootz, T.D. (2010) The global problem of antibiotic resistance. *Crit. Rev. Immunol.* 30, 79–93
2. Fournier, P.E. *et al.* (2013) Modern clinical microbiology: new challenges and solutions. *Nat. Rev. Microbiol.* 11, 574–585
3. Yoo, S.M. *et al.* (2010) Microarray-based identification of bacterial and fungal pathogens in bloodstream infections. *Mol. Cell. Probes* 24, 44–52
4. Tissari, P. *et al.* (2010) Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. *Lancet* 375, 224–230
5. Luo, X. *et al.* (2013) Electrical biosensors and the label free detection of protein disease biomarkers. *Chem. Soc. Rev.* 42, 5944–5962
6. Ahmed, A. *et al.* (2014) Biosensors for whole-cell bacterial detection. *Clin. Microbiol. Rev.* 27, 631–646
7. Cheng, C.I. *et al.* (2012) Biomolecular interactions and tools for their recognition: focus on the quartz crystal microbalance and its] diverse surface chemistries and applications. *Chem. Soc. Rev.* 41, 1947–1971
8. Harel, E. *et al.* (2008) Novel detection schemes of nuclear magnetic resonance and magnetic resonance imaging: applications from analytical chemistry to molecular sensors. *Annu. Rev. Anal. Chem.* 1, 133–163
9. Pires, N.M. *et al.* (2014) Recent developments in optical detection technologies in lab-on-a-chip devices for biosensing applications. *Sensors* 15458–15479
10. Vasan, A.S. *et al.* (2013) Point-of-care biosensor system. *Front. Biosci. (Schol. Ed.)* 5, 39–71
11. Pai, N.P. *et al.* (2012) Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS Med.* 9, e1001306
12. Bissonnette, L. *et al.* (2012) Infectious disease management through point-of-care personalized medicine molecular diagnostic technologies. *J. Pers. Med.* 2, 50–70

13. Pöhlmann, C. *et al.* (2014) A lateral flow assay for identification of Escherichia coli by ribosomal RNA hybridisation. *Analyst* 139, 1063–1071
14. Hossain, S.M. *et al.* (2012) Multiplexed paper test strip for quantitative bacterial detection. *Anal. Bioanal. Chem.* 403, 1567–1576
15. Ko, H. *et al.* (2014) Active digital microfluidic paper chips with inkjet-printed patterned electrodes. *Adv. Mater.* 26, 2335–2340
16. Funes-Huacca, M. *et al.* (2012) Portable self-contained cultures for phage and bacteria made of paper and tape. *Lab Chip* 12, 4269–4278
17. Jokerst, J.C. *et al.* (2012) Development of a paper-based analytical device for colorimetric detection of select foodborne pathogens. *Anal. Chem.* 84, 2900–2907
18. Lewis, G.G. *et al.* (2012) Quantifying analytes in paper-based microfluidic devices without using external electronic readers. *Angew. Chem. Int. Ed. Engl.* 51, 12707–12710
19. Shafiee, H. *et al.* (2015) Paper and flexible substrates as materials for biosensing platforms to detect multiple biotargets. *Sci. Rep.* 5, 8719
20. Tram, K. *et al.* (2014) Translating bacterial detection by DNAzymes into a litmus test. *Angew. Chem. Int. Ed. Engl.* 53, 12799–127802
21. Tokel, O. *et al.* (2014) Advances in plasmonic technologies for point of care applications. *Chem. Rev.* 114, 5728–5752
22. Bantz, K.C. *et al.* (2011) Recent progress in SERS biosensing. *Phys. Chem. Chem. Phys.* 13, 11551–11567
23. Torun, O. *et al.* (2012) Comparison of sensing strategies in SPR biosensor for rapid and sensitive enumeration of bacteria. *Biosens. Bioelectron.* 37, 53–60
24. Wang, Y. *et al.* (2012) Bacterial pathogen surface plasmon resonance biosensor advanced by long range surface plasmons and magnetic nanoparticle assays. *Anal. Chem.* 84, 8345–8350
25. Yoo, S.M. *et al.* (2015) Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species. *Talanta* 132, 112–117
26. Kim, D-.K. *et al.* (2011) Plasmonic properties of the multispot copper-capped nanoparticle array chip and its application to optical biosensors for pathogen detection of multiplex DNAs. *Anal. Chem.* 83, 6215–6222
27. Nusz, G.J. *et al.* (2009) Rational selection of gold nanorod geometry for label-free plasmonic biosensors. *ACS Nano* 3, 795–806
28. Nath, N. and Chilkoti, A. (2004) Label-free biosensing by surface plasmon resonance of nanoparticles on glass: optimization of nanoparticle size. *Anal. Chem.* 76, 5370–5378
29. Liu, F. *et al.* (2014) Effects of nanoparticle size and cell type on high sensitivity cell detection using a localized surface plasmon resonance biosensor. *Biosens. Bioelectron.* 55, 141–148
30. Kasera, S. *et al.* (2014) Quantitative multiplexing with nano-self-assemblies in SERS. *Sci. Rep.* 4, 6785
31. Kang, T. *et al.* (2010) Patterned multiplex pathogen DNA detection by Au particle-on-wire SERS sensor. *Nano Lett.* 10, 1189–1193
32. Yoo, S.M. *et al.* (2011) Combining a nanowire SERRS sensor and a target recycling reaction for ultrasensitive and multiplex identification of pathogenic fungi. *Small* 7, 3371–3376
33. Hennigan, S.L. *et al.* (2014) Detection and differentiation of avian mycoplasmas by surface-enhanced Raman spectroscopy based on a silver nanorod array. *Appl. Environ. Microbiol.* 78, 1930–1935
34. Zhou, H. *et al.* (2014) SERS detection of bacteria in water by in situ coating with Ag nanoparticles. *Anal. Chem.* 86, 1525–1533
35. Sivanesan, A. *et al.* (2014) Nanostructured silver-gold bimetallic SERS substrates for selective identification of bacteria in human blood. *Analyst* 139, 1037–1043
36. Kumar, S. *et al.* (2013) Microfluidic-integrated biosensors: prospects for point-of-care diagnostics. *Biotechnol. J.* 8, 1267–1279
37. Skowronek, V. *et al.* (2013) Particle deflection in a poly (dimethylsiloxane) microchannel using a propagating surface acoustic wave: size and frequency dependence. *Anal. Chem.* 85, 9955–9959
38. Martel, J.M. and Toner, M. (2013) Particle focusing in curved microfluidic channels. *Sci. Rep.* 3, 3340
39. Liu, Z. *et al.* (2013) Rapid isolation of cancer cells using microfluidic deterministic lateral displacement structure. *Biomicrofluidics* 7, 11801
40. Cheng, I.-F. *et al.* (2013) Rapid (<5 min) identification of pathogen in human blood by electrokinetic concentration and surface-enhanced Raman spectroscopy. *Sci. Rep.* 3, 2365
41. Lee, W. *et al.* (2015) 3D-printed microfluidic device for the detection of pathogenic bacteria using size-based separation in helical channel with trapezoid cross-section. *Sci. Rep.* 5, 7717
42. Ramalingam, N. *et al.* (2010) Real-time PCR-based microfluidic array chip for simultaneous detection of multiple waterborne pathogens. *Sens. Actuat. B.* 145, 543–552
43. Zhao, X. *et al.* (2012) Compatible immuno-NASBA LOC device for quantitative detection of waterborne pathogens: design and validation. *Lab. Chip* 12, 602–612
44. Connelly, J.T. *et al.* (2015) 'Paper machine' for molecular diagnostics. *Anal. Chem.* 87, 7595–7601
45. Foudeh, A.M. *et al.* (2014) Sub-femtomole detection of 16s rDNA from *Legionella pneumophila* using surface plasmon resonance imaging. *Biosens. Bioelectron.* 52, 129–135
46. Tokel, O. *et al.* (2015) Portable microfluidic integrated plasmonic platform for pathogen detection. *Sci. Rep.* 5, 9152
47. Coskun, A.F. *et al.* (2014) Lensfree optofluidic plasmonic sensor for real-time and label-free monitoring of molecular binding events over a wide field-of-view. *Sci. Rep.* 4, 6789
48. Liu, Y. *et al.* (2015) Surface plasmon resonance biosensor based on smart phone platforms. *Sci. Rep.* 5, 12864
49. Aćimović, S.S. *et al.* (2014) LSPR chip for parallel, rapid, and sensitive detection of cancer markers in serum. *Nano Lett.* 14, 2636–2641
50. Oh, B.R. *et al.* (2014) Integrated nanoplasmonic sensing for cellular functional immunoanalysis using human blood. *ACS Nano* 8, 2667–2676
51. Chen, P. *et al.* (2015) Multiplex serum cytokine immunoassay using nanoplasmonic biosensor microarrays. *ACS Nano* 9, 4173–4181
52. Mannoor, M.S. *et al.* (2012) Graphene-based wireless bacteria detection on tooth enamel. *Nat. Commun.* 3, 763
53. Xu, S. *et al.* (2014) Soft microfluidic assemblies of sensors, circuits, and radios for the skin. *Science* 344, 70–74
54. Zhu, A.Y. *et al.* (2014) Optoelectromechanical multimodal biosensor with graphene active region. *Nano Lett.* 14, 5641–5649
55. Chung, J. *et al.* (2015) Fast and continuous microorganism detection using aptamer-conjugated fluorescent nanoparticles on an optofluidic platform. *Biosens. Bioelectron.* 67, 303–308
56. Bouguelia, S. *et al.* (2013) On-chip microbial culture for the specific detection of very low levels of bacteria. *Lab Chip* 13, 4024–4032
57. Verbarg, J. *et al.* (2013) Catch and release: integrated system for multiplexed detection of bacteria. *Anal. Chem.* 85, 4944–4950
58. Tawil, N. *et al.* (2012) Surface plasmon resonance detection of *E. coli* and methicillin-resistant *S. aureus* using bacteriophages. *Biosens. Bioelectron.* 37, 24–29
59. Arya, S.K. *et al.* (2011) Chemically immobilized T4-bacteriophage for specific *Escherichia coli* detection using surface plasmon resonance. *Analyst* 136, 486–492
60. Singh, A. *et al.* (2011) Specific detection of *Campylobacter jejuni* using the bacteriophage NCTC 12673 receptor binding protein as a probe. *Analyst* 136, 4780–4786
61. Tawil, N. *et al.* (2013) The differential detection of methicillin-resistant, methicillin-susceptible and borderline oxacillin-resistant *Staphylococcus aureus* by surface plasmon resonance. *Biosens. Bioelectron.* 49, 334–340
62. Carey, J.R. *et al.* (2011) Rapid identification of bacteria with a disposable colorimetric sensing array. *J. Am. Chem. Soc.* 133, 7571–7576
63. Nguyen, V.T. *et al.* (2014) Multiple GO-SELEX for efficient screening of flexible aptamers. *Chem. Commun.* 139, 4696–4701
64. Dwivedi, H.P. *et al.* (2013) Selection of DNA aptamers for capture and detection of *Salmonella tyyphimurium* using a whole-cell SELEX approach in conjunction with cell sorting. *Appl. Microbiol. Biotechnol.* 97, 3677–3686
65. Kwon, Y.S. *et al.* (2014) An ultra-sensitive colorimetric detection of tetracyclines using the shortest aptamer with highly enhanced affinity. *Chem. Commun.* 50, 40–42

66. Millward, S.W. *et al.* (2011) Iterative in situ click chemistry assembles a branched capture agent and allosteric inhibitor for Akt1. *J. Am. Chem. Soc.* 133, 18280–18288
67. Longo, G. *et al.* (2013) Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors. *Nat. Nanotechnol.* 8, 522–526
68. Smith, G.P. (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228, 1315–1317
69. Wang, J. *et al.* (2014) Rapid detection of pathogenic bacteria and screening of phage-derived peptides using microcantilevers. *Anal. Chem.* 86, 1671–1678
70. Lee, S.Y. *et al.* (2003) Microbial cell-surface display. *Trends Biotechnol.* 21, 45–52
71. Abbaszadegan, M. *et al.* (2011) Cell surface display of poliovirus receptor on *Escherichia coli*, a novel method for concentrating viral particles in water. *Appl. Environ. Microbiol.* 77, 5141–5148
72. Richman, S.A. *et al.* (2011) Biosensor detection systems: engineering stable, high-affinity bioreceptors by yeast surface display. *Methods Mol. Biol.* 504, 323–350
73. Etayash, H. *et al.* (2014) Impedimetric detection of pathogenic Gram-positive bacteria using an antimicrobial peptide from class IIa bacteriocins. *Anal. Chem.* 86, 1693–1700
74. Yang, M. *et al.* (2011) Site-specific immobilization of gold binding polypeptide on gold nanoparticle-coated graphene sheet for biosensor application. *Nanoscale* 7, 2950–2956
75. Palafox-Hernandez, P.J. *et al.* (2014) Comparative study of materials-binding peptide interactions with gold and silver surfaces and nanostructures: a thermodynamic basis for biological selectivity of inorganic material. *Chem. Mater.* 26, 4960–4969
76. Rubino, J.T. *et al.* (2011) A comparison of methionine, histidine and cysteine in copper(I)-binding peptides reveals differences relevant to copper uptake by organisms in diverse environments. *Metallomics* 3, 61–73
77. Lee, S.J. and Lee, S.Y. (2004) Microarrays of peptides elevated on the protein layer for efficient protein kinase assay. *Anal. Biochem.* 330, 311–316
78. Hwang, I.Y. *et al.* (2014) Reprogramming microbes to be pathogen-seeking killers. *ACS Synth. Biol.* 3, 228–237
79. Saeidi, N. *et al.* (2014) Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen. *Mol. Syst. Biol.* 7, 521
80. Sohrabi, M. *et al.* (2014) Volatile organic compounds as novel markers for the detection of bacterial infections. *Clin. Microbial.* 3, 151
81. Tait, E. *et al.* (2015) Analysis of pathogenic bacteria using exogenous volatile organic compound metabolites and optical sensor detection. *RSC Adv.* 5, 15494–15499
82. Li, C.Z. *et al.* (2011) Paper based point-of-care testing disc for multiplex whole cell bacteria analysis. *Biosens. Bioelectron.* 26, 4342–4348
83. Bruno, J.G. (2014) Application of DNA aptamers and quantum dots to lateral flow test strips for detection of foodborne pathogens with improved sensitivity versus colloidal gold. *Pathogens* 3, 341–355
84. Fronczek, C.F. *et al.* (2013) Single-pipetting microfluidic assay device for rapid detection of Salmonella from poultry package. *Biosens. Bioelectron.* 40, 342–349
85. Zagorovsky, K. and Chan, W.C. (2013) A plasmonic DNAzyme strategy for point-of-care genetic detection of infectious pathogens. *Angew. Chem. Int. Ed. Engl.* 52, 3168–3171
86. Chang, Y.C. *et al.* (2013) Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles. *Sci. Rep.* 3, 1863
87. Wan, Y. *et al.* (2014) Multifunctional semiconducting polymer dots for imaging, detection, and photo-killing of bacteria. *J. Mater. Chem. B* 2, 4818–4825
88. Yang, M. *et al.* (2013) Highly specific and cost-efficient detection of *Salmonella paratyphi A* combining aptamers with single-walled carbon nanotubes. *Sensors* 13, 6865–6881
89. Joung, H.A. *et al.* (2014) An automatic enzyme immunoassay based on a chemiluminescent lateral flow immunosensor. *Biosens. Bioelectron.* 53, 330–335
90. Park, T.S. *et al.* (2013) Smartphone quantifies Salmonella from paper microfluidics. *Lab Chip* 13, 4832–4840
91. Xie, Y. *et al.* (2014) In situ fabrication of 3D Ag@ZnO nanostructures for microfluidic surface-enhanced Raman scattering systems. *ACS Nano* 8, 12175–12184