ACS Infectious Diseases

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Review

Recent Innovations in Bacterial Infection Detection and Treatment

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Cite This: *ACS Infect. Dis.* 2021, 7, 695−720

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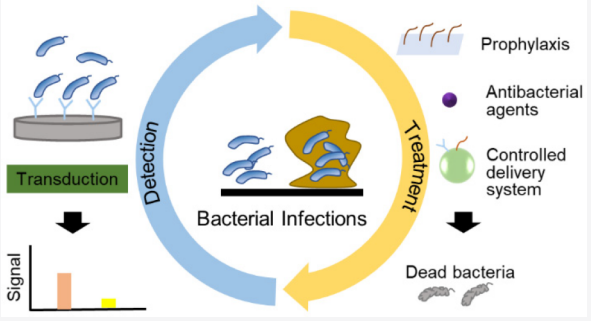
ABSTRACT: Bacterial infections are a major threat to human health, exacerbated by increasing antibiotic resistance. These infections can result in tremendous morbidity and mortality, emphasizing the need to identify and treat pathogenic bacteria quickly and effectively. Recent developments in detection methods have focused on electrochemical, optical, and mass-based biosensors. Advances in these systems include implementing multifunctional materials, microfluidic sampling, and portable data-processing to improve sensitivity, specificity, and ease of operation. Concurrently, advances in antibacterial treatment have largely focused on targeted and responsive delivery for both antibiotics and antibiotic alternatives. Antibiotic alternatives described here include repurposed drugs, antimicrobial peptides and polymers, nucleic acids, small molecules, living systems, and bacteriophages. Finally, closed-loop therapies are combining advances in the fields of both detection and treatment. This review provides a comprehensive summary of the current trends in detection and treatment systems for bacterial infections.



KEYWORDS: *bacterial infection, biosensor, point-of-care diagnostics, closed-loop therapy, antibacterial therapy, controlled drug delivery*

Bacteria are an essential component of the human microbiome, colonizing tissues including the gastrointestinal tract and skin.[1,2] Although a majority of bacteria are harmless and some strains even provide benefits in digestion and competition with opportunistic pathogens, bacterial infections are among the most common human illnesses.[2,3] Bacterial pathogenesis is caused by a disruption to the normal coexistence of bacteria and host cells observed in healthy individuals.[1,2] These infections most commonly occur when bacteria migrate from areas with an underlying microbiota (e.g., mucosal lining of the nose, mouth, intestine, and urethra) to areas of atypical colonization (e.g., lungs, pancreas, blood, brain, bladder, and kidney).[4−6] Temporary implants that connect these areas of the body (e.g., urinary catheters, intubation tubes, central lines, and needles)[4,5] and long-term implants (e.g., cardiac devices, joint replacements, and screws)[5−7] can facilitate this bacterial migration. Additionally, susceptibility to bacterial infections is enhanced when host immunity is preoccupied with underlying conditions,[8] inflammation,[9,10] or other infections.[3,11] Various factors specific to bacteria, including the production of toxins,[12,13] enhanced virulence due to cocolonization with other species,[12] and biofilm formation,[6] can enhance bacterial invasion, leading to severe infections that can require amputations, cause sepsis, and even lead to death.[3,14] Effective detection and treatment strategies are key to preventing and mitigating bacterial infections. There have been many advances in analytical technologies and fundamental microbiology that have enabled innovations in detection and treatment of bacterial infections over the last several years, which we will explore in this review.

Current clinical bacterial diagnostics primarily involve bacteria culture, colony formation methods, and molecular diagnostics.[15] These approaches isolate and identify bacteria from a patient sample, including blood, sputum, and urine, using swabs, aspirations, or tissue biopsies.[16] Although typically highly sensitive (able to detect down to 10^1−10^3 colony forming units per milliliter (CFU/mL)), these conventional methods require time, expensive equipment, and skilled operators, all of which limit utility, particularly in resource-limited settings.[17] Other factors, including the choice of culture media and culture conditions (aerobic or anaerobic), can also bias results.[15] Therefore, it is important to develop fast, cost-effective, and accurate methods capable of detecting bacteria at the early stages of infection. Biosensors have attracted attention as a method of bacterial detection that can address these needs. We will describe the various benefits and potential drawbacks of recent bacteria diagnostic biosensor designs.

Detection and identification of bacteria theoretically informs treatment practices. Oral and intravenous antibiotics are the

Received: December 18, 2020
Published: March 18, 2021

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

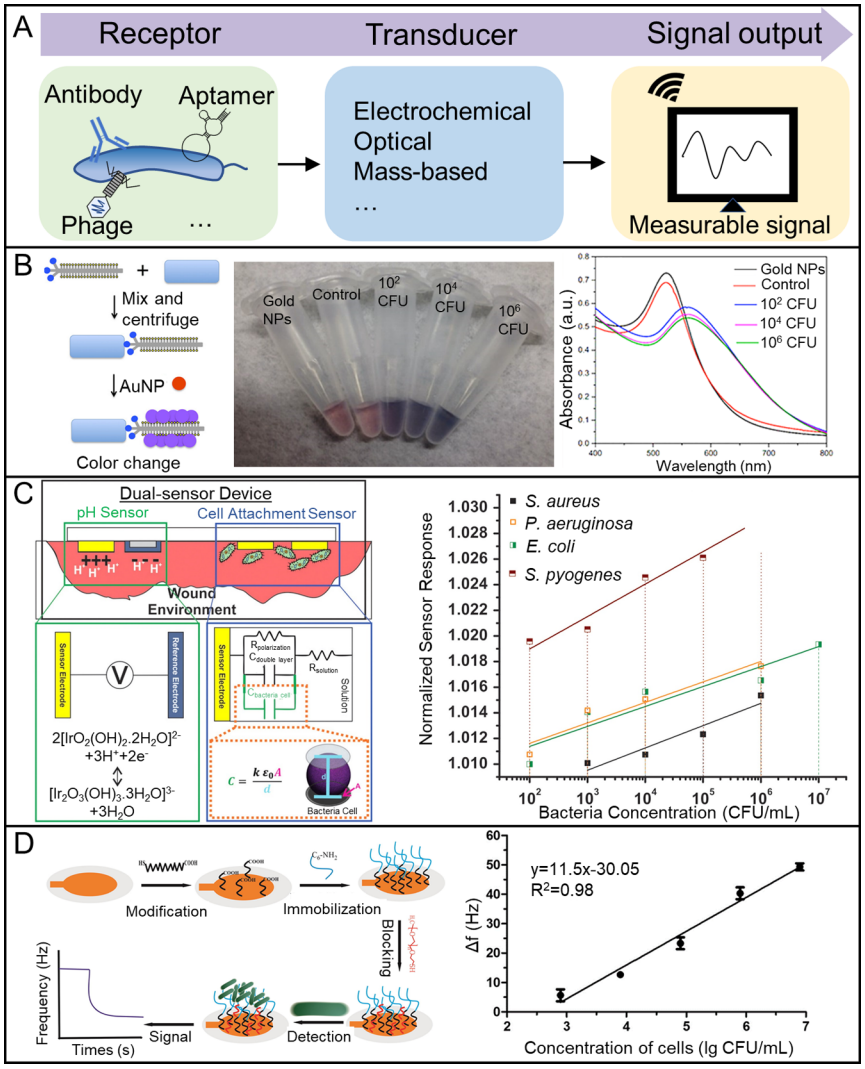


Figure 1. Recent biosensors for bacteria detection. (A) Schematic of a typical biosensor device containing a biological recognition element (e.g., antibody, aptamer, or bacteriophage) and a transducer that converts electrochemical, optical, mass, etc. changes upon binding of the analyte to a measurable signal that is read by a detector and converted into an observed output. (B) M13 bacteriophage modified to bind to *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, or *Xanthomonas campestris* and gold nanoparticles (left), resulting in a visible color change (center), and shift in the absorbance peak from ∼525 nm to ∼550 nm (right). Adapted with permission from ref 38. Copyright 2018, American Chemical Society. (C) Dual electrochemical pH and bacteria cell attachment sensor array schematic. For the cell attachment sensor, the relationship between capacitance, C; the projected bacteria area, A; and height, d (k is the relative permittivity of the dielectric and ε_0 is permittivity of space) is shown (left). The relationship between bacteria cell attachment sensor response and concentration of Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* (right). Adapted with permission from ref 39. Copyright 2016, Elsevier. (D) Modification of quartz crystal microbalance sensor to capture *Salmonella enterica* Typhimurium (left) and the relationship between frequency change (Δf) and the concentration of cells (right). Adapted with permission from ref 40. Copyright 2016, Elsevier.

most common therapeutics used for infections; however, they are often given without identifying the pathogenic bacteria. This inappropriate use of antibiotics has been linked to exacerbated antibiotic resistance.[18−20] The COVID-19 pandemic is expected to accelerate antibiotic resistance because of the extremely large number of hospitalized patients receiving antibiotics to treat potential secondary infections (∼72%).[11] Antibiotic alternatives[21] including antimicrobial peptides (AmPs),[22−24] synthetic and natural polymers,[25,26] and repurposed drugs[27−29] are promising antimicrobial agents[21] that potentially pose less of a concern regarding resistance. Additionally, biomaterials have been used to enhance the delivery of antibacterial agents and to functionalize surfaces to prevent bacterial colonization.[30−32] Drug delivery vehicles composed of biomaterials can be designed to enable targeted and localized release of encapsulated antimicrobial agents that may even overcome specific resistance mechanisms.[22,33] Here we will explore promising new antimicrobial agents, antimicrobial biomaterials, and bacteria-targeted and responsive drug delivery mechanisms.

As our knowledge of microbes has expanded, the overlap in the design of infection detection technologies with infection treatment technologies has also greatly expanded. Recognition elements used within bacterial sensors can also be used as targeting moieties within therapeutic delivery. The properties of sensor materials that enhance bacterial attachment for detection can also inform the development of antifouling and contact-killing surfaces. Additionally, a greater understanding of the diversity of microbes and their interaction with therapeutics has led to increased interest in combinations of

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

Table 1. Comparison of Common Bacterial Biosensor Recognition Elements

recognition element	advantages	disadvantages
antibody[61,63]	• high affinity and specificity • commercial availability	• high cost • lack of stability (easy damage/loss of activity in harsh environmental conditions)
nucleic acid aptamer[43,44,64,65]	• high specificity • reversible deformation • ease of being functionalized	• low stability
bacteriophage[46,49]	• specific interaction • easy production • tolerance of extreme environments	• limited functionalization due to large size (~100 nm)
charged ligands,[50−52] carbohydrates,[60] lectin,[59] and small molecules[56−58]	• high stability • strong binding affinity	• nonspecific recognition
antimicrobial peptide[53,54]	• intrinsic stability • low-cost synthesis • easy modification	• nonspecific recognition
molecularly imprinted polymers[55]	• simple preparation • low cost • superior chemical/physical stability • long shelf life • potential reusability	• long assay time • low sensitivity

new and old therapeutics and optimization of synergy to reduce resistance while enhancing antimicrobial efficacy. It is hypothesized that these trends in bacterial infection detection and treatment will continue expanding. Recent advances in infection detection aim to improve selectivity and sensitivity, while advances in treatment approaches aim to improve antimicrobial efficacy and address the concern of drug resistance. Collectively these advances in detection and treatment have enabled the integration of these fields into singular "closed-loop" technologies, an emerging field that is expected to grow. Closed-loop approaches are capable of monitoring an infection in real-time and administering a treatment as needed. In this review, we will describe recent developments in infection detection and treatment technologies and the development of closed-loop systems. Overall, we seek to summarize some of the most recent and promising approaches in this field (focusing on the last ~5 years) while reflecting on remaining challenges.

RECENT ENGINEERING APPROACHES FOR THE DETECTION OF PATHOGENIC BACTERIA

Over the last several decades, biosensors have emerged as effective tools for the detection of bacteria. These miniaturized devices are capable of amplifying the biological recognition of analytes into detectable electrical signals which are interpreted by the user. Compared with conventional diagnostics, biosensors can offer several advantages, including facile sample preparation, lower cost, and a high degree of sensitivity (i.e., $<10^1$ CFU/mL) and specificity (i.e., distinguishing between the target pathogen and the other species).[34] As shown in Figure 1A, a typical biosensor has three components: a biological recognition element (i.e., receptor) that binds to target analytes, a transducer that converts the binding event to a measurable signal, and a detector that yields a digital output related to the analyte concentration.[35] Biosensor performance is characterized by sensitivity, specificity, reproducibility, stability, and linearity.[36,37] Each of these metrics is greatly influenced by the design of the three main biosensor components. In the following section we describe some of the most recent biosensor strategies for the detection of pathogenic bacteria and the current trend toward point-of-care (POC) diagnostics.

Current Bacterial Biosensor Recognition Elements. Recognition elements are the key component of a biosensor that directly influences specificity. A comparison of common bacterial biosensor recognition elements is provided in Table 1. Antibodies are a naturally occurring recognition element produced by the immune system to detect antigens; these are the most commonly used biological recognition element for bacterial biosensors because of their naturally high specificity and commercial availability. However, antibodies often suffer from several inherent limitations, including high cost and poor stability. Recent technical advances, such as Systematic Evolution of Ligands by Exponential Enrichment (SELEX) for nucleic acid aptamer selection and understanding of bacteriophage targeting machinery, have allowed for the integration of new recognition elements for the sensitive and specific detection of pathogenic bacteria.[41−44] Compared to antibodies, aptamers have a lower cost and smaller size and can be synthesized at a larger scale. Zhang et al. compared different immobilization strategies to display DNA aptamers on the surface of iron oxide nanoparticles (Fe_3O_4 NPs), which improved the sensitivity of a colorimetric biosensor for the detection of *Streptococcus mutans*. A low limit of detection (LOD; 41 CFU/mL) was achieved when the aptamer was displayed on Fe_3O_4 NPs via affinity coupling (biotin−aptamer and streptavidin−Fe_3O_4 NPs), compared to 96 CFU/mL when the aptamer was adsorbed through electrostatic interactions onto Fe_3O_4 NPs.[45] Although more stable than their antibody counterparts, aptamers still face limitations of stability within the body. To achieve nuclease resistance, aptamers can be stabilized by chemical modifications, such as replacing the 2′ position with a fluoro, amino, or O-methyl group.[41] A better understanding of aptamer pharmacokinetics, pharmacodynamics, and toxicity is still needed to move these receptors into POC devices and closed-loop systems.

Bacteriophages are another naturally occurring entity that can be used as a biosensor recognition element.[46] These

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

bacteria-infecting viruses can specifically bind to the surface of target bacterial cells through displayed peptides and proteins that interact with the bacteria cell wall.[47] Apart from advantages including high specificity, facile production, and tolerance of extreme environments, it has also been suggested that bacteriophages may be able to distinguish between viable and dead cells without lysis of the bacteria.[48] For example, Fernandes et al. demonstrated that a broad-spectrum virulent bacteriophage (PVP-SE1) based magnetoresistive biosensor was able to sensitively discriminate viable from dead *Salmonella* Enteritidis based on different bacteriophage adsorption affinites.[49]

In contrast to highly specific recognition elements, recognition elements relying on generic chemical and physical properties such as electrostatic and/or hydrophobic interactions and/or hydrogen bonds have been used for the bulk detection of a range of bacteria. Positively charged nanomaterials (e.g., positively charged graphene oxide,[50] polyethylenimine-coated gold NPs (Au NPs),[51] and quaternary amine-coated Au NPs[52]) bind to the negatively charged surfaces of bacteria through electrostatic interactions. Similarly, AmPs containing cationic nucleic acids target the negatively charged head groups of lipids in the bacterial membrane.[53,54] Molecularly imprinted polymers work under a different mechanism by creating an imprint of the bacteria surface and use more specific polymer-ligand interactions to facilitate detection.[55] In addition, molecules like mannose,[56,57] vancomycin,[58] lectin,[59] and carbohydrates (α-mannoside and β-galactoside)[60] have attracted attention as biologically derived materials that can be used as recognition elements that have a tolerance to temperature and pH changes.[61]

Great progress has been made in developing and modifying recognition elements to increase stability and specificity, including chemical modifications and strategic display. However, there remains a need to investigate the specificity, stability, and speed of detection *in vivo*. Specifically, there is a need to better understand and optimize kinetics of interaction between receptor elements and target analytes to make rapid detection biosensors. Additionally, these technologies must be optimized for use in polymicrobial infections, which comprise a majority of wound-associated bacterial infections.[62] Future work may draw from the fields of targeted delivery and therapeutics that engage with the bacterial cell membrane to broaden the repertoire of recognition elements utilized.

Bacterial Biosensor Signal Transduction Approaches. Interactions between the bacteria and the biological recognition element must be translated to a signal that can be amplified. Biosensor signal transducers are most commonly classified into three categories: optical, electrochemical, and mass-based.[61] Each method has its unique advantages and disadvantages. In this section we describe recent developments in bacterial biosensor technologies categorized by these common transduction modes, summarized in Table 2.

Optical Biosensors. Optical biosensors rely on the measurement of light absorbed or emitted during the interaction of the receptor with target bacteria.[92] These biosensors can be further classified into colorimetric, fluorescence-based, surface plasmon resonance (SPR)-based, and surface-enhanced Raman scattering (SERS)-based biosensors.[92]

Colorimetric biosensors transduce the signal generated by bacteria−receptor interactions to a change in visible light absorption.[93] These changes in absorption are large enough to be visualized by eye without the use of additional analytical instrumentation, yielding a low-cost detection method. These biosensors can be roughly divided into solid substrate- and solution-based biosensors.[92] The former utilizes substrates such as paper or glass as the interface where bacteria interact with the receptor; these typically require small sample volumes. Readout from these designs often includes a visible line on the substrate, whose color is proportional to the analyte concentration. Examples currently on the market include those used for the detection of *Escherichia coli*, *Salmonella*, and *Streptococcus* infections among others; these biosensors are further described in a review by Yoo et al.[92] Solution-based biosensors rely on color change of a solution for detection and are often convenient and easily fabricated.[94] In a recent example by Peng et al., M13 bacteriophages were engineered to express thiol groups and a receptor-binding protein, which can target desired bacteria (including *E. coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and two strains of *Xanthomonas campestris*). Centrifugation is used to isolate the bacteriophage bound to bacteria. Then the thiol groups displayed on the bacteriophage bind to Au NPs causing aggregation and a vivid solution color change from red to purple (Figure 1B).[38] Aside from common solid- and solution-based substrates, hydrogels which consist of a highly hydrated cross-linked polymer network can also be used as color-changing substrates. We recently introduced a new colorimetric approach to bacteria detection, in which hydrogel biomaterials change color in the presence of bacterial β-lactamase enzymes. These biomaterials contain a chromogenic substrate, which can be cleaved by β-lactamases resulting in a change in hydrogel color from clear to yellow.[66] Low sensitivity remains the major drawback of colorimetric sensors, limited by the narrow visible light spectrum and ability of the human eye to detect changes in color. Sensitivity has been improved by varying recognition elements[38,67−70] and utilizing external equipment[95] to process the color changes, which ultimately eliminates the benefits of low cost and ease of use associated with colorimetric detection.[67]

As with colorimetric biosensors, fluorescence-based sensors also produce light changes; however, these sensors require specific wavelengths of light to excite fluorescent probes or labels to produce a signal.[72−74] These biosensors typically have higher sensitivity than most colorimetric biosensors because of the specificity of the emitted light and the required use of external instrumentation to detect this light. Various materials and recognition elements have been investigated in fluorescence-based biosensors for sensitive bacteria detection. For example, Cheeveewattanagul et al. utilized the unique emission properties of quantum dots (QDs) and the fluorescence-quenching capability of graphene oxide-coated nanopaper (GONP) to formulate a unique fluorescence-based biosensor. In the absence of bacteria, antibody conjugated-QDs (Ab-QDs) were adsorbed on the GONP and fluorescence was quenched. In the presence of the target bacteria, the bacteria served as a spacer between the Ab-QDs and GONP, leading to fluorescence recovery, which was quantified using a microarray scanner. This biosensor showed rapid (30 min) and sensitive detection of *E. coli* from river water without any prior enrichment (~70 CFU/mL).[96] In another example, Jin et al. developed a fluorescence resonance energy transfer (FRET) sensor that successfully detected *E. coli* in food and water samples with an LOD of 3 CFU/mL. This biosensor used aptamer-conjugated Au NPs as FRET-acceptors and complementary DNA (cDNA)-functionalized upconversion NPs

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

Table 2. Recent Biosensors for the Detection of Pathogenic Bacteria Categorized by Transducer Type

type	biosensor class	target pathogen	recognition element	assay time (min)	linear detection range (CFU/mL unless otherwise indicated)	limit of detection (CFU/mL unless otherwise indicated)
optical	colorimetric	*β*-lactamase produced by *Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*	cephalosporin[66]	N/A	N/A	4.6, 0.66, 0.11 units/mL
		Escherichia coli, *Pseudomonas aeruginosa*, *Vibrio cholerae*	bacteriophage[38]	<60	N/A	∼100 CFU
		Escherichia coli O157:H7	antibody[67]	N/A	2.0×10^4–2.0×10^7	1.95×10^4
		Escherichia coli O157:H7	antimicrobial peptides[68]	45	1×10^2–1×10^5	13
		Salmonella enterica Typhimurium, *Campylobacter jejuni* and *Salmonella* Enteritidis, *Staphylococcus aureus*	antibody[69]	N/A	N/A	10 and 100
		Staphylococcus aureus	D-amino acid[70]	N/A	1×10^2–1×10^8	3.2×10^2
		Bacillus cereus	cell wall binding domains from bacteriophage[71]	20	1×10^4–1×10^7	1×10^4
	fluorescence	*Escherichia coli* O157:H7	antibody[72]	120	N/A	14
		Vibrio parahemolyticus	aptamer[73]	N/A	15–1.5×10^6	15
		Salmonella enterica Typhimurium	aptamer[74]	∼120	10–5.0×10^5	8
		Escherichia coli	aptamer[75]	20	5–1×10^6	3
		Staphylococcus aureus, *Salmonella enterica* Typhimurium	aptamer[76]	15	4.5×10^1–4.5×10^6, 3.6×10^1–3.6×10^6	4, 8
		Escherichia coli, *Salmonella*, *Cronobacter sakazakii*, *Shigella flexneri*, *Vibrio parahemolyticus*, *Staphylococcus aureus*, *Listeria monocytogenes*	positively charged guanidine group[77]	N/A	1×10^3–1×10^8	1.30×10^2
	surface plasmon resonance	*Escherichia coli*	mannose[56]	N/A	N/A	$\sim 3.0 \times 10^2$
		Escherichia coli	antibody[78]	N/A	N/A	1.5×10^3
	surface-enhanced Raman scattering	*Vibrio parahemolyticus*	aptamer[79]	∼120	1.2×10^2–1.2×10^6	N/A
		Escherichia coli, *Staphylococcus aureus*	dual recognition by vancomycin and aptamers[80]	90	N/A	50 cells/mL, 20 cells/mL
		Escherichia coli, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*	vancomycin[58]	<30	N/A	5×10^2 cells/mL
electrochemical	amperometric	*Vibrio parahemolyticus*	antibody[81]	N/A	2.2–2.2×10^8	2
		Streptococcus agalactiae	antibody[82]	90	1×10^1 –1×10^7	10
		Salmonella enterica Typhimurium, *Salmonella enterica* Enteritidis	aptamer[83]	10	6.7×10^1–6.7×10^5, 5.5×10^1–5.5×10^6	6.7×10^1, 5.5×10^1
		Escherichia coli O157:H7	antibody[84]	N/A	3×10^1–3×10^7	∼30
	impedimetric	*Escherichia coli* B	bacteriophage[47]	N/A	1×10^3–1×10^7	1×10^3
		Streptococcus sanguinis	antimicrobial peptides[53]	<60	1×10^1–1×10^5	10
		Escherichia coli O157:H7	molecular imprinted polymers[55]	<60	10^3–10^8	10^3
		Escherichia coli O157:H7	antibody[85]	N/A	3×10^2–1×10^5	100
		Escherichia coli UTI89	imprinted organosilicate films[86]	N/A	1×10^0–1×10^4	1
		Escherichia coli	lectin[59]	N/A	1×10^2–1×10^5 cells/mL	75 cells/mL
	potentiometric	*Salmonella enterica* Typhimurium	antibody[87]	<60	5–12×10^3 cells/mL	5 cells/mL
		Vibrio alginolyticus	aptamer[88]	N/A	1×10^1–1×10^2	10
		Escherichia coli	D-mannose[57]	<60	1×10^2–1×10^3	10^2
		Listeria monocytogenes	antimicrobial peptides[89]	N/A	1.0×10^2–1.0×10^6	10
mass-based	piezoelectric	*Campylobacter jejuni*	antibody[90]	<30	N/A	20–30

https://doi.org/10.1021/acsinfecdis.0c00890
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Table 2. continued

type	biosensor class	target pathogen	recognition element	assay time (min)	linear detection range (CFU/mL unless otherwise indicated)	limit of detection (CFU/mL unless otherwise indicated)
		Aeromonas hydrophila	antibody[91]	5	6×10^6–10^8 CFU	6×10^6 CFU
		Salmonella enterica Typhimurium	aptamer[40]	<60	7.9×10^2–7.09×10^6	1×10^3

(UCNPs) as FRET-donors. In the presence of target bacteria, aptamer-Au NPs preferentially bind to bacteria and dissociate from cDNA-UCNPs, causing the recovery of upconversion fluorescence.[75] In addition to the need for specific identification of target bacteria, sensitivity is another challenge for fluorescence-based biosensors. Strategies to amplify the fluorescent signal produced by the biosensors are especially important when the number of microbes is low and can be used to improve sensitivity. For example, a novel multichannel structured three-dimensional (3D) chip was recently developed using hybridization chain reaction-based probes to amplify the fluorescence signal. This sensor was able to detect *Staphylococcus aureus* and *Salmonella enterica* Typhimurium as dilute as 4 and 8 CFU/mL, respectively.[76] Although fluorescence-based sensors have been widely used in bacteria detection, there are several remaining issues that must be addressed for future commercialization, POC use, and integration into closed-loop systems. Studies on simultaneous detection of multiple bacteria, assessment of the environmental safety of fluorescent sensor materials, and decreasing the cost of these sensors are required to improve fluorescence-based sensors.

One of the notable trends in bacteria-detecting plasmonic optical biosensors is the use of SPR and SERS transducers. The optical properties of plasmonic biosensors are highly dependent on the surfaces of the substrates used.[97] SPR-based biosensors measure the disruption of coupled incident light to surface plasmons due to the presence of particles (e.g., bacteria) on metal−dielectric interfaces.[98,99] The general SPR sensor has low sensitivity because of the limited penetration depth of the evanescent wave created at the interface into the volume of sample. In addition, the similarity of the refractive indices of bacteria and their medium also results in low sensitivity.[100] Multiple strategies have been explored to improve the LOD in SPR-based bacterial detection.[56,78] For example, Galvan et al. developed a sensitive SPR biosensor by maximizing bacteria proximity to the SPR region via dielectrophoretic (DEP)-enhanced mass transport. An LOD of ∼3.0×10^2 CFU/mL for *E. coli* was achieved, an ∼5 orders of magnitude improvement over conventional SPR chips without DEP.[56] SERS-based biosensors have also been widely used for the detection of pathogens because of their single-molecule-level sensitivity.[58,79,80] This method depends on the detection of Raman signals from SERS tags, such as Au/Ag NPs.[101] The main challenge with SERS biosensors is the interaction of NPs with nonbacteria content found in clinical samples. Recent studies have focused on improving specificity using functionalized SERS tags.[80,102−104] Zhang et al. developed an enrichment process using vancomycin-modified Fe_3O_4-coated Au NPs and used aptamer-functionalized Au NPs as SERS tags to quantify target bacteria. Results showed that aptamers could be exchanged to allow for either the capture of *S. aureus* or *E. coli* from urine samples within 15 min with an LOD of 20 and 50 CFU/mL, respectively.[80] Although these plasmonic biosensors are highly promising, the expensive and complex instrumentation involved remains the greatest challenge for clinical translation and integration into closed-loop devices.

Electrochemical Biosensors. Electrochemical bacterial biosensors interpret interactions of bacteria with recognition elements through changes in electrical signals (e.g., current, potential, impedance, and capacitance).[105] The low cost associated with these sensors and the potential for high

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

sensitivity and specificity make them a powerful and practical tool for the detection of pathogenic bacteria.[106] These biosensors are usually composed of three electrodes: a reference, a counter, and a working electrode. The working electrode, which can be fabricated from the nanometer to millimeter scale from various materials (e.g., Au and carbon), is the main component of the electrochemical biosensor.[107] It can also be functionalized to improve specificity and sensitivity. A recently reported amperometric immunosensor utilized a graphite electrode modified with a composite of polypyrrole, Au NPs, multiwalled carbon nanotubes (MWCNTs), chitosan, and antibodies to enable the detection of *E. coli* O157 with an LOD of 30 CFU/mL. Each component of the composite coating contributed to the performance of the biosensor. MWCNTs increased the sensor surface area and conductivity, increasing sensitivity. Polypyrrole enhanced the environmental stability and conductivity. Au NPs increased the conductivity within the coating, and chitosan promoted electron transfer to enhance sensor sensitivity. The use of antibodies in this sensor enhanced the specificity to detect *E. coli* O157:H7 from *E. coli* O124, *P. aeruginosa*, *Salmonella* Enteritidis, *Shigella* species, and *Burkholderia cepacia*, which are the most common sources of bacterial contamination in food samples.[84]

Compared to other electrochemical sensors, potentiometric bacterial biosensors are not as affected by the background noise of clinical samples.[108] Thus, these sensors have been widely used to detect bacteria for human health applications.[57,87−89] These sensors detect the change in electrical potential between two electrodes. Recent studies have focused on modifying these electrodes with novel receptors and signal amplification strategies to enhance attachment of bacteria and increase the change in electrical potential. For example, Zhao et al. modified a solid-contact polycation-sensitive membrane electrode with DNA nanostructure-modified magnetic beads, which amplify the potential signal for the detection of *Vibrio alginolyticus*. This potentiometric biosensor showed a linear range of 10−100 CFU/mL with a low LOD of 10 CFU/mL.[88] Approaches to transition these sensors into portable and low-cost devices to facilitate commercialization are also being explored. Silva et al. replaced traditional ion-selective metallic electrodes with a paper-based sensing platform to detect *S. enterica* Typhimurium.[87] In addition, incorporating anti-*Salmonella* monoclonal antibodies on the electrode surface via dendrimer coupling increased sensor sensitivity, reaching an LOD of just 5 *S. enterica* Typhimurium cells/mL.[87]

Impedimetric bacterial sensors capture interactions of bacteria with the sensor surface as increases in impedance.[109,110] Similar to amperometric sensors, biomaterials can be used to functionalize these sensors to increase sensitivity and specificity.[111,112] The current trend in impedimetric biosensors is to integrate low-cost receptors to further increase specificity.[53,113,114] For example, Zhou et al. integrated T2 bacteriophages within their electrode to specifically quantify the B strain of *E. coli* from a mixed population of other strains of *E. coli*.[47] Significant progress has been made in label-free impedimetric bacteria sensors, allowing for lower-cost detection.[39] There has also been a growing interest in combining various sensors to better monitor the microbial environment in addition to detecting bacteria. We recently reported a dual, label-free electrochemical sensor array that successfully monitors both the local pH and the presence of bacteria via impedimetric measurements in simulated wound conditions (Figure 1C). The pH sensor exhibited a high sensitivity of approximately −89 mV/pH over a wide pH range from 1 to 13, while the impedimetric sensor achieved a low LOD for several pathogenic bacteria species (1×10^3 CFU/mL for *S. aureus* and 1×10^2 CFU/mL for *P. aeruginosa*, *E. coli*, and *Streptococcus pyogenes*). The combination of these two high-performance sensors was demonstrated to provide accurate *in situ* monitoring of simulated infected wounds *in vitro*.[39] Integrating sensors utilizing different transduction methods is also an approach used to achieve more reliable results. Electrochemical biosensors have been combined with optical-[115,116] and mass-based[117,118] transducers. For example, Adkins et al. explored the individual and combined use of a bacterial enzyme responsive colorimetric biosensor and an amperometric biosensor for the detection of *E. coli* and *Enterococcus* species. Findings from this study indicated that using both methods together can overcome disadvantages associated with the individual techniques.[116]

Electrochemical biosensors have been widely applied to the rapid detection of bacteria. However, traditional electrochemical biosensors are typically unable to guarantee simultaneous detection of multiple pathogenic bacteria with high accuracy and stability, limiting their use in polymicrobial infections, and therefore limiting practical application. Novel electrochemical materials and signal amplification strategies are still needed to provide sensitive and specific detection. In addition, the next generation of electrode arrays with miniaturization and multiplexing capabilities will help commercialization of electrochemical biosensors for bacterial infection detection.

Mass-Based Biosensors. Mass-based bacterial biosensors generally utilize piezoelectric (PZ) materials that vibrate at a specific frequency under the influence of an electrical stimuli. When bacteria interact with the surface, the mass increases, leading to a decrease in sensor vibrational frequency and thus the electrical signal.[119] Quartz crystal microbalance (QCM) is a very common approach operating on this principle, based on the inherent PZ properties of quartz. QCM can be adapted to detect bacteria by adding a receptor element to the surface of the quartz crystal. Various methodologies can be used to attach receptors to the quartz crystal including coating the crystal with a metal or NPs that can then be conjugated with a receptor. The use of NPs specifically can improve sensitivity by amplifying frequency changes and increasing the surface area. Wang et al. reported a QCM-based biosensor for the rapid (within 1 h) detection of *S. enterica* Typhimurium with an LOD of 10^3 CFU/mL and high specificity due to the use of DNA aptamers as the receptor (Figure 1D).[40] To date, PZ bacterial biosensors are suitable only for laboratory-based detection,[90,91] and future approaches for these sensors are likely to focus on enabling field operation with new adaptable equipment.

Shift Toward Point-of-Care Pathogen Detection. Current bacterial diagnostic protocols require patient sample collection at a clinic and subsequent transfer to a laboratory for further processing using various equipment and techniques (e.g., culture-based and molecular diagnostics). The results are then analyzed by a technician and reported back to a physician, who makes decisions on patient treatment. Overall, this process is costly and time intensive, during which both the sample and the patient can undergo significant changes. As a promising alternative, POC detection devices aim to integrate the capabilities of high-performing biosensors into portable,

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

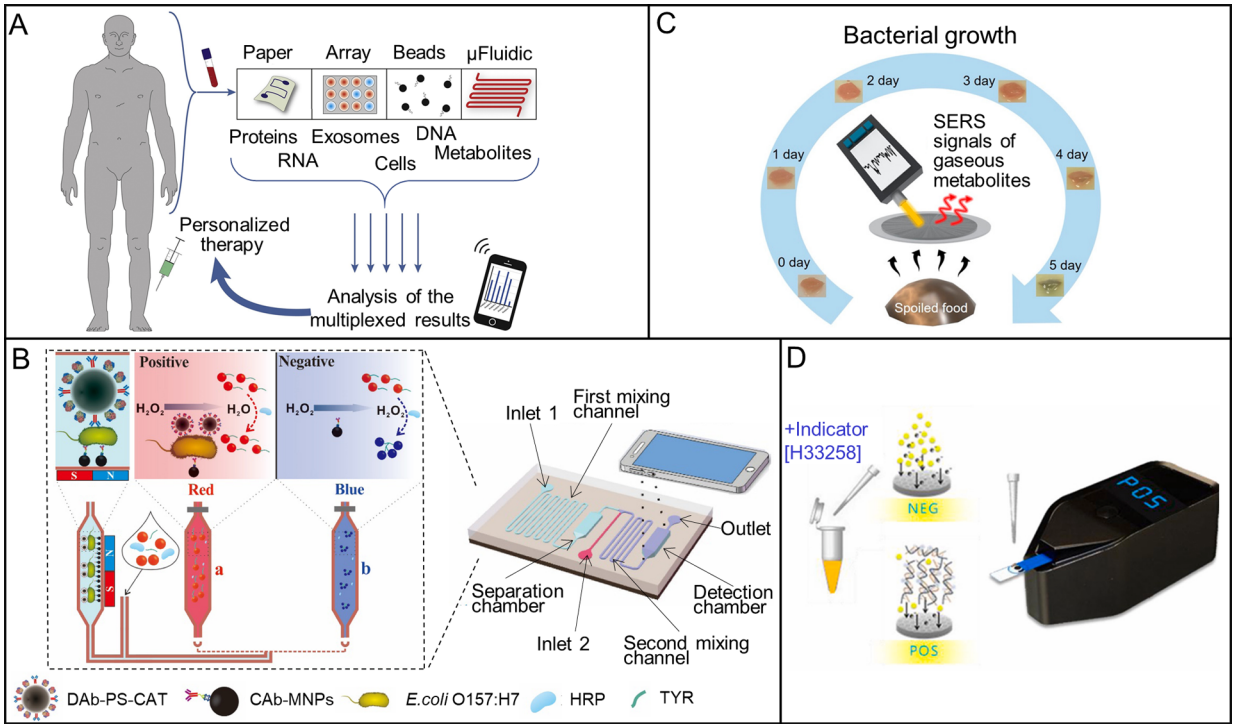

Figure 2. Point-of-care biosensors for bacterial detection. (A) Various approaches including paper, arrays, beads, and microfluidic devices have been used to develop point-of-care biosensors. These point-of-care biosensors can then allow for rapid analysis and personalized therapies. Adapted with permission from ref 120. Copyright 2017, Elsevier. (B) A microfluidic colorimetric biosensor in which *Escherichia coli* O157:H7 causes the capture of catalase modified polystyrene microspheres. The presence of catalase catalyzes hydrogen peroxide hydrolysis, thus hindering the aggregation of gold nanoparticles resulting in a color change from blue to red. Adapted with permission from ref 95. Copyright 2018, Elsevier. (C) A surface-enhanced Raman scattering (SERS) "nose" was fabricated by loading gold nanostars on a flat filter membrane. The SERS signals of gaseous metabolites were easily detected by a portable Raman spectrometer by placing the "nose" above the sample infected by bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Adapted with permission from ref 121. Copyright 2020, American Chemical Society. (D) An integrated point-of-care system consisting of loop-mediated isothermal amplification, screen-printed graphene electrodes, and portable mini-potentiostat for the detection of *Vibrio parahemolyticus*. Adapted with permission from ref 122. Copyright 2019, Elsevier.

rapid, and inexpensive tests that can be performed either in the patient or within close proximity to the patient (Figure 2A).[120]

Tremendous advances have been made in POC bacterial detection resulting from innovation in lab-on-a-chip technologies and hand-held readout devices. Lab-on-a-chip devices integrate and automate one or more laboratory functions using microfluidics into a millimeter−centimeter-scale chip.[123] Although there have been many applications of lab-on-a-chip devices for bacteria detection, shrinking price and size along with facile signal output remains challenging for these devices.[99,124−126] Smartphone technologies can enable the real-time analysis and sharing of diagnostic results from POC devices and allow for rapid onsite monitoring of a patient in various locations, including resource-limited settings.[127] For example, a recent microfluidic colorimetric bacteria biosensor used Au NP aggregation and smartphone imaging to detect *E. coli* O157:H7 in homogenized meat samples. This POC device achieved an LOD of 50 CFU/mL with good sensitivity and specificity (Figure 2B).[95] Current state-of-the-art smartphone-based analysis still lags behind traditional laboratory instruments in accuracy, and further technological advances are being pursued to improve upon this.[128]

In addition to colorimetric POC devices, other biosensors have also been successfully applied in POC systems.[129] For example, a convenient POC SERS device was fabricated by coating Au nanostars on a filter membrane for real-time monitoring of gaseous bacterial metabolites. As shown in Figure 2C, a portable Raman spectrometer was used to collect and quantify the SERS signals.[121] This SERS "nose" was used to monitor gaseous metabolites from *E. coli*, *S. aureus*, and *P. aeruginosa* in porcine tissue.[121] Electrochemical biosensors have also attracted attention for POC devices because of the ease of miniaturization. Kampeera et al. developed an integrated POC system composed of loop-mediated isothermal amplification (LAMP) and electrochemical biosensors for the detection of *Vibrio parahemolyticus* (Figure 2D). The LAMP DNA amplification products were mixed with the redox probe where an oxidation reaction occurred. A portable potentiostat was then used to measure current intensity coming from the oxidation reaction; this current was then correlated to the bacteria concentration. An LOD of 0.3 CFU *V. parahemolyticus* per 25 g of seafood was achieved by this sensor within 45 min, which could be extremely helpful in detecting this waterborne pathogen.[122]

Very few commercial POC bacteria detection devices have entered the global market. Most of these POC devices have implemented colorimetric biosensors. For example, AZO, Inc. (Memphis, TN) provides test strips that can detect urinary tract infections (UTIs). This sensor relies on the nitrate reductase produced by bacteria such as *E. coli* that enter the urinary tract, which converts urine nitrates to nitrites. When the nitrates interact with an aromatic amine within the test strip, they form an aryl diazonium salt, which can further react with the tetrahydrobenzoquinoline within the test strip to

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

Table 3. Recent Developments in Antibacterial Therapeutics Categorized by Mechanism of Action[a]

		Acinetobacter baumannii	*Aggregatibacter actinomycetemomitans*	*Aspergillus flavus*	*Bacillus subtilis*	*Clostridium difficile*	*Enterobacter cloacae*	*Enterococcus faecalis*	*Enterococcus faecium*	*Escherichia coli*	*Helicobacter pylori*	*Klebsiella pneumoniae*	Methicillin Resistant *Staphylococcus aureus*	*Micrococcus luteus*	*Mycobacterium abscessus*	*Photobacterium phosphoreum*	*Porphyromonas gingivalis*	*Pseudomonas aeruginosa*	*Rhodobacter sphaeroides*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Streptococcus mutans*	Vancomycin Resistant *Enterococcus*
Biomimetic	Gallium[136]																	X					
	DNA-histone microwebs[137]									X													
	Artificial super neutrophils[138]																			X			
Cell lysis	Bacteriophage cocktail[139]																			X			
	Bacteriophage EFDG1, EFLK1[140]							X															
	MMI-Ps1 and Caudovirales bacteriophages[141]																	X					
	Muddy, BPs*33*ΔHTH-HRM10, ZoeJΔ*45*[142]														X								
Competition	*Bacillus subtilis* exopolysaccharide[143]																			X			
	Fecal transplant[144]					X																	X
	Lucilia sericata[145]					X				X										X			
	Probiotics (*Saccharomyces*, *Lactobacilli*, *Bifidobacteria*, *Streptococci*)[146]					X																	
Gene expression	Mitomycin C[147]	X																					
	Niclosimide[27,148]					X							X										
	anti-*DNAe*[149]					X																	
	anti-*mazE*, anti-*thyA*, anti-*hipB*, anti-*glt*[150]		X							X													
	anti-groEL, anti-acpP[151]																X						
Immunomodulation	Fusobacterium associated defensin inducer[152]																X						
	anti-*Irf5*[153]																			X			
Intracellular	SkQ1[33]				X										X	X			X	X			
	VB-82252[154]					X																	
	Promysalin[155]																	X					
	Glycine-pendent poly(2-oxazoline)[156]												X										
Membrane disruption	α-helical peptides[157]																	X		X	X		
	D4E1[158]			X																			
	Cecropin B[159]				X					X				X				X		X		X	
	Poly-β-peptides[160]																	X		X			
	Block co-β-peptide, poly-amido-saccharide-block-poly-dimethyl β-lactam[161]	X					X					X						X					
	Block co-β-peptide, poly(amido-D-glucose)-block-poly(β-L-lysine)[162]												X					X					
	Main chain cationic polyimidazolium[163]	X					X	X		X		X						X		X			
	Polymer side chains (imidazolium, DABCO-diium, quaternary ammonium)[164]									X										X			
	Polymers (NBD-SMAMP or BP-NBD-SMAMP)[165]									X										X			
	AR-12 and polymxins[166]																			X			
	Inhibitor Ia[167]																	X					
Protein synthesis	Ebselen[168]	X											X										
	Auranofin, ebselen, PX12[169]						X		X			X						X		X			
Stress response	TC19, TC84, BP2[170]				X																		
	1018, 3001, 3002, 3003, 3004, 3005, 3006, 3007[171]	X											X										
	Antibiotics and antimicrobial peptides[172]						X		X			X						X		X			
	α-defensin-1, β-defensin-3, LL-37, histatin-5[173]																					X	

Therapeutics	Living organisms & biomimetic systems
AmPs /Host defense peptides	Nucleotides
Antimicrobial polymers	Repurposed drugs
Bacteriophages	Small molecules

[a]x indicates that the therapeutic was effective against the indicated pathogens.

ultimately produce a pink azo dye, visible to the human eye, indicating the presence of bacteria. Given the promising advances that have been made in biosensors, POC devices may soon be the standard of care for many types of bacterial infections. Additional advances in new nontoxic, environmentally friendly materials for biosensor fabrication will also contribute to the development of economical, ultrasensitive, and stable POC devices for monitoring long-term human health.

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

Bacterial Detection: Limitations and Future Directions. Many advances have been made in the field of detection of bacterial pathogens, which is critical for rapidly and accurately diagnosing an infection. These advancements include improvements in the stability and specificity of bacterial recognition elements for biosensors. Combining these with new techniques for quantifying receptor−analyte interactions has led to the development of many new bacterial biosensors. Several challenges still need to be overcome, however, including reducing costs, increasing sensitivity, and ensuring effective operation in polymicrobial environments. In addition, many new biosensors rely on interpretation of complex readouts, such as Raman spectra. Additional work is needed to simplify both the preparation of samples and the analysis of the data from these complex systems in order to develop these technologies into commercial use devices specifically for POC. Furthermore, there are many challenges in translating the transduced signals into triggers to initiate drug delivery, requiring additional optimization in order to facilitate detection and treatment integration into closed-loop systems.

RECENT ADVANCES IN TREATMENTS FOR BACTERIAL INFECTIONS

Along with the numerous innovations that have been reported in bacteria detection technologies, research in new treatment approaches has also opened up the possibility for improved patient outcomes in the management of infectious diseases. In this section, we will review many of the most recent antibacterial therapeutics, followed by a discussion on using biomaterials to incorporate and/or deliver these agents to provide antibacterial therapy.

Therapeutic Agents for Bacterial Infections. Antibiotics remain the most common treatment for bacterial infections. The mechanism of action for these and other antibacterial therapeutics can broadly be divided into two categories: membrane and intracellular dysregulation. Membrane dysregulation involves therapeutic interactions that facilitate changes in membrane rigidity, charge, and permeability that result in membrane collapse, disruption of nutrient uptake, and/or changes in intracellular ion concentration.[130−132] Antibiotics like carbapenems specifically bind to the penicillin-binding protein within the cell membranes of many bacteria, preventing cell wall synthesis, while antimicrobials such as positively charged AmPs interact with the negatively charged teichoic acids of the cell membrane to form pores. Intracellular dysregulation occurs when therapeutics interact directly with intracellular components to disrupt homeostasis. Quinolones, for example, interfere with critical intracellular enzymes involved in DNA synthesis. Antibiotic resistance, which can develop for drugs functioning via either of these mechanisms, is a growing public health threat that is challenging the utility of existing therapeutics. There are numerous mechanisms by which bacteria exhibit antibiotic resistance. These include the production of enzymes like β-lactamases, which can cleave the β-lactam ring structure within β-lactam antibiotics (e.g., penicillins, cephalosporins, and carbapenems) rendering them nonfunctional. Another mechanism of resistance includes changes to cell wall composition, such as the production of penicillin-binding protein 2 or other positively charged proteins on the surface that reduce the affinity for certain antibiotics, and thus their efficacy. Bacteria also possess multiple efflux systems, and these efflux pumps can facilitate extracellular transport of therapeutics that act intracellularly, thereby imparting resistance.[19] It is estimated that over 2.8 million antibiotic-resistant infections occur annually in the United States alone.[18] Unfortunately, a mere 14 antibiotics, only 3 of which have been classified as novel by the United States Food and Drug Administration (FDA), have received FDA approval between 2014 and 2019.[133] With a dwindling pipeline of new antibiotic discovery and increasing antibiotic resistance, the complexity of treating bacterial infections is rapidly increasing. For example, antibiotics such as fluoroquinolones, third-generation cephalosporins, macrolides, and amoxicillin/clavulanic acid have all been linked with increased incidence of health care-associated methicillin-resistant *S. aureus* (MRSA).[134]

In recent years, various sources of antimicrobial agents have been investigated in an attempt to increase the antibacterial drug repertoire and delay resistance development. Additionally, combination therapeutics have been examined in order to enhance bacterial killing, and potential mechanisms of action and synergy have been explored. These mechanisms of synergy include parallel pathway inhibition, mutual stabilization, sequential blockade, bioavailability modulation, and inhibitor inhibition.[135] Table 3 summarizes many of these recent therapeutics, their mechanisms of action, and some of the bacterial species that have demonstrated antimicrobial susceptibility to these therapeutics.

Prophylactic Therapeutics. The human body has multiple barriers against invading microbes, including the skin, mucus, changes in temperature, and the innate and adaptive immune systems. In healthy individuals, these mechanisms work together to prevent these microbes from establishing an infection. Prophylactic actions are preemptive attempts to reduce interactions with microbes and/or aid these host defense mechanisms in preventing infections. Prophylaxis with antibiotics is common in a variety of scenarios, including patients undergoing surgery or chemotherapy. Recent reviews investigating hospitalized COVID-19 patients show that 72% of these individuals (of 2010 total) received one or more antibiotics, where only 8% had indications of coinfection with bacteria or fungi.[11] Clinical trials investigating antibiotic prophylaxis show a mix of positive and negative outcomes, depending on the antibiotic, the procedure, delivery method, and duration.[9,174] The prophylactic approach also encourages overuse of antibiotics, which can exacerbate antibiotic resistance and dysbiosis. The current trend for prophylactic therapies which aims to address these concerns is twofold: (1) development of targeted antibiotic delivery systems and (2) investigating systemic nonantibiotic therapeutics. One example of the first approach by Chen et al. was to develop a polymeric ciprofloxacin pro-drug with targeted delivery to infected immune cells via mannose-mediated interactions. These prodrugs are hydrolyzed in the intracellular space of macrophages, activating the ciprofloxacin to treat intracellular infections.[175] Examples of the second approach involve prophylaxis with compounds such as vitamin D[176] and proanthocyanidins.[177] In a 2017 retrospective study of 10 933 patients, Martineau et al. found independent associations between low serum concentrations of the vitamin D metabolite, 25-hydroxyvitamin D, and susceptibility to acute respiratory tract infections. Therefore, there has been increased interest in delivering vitamin D metabolites to induce bacterial autophagy and cell death through the production of reactive nitrogen and oxygen species.[176] Proanthocyanidins, which are

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

polyphenols found in many fruits including cranberries, have shown promise in inhibiting the adherence of highly virulent P-fimbriated *E. coli* to the uroepithelial cells of the urinary tract.[177] Optimized dosages and delivery are important obstacles that still need to be addressed for these promising antibiotic alternative prophylactic therapeutics.

Repurposed Drugs. Repurposing therapeutics for indications different from their original target is not a new concept; many drugs have been found to exhibit interesting side effects that lead to their development for new conditions. Current research on repurposing drugs for antimicrobial applications comes from a variety of drug classes, including anticancer, anthelmintic, immunomodulatory, antipsychotic, and antidepressant therpeutics.[29] A large benefit of repurposing drugs is that they have already undergone extensive safety and efficacy testing for FDA approval, reducing the cost and toxicity concerns of developing these drugs as antimicrobials. Similar to tumors, pathogenic bacteria have high replication rates and resistance mechanisms. These similarities have inspired the translation of cancer drugs to bacterial infections. Mitomycin C, for example, is a bladder, gastric, and pancreatic cancer drug that works by cross-linking DNA and preventing replication and gene expression.[147] Cruz-Muñiz et al. showed higher survival rates over 5 days in an *Acinetobacter baumannii* infected *Galleria mellonella* larvae model when treated with mitomycin C (80% and 53% *Galleria* survival for ATTC BAA-74 and multidrug resistant strain A578, respectively) compared to survival rates when treated with antibiotics, ciprofloxacin (70% and 0% *Galleria* survival for ATTC BAA-74 and A578, respectively) and ceftazidime (100% and 0% *Galleria* survival for ATTC BAA-74 and A578, respectively).[147] Similarly, anthelmintic drugs that restrict anaerobic respiration in nematodes, such as niclosamide, have been repurposed to eradicate and prevent infections from various pathogens such as *Helicobacter pylori*,[28] *Clostridium difficile*,[148] and MRSA.[27] We demonstrated the potent antibiofilm and antibacterial efficacy of niclosamide coatings against several common sources of medical device-associated infections, including *S. aureus*, MRSA, and *Staphylococcus epidermidis*.[27] Another susceptible genetic interaction is the highly conserved thioredoxin system, which aids in DNA synthesis. Drugs such as auranofin,[178] originally developed for treating osteoarthritis and ebselen,[168] an anti-inflammatory, antioxidant, and cytoprotective drug, have both been shown to irreversibly bind to thioredoxin constituents resulting in eradication of MRSA infections.[169] We recently reported a polyurethane catheter coating containing auranofin which was able to inhibit the growth of MRSA over 26 days.[178] A limitation of repurposed drugs in the context of antimicrobials is that microbes may have already come in contact with the repurposed drugs, therefore leading to the risk of drug resistance. One potential avenue to mitigate this limitation is to use a delivery system that enhances the drug's antimicrobial activity. Additionally, if the drug has a wide range of activity, targeted and responsive delivery (further evaluated in Targeted Biomaterials and Responsive Biomaterials) is essential to prevent side effects.

AmPs and Polymers. The discovery and development of antibiotics start with extraction and isolation of compounds from bacterial and fungal cultures; these compounds are screened for potential antibacterial activity and altered synthetically as new antibiotics. Using a similar approach, researchers have explored a variety of defense mechanisms employed by different organisms to prevent infections, including the production and display of peptides, to identify new antimicrobial therapeutics. AmPs, also termed host defense peptides, are short amphipathic, and cationic peptides (<50 amino acids) exhibiting antimicrobial activity.[179] These peptides, originally identified in the defense mechanisms of humans,[157,180] animals,[157,159,181] and bacteria,[152] have generated significant interest as an alternative to antibiotics. Recent advances in computational modeling[171,180,182,183] and machine learning[157] have facilitated the identification of residue patterns, including hydrophobic regions[157] and amine residues,[164] that enhance bacterial membrane interactions and facilitate antibacterial activity. Furthermore, these methods can be used to design AmPs that remain inactive and thus nontoxic to host cells while in aqueous environments and transition to an α-helical, active conformation upon entry into bacterial membranes.[157] Many different mechanisms of action have been proposed for AmPs, including pore formation in the bacterial membrane,[159] depolarization of the bacterial membrane, and stimulation of stress responses.[170,172] In addition, several AmPs have been shown to work synergistically with the host immune system[152,184,185] or with antibiotics,[172,186] thus lowering the risk of resistance.[187] Recently, Lázár et al. explored the resistance mechanisms of *E. coli* and how to synergistically utilize antibiotics and AmPs to exploit these mechanisms. Ultimately, these studies found that cross-resistance to AmPs and antibiotics was rare and that the development of resistance to the AmP, 18-kDa cationic antimicrobial protein (CAP18) in *E. coli* led to an increase in sensitivity to conventional antibiotics. Additionally, antibiotic resistance development in *E. coli* led to an increase in sensitivity to the AmP, peptidyl-glycylleucine-carboxyamide (PGLA).[187] With continued interest in discovering new naturally occurring antimicrobials, the future of AmPs is moving toward predictive computational analysis of antimicrobial properties and combination therapeutics to enhance antimicrobial efficacy and reduce the risk of resistance. Additionally, antimicrobial peptoids (i.e., N-substituted glycine oligomers) have been investigated in order to overcome the challenges of scalability and stability associated with many AmPs.[188]

Natural and synthetic polymers and their derivatives also have great potential as antibacterial agents. Because of the versatility of synthetic approaches, recent innovations have focused on synthetic antimicrobial polypeptides and polymers. These macromolecules often mimic the physiochemical properties of AmPs leading to their antibacterial activity, while being more cost-effective and scalable.[164] Some common examples include quaternary ammonium polymers,[189] polymethacrylates,[190] poly[2-(dimethyl decyl ammonium)ethyl methacrylate],[191] poly-β-peptides,[160−162] and polyimidazolium.[163,164] Guo et al. investigated the relationship between polymer structure and antimicrobial properties of several antimicrobial compounds, finding a greater antibacterial efficacy in main-chain cationic polymers than side-chain cationic polymers. Overall, this study found that the side-chain/main-chain cationic polymers synthesized with small molecule cationic compounds, including imidazolium, quaternary ammonium, and 1,4-diazabicyclo[2.2.2]octane-1,4-diium, had greater antibacterial efficacy than their corresponding small molecule cationic constituents. Specifically, these polymers effectively inhibited the growth of Gram-negative *E. coli* and Gram-positive *S. aureus*.[164] Synthetic polymers can also be of interest in terms of their stability and scalability, as

https://doi.org/10.1021/acsinfecdis.0c00890
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demonstrated by Si et al., who effectively used a block copolymer of glycosylated poly amido-saccharide amphiphiles and cationic poly dimethyl β-lactam in synergy with the antibiotic novobiocin, which is used against Gram-positive bacteria. Together this combination approach reduced bacterial load by 99.99% for drug-resistant strains of *E. coli*, *Klebsiella pneumoniae*, and *A. baumannii* in a murine systemic infection model.[161]

Synthetic polymers used in other applications have also been tuned to enhance their inherent antimicrobial properties, while focusing on increased physiologic stability. In a recent example, Zhou et al. synthesized a glycine-pendant poly(2-oxazoline) (Gly-POX) to mimic host defense peptides, taking advantage of the biocompatibility of glycine and the FDA-approved indirect food additive with inherent proteolytic resistance, POX. Gly-POX was found to be a potent antimicrobial polymer capable of permeating the bacterial cell membrane and inducing the SOS response, killing MRSA, as well as MRSA persister cells, which are typically highly resistant to treatment with antibiotics.[156] Although exploration of naturally found AmPs and antimicrobial polymers is important to continue to diversify the depot of antimicrobial agents, recent advances emphasize the importance of understanding the properties that enable antimicrobial activity and synergy. These properties can then be mimicked in more stable configurations or with synthetic materials to increase the ability of these therapeutics to be translated to clinical applications.

Nucleic Acid Therapeutics. Gene expression and translation are essential processes in both host and bacterial cell homeostasis. Many antibiotics function by targeting these mechanisms. Nucleic acid therapeutics such as single-stranded oligonucleotides,[149] double-stranded small interfering RNA (siRNA),[192] and synthetic peptide nucleic acids[150,193,194] can directly bind to RNA and disrupt the gene expression and translation balance, thus redirecting cell behavior. Nucleic acid therapeutics are also utilized to directly modulate the host immune system as another approach to combating bacterial infections. Given their important role in the host immune system, phagocytic cells, namely, neutrophils[195] and macrophages,[153] are the main targets of this immunomodulatory approach. The specificity of nucleic acid interactions helps avoid harmful interactions with host cells and the commensal microbiota. Równicki et al. investigated the delivery and mechanisms of anti-*mazE*, anti-*thyA*, anti-*hipB*, and anti-*gltX* peptide nucleic acids (PNAs) for inhibiting the growth of *E. coli*, finding that these PNAs effectively reduced the expression of these bacterial target genes. As expected because of their specificity for these bacterial genes, these PNAs were found to be nontoxic to human cells, namely, human embryonic kidney cells 293.[150] Computational modeling suggests that nucleic acid therapeutics are less likely to cause resistance, yet efflux still remains a major complication for this type of treatment.[196] New methods for accurate and efficient delivery of these therapeutics into the intracellular space of bacteria are also needed.

Small Molecule-Based Disruption of Bacterial Nutrient Metabolism. In addition to gene expression, cellular metabolism is another promising target for antimicrobial therapeutics. Naturally occurring,[155] synthetic,[197] and biomimetic[136,198] small molecules are some of the main antimicrobial therapeutics that are used to disrupt bacterial nutrient metabolism, causing cell death. Keohan et al. showed that promysalin, a secondary metabolite of many *Pseudomonas* species, inhibits succinate dehydrogenase activity, thereby inhibiting glycolysis and disrupting homeostasis in *P. aeruginosa*. Treatment with promysalin created equal zones of inhibition, similar to that of gentamicin against *P. aeruginosa* PA14, PAO1, KT2440, and RW10S1 under minimal media conditions forcing metabolism to use succinate dehydrogenase in the tricarboxylic acid cycle.[155] Molecules such as gallium mimic the properties of iron, a common nutrient taken up by *P. aeruginosa*. Unlike iron, however, gallium cannot be processed to provide nutritional value; thus, bacterial cell growth is suppressed when gallium is present along with iron.[136] Because of the robust stress response of bacteria, these types of therapeutic approaches are typically combined with current antibiotic therapeutics.[166,167] Alternatively, small molecules can also be used to directly modify bacterial toxins, preventing host cell damage.[154,199] For example, Strokes et al. developed a small molecule inhibitor of *C. difficile* toxin A and B's uracil diphosphate-glucose hydrolysis activity, VB-82252. Treating with VB-82252 reduced *C. difficile* infection symptoms in a murine model while not harming commensal *Bacteroides fragilis*, *Bifidobacterium longum*, and *Lactobacillus reuteri*. VB-82252 treatment also extended the survival of *C. difficile* infected hamsters from 3 days to 12 days.[154] Resistance mechanisms like efflux pumps remain a formidable challenge hindering the success of these small molecule therapeutics. Therefore, continued research on the synergistic application of these drugs with existing therapeutics or inhibitors of resistance mechanisms is needed. In addition, as these antimicrobials work intracellularly, carrier systems and drug delivery approaches can play an important role in enhancing their efficacy.

Cellular Therapeutics. Cellular therapeutics ranging from the commensal microbes to host cells are also being explored for their potential benefit in combating infections. There has been a recent explosion of interest in the impact of the gut microbiome on systemic health.[200,201] In particular, probiotics have shown positive results in preventing infections. These beneficial bacteria, including those of the genus *Saccharomyces*, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, assist with nutrient absorption and are thought to maintain a balance within the microbial composition of the gut.[146,202−204] Antibiotic use often disrupts this balance by eliminating commensal bacteria, allowing for opportunistic pathogens such as *C. difficile* to take over.[205] Therefore, probiotics have gained attention as treatments for opportunistic infections and their associated pathologies. Recent studies have shown that patients who took oral probiotics[146,204] or had direct fecal transplants[144,206] after antibiotic treatment were at lower risk of contracting *C. difficile* infection and had fewer antibiotic-associated adverse effects than those who took placebos or were given no treatment. With growing knowledge of the human microbiome, research in probiotics and their therapeutic potential will undoubtedly expand.

Host immune cells perform many functions, including recruiting additional immune cells, modulating the infection microenvironment, and killing bacteria.[203,207] These cells can also work with commensal microbes to protect the host.[203,207] For example, extracellular polymeric substances (EPS) produced by *Bacillus subtilis* aid macrophages in protecting against systemic *S. aureus* infection.[143] There is an interest in mimicking the antibacterial mechanisms of host immune cells, including the production and release of neutrophil extracellular traps (NETs)[137] and hypochlorous acid.[138] For example, Song

https://doi.org/10.1021/acsinfecdis.0c00890
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Table 4. Recent Examples of Antifouling and Contact-Killing Biomaterials Approaches[a]

	Type of Material	Interface Material	*Escherichia coli*	Methicillin Resistant *Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Staphylococcus aureus*	*Staphylococcus saprophyticus*	*Streptococcus mutans*	*Streptococcus oralis*, *Actinomyces naeslundii*, *Veillonella dispar*, and *Porphyromonas gingivalis*
Anti-fouling	Topography (Brush)	Polyurethane monolayer with pendant azide displaying propargylic modified polyethylene glycol[210]	X				X			
	Topography (Nanodiamonds)	Nanodiamond coating on selective laser melted titanium scaffold[218]								
	Topography (Nanobush)	Zinc oxide nanobush on gold-coated polystyrene microneedles[219]				X	X			
	Co-polymer coating	Dopamine methacrylamide and 2-methacryloyloxyethyl phosphorylcholine covalently bound to polydopamine/polyethyleneimine with embedded silver nanoparticles[211]	X				X			
		Crosslinked chitosan and carboxymethylcellulose on polyethylene terephthalate[212]							X	
	Slippery liquid-infused porous surfaces	Layer-by-layer assembly of poly(vinyl-4,4-dimethylazlactone) and branched poly(ethylenimine) loaded with biofilm inhibitors and infused with silicone oil[216]			X					
		Laser-induced titanium spikes silanized with a fluorinated polymer and infused with perfluoropolyether lubricant Krytox GPL 104[214]								X
Contact-killing	Polymer coating (Brush)	Polyurethane modified poly-(N,N-dimethylacrylamide-co-N-(3-aminopropyl)-methacrylamide hydrochloride) brushes decorated with antimicrobial peptide, E6[220]			X		X	X		
	Polymer coating	Based on electrostatic complexation of ε-poly-L-lysine and 1,4-bis(2-ethylhexyl) sodium sulfosuccinate[221]					X			
		Layer-by-layer film consisting of poly(diallyldimethylammonium) and poly(styrene sulfonate) and hetero-bifunctional polyethylene glycol terminated with a carboxyl group and ε-poly-l-lysine[222]	X				X			
		Layer-by-layer film of α-Poly-L-lysine and hyaluronic acid[229]	X	X	X		X			
		Poly(hexamethylene biguanide) hydrochloride–sodium stearate[223]	X				X			
		Polyimidazolium salt film coated polydimethylsiloxane[225]		X						
	Non-polymeric coating	Supersonic cluster beam deposited Ag, Cu, and Mg nanoparticle film[226]	X				X			
	Antibiotic functionalization	Branched polyethylene glycol diglycidyl ether and gentamicin functionalized titanium[224]	X				X			

[a]x indicates that the therapeutic was effective against the indicated pathogens

et al. reported a novel microweb of DNA–histone complexes which mimic NETs to trap and kill pathogenic bacteria, such as *E. coli*.[137] Recently Zhang et al. reported an artificial "super neutrophil," which generates hypochlorous acid like natural neutrophils. These artificial neutrophils consisted of a glucose oxidase and chloroperoxidase loaded metal–organic framework as the core material, coated with rat neutrophil membranes. Treatment with artificial neutrophils led to a reduction in CFU and greater wound closure compared to treatment with phosphate buffer solution in an *S. aureus* infected mouse model.[138] Successful use of cellular therapeutics to combat infections requires stringent delivery systems and approaches to prevent adverse effects as is often seen with antibiotic overuse.

Bacteriophages. Bacteriophage, which we have discussed as a promising biosensor receptor for bacteria detection, are more commonly known for their viral activity against bacteria. Briefly, bacteriophages attach to their bacterial targets and introduce their genome into the bacteria, followed by exploitation of the bacteria's DNA replication machinery in one of two ways. In a lytic replication cycle, additional bacteriophages are produced within the cell, overwhelming the bacteria in regard to both intracellular space and energy, ultimately leading to cell death and release of the phage. In a lysogenic replication cycle, the bacteriophage genome is combined into the bacterial chromosome, replicated, and passed on to daughter cells. The species-specific nature of bacteriophages limits off-target killing; however, because of a lack of broad-spectrum activity, bacteriophages are typically explored in combination with antibiotics[140] and/or additional bacteriophages.[139] The clinical use of bacteriophage has currently been limited to individual cases of personalized therapy in the United Kingdom and as an emergency investigational new drug (eIND) in the United States.[142,208] However, many bacteriophage therapies are moving toward FDA approval, such as LBP-EC01 for urinary tract infections, developed by Locus Biosciences.[205] In addition to killing bacteria, bacteriophages have also been shown to enhance host immunity against infections.[141] Finally, with advances in genetic engineering, there is tremendous potential for bacteriophage manipulation to tailor these therapeutics for combination therapies and as drug carriers.[142,205,209]

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695–720

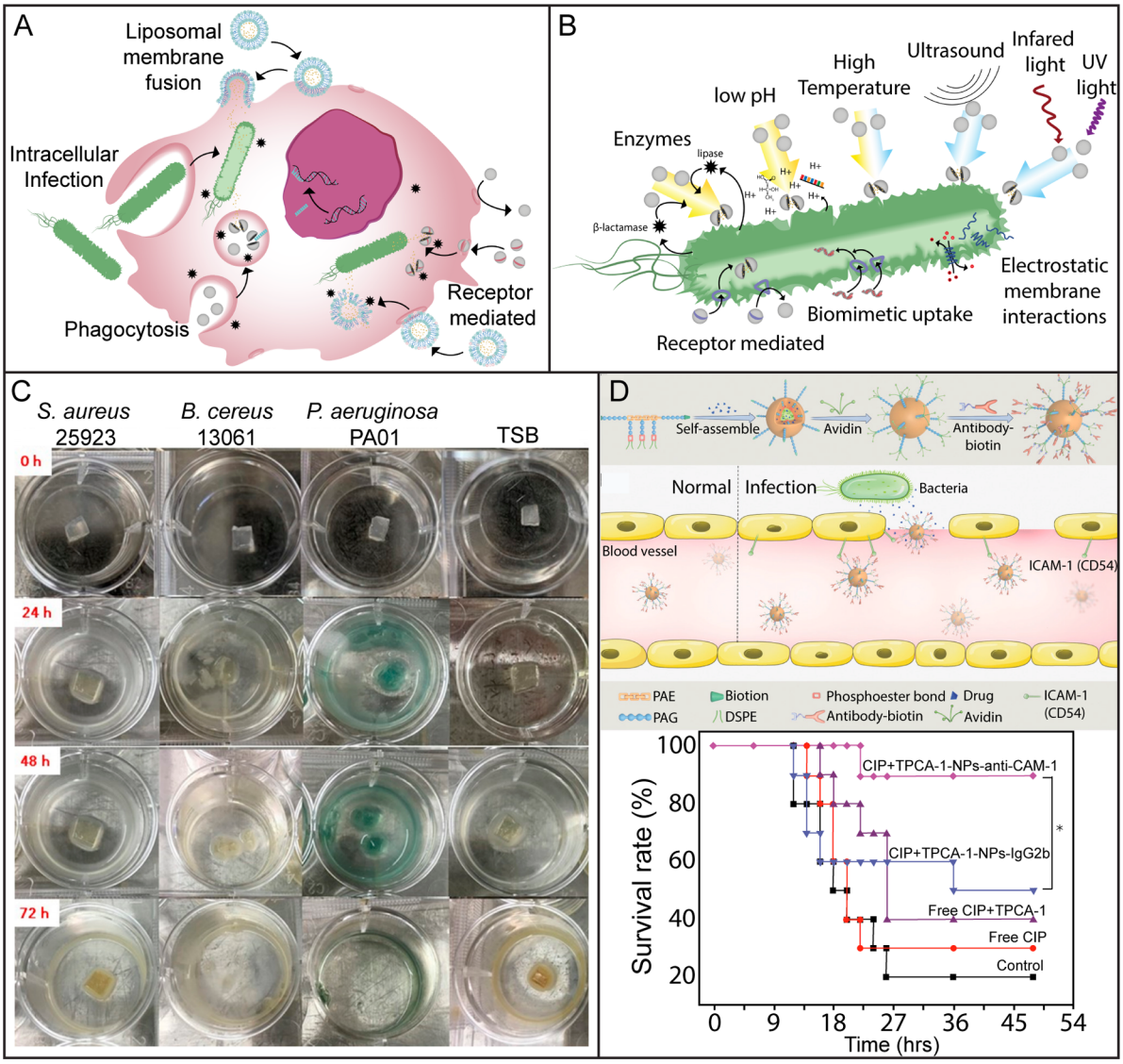

Figure 3. Mechanisms and examples of targeted and responsive antibacterial therapeutic delivery. (A) Host cells can take up bacteria and nanoparticle drug carriers via phagocytosis. For the example shown of a therapeutic liposome, these can be targeted to the host cell via various mechanisms, including receptor-mediated uptake and membrane fusion. (B) Similarly, bacteria can be targeted through receptors, biomimetic carriers, and electrostatic membrane interactions. Responsive delivery systems can respond to the infection microenvironment (yellow arrows), particularly enzyme activity, low pH, and higher local temperatures. Additionally, external activation (blue arrows) using ultraviolet light, infrared light, ultrasound, and high temperatures can be used to initiate therapeutic release. (C) Representative images (n = 3) of β-lactamase responsive supramolecular host−guest interpenetrating polymer network hydrogels containing polymeric cyclodextrin host moieties and adamantane guest cross-links with a β-lactam core incubated in *Staphylococcus aureus* 25923, *Bacillus cereus* 13061, *Pseudomonas aeruginosa* PA01, and tryptic soy broth (TSB) over time at 37 °C. Adapted with permission from ref 233. Copyright 2020, American Chemical Society. (D) Fabrication of pH and enzyme responsive copolymer PEGylated nanoparticles decorated with intercellular adhesion molecule-1 (ICAM-1) antibodies used to target infection microenvironments for the responsive delivery of ciprofloxacin (CIP) and an anti-inflammatory drug (2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide) (TPCA) (top). Increased survival rate of mice when inoculated with a lethal dose of *Pseudomonas aeruginosa* and treated with the drug loaded pH and enzyme responsive, ICAM-1 targeted nanoparticles (CIP+TPCA-1-NPs-anti-ICAM-1) compared to IgG2b targeted, drug loaded nanoparticles (CIP+TPCA-1-NPs-IgG2b), free drugs (Free CIP+TPCA-1), free CIP, and no treatment controls. Statistical analysis was done using Kaplan−Meier method (n = 10). *p < 0.05 (bottom). Adapted with permission from ref 234. Copyright 2018, John Wiley and Sons.

Antibacterial Therapeutics: Limitations and Future Directions. Recent advances in computational modeling and analysis have aided the development of new antimicrobial therapeutics, and expanded methods for testing their therapeutic efficacy and mechanisms of action. With an increased understanding of how new compounds can be designed to mimic the properties of existing synthetic and naturally derived antimicrobials, limitations like stability and activity *in vivo* can be overcome. Furthermore, these computational approaches can be used to design therapeutics that are better able to target specific bacterial components. Despite the advances that have been made, many studies have focused on assessing therapeutic activity only within isolated bacterial cultures. In actuality, microbial infections are often polymicrobial, containing more than one pathogenic bacteria species and may also be composed of biofilms and even viruses and fungi.

https://doi.org/10.1021/acsinfecdis.0c00890
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Future directions for new antimicrobial therapeutic and combination therapies require a more robust exploration of how well these therapeutics perform against these multi-pathogen infections. Concurrently, with the increasing appreciation for the fine balance of the human microbiome, the impact of new therapeutics on the beneficial microbiome must be elucidated. Many strides have been made to develop combination therapeutics to mitigate the development of resistance mechanisms; these analyses will undoubtedly continue to be a crucial component of future therapeutic development. Lastly, limitations such as effective intracellular delivery, host cell toxicity, and targeted delivery can be addressed with the proper design of drug delivery technologies using biomaterials.

Antibacterial Biomaterials. Biomaterials have been used in antimicrobial therapies both as an antimicrobial agent and/or as a carrier of antibacterial therapeutics, such as the ones we described in Therapeutic Agents for Bacterial Infections. Additionally, as carriers of antimicrobial drugs, biomaterials have the potential to increase drug efficacy, reduce the occurrence of infection, and mitigate host toxicity. A majority of innovations in this field have focused on antibacterial surface coatings and drug delivery approaches. These include nano- to macro-scale materials, from polymer and metal NPs, to thin films and hydrogels. Here we will specifically discuss recent developments in antifouling and contact-killing surfaces and responsive and targeted drug delivery systems.

Antifouling and Contact-Killing Surfaces. Biofilms develop when bacteria under various forms of stress produce EPS, creating a protective network that tethers the bacteria to surfaces.[6,210,211] Biofilms are highly prevalent on implanted materials and are well-known for their tolerance to antibiotics and recurrence.[6,210,211] In order to prevent biofilm formation there has been increasing interest in engineering surfaces that prevent bacterial attachment (i.e., antifouling surfaces) and/or kill bacteria upon interaction with the surface (i.e., contact-killing surfaces).[26] Table 4 summarizes these approaches and provides recent examples.

Antifouling surface strategies include manipulating the nanotopography of surfaces as well as displaying bacteria repellant polymer brushes[212] and copolymers.[213,214] Surface coatings displaying superhydrophilic polymers[213,214] or hydrophilic brushes[212] lead to the formation of a densely hydrated layer that acts as a physical barrier preventing bacteria attachment. Slippery liquid-infused porous surfaces (SLIPS) formulated from nanoporous materials infused with inherently "slippery" oils, which cause most liquids and even bacteria to slide off, have shown particularly promising results as antifouling surfaces.[215−217] Kratochvil et al. took advantage of the versatility of layer-by-layer assembly to form multifunctional SLIPS using a porous poly(vinyl-4,4-dimethylazlactone) and branched poly(ethylenimine) hydrophobic multilayer coating infused with silicone oil and loaded with quorum sensing inhibitors. These drug-eluting SLIPS multilayers were found to prevent *P. aeruginosa* biofilm formation on coated materials and surrounding surfaces by 60% and 50% relative to untreated controls, respectively.[218] Many of these antifouling mechanisms can also prevent host cell interaction, and therefore, these approaches are often best suited for materials that do not require integration into tissues such as mouthguards[214] and cotton fabrics[219] for clothing. In contrast, Rifafi et al. developed a rough surface through selective laser melting followed by nanodiamond dip coating that prevented bacteria attachment while allowing for beneficial host cell interactions. These nanodiamond-coated titanium surfaces enhanced fibroblast and osteoblast cell density by 32% and 29% respectively, while reducing *S. aureus* attachment by 88%, compared to untreated selective laser-melted titanium surfaces.[220]

Contact-killing surfaces are specifically engineered to elicit a stress response in bacteria upon interaction, leading to cell death. These surfaces can employ various tethered antibacterial agents including nanodiamonds,[221] AmPs,[222−224] antimicrobial polymers,[225] small molecules,[226] and NPs.[227−229] Optimized shape, orientation, and material properties can also facilitate puncturing of bacterial cell membranes.[230] Other surface modifications utilize ionic interactions to create disturbances in the bacterial membrane. These ionic interactions can be facilitated through AmP brushes,[222] interactions with antimicrobial polymers in coatings such as poly-L-lysine,[223,224,231] or coatings with embedded salts[227] and metals.[228,229] Adsorption and fouling of nonmicrobial content including host proteins and stimulation of the foreign body response, however, can reduce the antibacterial properties of many existing coatings.[6,7] Changing coating composition and improving fabrication to reduce defects can help overcome this limitation.

Targeted Biomaterials. Targeted biomaterials are designed to encourage therapeutic or therapeutic carrier interactions with a target of interest. In the case of antimicrobial therapies, this target is usually either bacteria or host cells. Figure 3A,B summarizes some of the mechanisms involved in targeting these cell types. Most targeted approaches rely on nanoformulations as vehicles for intracellular delivery.[211,232] Targeting ligands need to be carefully selected, while additional components can be added to reduce uptake by other cells.

Host immune cells are an obvious choice for targeted therapeutics to combat infections. Recent work has involved development of macrophage-targeting delivery systems to enhance macrophage-killing of bacteria[153] and to treat intracellular bacteria.[235,236] These NP delivery systems are able to target macrophages by coating with lipid bilayers[153,235] and displaying moieties that macrophages have receptors for, such as mannose.[175,236] Once phagocytosed these delivery vehicles can release their therapeutic payload. This methodology has been effective against various different intracellular bacteria, including *S. aureus*,[235] *E. coli*,[235] and *Francisella novicida*.[175,236] NP facilitated intracellular delivery of siRNA against the *Irf5* gene (si*Irf5*) has been shown to knockdown the proinflammatory expression of *Irf5* in macrophages and effectively eliminate a lethal *S. aureus* lung infection in a murine model. It was hypothesized that reduced inflammation enhanced macrophage antibacterial activity through one of two possible methods: by either extending the time that macrophage dominate bacteria clearance or by transitioning macrophage to a tissue-restorative phenotype and recruiting other immune cells to clear bacteria.[153]

Mimicking the properties of materials involved in quorum-sensing and horizontal gene transfer can encourage uptake of drug delivery vehicles by bacteria. Zhang et al. used noncoding DNA to form tetrahedral nanoconstructs as a delivery system. These DNA constructs are recognized by bacteria as beneficial and taken up into the bacterial cell. Once internalized, these DNA constructs can dissociate, releasing antisense DNA oligonucleotides[237] or synthetic peptide nucleotides[149] to

https://doi.org/10.1021/acsinfecdis.0c00890
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disrupt bacterial homeostasis. Other mechanisms employed to enhance bacterial uptake include displaying peptides[238−240] and ligands[138] on NP surfaces that are known to attach to bacterial membranes and receptors. Another strategy is to coat NPs with the membranes of cells that bacteria want to infect such as gastric epithelial cells[241] and macrophages.[242] Angsantikul et al. showed that *H. pylori* preferentially attaches to gastric endothelial membrane coated, clarithromycin loaded, polyethylene glycol (PEG) NPs. In an *H. pylori* infected mouse model, these targeted NPs reduced bacterial burden by ∼3 orders of magnitude compared to untreated mice, while free clarithromycin reduced bacterial burden by only ∼0.5 orders of magnitude.[241] Targeted delivery systems are essential not only to enhance bactericidal activity but also to reduce off-target accumulation. Additional studies investigating the preferential interaction of these delivery systems with the intended cell types compared to relevant nontarget cells (e.g., fibroblasts, endothelial cells, stem cells, etc.) could improve designs before moving to preclinical investigation.

Responsive Biomaterials. Responsive systems establish control over delivery by requiring trigger mechanisms that coordinate the release or activation of a therapeutic. As shown in Figure 3B, many possible triggers exist for antibacterial delivery systems, most commonly enzymes, pH, membrane interactions, and light. Bacteria produce enzymes as a part of their natural metabolism, and in some species as a defense mechanism. As bacteria proliferate, the quantity of enzymes, such as lipases,[243−245] hylaluronidases,[246,247] and β-lactamases,[248] increases proportionally. Researchers have engineered antibacterial biomaterials to respond to these enzymes by integrating enzyme-degradable polymers, polymer linkers, and lipids in NPs and pro-drugs. For example, enzyme-degradable polymers such as poly(ethylene succinate)-(PES),[243] polycaprolactone (PCL),[243] and hyaluronic acid[246] have been used to form responsive NPs[245−247,249] and nanofibers.[243] Many of these enzyme triggers however are not specific to bacteria and can also be produced by host cells. We recently reported a hydrogel matrix specifically cleaved by β-lactamase enzymes, which is a family of enzymes produced only by bacteria and one of the leading causes of antibiotic resistance; this biomaterial has the potential to be used as a highly bacteria-specific responsive antimicrobial carrier (Figure 3C).[233]

The high metabolic rate and rapid proliferation of bacteria generally create an acidic environment within infected regions.[250] Biomaterials can be designed to respond to this low pH, including disruption of biomaterials in the carrier system[243,246,251] or release of active therapeutics from a pro-drug formulation.[252] Various pH-responsive antimicrobial biomaterials have recently been reported, including electrospun fibers,[243] NPs,[246] hydrogels,[252] and microspheres.[251] There are multiple approaches to fabricate these materials, including integrating pH-responsive materials within non-responsive constructs[251] or coating a nonresponsive core with a pH-responsive shell.[243,246] In an example of the later approach, Abdali et al. developed a lipase and pH-responsive electrospun core−shell nanofiber composed of a poly-(vinylpyrrolidone) and biocidal drug, benzyl dimethyl tetradecyl ammonium chloride coated in a PCL/PES shell. Incubation with these fibers resulted in >1 log reduction in gram-positive *S. aureus* and gram-negative *E. coli* within 6 h.[243] Although pH-responsive antimicrobial systems have been demonstrated to be successful in terms of intravenous stability and delivery, the potential for off-site delivery due to similar acidity at other sites in the body must be considered (e.g., a tumor site).

Responsvie drug delivery approaches can also utilize external triggers to release therapeutics on-demand. These may include infared,[253−255] visible,[256,257] and ultraviolet light;[258,259] temperature;[260] and ultrasound.[245] Implant and device coatings,[256,257,260] hydrogels,[258] and NPs[77,83,84,91,261] have all been designed to respond to these types of external triggers for antibacterial applications. The external control of these systems can allow for repeatable and on-demand self-sterilizion.[256,258,260] Applications for these on-demand and self-sterilizing mechanisms extend beyond infections, and they can be implemented as a prophylactic approach. Shen et al., for example, developed a graphite carbon nitride photocatalyst material that prevented and eradicated *S. epidermidis* biofilms under activation of white light-emitting diodes.[257]

Recent work has examined the combination of both targeted and responsive approaches to enhance drug delivery. Zhang et al. reported an antimicrobial NP formulation loaded with ciprofloxacin that was both pH- and lipase-responsive and also coated with an intercellular adhesion molecule-1 antibody for targeting (Figure 3D, top). Treatment with these NPs increased survival from 50% to 90% in a murine peritonitis-induced sepsis model (Figure 3D, bottom).[234] Such approaches are promising to limit toxicity of antimicrobials and also increase efficacy, potentially helping to eliminate unnecessary exposure which is linked with resistance, but care must be taken to ensure that both the targeting and responsive behaviors are specific to the target of interest.

Antibacterial Biomaterials: Limitations and Future Directions. Biomaterial technologies for antibacterial therapy are highly versatile in design and function. Contact killing surfaces rely on the interaction of microbes directly with the modified surface, while antifouling surfaces prevent direct microbial interaction. For both types of surfaces, a major limitation is potential adsorption of proteins and other components from the physiological microenvironment, preventing antimicrobial activity or changing surface properties, allowing for microbial attachment. Omniphobic surfaces, such as many SLIPS, are able to overcome this limitation. However, each of these surface modifications is limited by the fabrication process, as defects in the surface coating can allow for bacteria attachment. Targeted biomaterials for infection treatment are expected to become an essential strategy used to mitigate antibiotic resistance. These biomaterials can not only improve antimicrobial activity by directly delivering therapeutics to or within the target bacteria but can also aid in preventing off-site exposure and activity. Similar to targeted biomaterials, responsive biomaterials can also be used to facilitate localized delivery and control exposure. The specificity and rate of the responsive behavior is the major challenge facing these delivery approaches. Although promising antibacterial activity has been observed with both targeted and responsive antibacterial drug delivery systems, a strong effort to study resistance development in the presence of these materials must be made in order to optimize these technologies to thwart antibiotic resistance. Current efforts are focusing on combining advancements in targeted and responsive biomaterials with advances in detection methods for the most effective control of infection in closed-loop therapies.

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

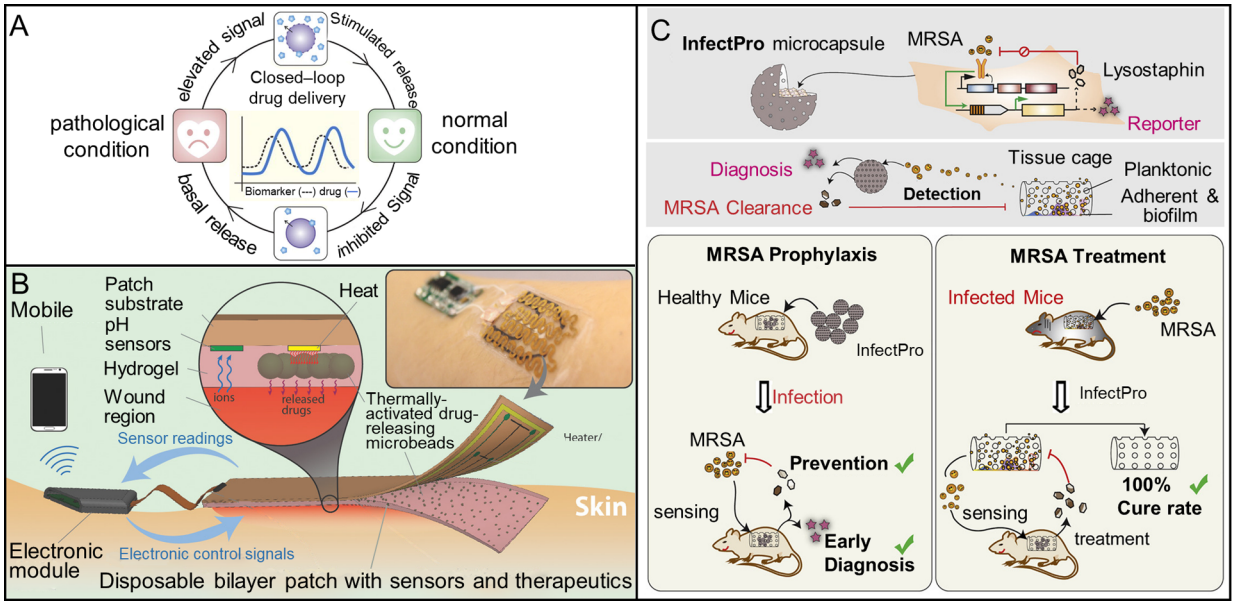

Figure 4. Closed-loop systems for bacterial infections. (A) Closed-loop systems combine detection and treatment to achieve real-time monitoring of normal and pathological conditions to initiate treatment on demand. Adapted with permission from ref 262. Copyright 2017, Elsevier. (B) A closed-loop smart bandage that monitors pH changes, initiating thermally activated release of cefazolin from microbeads at a wound site with mobile device monitoring. Adapted with permission from ref 263. Copyright 2018, John Wiley and Sons. (C) Schematic of microencapsulated cells with closed-loop gene circuit (top). Mechanism of designer cells for the clearance of methicillin-resistant *Staphylococcus aureus* (middle). Schematic of prophylaxis with designer cells prior to infection with methicillin-resistant *Staphylococcus aureus* (left) and treatment following infection (right) in a murine model (bottom). Adapted with permission from ref 264. Copyright 2018, Elsevier.

CLOSED-LOOP THERAPY

In this review we have described some of the most recent innovations in infection detection and treatment. Closed-loop therapies have the potential to effectively combine these methods. As shown in Figure 4A, these self-regulated approaches continuously monitor and respond to the presence or absence of a pathologic condition.[262] Closed-loop therapy is a precision medicine approach that autonomously detects and eliminates bacterial infections. An example of a closed-loop smart bandage technology is shown in Figure 4B. Here, a pH sensor monitors the infection status; under low pH conditions (indicating infection), the bandage triggers a heating element which subsequently causes drug release from a thermoresponsive carrier. *In vitro* studies using this bandage showed effective pH monitoring and triggered release of cefazolin causing 90% reduction of *S. aureus*.[263] In addition to macroscale devices, there is also interest in engineering cells to facilitate closed-loop therapy. For example, Fussenegger et al. genetically modified embryonic kidney cells to produce the antimicrobial enzyme lysostaphin, following human Toll-like receptor (TLR)-mediated interactions with MRSA (Figure 4C). The genetic circuit employed in this system utilzes the natural nuclear factor κB (NF-κB) signaling cascade initiated by TLRs that recognize peptidoglycan, lipoteichoic acid, and lipoproteins from the bacterial cell walls. Activation of this circuit results in production of lysostaphin. *In vivo* studies showed that this cell-based, closed-loop approach can effectively prevent 91% and eradicate 100% of MRSA infections in a murine implant-associated infection model.[264]

With the promising recent advances made in this field, further preclinical investigation will help to translate closed-loop technologies. In particular, the biomaterials used in these technologies need to be carefully evaluated in regard to maintaining function in relevant physiologic conditions. Futhermore, sustained biocompatibility is also required for long-term usability. Additionally, for clinical use it is important to consider human variability, the variability of bacterial signals, and how these will influence the efficacy and specificity of the therapy.

CONCLUSION

As bacterial resistance toward present-day antibiotics continues to rise, it is essential, now more than ever, that we continue to develop innovative and effective methods of detecting and treating bacterial infections. In recent years there has been great progress in developing faster, more accurate, and more cost-effective detection modalities for monitoring bacterial infections. Many detection methods have utilized advances in nanotechnology and chemical modifications to stabilize receptor elements, increase tranducer sensitivity, and produce POC devices. However, there are still significant hurdles to translating these technologies into commercial devices while maintaining low costs. Trends for future biosensors include advances in wearable devices, microfluidic devices, and self-powered technologies providing more accurate detection and real-time transmission of information. With better detection approaches, we gain a better understanding of pathogen populations and their microenvironments. Subsequently, this understanding aids in the evolution of antimicrobial therapeutics to target different mechanisms of action and develop combinations of therapeutics to avoid drug resistance. Recent emphasis on targeted and responsive delivery has improved efficacy of therapeutics; however, further improvements are needed to prevent off-target effects. Additional investigation of the biocompatibility, stability, and efficacy of new therapeutics and their delivery systems, particularly in preclinical models, is needed to enable successful translation.

https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720

Closed-loop therapies have begun to combine these detection and treatment approaches in exciting early phase technologies. The goal of antibacterial closed-loop therapies is to continuosly monitor bacterial infections and release antimicrobial drugs as needed. Limitations that these technologies face include the stability of the systems over long-term use and need for rapid communication between the integrated detection and treatment technologies. In addition to the development of these new approaches, there is a need to improve our testbeds for bacterial detection and therapeutic activity assessment of antibacterial agents and delivery systems, with a goal of enabling more predictive capability when advancing from *in vitro* to *ex vivo* and finally *in vivo* study. Lastly, the feasibility of manufacturing, scale-up, and costs of these systems must all be considered to truly enable translation. Ultimately, it will require combined expertise from various fields, including medicine, chemistry, materials science, and engineering, to overcome current challenges associated with the prevention, detection, and treatment of bacterial infections.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the Office of Naval Research (N000141712120); the Dr. Ralph and Marian Falk Medical Research Trust Bank of America, N.A., Trustee; and Brown University.

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https://doi.org/10.1021/acsinfecdis.0c00890
ACS Infect. Dis. 2021, 7, 695−720