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CHAPTER THREE

Advances in aggregation induced emission (AIE) materials in biosensing and imaging of bacteria

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Abstract

With their ubiquitous nature, bacteria have had a significant impact on human health and evolution. Though as commensals residing in/on our bodies several bacterial communities support our health in many ways, bacteria remain one of the major causes of infectious diseases that plague the human world. Adding to this, emergence of antibiotic resistant strains limited the use of available antibiotics. The current available techniques to prevent and control such infections remain insufficient. This has been proven during one of greatest pandemic of our generation, COVID-19. It has been observed that bacterial coinfections were predominantly observed in COVID-19 patients, despite antibiotic treatment. Such higher rates of coinfections in critical patients even after antibiotic treatment is a matter of concern. Owing to many reasons across the world drug resistance in bacteria is posing a major problem. According to Center for Disease
control (CDC) antibiotic report threats (AR), 2019 more than 2.8 million antibiotic resistant cases were reported, and more than 35,000 were dead among them in USA alone. In both normal and pandemic conditions, failure of identifying infectious agent has played a major role. This strongly prompts the need to improve upon the existing techniques to not just effective identification of an unknown bacterium, but also to discriminate normal Vs drug resistant strains. New techniques based on Aggregation Induced Emission (AIE) are not only simple and rapid but also have high accuracy to visualize infection and differentiate many strains of bacteria based on biomolecular variations which has been discussed in this chapter.

1. Introduction

Public health remains a major concern in developing and developed nations alike. Human race is threatened by different infections caused by pathogens like bacteria and fungi, which account for more than 2 million lives every year worldwide. In other way, intestinal bacteria play important roles, including food digestion, generation of essential micronutrients (e.g., Vitamin K) and contribute to the overall immunity in adults. Contrarily, bacteria remain a major cause of fatal infectious diseases globally. Emerging antibiotic resistant bacterial strains further exaggerates this problem. In case of bacterial infections, diagnosis, and treatment at the early stage will be more effective to arrest the growth of diseases. Further, identification of causal organism is crucial, and is the foremost step, in clinical practice which helps in making informed decisions such as selection of drugs. Another area where bacterial identification remains a high priority is the food industry, for detection of food contamination. Unfortunately, emergence of drug-resistant bacterial pathogens, non-availability of suitable biomarkers and time-consuming screening of infections in patients pose a grave challenge to human health. Identification and classification of microbes with high efficiency and accuracy will facilitate rational use of antibiotics in clinical settings.

Recently, due to environmental pollution and climate change, the prevalence of water-borne pathogens has risen significantly. For the detection of bacterial contamination, different methods are available. However, the currently available methods have some limitations such as longer incubation periods (e.g., standard plate count) and requirement for trained personnel (e.g., polymerase chain reaction). Additionally, development of methods for fast, reliable and instrument free detection of bacteria and other pathogens remains challenging. To overcome these limitations, there is an urgent
need to develop whole bacterium analysis without involving complicated microbiological preparations. This not only provide greater understanding of the pathogen under investigation, but also, guide the development of novel therapeutic strategies. Theranostics is an emerging concept of combining diagnostic imaging and therapeutic intervention which is widely used in the treatment of bacterial diseases and systemic diseases like cancer. Various radiological, fluorescence, and magnetic resonance imaging (MRI) techniques have been developed based on the specific interactions of ligand-receptor or antigen-antibody interactions and are commonly used diagnostic techniques for more accuracy. Nevertheless, these are relatively expensive and have limited usage in real-time pathogen detection and tracking of tumor in intraoperative surgeries. In many instances, the variety of microbes to be detected may be novel and not detected by the probes with specific recognition moieties making the techniques ineffective. Thus, a simple light based turn on or off like rapid diagnosing method which can distinguish differentially expressed biomolecules on different strains of same microbe, are high on demand for efficient and accurate detection of microbes.

The classification and identification of bacteria and fungi relies on a labor intensive battery of tests such as Gram staining, PCR, genome sequencing, Raman Spectroscopy. These techniques are not only complicated, but also, require sophisticated equipment. Additionally, integration of therapeutic agents is very much complicated process for cancer treatment, especially in coinfection cases. Therefore, developing new strategies based on single component will largely minimize the design process of the targeted system for both imaging and killing of bacteria and cancer cells, and enhancing the theranostic purpose. Most bacterial surfaces are negatively charged, and are responsible for changes in the infected site microenvironment such as alterations in pH, and temperature. Additionally toxins and lipases released from bacteria also contribute to the unique characteristics of the infected site microenvironment. Recently, the dependency on fluorimetry has become common in screening, and imaging applications due to high sensitivity and selectivity, easy to operate, noninvasive and rapid response. In the recent past, a special class of luminogens with aggregation induced emission (AIEgens) characteristics has received global attention among researchers. Further, AIEgens exhibit good biocompatibility, high quantum efficiency, and are photo stable. Thus, AIEgens are suitable for diverse applications such as biosensors, cell imaging, electroluminescent materials, and thus have great potential to use in biological and biomedical applications. In this chapter, we
have reviewed the principles, properties, and applications of different AIE materials with respect to imaging or sensing of bacteria.

### 2. Aggregation induced materials

Individual molecules yet times end to aggregate into well-ordered structures supported by non-covalent interactions including hydrogen bonds, electrostatic interactions, π-π (pi-pi) interaction, hydrophobic interactions, and charge transfer effects.\(^\text{12,13}\) Such aggregation at high concentrations is known to cause quenching of traditional fluorophores, a phenomenon called as Aggregation Caused Quenching (ACQ). The notorious ACQ effect has limited the use of luminogens at very dilute concentrations, which leads to serious photobleaching, effecting the imaging and other applications.\(^\text{14}\) AIE is a photophysical phenomenon to monitor the light emitting processes from the aggregation of weakly or non-emissive luminogens.\(^\text{11,15}\) In the solution, AIEgens are in monomeric form and remain non-emissive, but when aggregated restricted intra-molecular rotations (RIR)\(^\text{10}\) cause proscription of energy dissipation resulting in high emission. The RIR restricts the energy dissipation of AIEgens through the nonradiative decay pathways, thus overcoming the ACQ effect and provide a way to develop efficient biochemicals.\(^\text{16,17}\) Besides being bio-compatible AIEgens have high quantum efficiency and photo-stability. These characteristics make them suitable for multiple applications in diverse fields such as therapeutics, biosensors, electroluminescent materials, bioimaging, optical devices etc.\(^\text{11}\)

High scientific value and greater application potential for AIE phenomenon has attracted growing research efforts in various application fields. The development of AIEgens have opened new avenues for the applications in biosensing, chemotherapy, bioimaging and optoelectronics.\(^\text{18}\) Additionally, some AIEgens have demonstrated production of reactive oxygen species (ROS) upon aggregation. This offers an unique opportunity toward development of light-up probes for image guided photodynamic therapy (PDT) for eliminating bacteria and cancer cells.\(^\text{19–21}\) The fluorescence strength of AIEgens could be fine-tuned by using mixed solvent system containing organic water. Because most of the assays are carried out in aqueous buffer solutions, water solubility is a key requirement for the AIEgens along with the biocompatibility.\(^\text{20}\) Among the AIEgens, owing to its simple synthesis and functionalization methods, Tetraphenylethene (TPE) is the smartest AIE-fluorogen reported so far to use in a variety of contexts\(^\text{16}\) such
as bioimaging, sensing, mechanochromism and as light harvesting material.\textsuperscript{22,23} A variety of functionalized TPE-derivatives were reported for their use in bioassays and imaging of macromolecules in the organisms\textsuperscript{24} (Fig. 1). A new promising strategy to eradicate pathogenic organisms have been developed using photosensitizers and AIEgens, this process is called photodynamic inactivation (PDI) or antimicrobial photodynamic therapy (PDT).

\section*{3. Photodynamic inactivation}

Photodynamic inactivation (PDI) the phenomenon applied in photodynamic therapy (PDT) has become the recent promising tool for antibacterial activity and clearing cancer cells. PDI depends on light-sensitive, non-toxic photosensitizers (PS), which generate ROS under light irradiation.\textsuperscript{25} The generated ROS can damage the bacterial cell walls by oxidation and subsequently destroy the bacteria. Photosensitizers are fluorogenic and could be utilized for image guided antibacterial functions. However, use photosensitizers require repeated washing steps which limits their use in real time detection of bacteria. Most of the photosensitizers used for PDI are hydrophobic, their interaction with bacterial cell wall causes aggregation resulting in fluorescence quenching and reduced ROS generation. Compromising the quality of imaging and PDT.\textsuperscript{26} The effects of quenching were overcome by the recently developed AIEgens that could efficiently generate ROS under aggregated conditions. AIEgen based

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{Micromorphological changes of \textit{E. coli} (A–D) and \textit{S. aureus} (A’–D’) incubated with TPE-Cn Im compounds. The scanning electron microscopy (SEM) images shows the collapsed and distorted cell walls of the bacteria (indicated by arrows). Scale bar 2 μm. Image from Shi J, Wang M, Sun Z, et al. Aggregation-induced emission-based ionic liquids for bacterial killing, imaging, cell labeling, and bacterial detection in blood cells. Acta Biomater. 2019;97:247–259.}
\end{figure}
photosensitizers therefore would revolutionize the development of light up probes utilized in image guided PDT \(^8\) (Fig. 2).

Being PDI targets are mostly external components of the bacteria, it does not require the photosensitizers to enter the bacterial cytosol all the time unlike the antimicrobial agents,\(^27\) therefore bacteria cannot effectively develop resistance against AIEgens used in PDI. So far, several cationic AIEgens have been shown the activity of killing bacteria based on electrostatic interactions. To give a positive charge to the AIEgens, ammonium slats and Zinc (II)-dipicolylamine (ZnDPA) are often employed in a coordinated complex, among these ZnDPA is the most popularly used, it has higher positive charge and has stronger binding affinity for bacteria.\(^{28,29}\) Elevated ROS generation was reported with some AIEgens, which may be effectively used for light induced cancer cell ablation.\(^{30}\)

### 4. Natural biocompatible AIE materials

The most abundant natural polysaccharides including starch, cellulose and chitosan have been studied extensively in the industrial material applications and biomedicine. The fluorescent properties of biomacromolecules
have received very little attention. The versatility, and biocompatibility of biomacromolecules will aid the development of new generation of biosensors with potential applications in a wide range of domains including diagnostics.\textsuperscript{31–33} Chitosan, a product of chitin deacetylation, is abundant in nature. Chitosan is a non-toxic bioactive polymer with bactericidal property. Further, chitosan is easy to modify, and biodegradable. All these factors render chitosan to be used in a wide variety of fields including food and water treatment, and medicine.\textsuperscript{34,35} But, the AIE effect of chitosan has not been reported without chemical modification.

Recent studies have made attempts to utilize chitosan as AIEgen in bacterial imaging, and metal ion concentration detection.\textsuperscript{36} The fluorogenic activity of chitosan mainly depends on two aspects, modification of the fluororescent group and crosslinking with aldehyde groups.\textsuperscript{34,37} The Schiff base intermediate formed during the crosslinking of aldehyde to chitosan causes autofluorescence due to $\pi$-$\pi$ transition of the CN bond. This has provided a possibility of using chitosan as a AIE material.\textsuperscript{36} It was also found that chitosan emits a range of colors from blue to red in an excitation wavelength dependent manner, offering excellent opportunities for cellular imaging. Moreover, chitosan is capable of efficiently detecting Fe$^{3+}$, making it a potent material for the detection of Fe$^{3+}$ concentration in the environment and inside the living systems. Additionally, chitosan was used to detect \textit{E. coli} by aggregation of chitosan on the \textit{E. coli} surface, which makes chitosan a better option for real-time image guided monitoring of antibacterial processes.\textsuperscript{36} Together, Chitosan offers a novel platform for multifunctional applications.

### 5. Applications in biosensing

Gram’s staining is the first and gold standard to discriminate bacteria from all microbes and on broad, which is simple staining method based on bacterial surface changes. With the emerging bacterial diversities and surface adaptations, it became a tedious task to use suitable technique identify the bacterial species, which further laid the foundation for the use of targeted and sensitive fluorescence based probes in diagnosis of the disease. Qualitative and quantitative staining of live bacteria and imaging the intra-cellular bacteria, distinctly which are viable but not culturable in laboratory have attained main focus in the era of high-resolution microscopes to aid the diagnosis and treatments.\textsuperscript{38} In contrast to the time-consuming
methods, rapid, instantly staining and live imaging/diagnostic methods have many advantageous in monitoring sudden changes in environment, pharmaceutical developments, food processing, biomedical research and clinical diagnosis aspects. Faster, economic, and sensitive diagnosis in resource limiting conditions demand a simpler way like color or light turn on/off reactions as a read out, just by adding a chemical compound at room temperatures. AIEgens are unique probes that offer highly sensitive detection process rapidly by turning off/on light upon binding to the target. AIEgens are tunable, targetable to modulate according to the requirement.

Rapid detection of bacteria is valuable in different fields such as clinical diagnosis, pharmaceutical development, food processing and environmental sciences.\textsuperscript{39,40} In all these applications, viability of the bacteria is an important aspect which offers significant improvement for simple, rapid, and accurate detection. The methods involved in the detection and identification of bacteria such as standard plate count method, PCR and high-resolution microscopy are time consuming and laborious processes. Moreover, these techniques are based on the indirect recognition of alterations in bacterial morphology and membrane potential.\textsuperscript{41,42}

In this regard, fluorescence-based methods may offer direct method for real-time bacterial detection and identification, without requiring sophisticated instrumentation.\textsuperscript{43,44} For this purpose, the most essential criterion for achieving rapid detection of bacteria using fluorescence methods is the development of fluorescent dyes with high sensitivity and specificity. Fluorescent detection of wild type bacteria could be readily achieved by using positively charged dyes that electrostatically bind to negatively charged bacterial surfaces. However, binding of multiple charged fluorescent dyes may cause severe alterations in bacterial states through induction of metabolic changes or by aggregation of the bacteria. Additionally, electrostatic adsorption based fluorescent labelling cannot distinguish between live and dead bacterial cells. Further, in contrast to small molecule antibiotics, macromolecular antibacterial compounds exhibit sustained bacterial inhibition with broad specificity. Also, such macromolecules are easy to be functionalized through covalent or non-covalent approaches.\textsuperscript{45} In line with this, TPE core containing amphiphilic/cationic arms (TPE-star-P (DMA-co-BMA-co-Gd)) have been shown to exhibit fluorescence emission upon binding to bacteria. In addition, these molecules were reported to have anti-bacterial properties against both gram-positive and gram-negative strains.\textsuperscript{21}
6. Potential application of AIEgens in imaging and killing of bacteria

Ubiquitous bacteria are intricately associated with every aspect of human ordinary life, in particular public health and human welfare. Globally, millions of people are affected by bacterial infections every year. To overcome the tragedies, several antibiotics have been developed for treating the infections and prevention of pathogens. Inappropriate use of antibiotics is the leading cause for the rapid emergence of antibiotic resistance, which further increased the need for novel antibiotics. However, antibiotic development is an expensive and time-consuming process. Considering these facts, real-time visualization and elimination of bacteria that cause infections and contaminations is an important task necessary for the human health and food security.

6.1 Detection of bacterial viability

To date, various approaches have been established for the imaging and identification of bacteria. Among them, the methods based on fluorescent probes have been widely used because of their easy operation and high sensitivity. For any fluorescence based rapid detection, the key is to be highly responsive and bacterial specific dye. In common, a counter ionic fluorescent dye is used to the charge on bacterial surface. But, normal fluorescent dyes, have been known for inducing metabolic changes and bacteria cell aggregation. Also, these dyes cannot distinguish between live and dead bacteria because of their electrostatic adsorption to the surface.

Most bacterial species contain abundant polysaccharides and peptidoglycan on their surfaces and cell wall. Peptidoglycan accounts for 50–80% of the dry weight of gram-positive bacterial cells and 5–20% of gram-negative cells. The multiple hydroxyl groups of the surface polysaccharides present in various configurations are used as binding sites for fluorescent compounds. The mechanism of specific binding of phenylboronic acids to the diols of the carbohydrates has been adapted to develop phenylboronic acids conjugated to AIEgens resulting in the development of efficient indicator compounds such as TBE-2BA for the detection of polysaccharide surfaces of the bacteria. TPE-2BA emitted fluorescence only when aggregated on binding to the cis-diols of D-glucose representing binding induced aggregation, which demonstrated the feasibility of specific imaging and detection.
of predetermined targets such as detecting live bacteria. Using similar approach, hybrid systems containing β-galactosidase and cationic gold nanoparticles were fabricated for bacterial detection. Also, a FRET system using quaternary ammonium functional group conjugated polymer was developed to probe the antimicrobial susceptibility, and to screen for potent antimicrobial compounds. Thus, competitive supramolecular interactions between negatively charged bacterial surface and synthetic materials could be extensively utilized for sensing bacteria.

To detect live bacteria, chemical specificity based methods are essential like targeting polysaccharides, or receptors on bacteria unlike whole surfaces. Bacterial surface polysaccharides possess several hydroxyl groups in different configurations and thus could be used as fluorescent binding sites. For instance phenylboronic acid specifically binds to diols present on carbohydrates. This affinity inspired the design of biosensors to specifically target carbohydrates on bacteria. Upon conjugating phenylboronic acid to an unique indicator molecule, 4,4′-(1,2-diphenylethene-1,2-diyl)-bis-(4,1-phenylene) diboronic acid (TPE-2BA), emitted fluorescence only when bound to cis-diols of D-glucose to form aggregates. Tang and coworkers, in 2001, for the first time, have explained this phenomenon as AIE.

Same Tang group in 2018, have successfully designed and demonstrated that, a morpholine-containing 2-(diphenylmethylene) hydrazono methyl naphthalene (DPAN), a derivative of M1-DPAN could specifically discriminate gram-positive bacteria from other bacteria, and when bound, it could yield 24 h long fluorescence signal which allowed to trace their infectivity toward mammalian cells. DPAN-based AIEgens are not only applied to discriminate and visualize live pathogens, but also, provided platform to develop better tracers that could be used in real-time monitoring of infection dynamics under different treatments as shown in Fig. 3.

### 6.2 Single platform array to detect multiple bacterial species

AIEgens are used for the bacterial imaging. For instance, vancomycin based light up probe (a red emissive fluorescence probe), AIE-2Van, for specific recognition, and image guided photodynamic elimination of Gram-positive bacteria. For visualization and elimination of both gram positive and gram-negative bacteria, TPE derived cationic amphiphilic molecules with ammonium groups are used which uses electrostatic interactions for binding. In another approach, for developing wash free detection tools for
imaging of bacteria, the abundance of lipoteichoic acid (LTA) on the cell walls of gram-positive bacteria have been exploited. LTA, an amphiphilic molecule with NH$_3^+$ containing backbone offers a scope to develop an array of negatively charged AIE-probes as potential turn-on fluorescent molecules for effective imaging.

An array of TPE based AIE materials with positive, negative, or neutral charges were synthesized with capability for broad spectrum bacterial imaging which were tested successfully on different gram-positive and gram-negative bacterial species. The array of TPEs provided differential fluorescent responses due to multivalent nonspecific interactions between the bacterial cell surface molecules and TPEs provided an excellent platform for bacterial discrimination without the use of radioactive markers and antibodies.

Fig. 3 The CLSM images of HeLa cells infected by M1-DPAN-labeled (A) living *S. aureus*, (B) *S. aureus* treated by 75% EtOH and (C) *S. aureus* treated with cephalothin and PI. (D) The CLSM images of NIH 3T3 cells infected by M1-DPAN-labeled living *S. aureus*. Reproduced with permission from Hu R, Zhou F, Zhou T, et al. Specific discrimination of gram-positive bacteria and direct visualization of its infection towards mammalian cells by a DPAN-based AIEgen. Biomaterials 2018;187:47–54. Copyright 2018 Elsevier.
6.3 pH dependent detection and clearance of microbes

Generally, synthesized chemical compounds exhibit antimicrobial activity by stress-induced cell death which is dependent on the binding of the compounds to the cell wall receptors. The same mechanism has been exploited to develop KB1, active site’s OH group of KB1 might interact with the cell wall or plasma membrane proteins, upon adherence it will increase the cell membrane permeability by interacting with membrane proteins and other microbial molecules. The increased permeability will lead to the loss of intracellular electrolytes such as Na+, Ca2+ and K+ resulting in the cell death. In other instances, decrease in pH occurs at the site of infection due to local acidosis caused by the infiltration of macrophages and neutrophils during infection induced inflammatory response. The usage of pH responsive agents with enhanced anti-bacterial activity can efficiently control infections with minimal side effects and high therapeutic efficacy. The local acidosis principle has been adapted by Yang et al. to fabricate pH sensitive polymers such as quaternary pyridinium with on demand antimicrobial activity. However, the potential toxicity associated with pyridinium units limit their use for biomedical applications. As an alternative poly (vinyl alcohol) (PVA), which has excellent biocompatibility and bio adhesive properties can be used as precursor to develop alternative pH sensitive polymeric materials for bacterial killing.

Various fluorescent probe-based approaches are available for bacterial imaging and killing such as molecule-, polymer- and nanomaterial-based probes, which have been intensively used because of their easy handling and high sensitivity. The AIE active materials (AIEgens) are of great scientific value and practical implications. AIEgens with light induced ROS generation capability have been applied to light enhanced bacterial elimination during photodynamic therapy. A small AIEgen molecule TPE-Bac is an effective bacterial imaging and antibacterial material. Owing to their positively charged surfaces, these molecules were successfully able to penetrate the plasma membranes. Another molecule, AIE-active DBPE polymerized to DBPE-DBO possess multiple positive charges on the polymers which provide multiple interaction points with bacterial cell surface. The hydrophobic alkyl chains of the polymers intercalate into the membrane components of the bacterial cell surface. Thus, synergistically increasing the membrane permeability for the AIE active polymer DBPE-DBO into the bacteria. Additionally, DBPE-DBO could distort phospholipid arrangement on bacterial membranes which can increase bacterial toxicity. DBPE-DBO exhibited excellent photosensitizing properties generating
ROS (singlet oxygen), which exerted potential antibacterial activity after irradiating at room light conditions suggesting DBPE-DBO as an excellent candidate for PDT.

6.4 Phage-guided AIE bioconjugates for imaging and killing of bacteria

Meanwhile, in the last year, i.e., 2020, Tang and coworkers came up with much curious working principle, where AIE bioconjugates can be designed and utilized through the guidance of bacteria phage, which has natural capability to selectively target particular bacteria type. This group has showed that these conjugates are not only useful in tracing bacterium, but also kill them by using their exceptional capability of photodynamic therapeutic action. This novel strategy is based on integrating AIEgens like TVP-S with bacteriophage targeting P. aeruginosa. These molecules form a new class of antimicrobial bioconjugates (TVP-PAP) with AIE properties and excellent PDI activity. AIEgens have been conjugated through an amino carboxyl reaction, without affecting the properties of both AIEgen and phage, thus allowing the real time monitoring of phage-bacterium interaction with high specificity as shown in Fig. 4.

![Fig. 4](Image)

Fig. 4 Pictures depict the mechanism of phase guided bacterial targeting by discriminative imaging and synergetic killing through AIE bioconjugates. The phage guided targeting and killing include: (I) Identification of bacteria by AIEgen conjugated specific bacteriophage; (II) lighting up and imaging of the targeted bacteria and infectious phage by AIE generated fluorescence; and (III) the synergistic killing of the targeted bacteria by phage infections and AIE-base photodynamic inactivation. Reprinted with permission from publishers.
Together, phage conjugated AIE-based photodynamic inactivation pave a wonderful exciting platform for killing bacteria which has benefits both fluorescence properties, and efficient $^1\text{O}_2$ generation capability. TVP-PAP AIEgens showed good visualization and selective bacterial elimination properties, with minimal or no effects on other bacteria and normal mammalian cells as reported by Tang group. The in vitro test provided the evidence that even multidrug resistant $P$. aeruginosa could be killed with near 100% efficiency. This could be attributed to the synergistic effect within phage guided AIEgen, i.e., TVP-PAP.

## 7. Toxicology aspects of AIEgens

Though the AIE materials offer color tunability and structural diversity, the sources of AIEgens are organic synthesis with non-planar conjugated molecular confirmations and thus have poor water solubility making them environmentally disadvantageous because of the toxicity and poor degradation.\textsuperscript{74–76} AIEgens are generally non emissive and non-toxic in dark but upon aggregation can produce toxic ROS, the ROS destroys the bacteria by oxidizing the bacterial cell walls. This unique feature provides a better chance to develop novel light up probes for image guided PDI of bacteria.\textsuperscript{77} There is an urgent need to develop new class of AIEgens with good water solubility, degradation, and biocompatibility to reduce environmental toxicity.

The AIEgens can be effectively used for killing bacteria, as AIEgens such as TPETH–2Zn, AIE-ZnDPA and TPE-amphiphiles selectively images and exerts phototoxicity to bacteria over mammalian cells, this has been evident from the mammalian cell viability analysis using MTT assay on HeLa cells and Jurkat T cells. No significant mammalian cell cytotoxicity and binding by TPETH–2Zn to the mammalian cells was observed, whereas the bacterial killing efficiency was more than 95% at the same concentration.\textsuperscript{61,78,79} This could be due to the selective binding affinities of the AIEgens toward bacteria. Moreover TPETH–2Zn is very selective toward targeted pathogenic bacteria than the nonpathogenic bacteria present in the solution for photoinactivation.\textsuperscript{80} In vivo toxicity analysis has revealed that TPE based AIEgens have exhibited no or negligible toxicity.\textsuperscript{81}

Certain copolymers have showed relatively high cytotoxicity against human red blood cells (RBCs) making them unfavorable for their potent applications. But, after quaternization reactions the cytotoxicity to RBCs has decreased drastically assessed by hemoglobin release assay, and these
copolymers possess pH independent water solubility to accomplish bacterial detection under physiological conditions. The relative low hemolysis rate and cell toxicity of quarternized copolymers of TPE-C1 allow them to act as light-up sensors for the visualization and elimination of pathogenic bacteria in blood cells.²¹,⁸²

8. Future perspectives

Identification of bacteria and diseased cells plays crucial roles in clinical practice, which helps in the understanding of origin of the disease. A new class of AIEgens with good photostability, sensitivity and improved signal to noise ratio, are being used for various bioanalytical techniques, biosensing and cell biology applications. The studies also validate the capabilities of the AIEgens to differentiate between Gram-positive and Gram-negative bacteria, and cancer cells from normal cells.⁸¹,⁸³ The AIEgens can be further developed for comprehensive cancer and infectious disease therapies, with an approach to design multifunctional systems with accurate in vivo localization, diagnosis and killing of cancer cells and pathogenic bacteria.

The traditional AIEgens have the excitation wavelengths in the UV range with poor tissue penetrations.⁸⁰,⁸¹,⁸⁴ This limitation can be overcome by the development of red and near-infrared emissive AIEgens. Such molecules benefit the study of in vivo localization of bacteria and cancer cells, and treatment. The anti-bacterial activity of the AIEgens can be enhanced by developing easy synthetic methods for generating fluorophores coupled with antimicrobial agents which will revolutionize the field of nano antibiotics.

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