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TRENDS

Bacterial bioluminescence assay for bioanalysis and bioimaging

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Abstract

Bioluminescence occurs through a chemical reaction in organisms that spontaneously produce light. Luminescent bacteria are unique among bioluminescent organisms. Their bioluminescence intensity is an indicator of their metabolic activity, which can directly reflect the influence of environmental factors on cell viability. Moreover, the whole bioluminescence process is totally gene encoded without the addition of extra substrates. As a result, bacterial bioluminescence has been a powerful tool for whole-cell biosensors and bioreporters in bioanalysis and bioimaging. This review aims to cover the applications of wild-type and recombinant luminescent bacteria to detect the toxicity of environmental pollutants and biological molecules. The bacterial bioluminescence analytical assay has characteristics such as high sensitivity, short-term detection, and easy operation. Meanwhile, due to the development of gene engineering and optical technology, bacterial luciferase as a reporter protein has been successfully expressed in prokaryotic and eukaryotic cells, tissues, and organs of animals. The major applications for bacterial luciferase-based bioluminescence imaging, such as infectious diseases, cancer therapy, and stem cell tracing, are discussed in this review.

Keywords Bacterial bioluminescence · Bioanalysis · Luciferase · Whole-cell biosensor · Bioimaging

Introduction

The phenomenon of bioluminescence is widely observed in nature and there are many auto-luminescent organisms, such as bacteria, fungi, fish, and insects [1]. This is a result of a chemiluminescent reaction catalyzed by enzymes in the living organisms. Depending on the type of bioluminescent organisms, different substrate molecules are oxidized to form product molecules in the excited state that decay to the ground state and emit light. Bioluminescent proteins are usually divided into two categories: luciferases and photoproteins. In bacteria, firefly, *Renilla*, and *Gaussia*, luciferase catalyzes an enzymatic reaction in which luciferins are oxidized by oxygen molecules to form a product in the excited state. These luciferases have extensive applications in protein-protein interactions [2], immunoassays [3], and bioluminescent imaging [4]. Another type of bioluminescent protein is the photoprotein, a stable complex of enzymes, where substrates when triggered by Ca^{2+} ions emit a flash of light. Aequorin from jellyfish containing apoaequorin and coelenterazine is the first photoprotein to be discovered and extensively studied as a Ca^{2+} ion cellular probe [5]. Obelin from the hydrozoa *Obelia longissimi* is another type of photoprotein with a wide range of applications.

Among these bioluminescent organisms, luminescent bacteria are the most abundant. These bacteria usually inhabit the ocean as planktons or symbionts in the photophore of other organisms, such as fish and squid. Not only do they inhabit the sea but are also found in freshwater and terrestrial environments. All luminescent bacteria are rod-shaped, gram-negative anaerobes that have flagella for swimming. They are divided into three categories: *Vibrio*, *Photobacterium*, *and Xenorhabdus* based on their luciferases, *lux* genes, and luminescent systems. *V. harveyi*, *V. fischeri*, *P. phosphoreum*, *P. leiognathi*, and *Xenorhabdus luminescens* are some of the commonly studied organisms.

At present, the biochemical processes and genetic regulation of bacterial bioluminescence have been largely understood. The bacterial luciferase catalyzes a bioluminescent chemical reaction, in which a long-chain aliphatic aldehyde reacts with the complex formed by reduced flavin mononucleotide ($FMNH_2$) and oxygen to emit light at approximately 490 nm. The bioluminescence reaction is mentioned as follows [1]

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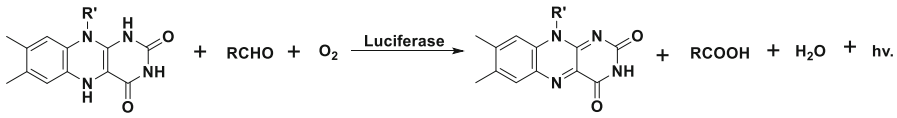

(1)

During this reaction, the reactants are circularly produced by the bacterial metabolic system. FMN and RCOOH are reduced by flavin reductase and the fatty acid reductase complex to form $FMNH_2$ and RCHO, respectively.

$$FMN + NAD(P)H + H^+ \longrightarrow FMNH_2 + NAD(P)^+ \quad (2)$$

$$RCOOH + ATP + NADPH + H^+ \rightarrow RCHO + AMP + PPi + NADP^+ + H_2O \quad (3)$$

A possible mechanism for bacterial bioluminescence is speculated. This reaction can be divided into two steps. First, reduced FMN binds to bacterial luciferase and then reacts with oxygen to form the peroxyflavin intermediate. Second, the aldehyde reacts with peroxyflavin to form a flavin peroxyhemiacetal intermediate. This subsequently decomposes to produce carboxylic acid and bioluminophore in an electronically excited state that decays to the ground state and emits light [6]. The overall reaction mechanism is illustrated in Fig. 1. The intensity of bioluminescence depends on the whole quantum efficiency of this chemiluminescence reaction, which consists of three parts: the chemical yield of the reaction (φ_C), excited state production yield (φ_{EX}), and emission quantum efficiency of the excited state (φ_F) [7]:

$$\varphi_B = \varphi_C \times \varphi_{EX} \times \varphi_F$$

The quantum efficiency of luminescent bacteria ranges from 0.1 to 0.3, which is slightly lower than that of the firefly (φ_B = 0.4–0.6).

From a genetic standpoint, bacterial bioluminescence is regulated by the core gene cluster *luxCDABE(G)* as an operon. Luciferase, composed of the heterodimer protein (α and β subunits), is encoded by the *luxAB* gene. To provide a sufficient quantity of long-chain aliphatic aldehydes for the bioluminescence reaction, *luxC*, *luxD*, *and luxE*, constituting a fatty acid reductase complex, participate in the reduction of carboxylic acids to aldehydes. These three genes encode an NADPH-dependent acyl-protein reductase, acyl-transferase, and acyl-protein synthetase, respectively. Furthermore, *luxG* encodes NAD(P)H-dependent flavin reductase, which converts the FMN to $FMNH_2$. Because the genes associated with luciferins and luciferase are known, bacterial bioluminescence can be realized by transfecting the *luxCDABE(G)* operon into various types of cells. The biochemical processes and genetic regulation of bacterial bioluminescence have been understood, which promote their application as reporter genes in analytical chemistry, environmental science, and life science.

While several excellent reviews have summarized various aspects of bioluminescence ranging from biochemical mechanism, biosensing by developing bioluminescent probes to image tissues and cancer in vivo, they mainly focused on the firefly and *Gaussia* luciferase bioluminescent systems which require the addition of exogenous substrate such as D-luciferin, coelenterazine [8–11]. In contrast, the natural gene encoding ensures the endogeneous production of the

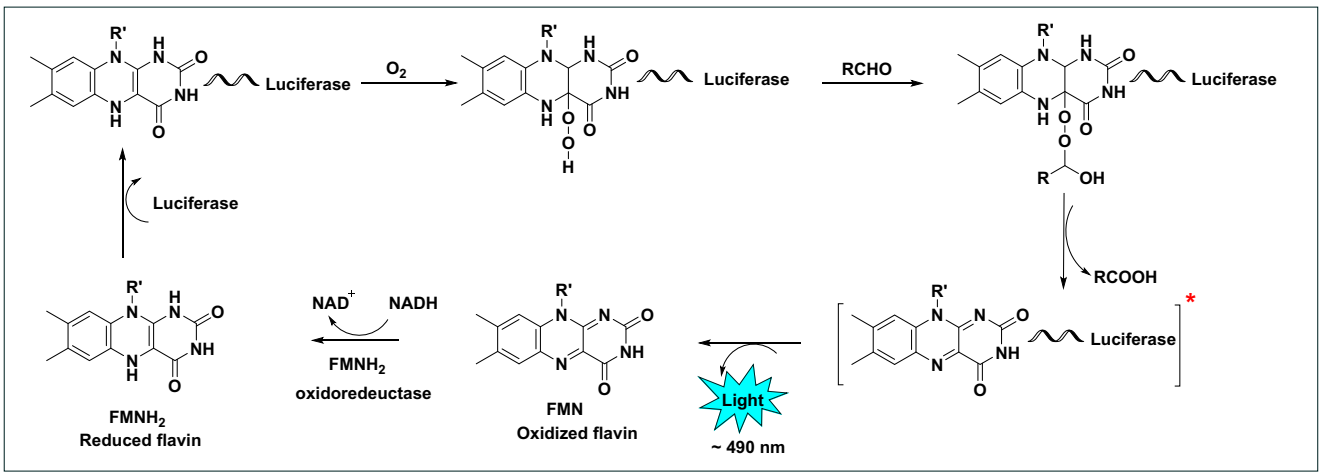

Fig. 1 The bacterial luciferase bioluminescence reaction mechanism [6]

substrates (RCHO and $FMNH_2$) in bacterial bioluminescence. Consequently, bacterial bioluminescence has received particular attention, and a review article is desirable to cover both bioanalysis and bioimaging by using bacterial luminescence system. In this review, species and characteristics of luminescent bacteria, together with the mechanism and genetic constitution of bacterial bioluminescent system, have been discussed in the "Introduction" section. In the following sections, bioanalysis and bioimaging applications have been further elaborated, including the toxicity detection of environmental pollutants and biologically relevant molecules, and in vivo imaging of infectious disease, cancer therapy, and tracking the stem cells. Finally, the challenges and further development directions of bacterial bioluminescent imaging have been provided.

Bacterial bioluminescence intensity as an indicator for bioanalysis

Toxicity evaluation of environmental pollutants

The main bioanalysis application of luminescent bacteria is as a whole-cell biosensor to detect environmental pollutants and biologically relevant molecules [12]. Compared with protein-based assays, bacterial whole-cell assays can reflect the cytotoxicity, genotoxicity, and synergistic toxic effects of the tested samples.

The toxicity of pollutants, such as heavy metals and organic compounds, often is evaluated using chemical analytical methods, such as liquid chromatography, gas chromatography, and mass spectrometry, to report the concentration of specific toxicants. However, these methods have some limitations, such as expensive equipment, longer analysis time, and requiring skilled experimental operators. Alternatively, researchers have started to use living organisms, such as rat, fish, algae, and bacteria, as biosensors to monitor pollutants. Among these bioassays, the bacterial bioluminescence assay has characteristics, such as high sensitivity, short-term detection, and easy operation. Its core principle is that the luminescence intensity of living bacteria is directly related to their metabolic activities. Based on the distinct responses of luminescence intensity upon the addition of analytes, bacterial bioassays are divided into two categories: "light-off" and "light-on" (Fig. 2).

"Light off" shows that light intensity of native luminescent bacteria would decrease when incubated with pollutants. For example, *Vibrio fischeri* bioluminescence inhibition bioassay (VFBIA) has been widely used for detecting toxicity because it is non-pathogenic and has high bioluminescent intensity. To date, a number of reagents based on the VFBIA have been developed in commercial test systems, such as BioTox™, Microtox®, and LUMIStox® tests, for the detection of various analytes [13]. VFBIA is performed by simply mixing bacterial suspensions with the same volume of a 2% NaCl solution containing the dissolved analyte. The cells were then incubated for different durations, such as 10, 20, and 30 min. The decrease in luminescent intensity was subsequently compared with the control sample (bacterial suspension in the absence of test samples) to calculate the inhibition efficiency. For example, the EC_{50} value, which is the amount of sample that reduces light by 50%, is commonly used to assess toxicity.

However, "light-off" assay is used to detect toxicity without specificity to sample compounds. Furthermore, these experiments were carried out in a 2–3% NaCl solution, which will influence the detection results in some situations. "Light-on" assay is an excellent alternative for specific detection. This refers to the significant enhancement of the bioluminescence intensity when specific analytes are added to bacterial suspensions. This assay usually employs recombinant bacteria containing antagonism and lux genes as reporters. In general, antagonism genes act as promoters, which are based on different resistance mechanisms against chemical compounds. Thus, high expression of the lux gene is obtained in the presence of some specific stress situations or compounds [14].

Heavy metals and organic compounds are the most common environmental pollutants. Their toxicities are usually divided into acute and chronic toxicity based on the exposure time with organisms. Bacterial bioluminescence as a whole-cell biosensor is a good choice to assess their toxicity. In some emergencies, the luminescent bacteria inhibition assay is used to test the acute toxicity of environmental pollutants within 5–30 min, owing to its rapid, convenient, and reliable nature [15]. Acute toxicity assesses high concentration of pollutants (ppm level), whereas chronic toxicity measures low concentration of pollutants (ppb level). As a result, chronic toxicity evaluation requires more sensitive detection assays. Bacterial bioluminescent bioanalysis compared with other toxicity tests was more sensitive to evaluate the sub-lethal effects of toxicants in some circumstance [16, 17]. In order to explore toxicity mechanism of specific pollutants, "light on" method which relied on recombined bacteria expressing *luxCDABE* was often used [18].

In recent years, nanomaterials have been widely used in daily life. In addition, their safety has attracted public attention. Luminescent bacteria as a whole biosensor can conveniently assess the toxicity and bio-viability of nanomaterials, including metal, metal oxide nanoparticles, carbon nanotubes, and quantum dots (QD). The bioluminescence inhibition bioassay is one of the most common assays used to test the toxicity of nanomaterials and the factors affecting toxicity, such as the composition and surface modification of nanomaterials. The experimental procedures for toxicity evaluation are similar to those of heavy metals in wastewater. However, because of the high molar absorption coefficient of some

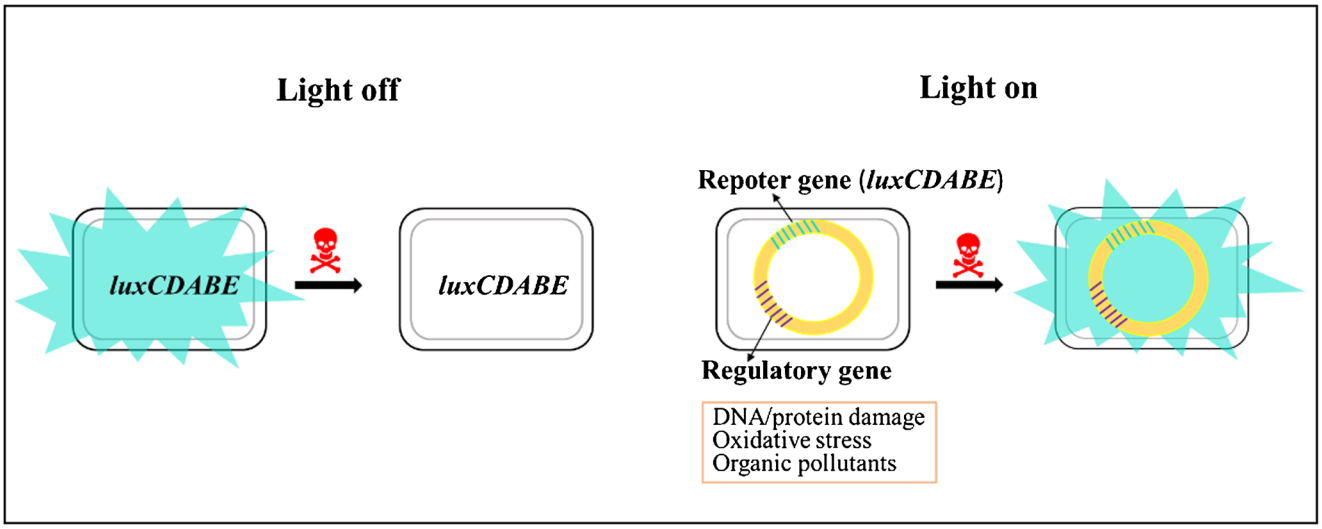

Fig. 2 Two types of bacterial bioassay-based methods for the toxicity detection of pollutants

nanomaterials at specific wavelengths as well as strong scattering, the background absorption needs to be calibrated using a standard method [19]. What's more, the single and combined toxicity of nanomaterials could also be evaluated. Xie et al. employed a model luminescent bacteria, *V. fischeri*, to evaluate the acute toxicity of individual and mixture nanoparticles. Interestingly, they found that the combined toxicity of different binary nanoparticles had different effects, such as antagonistic, synergistic, and additive effects. And the difference of toxicity effect came from the dissolved metal ions. For example, the concentration of dissolved metal ions from combined nanoparticles was higher than that from individual nanoparticles. It provided a better understanding about the risk of nanomaterials in the environment [20].

Biologically relevant molecules

Quorum sensing inhibitor

Quorum sensing is regulated by gene expression, which represents fluctuations in cell population density. Quorum sensing is vitally important for bioluminescence, virulence, biofilm formation, and sporulation [21, 22]. Autoinducers are a type of small molecules produced and released by cells. They are responsible for bacterial density administration. Bioluminescence quorum sensing is regulated by *luxR/luxI* genes to release the N-acylhomoserine lactone (AHL) family molecule [23] as shown in Fig. 3. Disturbance in bacterial quorum sensing is an effective antimicrobial strategy. Several bioluminescence biosensors have been employed to recognize quorum sensing signals and monitor infectious diseases. Different types of compounds have been developed to recognize molecules released by quorum sensing. Consequently, it has been another effective antibacterial strategy [24–26].

Antibiotics and antibacterial agents

Bacterial infections can cause serious threats to human health. Antibiotics and antibacterial agents [27] are effective drugs for the treatment of bacterial infections. Li et al. monitored the acute toxicity of 21 quinolone antibiotics with luminescent bacteria. The minimum IC_{20} (20% inhibitory concentration) of these tested quinolones was 18.86 μmol/L. To interpret the mechanism of acute toxicity, they established a quantitative structure-activity relationship model. With this model, researchers speculated that electron transfer might occur between the quinolones and *V. fischeri*; chlorination during this process causes toxicity elevation. As a result, it is necessary to treat quinolone-containing water to disinfect for chlorine [28]. To kill bacteria more effectively, a variety of antibiotics are required to work together. Tong et al. evaluated the combined toxicity of tetracycline (TC), copper (II), and cadmium (II) caused by *V. fischeri.* The results showed that TC and Cu (II) had an antagonistic effect, whereas TC and Cd (II) showed a synergistic effect. Since the traditional joint toxicity

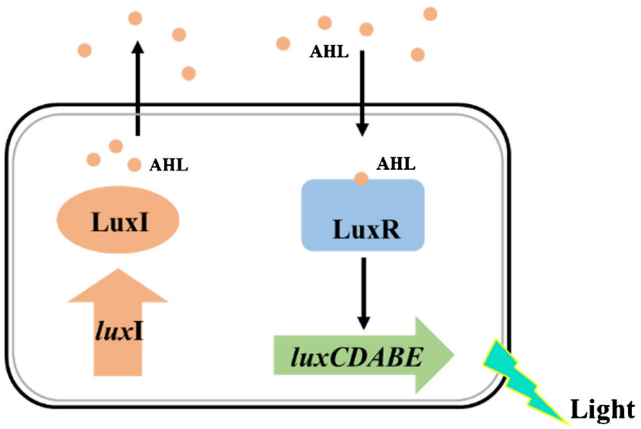

Fig. 3 LuxI/LuxR regulatory system of quorum sensing

prediction method was not suitable for tetracycline and metal ions, they also developed a new method based on speciation calculations for joint toxicity evaluation [29].

Bacterial luciferase as reporter protein for bioimaging

Bioluminescent reporter proteins are powerful tools for imaging cellular activity and biological processes. Thus, they are commonly used to study infection, tumor location, and gene therapy. Compared to fluorescence, bioluminescence is advantageous for in vivo imaging. First, bioluminescence is a spontaneous light emission phenomenon that requires no excitation light source. Thus, it can remarkably decrease the background noise and improve the signal-to-noise ratio. Second, bioluminescence is not affected by phototoxicity and photobleaching, which favors long-term imaging. In particular, bioluminescence requires energy produced by aerobic respiration, which reflects cellular metabolism level variation. Luciferases, including Fluc, Rluc, and Gluc, are the most frequently employed molecules for in vivo imaging with relatively high light intensity and variable wavelengths. Despite these applications, the above-mentioned luciferase systems from eukaryotes have encountered some challenges. First, exogenous luciferins are required for in vivo imaging. The quantity of luciferins gradually decreased along with the luminescence reaction, which would compromise the quantitative analysis of bioluminescence intensity. Second, the luciferins themselves are unstable when exposed to light, oxygen, and moisture. Third, the luciferins have limited solubility, which makes it difficult for them to permeate cells.

In contrast to bioluminescence from eukaryotes, bacterial bioluminescence does not require the external addition of luciferin because the synthesis of their luciferase (Lux) and luciferin substrates is genetically encoded. In addition to bacterial bioluminescence, fungi can also emit photons controlled by gene expression, but their luciferase shows no activity at high temperature [30]. As a result, studies on fungal bioluminescence used in mammalian cell imaging have rarely been reported. Nevertheless, the bacterial bioluminescence system has also been successfully expressed in other prokaryotic and eukaryotic cells and has bright prospects in the field of bioimaging.

Improving bacterial bioluminescence imaging quality

Even though bacterial bioluminescence has autonomous luminescent functionality, its light intensity in vivo is lower than that of the external substrate system (such as firefly luciferase). Therefore, to obtain higher imaging quality, the bacterial bioluminescence intensity must be enhanced. Researchers have attempted to improve the brightness of bacterial bioluminescence. Yagur-Kroll et al. split the large lux operon (*luxCDABE*) into two small functional units: *luxAB* and *luxCDE*. The inducible and constitutive promoters were randomly combined with these two units. The performance of different combinations expressed in *E. coli* was evaluated in the presence of specific chemicals. Inducible *luxAB* and constitutive *luxCDE* showed a significantly improved performance in terms of signal intensity, detection sensitivity, and response times than the complete *luxCDABE* operon [31]. Gregor et al. used the *luxCDABE* operon from *P. luminescens* and FMN reductase gene frp from *V. campbellii* to co-express in *E. coli* cells. Then, luxCDABE+ frp genes were manipulated by error-prone PCR for mutagenesis. Finally, the researchers found a new operon named *ilux*. When this *ilux* operon was expressed in a single *E. coli* cell, the bioluminescence was brighter and more stable than firefly luciferase (FLuc) and *luxCDABE* WT in vivo, shown as Fig. 4(a). The *ilux* operon expressed in bacterial cells is the brightest to date and has huge potential for imaging applications [32]. The bacterial bioluminescence *lux* operon has been well expressed in prokaryotic cells, but its expression in mammalian cells has some difficulties, such as fewer types of cell choices and lower light intensity. Consequently, Gregor et al. optimized the codon of the lux gene from an individual plasmid and adjusted the codon ratio. The light intensity expressed by the recombinant lux gene was similar to that of the firefly luciferase, and bioluminescence was expressed in different mammalian cell lines for a long period, shown as Fig. 4(b) [33]. This study paved the way for the widespread use of bacterial autonomous luminescence in medical research and tumor therapy.

Bacterial bioluminescence imaging (BLI) application

Since the 1980s, researchers have successfully transfected the *luxCDABE* gene cassette to be exogenously expressed in other host organisms, such as *E. coli*, which broadens the applications of bacterial bioluminescence [34]. With a thorough understanding of biochemical reactions involved in the bioluminescence phenomena and the development of genetic engineering, the *lux* operon from bacteria can be expressed in different prokaryotic cells (such as *E. coli*, *Bifidobacterium*, and *Brucella* [35]), eukaryotic cells (such as mammalian cells [36]), and plant cells [37]. Based on this progress, these recombinant cells are injected into animals for imaging infectious diseases and targeting tumors (Fig. 5).

Infectious disease research

Traditional assays for infectious disease studies would kill many experimental animals at multiple time points for real-

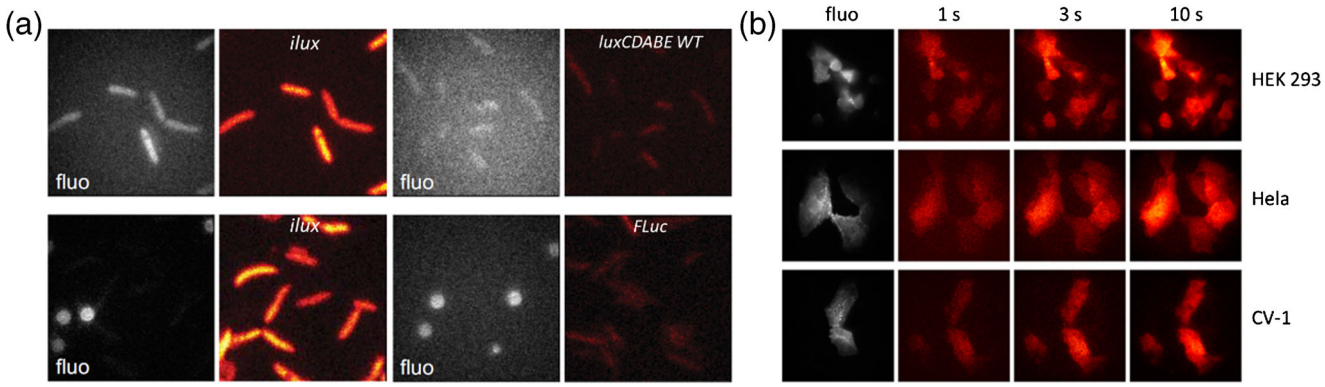

Fig. 4 **a** Comparing the brightness of single *E. coli* expressing *ilux* or *luxCDABE WT* (on the top). Comparing the brightness of single *E. coli* expressing *ilux* or FLuc (in the bottom) [32]. **b** Co lux genes were expressed in different cell lines [33]

time imaging analysis. These assays require large numbers of animals to obtain effective data and analyze the pathogenic processes. To solve this problem, BLI has attracted attention owing to its continuous imaging potential, high sensitivity, and good signal-to-background ratio. Luciferase proteins have been transfected into bacteria as reporters for imaging infections caused by pathogens. In 1995, Contag et al. confirmed the feasibility of bioluminescence imaging in microbial infection processes. They managed to transfect the *lux* operon from *P. luminescens* to three strains of *Salmonella typhimurium* and used recombined bacteria to infect mice. After a period of time, the bioluminescent signal of recombined bacteria can be detected in infected mice [40]. This imaging method sheds new light on the study of various infectious diseases, such as plague [35], and those of the respiration [41]. In these infectious diseases, infection sites, the degree of virulence, and bacterial pathogenesis can be uncovered using BLI. Recently, Tian et al. employed BLI to screen antibacterial drugs, which have a lesser reaction time and lower cost. They constructed a strain of *Klebsiella pneumoniae* with *luxCDABE,* combining a Tn7 transposon and a Xer-dif system. The antibacterial drugs were screened by bioluminescence intensity of recombined *K. pneumoniae* in a chemotherapy mouse model. It is a marker-free, auto-luminescence-based imaging method for high-throughput drug screening [38]. In addition to bacterial infection, the response of the host is also important. Kadurugamuwa et al. developed a method to simultaneously monitor the pathogenesis of pneumococcal meningitis and neuronal injury. Auto-luminescent *Streptococcus pneumoniae* with *luxCDABE* was used to study

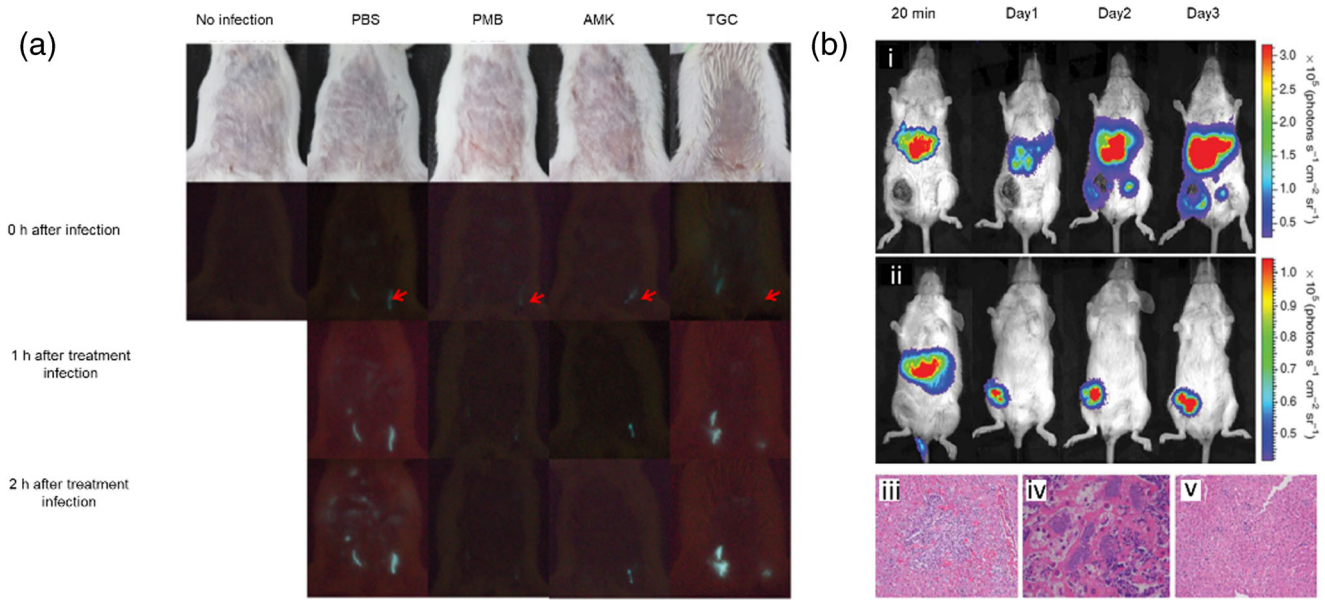

Fig. 5 **a** Different drug efficacy was evaluated with infected mice by SfAIKp that could express *luxCDABE*. The red arrows referred to injection sites. The bioluminescent images of 0 h, 1 h, and 2 h after infection were taken by a home digital camera [38]. **b** (i)–(ii) Highly and non-metastatic 4T murine breast cancer cells were implanted in a BALB/c mouse, respectively. Histological observation confirmed that a BALB/c mouse implanted with highly metastatic breast cancer cells was in presence of metastatic lesions (iii) and bacterial colonies (iv). (v) Metastatic lesions and bacterial colonies were not observed in a BALB/c mouse implanted with non-metastatic cancer cells [39]

the infection process of pneumonia and the function of antibacterial drugs. An inducible firefly luciferase (F*luc*) was used to monitor the response of the mouse [42]. This study validated that bacterial infection and host response can be simultaneously monitored in the same animal model.

Cancer therapy

In the 1890s, Dr. William B. Coley was the first person to find that cancer patients experienced tumor regression and symptomatic relief after infection by bacteria. The reason for this phenomenon was speculated to be that infectious bacteria could activate the immune system to kill tumor cells [43]. Thereafter, a variety of bacteria was found to accumulate near both primary tumors and metastases. For example, Yazawa et al. found that *Bifidobacterium longum* selectively localized and proliferated in rat mammary tissue. They further compared the effects of wild-type and engineered *B. longum* for the tumor therapy. This is the first demonstration of *B. longum* as a specific gene delivery vector for gene therapy in solid breast tumors [44]. However, investigating the sites of bacterial aggregation requires killing of many animals. To investigate the bacterial therapy process in real time, fluorescence and bioluminescence were used. Although the fluorescence label method has several advantages, there are still some limitations. For instance, the imaging quality depends on the number and location of the cells. Another alternative imaging method is bioluminescence, which has low background noise and high sensitivity. Therefore, various bacteria expressing bioluminescent proteins have been developed to localize and image tumors [45, 46]. Especially, these bacteria can be drug carriers to relieve tumor progression [47]. Bacterial bioluminescence due to adding no substrates plays an important role in cancer therapy. Min et al. developed a quantitative and noninvasive BLI technique to record the growth of *E. coli* during tumor progression. This engineered *E. coli* expressed the plasmid (pLux) that contained the *luxCDABE* operon from *Photobacterium leiognathi*. A bioluminescence signal was detected around the tumor approximately 24 h after the intravenous injection of pLux-expressing *E. coli*. This engineered *E. coli* targeted not only primary tumors but also metastatic cells, which was an improvement over previous studies on light-emitting bacterial tumor imaging [39]. BLI of bacterial tumor accumulation is a traditional 2D modality, which is limited due to spatial resolution. To obtain exact spatial location information, Cronin et al. combined 3D diffuse optical tomography with an anatomical imaging modality, such as micro-computed tomography (mCT), to monitor the commensal bacteria *E. coli*K-12 MG1655, *Bifidobacterium breve* UCC2003, and *S. typhimurium* SL7207 targeting xenograft tumors. These bacteria and xenograft tumors were transfected with the *luxCDABE* operon and FLuc, respectively. Using this imaging technique, the position of the bacteria expressing Lux co-localized with tumors expressing FLuc can be precisely detected. Imaging analysis using 3D BLI and micro-CT revealed the distribution of bacterial clusters within the tumors. Investigation of the spatial resolution of 3D optical imaging was supported by ex vivo histological analyses. This study demonstrated the potential for simultaneous imaging of multiple BLI reporter genes in vivo and provided new insights on 3D spatial locations [48].

Tracking the stem cells

Bacterial luciferase has been successfully expressed in mammalian cells, but its expression in stem cells remains challenging. This was until recently, when Conway et al. artificially subdivided bacterial luciferin and luciferase pathway subcomponents, that the luciferin pathway was overexpressed in a 20–30:1 ratio, with continuous or inducible bioluminescence occurring in stem cells. Bioluminescence requires the utilization of metabolic energy; therefore, the change in light intensity was employed to study the cellular biological process. Thus, they studied the proliferation, viability, and toxicological responses of iPSCs using BLI. The results showed that these assays were comparable in performance to established colorimetric assays [49].

Outlook

A number of whole-cell biosensors and in vivo imaging assays based on bacterial bioluminescence have been applied to evaluate the toxicity of environmental pollutants, biological molecules, infectious diseases, and cancer therapy. For toxicity detection, this method has high sensitivity, low limit of detection, low cost, and ease of operation. Many bacterial biosensors have been commercialized to monitor the toxicity of wastewater online. In order to meet the needs for the detection of large quantities of a sample, high-throughput methods are becoming increasingly popular. The sample chambers have been developed from single vials to thousands of holes in microplates, even microfluidic chips. The bacterial bioluminescence bioassay would have a larger application market in the future. As for in vivo imaging, bacterial bioluminescence does not require the addition of extra luciferin, so it has obvious advantages over other luciferases, such as Fluc, Rluc, and Gluc. In addition, bioluminescence has been an alternative to fluorescence because of its high sensitivity and lack of photobleaching. Compared with other luciferases and chemiluminescence probes imaging, bacterial luciferase has its own characteristics in single cells imaging. Bioluminescence intensity can indicate the metabolic level and cell viability. As a result, not only does bacterial luciferase

image biological processes but can also monitor and image metabolic change of single cells. For example, the bactericidal effect of antibiotics can be evaluated by imaging bioluminescence of single cells, which can help to discover single-cell heterogeneity and dormant cells.

In terms of the further development of bacterial bioluminescence bioimaging, it is extremely necessary to improve bacterial luciferase expression levels in different cells, tissues, and animals. Even though there are some progresses in enhancing bacterial luciferases expression, such as *ilux* gene developed by Hell's group [32], compared with fluorescence and other bioluminescence luciferase imaging, it is still required to further enhance bioluminescence intensity and amplify bioluminescence emission.

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Declarations

Conflict of interest The authors declare no competing interests.

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