Self-luminescent photodynamic therapy and pathogen detection for infectious diseases

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Abstract
The importance of detection and treatments of infectious diseases has been stressed to the world by the ongoing COVID-19 pandemic. As a substitution of an external light source, self-luminescent therapeutics featuring in situ light emission aims to address the lack of tissue penetration in conventional photodynamic therapy (PDT). Luminol-based self-luminescent systems are successfully incorporated in PDT and detection of pathogens in infectious diseases. In these systems, luminol/hydrogen peroxide is served as luminescence source which can be activated by horseradish peroxidase (HRP). As a supplement strategy to the HRP-based bioluminescence, electrochemiluminescence (ECL) provided an electric-driven therapeutic solution and demonstrated potential capabilities of wearable healthcare devices with properly constructed transparent flexible hydrogels. Besides the diagnosis of infection and detection of bacteria, fungi and virus in solution or powder samples have been achieved by ATP-derived self-luminescence as the light source. In this inspirational note, we provide an overview on latest progress in the PDT and microbial detection by self-luminescent systems with an emphasis on the bioluminescence and ECL.

Keywords Photodynamic therapy · Self-luminescence · Antimicrobial · Pathogen detection

Background
In 2020, the COVID-19 pandemic caused by infection of the deadly virus SARS-CoV-2 has tremendously threatened global healthcare with high pathogenicity and infectiousness. To combat the outbreak of infectious diseases, it is imperative to develop rapid and effective therapeutics, particularly before specific drugs and vaccines become available. Recently, photodynamic therapy (PDT) as a promising alternative intervention has been employed for lung cancer, esophagus cancer, and non-melanoma skin cancer [1]. Three essential components are typically involved in PDT process: (1) a non-toxic photosensitizer, (2) light irradiation at wavelengths the photosensitizer can absorb, and (3) surrounding reactive oxygen species (ROS) [2]. However, molecular photosensitizers are usually excited by UV or visible light with limited tissue penetration (< 1 cm) [3]. Although some reported photosensitizers can be activated by the near-infrared light or via two-photon absorption, the penetration only extends to 2–3 cm [4]. To overcome these difficulties, the external light source needs to be replaced by an internal component capable of generating in situ self-luminescence. Such self-luminescent systems based on bioluminescence and electrochemiluminescence (ECL) have emerged as alternative approaches for treating infectious diseases.

Bioluminescent PDT systems
Commonly applied in forensic detections, the luminol-based chemiluminescence provided a well-understood example of chemical transformation that results in light emission. Under basic conditions, the deprotonated luminol di-anion intermediate can be oxidized by O$_2$ to yield the excited state of 3-aminophthalate, which emits striking blue chemiluminescence upon decay to ground state. Various iron-complexes can catalyze the reaction, and the resulting chemiluminescence can be utilized as an internal light source.
Based on this principle of self-luminescence, Wang and co-workers [5] pioneered a “light-free” bioluminescence resonance energy transfer (BRET) system for photodynamic antimicrobial applications (Fig. 1), demonstrating the strong capability of the luminol/HRP/H2O2/OPV system for clinical analysis and detection. In this system, catalytic oxidation of luminol occurred with the addition of H2O2 and HRP, and the resulting luminescence near 425 nm was well-overlapped with the broad absorption of the photosensitizer oligo(phenylenevinylene) (OPV). Electrostatic attraction between the anionic 3-aminophthalate and cationic OPV ensured efficient BRET and excitation of OPV, thus generating abundant cytotoxic ROS. While the individual biocompatible components would not elicit high toxicity, their combination in a BRET system achieved an antifungal efficiency of 98%.

Subsequently, Wang and co-workers [6] further constructed an organic-inorganic assembled network for disinfection of both bacteria and fungi based on the glucose-powered bioluminescence. To circumvent the cytotoxic side effects caused by H2O2, a cascade catalytic system was built with two enzymes, glucose oxidase (GOx) and HRP, which were co-immobilized in situ by 5′-adenosine monophosphate (5′-AMP) and Gd3+ ions. In the presence of GOx and glucose, oxygen can be reduced to H2O2 that triggers the BRET-based PDT system. The hybrid assemble network achieved high inhibition efficiency of 80% against E. coli and 70% against C. albicans. In spite of the broad spectrum antimicrobial activity, efficiency of energy transfer was compromised due to the mismatching between the CL emission of luminol and the absorption of photosensitizer. Then, Huang group [7] fabricated the chemiluminescent nanoparticle consisted of luminol/HRP/PLGA/DSPE-mPEG2000 as a nanophotosensitizer to activate PDT, thus circumventing the issue of energy transfer efficiency. Under H2O2-rich conditions, the chemiluminescent nanoparticles could directly produce more 1O2 without external photoexcitation and achieved an antibacterial efficiency around 70%.

Self-luminescent PDT systems have also shown potentials against viral infections. Since various viruses may infect deeper tissues and organs that are optically opaque, acquisition of photon counts becomes increasingly difficult. Furthermore, considering the high mutant potentials of the RNA virus (e.g., COVID-19), some virus may attack immune cells to damage the immune system, for which the vaccine might not be effective in the long term [8]. Ghiladi and co-workers [9] identified DIMPy-BODIPY that showed broad-spectrum antiviral effects in vitro at nanomolar concentrations and short illumination times. In addition, MXenes as good candidates for virus inactivation are utilized in PDT system, as well as porphyrin, chlorins, porphin, and phthalocyanines materials [10]. Fullerene and graphene with two-dimensional carbon allotrope also show PDT effects against various viruses, including influenza A virus, HIV-1, HSV-1, vesicular stomatitis virus, Semliki Forest virus, mosquito iridovirus, and phage MS2 [11]. As an effective antiviral method, “non-specific” PDT bypassed the hysteresis, specificity, and selectivity of drugs or vaccines [12]. Hence, applying the self-luminescent PDT systems to the inactivation of COVID-19-associated virus could be an area of great interests and expectations.

**ECL PDT systems**

Despite the promising results, bioluminescent PDT systems suffered from two major drawbacks: (1) difficulties to achieve the temporal and spatial control of the ROS release and (2) limitations of the PDT efficiency due to enzyme activity. As an alternative strategy to enzymatic catalysis, luminol oxidation and the self-luminescent can be achieved by electrochemical methods, thus enabling the electric-driven ECL antimicrobial systems.

In 2018, Wang and co-workers [13] constructed an ECL PDT system that oxidized luminol intermediate on the anode of glassy carbon electrode and reduced H2O2 to generate superoxide radical anions (O2•−) on the cathode. Then, excited-state 3-aminophthalate anion was generated by the reaction of O2•− and the oxidized luminol intermediate. Along with excited aminophthalate returning to the ground state, the blue luminescence was emitted to accomplish the energy transfer to surrounding OPV (Fig. 2a). Compared to the commercially available porphyrin sensitzers, OPV exhibited better ROS generation ability because of its higher energy transfer efficiency. Built on these principles, a transparent elastic polyacrylamide hydrogel loaded with luminol/H2O2/OPV was fabricated into an electric-driven antimicrobial therapeutic device. The flexible porous hydrogel was sandwiched between two thin sheets of copper electrode to apply direct current (Fig. 2b).
The device was remarkable in that merely 5-s charging provided an immediate luminescence that lasted for more than 10 min. (Fig. 2c). The characteristics of long afterglow lifetime and flexibility endowed the device with promising potential in developing wearable device or implanted-platform into organs.

Self-luminescent sensors for pathogen detection

Once the pandemic has been gradually brought under control, the priority may shift from treating numerous patients to detecting sporadic emerging cases and preventing recurrence of widespread infection. Early diagnosis provides valuable guidance for subsequent medical treatments, especially when the pathogen concentration is relatively low and difficult to detect. High sensitivity, specificity, and speed are all urgently needed from the detection method. However, current pathogenic detection technologies heavily relied on trained professionals and sophisticated diagnostic instruments, which placed severe restrictions on the therapeutic efficiency. Studies have demonstrated the detection of pathogens by ATP-derived self-luminescence in drinking water [14], beach water [15], and food specimens [16]. Researchers have also developed a screening method for the detection of viable spores in powder samples by ATP bioluminescence combined with a heat shock [17]. The method featured a rapid procedure within 5 min and a high sensitivity with detection limit of less than 100 spores. In order to enhance the limit of bioluminescence detection, a novel mutant enzyme Photinus pyralis luciferase with high luminescence intensity [18] was investigated and utilized in detecting E. coli and B. subtilis. Moreover, multi-enzyme bioluminescent systems [19] were also designed and constructed for amplifying bioluminescence signal by employing...
regenerating ATP strategy. Adenylate kinase could catalyze the reaction of converting AMP + ATP to two molecules of ADP, which then was catalyzed by polyphosphate kinase (PPK) for ATP amplification. In addition to enhancing the bioluminescence signals, detection efficiency can be improved by rapid and efficient separation of pathogens. Immunomagnetic beads coated with the targeted bacterial cell antibody were designed and synthesized [20]. The beads that captured the targeted bacteria were subjected to an external magnetic field, and the interferent bacteria in supernatant were removed by centrifuging. Then, the detection was achieved by bioluminescent reaction of firefly luciferin and ATP.

Combined with a highly sensitive CCD camera, in vivo detection of virus such as vaccinia, herpes simplex, hepatitis B/C, and influenza based on bioluminescence in living animals has been studied [21]. Schultz-Cherry and co-workers rationally designed and constructed a replication-competent influenza reporter to visualize spatiotemporal dynamics of virus infection and transmission [22]. The A/California/04/2009 virus strain encoded with engineered NanoLuc was utilized to track the real-time intra-host dissemination and inter-host transmission in ferret lungs. It also achieved quantification of the virus loading in larger animal models by bioluminescence imaging. The reporter virus was sufficiently stable and sensitive to be implicated for responses of antiviral drug/vaccine susceptibility. In another work, Qin and co-worker described a recombinant reporter Japanese encephalitis virus (JEV) stably expressing Renilla luciferase. The dissemination and transmission of JEV were detected in both brain and abdominal organs in vivo using bioluminescent imaging technologies.

Therefore, because of high throughput, high sensitivity, and low background signal without external light, ECL triggered by electron transfer between electrogenerated species could be applied to pathogen detection [23]. Previous works have reported bacteria and virus detection by using ECL technology [24]. Pang and co-workers constructed sandwich-structured immunoreactors for electroluminescent detection of 2014–2016 Ebola virus (EBOV) based on polyclonal antibody (pAb)-modified electroluminescent nanospheres (IENs). While in the presence of EBOV, a gold nanoisland film electrode magnetically bridges the pAb-modified magnetic nanobeads, which could capture EBOV by identification of antibody. Then, IENs could couple with EBOV as well. Due to the steric effect between the IENs and electrode, ECL signal was obtained. Since IENs encapsulated plenty of quantum dots (QDs), ECL signal was enhanced to 85-fold in comparison to normal QDs.

Conclusion

The COVID-19 outbreak has hit over 200 countries, and the viruses of new variant continued to spread rapidly worldwide. To address such urgent and complex challenge against crisis of infectious diseases, self-luminescent therapeutic system exhibits qualified capabilities of counteracting the pathogens. Here, we noted bioluminescence and ECL-involved self-luminescent PDT and detection in diseases associated with bacterial or viral infections. As one of the promising clinical strategies, self-luminescent systems that can replace the external light source have been developed in imaging, detection, and therapy for deep-tissue cancer and infectious diseases. Since our group pioneered BRET self-luminescent system for anticancer and antimicrobial activities, numerous researches of self-luminescent PDT had been studied in succession. Taking the advantages of technological progress and drug developments, future investigations will entail outstanding self-luminescent systems for sensitive detection of pathogens and effective treatment of infectious diseases.

Author contribution Both E.Z. and Y.H. contributed to write the manuscript. S.W. supervised the project.

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Availability of data and materials The authors agree that any materials and data that are reasonably requested by others will be made available for noncommercial purposes.

Declarations

Ethics approval and consent to participate This work complies with the current laws of the country in which it was conducted. This work does not involve human subjects.

Consent for publication All authors approve for publication.

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References

1. Fan WP, Huang P, Chen XY. Overcoming the Achilles’ heel of photodynamic therapy. Chem Soc Rev. 2016;45:6488–519.
2. Miao QQ, Pu KY. Organic Semiconducting agents for deep-tissue molecular imaging: second near-infrared fluorescence, self-luminescence, and photoacoustics. Adv Mater. 2018;30(49):1–23.
3. Lucky SS, Soo KC, Zhang Y. Nanoparticles in photodynamic therapy. Chem Rev. 2015;115(4):1990–2042.
4. Shen YZ, Shuhendler AJ, Ye DJ, Xu JJ, Chen HY. Two-photon excitation nanoparticles for photodynamic therapy. Chem Soc Rev. 2016;45:6725–41.
5. Yuan HX, Chong H, Wang B, Zhu CL, Liu LB, Yang Q, Lv FT, Wang S. Chemcial molecule-induced light-activated system for anticancer and antifungal activities. J Am Chem Soc. 2012;134(32):13184–7.
6. Yuan HX, Bai HT, Liu LB, Lv FT, Wang S. A glucose-powered antimicrobial system using organic–inorganic assembled network materials. Chem Commun. 2015;51(4):722–4.
7. Tang YF, et al. Chemiluminescence-initiated and in situ-enhanced photoisomerization for tissue-depth-independent photo-controlled drug release. Chem Sci. 2019;10(5):1401–9.
8. Almerda A, Faustino MAF, Neves MGPMS. Antimicrobial photodynamic therapy in the control of COVID-19. Antibiotics. 2020;9(6):320–330.
9. Carpenter BL, et al. Antiviral, antifungal and antibacterial activities of a BODIPY-based photosensitizer. Molecules. 2015;20:10604–21.
10. Wiehe A, O’Brien JM, Senge MO. Trends and targets in antiviral phototherapy. Photochem Photobiol Sci
11. Weiss C, et al. Toward nanotechnology-enabled approaches against the COVID-19 pandemic. ACS Nano. 2020;14:6383–406.
12. Khorsandi K, et al. Antiviral photodynamics therapy: a probable biophysicochemical management modality in SARS-Cov-2. Expert Opin Drug Del. 2020. https://doi.org/10.1080/17425247.2021.1829591.
13. Liu SS, et al. Electrochemiluminescence for electric-driven antibacterial therapeutics. J Am Chem Soc. 2018;140(6):2284–91.
14. Frundzhyan V, Ugarova N. Bioluminescent assay of total bacterial contamination of drinking water. Luminescence. 2007;22(3):241–4.
15. Lee J, Deininger RA. Detection of E. coli in beach water within 1 hour using immunomagnetic separation and ATP bioluminescence. Luminescence. 2004;19(1):31–6.
16. Whitehead KA, Smith LA, Verran J. The detection of food soils and cells on stainless steel using industrial methods: UV illumination and ATP bioluminescence. Int J Food Microbiol. 2008;127(1–2):121–8.
17. Lee J, Deininger RA. A rapid screening method for the detection of viable spores in powder using bioluminescence. Luminescence. 2004;19(4):209–11.
18. Noda K, Matsuno T, Fujii H, Kogure T, Urata M, Asami Y, Kuroda A. Single bacterial cell detection using a mutant luciferase. Biotechnol Lett. 2008;30:1051–4.
19. Sato T, Kato J, Takiguchi N, Ohtake H, Kuroda A. ATP amplification for ultrasensitive bioluminescence assay: detection of a single bacterial cell. Biosci Biotechn Bioch. 2004;68(6):1216–20.
20. Cheng YX, Liu YJ, Huang JJ, Li K, Zhang W, Xian YZ, Jin LT. Combining biofunctional magnetic nanoparticles and ATP bioluminescence for rapid detection of Escherichia coli. Talanta. 2009;77(4):1332–6.
21. Avci P, et al. In-vivo monitoring of infectious disease in living animals using bioluminescence imaging. Virulence. 2018;9(1):28–63.
22. Kaelsson EA, et al. Visualizing real-time influenza virus infection, transmission and protection in ferrets. Nat Commun. 2015;6:6378.
23. Chen SF, et al. Electrochemiluminescence detection of Escherichia coli O157:H7 based on a novel polydopamine surface imprinted polymer biosensor. Acs Appl Mater Inter. 2017;9(6):5430–6.
24. Hao N, et al. AgBr nanoparticles/3D nitrogen-doped graphene hydrogel for fabricating all-solid-state luminol-electrochemiluminescence Escherichia coli aptasensors. Biosens Bioelectron. 2017;97:377–383.

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