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# A Light To Our Darkness: Bioluminescence and Its Uses In Medical Research

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**ABSTRACT**

Bioluminescence refers to the production of light without the need for an external light source, and as such, it differs from the concept of fluorescence. After this phenomenon became reproducible and measurable, bioluminescence began to be used as an investigation and research method in scientific research. Today, bioluminescence is used in most of the medical research fields, especially in cancer and cell culture. Improvements in brighter luminescence and imaging provides much wider areas of non-invasive research in different fields of medical research. Currently research articles can be found in the subjects of neuroscience, immunity, muscular regeneration, dermatology, surgery, microbiology, drug discovery, cardiovascular research and aging. In this review, the concept of bioluminescence and examples of its use in medical research are presented. Scientific literature was reviewed in June 2022. The articles were scanned according to their titles and abstracts, and articles from 2018 and later were included for findings on the use of bioluminescence in medical research. Only English articles are considered for the review. A total of 59 articles were selected and included.

**Keywords:** Bioluminescence, luciferin, luminescence, light emission, medical research

## Introduction

Bioluminescence is a phenomenon that has attracted the attention of people throughout history. It is an important natural phenomenon that continues to be used in scientific fields today. The purpose of this review is to present use of bioluminescence in medical research.

**Bioluminescence That Illuminates The Dark:** The term bioluminescence is a combination of the words "bios" which means life and "lumen" which means light. Bioluminescence in living things requires a chemical reaction, but unlike that of a burning wood, it involves the production of light without producing a burning heat. Firefly is among the most known animal for bioluminescence however bioluminescence can be observed in a variety of different organisms such as worms, insects, vertebrates, aquatic organisms of different taxa, plant and fungi as well as extinct species (1-6). When we look at the concepts related to light production in living things in more detail, we see that the forms of radiation seen in the world are generally referred to as luminescence. We come across types such as electroluminescence, photoluminescence, chemoluminescence, radioluminescence, sonoluminescence and thermoluminescence as its subtypes. Within the scope of this review, we will be restricted to chemiluminescence event that is more common than others in the world and more used in medical research.

**Molecular Explanation of Bioluminescence:** After the luciferin molecule reacts with oxygen, an oxidation occurs. After this oxidation, the molecule becomes excited and releases the energy it has loaded as light. This reaction is catalyzed by the enzyme luciferin. Following studies on luciferin molecule in 1940s and 50s it was revealed that this chemical is an ATP dependent light emitting molecule without heat production (7-11). However, the light release here differs from the process occurring in fluorescence. In fluorescence, the light that provides stimulation in terms of energy in the molecule is released when the excitation ends, while in bioluminescence, light is released during the conversion of D-luciferin to oxyluciferin by the activity of components such as oxygen, ATP and magnesium ion and luciferase enzyme (12).

In other words, bioluminescence refers to the production of light without the need for an external light source. However, firefly is not the only bioluminescent living being in nature. Apparently, this bioluminescence property is not limited to just aquatic organisms and number of bioluminescent organisms

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from aquatic or terrestrial environments covers more than 700 genera (13).

Within the scope of this review, first of all, current articles about the concept of bioluminescence and its use in scientific and especially medical research have been researched, and examples from different medical research areas are selected from different disciplines and presented together.

**Uses of Bioluminescence in Medical Research:**

**Cardiovascular and Respiratory Research**

Although advances such as better understanding of the relationship between nutrition and heart health in the past century and the development of surgical intervention techniques for heart vessels and valves have made heart diseases more controllable, they are still among the leading causes of major causes of deaths (14).

A blockage in the coronary vessels feeding the heart muscle causes the myocardial muscle to enter an ischemic state and to suffer reversible or permanent damage. Although vascular intervention is more common, intramyocardial injection options have been tried for regeneration of the myocardium. However, there are limits for the effectiveness and permanence of this injection. Bioluminescence has been used to show that fluorescently labeled cardiomyocytes are homogeneously distributed on the 28$^{th}$ day of the surgery, using special needles developed by bioengineering and Adenovirus (15).

Closely related with cardiovascular system is the pulmonary system. Diseases such as pneumonia can lead to death which was commonly observed in the covid-19 pandemic. Imaging of the pulmonary tissue and progress of infection in lungs is very informative in such research. Establishing a pneumonia in mice was performed with bioluminescent Streptococcus pneumoniae in a study. Therefore, bioluminescence was used to evaluate bacterial burden in these experimental diseases progress (16).

**Ageing**: In a study on the effect of aging on productivity-related functions compared to younger ages, it was investigated how mitochondrial protein synthesis and autophagy in mature oocytes of aged mice are affected in the presence of melatonin *in vitro*. ATP-dependent luciferin luciferase bioluminescence assay was used to measure the ATP content of the cells (17). Age related Mitochondrial membrane changes are monitored in a study by Bazhin et al 2020 (18). In another study impact of aging on bioluminescence in the retina, retinal pigment epithelium, and cornea was studied with a mice species (19). In a study, bioluminescence of intervened human adipocytes was observed for 3 days in an *ex vivo* setup showed endogenous circadian oscillators though they are devoid of suprachiasmatic nucleus signaling (20). It is known that aging causes various alterations in the body and mostly leads to insufficiency of functioning. Bioluminescence study with NG2-firefly luciferase transgenic rats revealed a decrease of selected cell type in the stomach of rats (21). Another study focus on senescence in mice showed alterations in bioluminescence steadily with age and this increase was more prominent in lung, thymus, and pancreas (22).

**Cancer Research:** Bioluminescence is also widely used in cancer research. Cancer research has shown that circular RNAs, abbreviated as circular RNAs, play a role in the regulation of cancer development. Xie et al. (2020) used mouse xenograft and bioluminescence imaging to evaluate the clinical relevance of cirSHKBP1 *in vivo* (23). Some methods of cancer treatment also involve bone marrow transplantation. Therefore, evaluating the success of this transplantation is crucial. Research on this subject is supported with light coming from bioluminescence. Oliveira et al (2021) used grafting of hematopoietic stem cells from young and old mice in a bone marrow transplant model (24). In another study on cancer research, bioluminescence imaging was used to determine antitumor activity in a mouse xenograft model for aggressive acute myeloid leukemia (25). Bioluminescence is also used together with immunohistochemistry when the tumor grows or metastasizes after the treatment and to decide whether the administration will work or not in various cancer treatments (26).

**Drug Discovery:** Bioluminescence is becoming an important component of drug discovery and as an example imaging of the activation state of an androgen receptor with a genetically encoded nanosensor can be screened by this methodology (27). Such a technology attracts interest but there are still various unknown aspects of the subject and further research may provide information on different components of bioluminescence such as patterns of emission (13). Although bioluminescence from original sources are important for medical research, new bioluminescence emitting combinations including engineered railroad worm luciferase shows promising results since they provide augmented thermostability, attenuated bioluminescence decay period and higher penetration to bacterial cell membranes (28). So, search for different mode of bioluminescence from different organisms is an ongoing research issue. Fungi are known to possess bioluminescence in the eukaryotic world. They can emit green wavelength. Biochemical mechanism was explained due to reactions of luciferin however, recent research reveals different aspects of this emission. Further research may provide different mechanism on this subject (29). Another molecule which required further investigation is polynoidin which is a membrane enzyme found in some worms of the polynoidae family (known as scale worms). Although it was isolated nearly 40 years ago in 1980's from *Malmgrenia lunulate* biochemical details of this bioluminescence still needs further research (30). Problems related with luminescence of different sources include phenomenon such as penetration depth or interference of tissue autofluorescence. Solutions for those problems focus on near infrared or self-luminescence imaging with organic luminophores (31).

**Imaging:** Medical uses of bioluminescence mostly focus on analysis and imaging studies which aim to clarify the nature of different biological mechanisms. However, some recent advances also give way to bioluminescence dependent photodynamic therapies or use of bioluminescence in the field of neuroscience to control neurons (32). Optogenetics is a method recently used in neuroscience. Bioluminescence-optogenetics are evaluated for their potential superiority over conventional optogenetics (33). Assays used for exploration of therapeutic options for channelopathy-associated diseases now evaluate potentials of bioluminescence studies (34). Not only medical research is inspired by bioluminescence of different organisms but technology also uses information coming from medical research. Bioluminescence of marine dionflagellates also shed a light on polymerosome nanoreactors (35).

**Infectious Diseases and Microbiology:** A new area where bioluminescence is used is the detection of pathogenic microorganisms in the urine sample. In routine clinical procedures, there is a urine culture process that takes approximately 48 hours to detect urinary tract infection. However, instead of this time-consuming and sometimes misdiagnosed procedure, two new technologies have been developed; tube bioluminescence extinction technology urine (TuBETUr) and cellphone-based UTI bioluminescence extinction technology (CUBET). With these techniques, the diagnosis of *Escherichia coli, Proteus mirabilis, Staphylococcus aureus*, and *Candida albicans* has been confirmed (36).

Bioluminescence measurements can find certain study areas for practical used such as assessment of hand hygiene against bacteria (37) or to evaluate cross contamination (38).

Since discovery of microscope and studies of pioneer scientists such as Koch and Pasteur field of microscopic imaging diversified. Bioluminescent pathogens make further revolutions in this field. Bioluminescent *Escherichia coli* was used to track and monitor ascending vaginal infections in rats and yield promising results for further research studies (39).

Although the modern world suffers from degenerative diseases mostly related with aging or life-long habits, still millions of people in developing countries diseases such as Chagas disease, which is caused by the intracellular protozoan *Trypanosoma cruzi*, affects millions of people. Detection as well as monitoring disease is an important step for elucidating true nature of this disease. Therefore, researchers infected Swiss Webster mice luminescent trypomastigotes. Bioluminescence procedures helped researchers for 126 days of monitoring on this species (40).

**Endocrine, Obesity and Reproduction:** Although some part of the world population is suffering from infection diseases obesity is becoming a non-contagious pandemic. Therefore, finding new chemicals or treatments against obesity is becoming more common. Im et al (41) administered thermogenic and anti-obesity effects of heat-transformed green tea extract on mice. Researchers injected D-luciferin (150 mg/kg, i.p. USA). Bioluminescence signals were detected with Ami-X imaging systems and image processing was performed with Aura software.

Endocrine disrupting chemicals is a growing problem due to increased human manufactured toxic chemicals in the ecosystems and food web which ends up in human consumption. Lee et al (42) have shown that transfected human cell lines using a bioluminescence resonance energy transfer system is comparable with conventional methods to study human androgen receptor-mediated endocrine disruption. Similarly, biological effects of chemicals which exert harmful impact on female reproductive system can also be studied with bioluminescence in an in vitro experimental setup (43).

**Neuroscience:** Optogenetics which is using modification of the neurons and stimulating them with appropriate light opened a wide range of research. Luciferase using bioluminescence brought promising alternatives for this research field. External light for opsin stimulation may alter the functions of the neurons in an unwanted manner. On the other hand, luciferin incorporated to cells and presence of luciferase can produce required light for activating the opsin molecule without the aforementioned technical problem (44).

Optimization of this biological light source rather than an external light source is still continuing. Protocols for optimum culture, test protocols and parameters for target effects of luciferin are among parameters on which future research will focus more (45).

Combination of bioluminescence and optogenetics provide promising results both for imaging of biological systems and therapy for neurological diseases. Zenchak et al (46) showed that an improvement of motor deficits in a Parkinson's disease mouse model by the help of bioluminescence-driven optogenetic activation of transplanted neural precursor cells.

**Future Aspects in Bioluminescence research:** Up to this point it is clear that bioluminescence occupies an important technique for present but future possibilities is also fascinating when we consider 11 over 30 known bioluminescent systems are characterized to-date. Therefore, further research on the subject may present new possibilities (32). New imaging possibilities are also under development [47] which may incorporate different wavelengths in light spectrum (48). Further research also includes augmentation of the brightness of luminescence energy to enhance imaging (49). Further research field of bioluminescence also includes potential treatments with bioluminescence activated photodynamic therapies (50). Imaging of during in vivo conditions reflect life systems more appropriately. Thus, development of smaller systems and probes allowing imaging of alive organisms may incorporate in a variety of research fields such as gene expression to behavior (51, 52).

Use of bioluminescence in medical research is not limited to the mentioned research fields in this review. There are various fields using bioluminescence in medical research. Immunity (53), muscular regeneration (54), dermatology (55), anti-sepsis procedures in surgery (56), orthopedic implant antimicrobial prophylaxis and therapy (57), wound healing and infections (58) are just a brief presentation of this very large research areas.

It is clear that bioluminescence, with its wide use areas in medical research will be much commonly used in the future. Therefore, techniques focusing on imaging with bioluminescence will be much more studied in the professional level and also it will find more opportunities even at undergraduate education level with improvements in probes, methods and imaging devices (59).

Following the understanding the biological mechanism of that natural phenomenon, the use of bioluminescence has expanded in medical research fields. Nowadays, it is moving towards being a routine examination method such as bioluminescence immunohistochemistry in many areas, from the validation of xenograft-containing methods used for regeneration of a tissue to assessment of cancer treatments. In addition to existing forms of bioluminescence, perhaps new bioluminescence forms will be found among the millions of species still waiting to be discovered on our planet. Further scientific research will provide more accessible, diversified and better imaging methods of bioluminescence for medical research.

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