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The power of bioluminescence imaging in understanding host-pathogen interactions

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ABSTRACT

Infectious diseases are one of the leading causes of death worldwide. Modelling and understanding human infection is imperative to developing treatments to reduce the global burden of infectious disease. Bioluminescence imaging is a highly sensitive, non-invasive technique based on the detection of light, produced by luciferase-catalysed reactions. In the study of infectious disease, bioluminescence imaging is a well-established technique; it can be used to detect, localize and quantify specific immune cells, pathogens or immunological processes. This enables longitudinal studies in which the spectrum of the disease process and its response to therapies can be monitored. Light producing transgenic rodents are emerging as key tools in the study of host response to infection. Here, we review the strategies for identifying biological processes *in vivo*, including the technology of bioluminescence imaging and illustrate how this technique is shedding light on the host-pathogen relationship.

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1. Introduction

Bioluminescence imaging (BLI) is a powerful technology for studying microbial pathogenesis, immune response to infection and the effectiveness of anti-infective therapy. It has gained popularity, because unlike conventional methods which require the analysis of multiple cohorts of animals at different time points, BLI allows for continual analysis in the same cohort. Continual BLI for pathogen colonization and treatment response is a well-established tool but its use in determining how animals respond to infection is an emerging technology. Reporter genes

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are invaluable for studying cellular immune responses *in vivo*. They can be used, for example, to monitor specific inflammatory signalling pathways and immune cell recruitment to areas of infection. This review explores the numerous strategies for identifying biological processes *in vivo* and how the use of bioluminescent pathogens and the luciferase enzyme reporter is being applied to the study of host response to infection.

2. Models of infectious disease

Infectious diseases are one of the leading causes of death worldwide, accounting for over 15% of global mortality, according to the WHO [1]. Therefore, modelling human infection is imperative to understanding and developing treatments to reduce the global burden of infectious disease. Disease models of infection include *in vitro* modelling in cultured cells, *in vivo* animal models as well as *ex vivo* models of human organs or organoids [2–5]. Today, rodents account for the majority of models used in infectious disease research and they model a wide range of infectious disease agents [6].

To determine infection and the host response to infection using traditional disease models of infection, conventional markers such as blood samples or swabs are taken. The infected animals may then be sacrificed at defined time points and infected tissues harvested. An example of subsequent analysis is serial plating and colony counting to estimate pathogen numbers and to determine localisation. This whole process is highly invasive, end-point analysis intensive and expensive. To address this invasive and time-consuming process, imaging and reporter genes have been employed for use in *in situ* detection of specific infectious pathogens and inflammation as well as gene activities responsible for the immune response. The use of reporter genes provides a means of expedient, simple and highly-sensitive endpoint analysis compared to conventional infection markers.

3. Pre-clinical imaging modalities in infectious disease research

Pre-clinical imaging modalities are integral to translational research and they constitute a means of assessing biological structures and processes using non-invasive techniques. They enable longitudinal studies in individual animals. A main advantage is the reduction of biological variability as each animal can function as its own control. These imaging modalities also support two of the 3Rs of animal research; reduction and refinement, by minimising numbers of animals sacrificed and the intrinsic non-invasive nature of imaging which help to improve animal welfare [7]. Numerous non-invasive imaging modalities have been used for infectious disease research as discussed below.

Positron emission tomography (PET) labels biologically active molecules with positron-emitting radioisotopes to image *in vivo* pathophysiological processes. Using the clinically-established ^{18}F-FDG as a surrogate marker for infection-induced inflammation it was possible to monitor response to *Staphylococcus aureus* vascular graft infection [8]. Following tuberculous meningitis infection in a young rabbit model, activated immune cells in the brain were detected non-invasively using the ^{124}I-DPA-713 radio-isotope tracer [9].

Single photon emission computed tomography (SPECT), is a similar imaging modality to PET, whereby administered radioisotopes emit gamma radiation. SPECT combines multiple images to create a 3-dimensional image. It has an established role in the imaging of myocardial and cerebral perfusion but its role in infection is evolving. For example, the radioisotope, [99mTc]annexin V-128, is an *in vivo* marker of cellular stress and apoptosis, and can be used to detect and trace bacterial infection and response to treatment by SPECT imaging [10].

Computed tomography (CT) uses X-rays to measure and compare differences in tissue densities. It is helpful in detecting tissue or organ morphological changes caused by infection and inflammation, such as pulmonary fibrosis [11].

Magnetic resonance imaging (MRI) is a non-ionizing 3D imaging modality that uses the magnetic properties of tissues and their interactions with external magnetic fields. MRI not only provides anatomical information but it can also provide physiological data such as organ perfusion, molecule diffusion and tissue chemical composition [12]. Unsurprisingly MRI is an important tool in infectious disease research and has been used in multiple ways including the monitoring of inflammation [13], the quantification of blood flow to infected sites [8] and the imaging of host abscess formation [14].

Photoacoustic tomography works on the natural property of tissues to thermoelastically expand when stimulated with pulsed laser. This leads to ultrasound waves being emitted from the tissues which can be detected using an ultrasound transducer. It produces real-time high resolution scans and 3D reconstructions [15]. Its use in infectious disease research is limited but recently a new photoacoustic contrast agent that is highly specific for detecting certain bacteria *in vivo* has been described [16].

Optical imaging includes a variety of imaging techniques that rely on light production in the visible, ultraviolet or infrared electromagnetic spectrum. These optical imaging modalities usually require suitable reporter genes to be tagged in cells or pathogens of interest. The most relevant to infectious disease research are BLI, fluorescence imaging and two-photon intravital microscopy. Two-photon intravital microscopy, for example, contributed to detection of a pathway involved in the intravascular coagulation process which occurs during sepsis [17].

4. Conventional reporter gene methods

Monitoring biological processes *in vivo* is challenging therefore reporter genes may be used as surrogate markers to localise and quantify molecular signals. This technology relies upon the control of reporter genes by selected regulatory sequences; this confers the organism with a marker that can be easily detected and quantified, such as luminescence or fluorescence.

Numerous reporters have been developed. The first to be exploited were the bacterial enzymes chloramphenicol acetyltransferase (CAT) and beta-galactosidase (β-gal). CAT catalyses the transfer of the acetyl group from acetyl-coenzyme A to chloramphenicol [18]. Its popularity is limited by the need for radioisotopes and relatively elaborate purification and enzymatic assays to detect CAT reporter expression. β-gal, which recognises and cleaves X-gal to generate an intense blue stain, was first described by Jacob and Monod [19]. β-gal, the enzyme encoded by the LacZ gene in *E. coli*, became one of the most commonly used reporter genes for quantifying gene promoter activity [20].

These reporters have now been superseded by fluorescent markers. This began with the cloning of green fluorescent protein (GFP) in the 80s and the development of enhanced mutants of GFP [21–23]. The two best characterised GFPs are from the marine invertebrates; *Aeqourea victoria* and *Renilla reniformis*. Other GFP-like green, yellow and red proteins have been subsequently cloned [24]. The great advantage of these GFP-like proteins is their ability to form internal fluorophores without requiring accessory enzymes or substrates. They are also highly stable and are non-toxic in most cases. They are widely used to visualise transcriptional activities of promoters and to locate proteins in live cells [25].

4.1. Disadvantages of conventional reporter gene methods

Conventional assays of host-pathogen interactions require that experimental animals be euthanized at multiple time points to