In vivo bioluminescence for tracking cell fate and function

Patricia E. de Almeida,1,2 Juliaan R. M. van Rappard,4 and Joseph C. Wu1,2,3

Departments of 1Medicine and 2Radiology and 3Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, California; and 4Leiden University School of Medicine, Leiden, The Netherlands

Submitted 4 April 2011; accepted in final form 1 June 2011

de Almeida PE, van Rappard JR, Wu JC. In vivo bioluminescence for tracking cell fate and function. Am J Physiol Heart Circ Physiol 301: H663–H671, 2011. First published June 10, 2011; doi:10.1152/ajpheart.00337.2011.—Tracking the fate and function of cells in vivo is paramount for the development of rational therapies for cardiac injury. Bioluminescence imaging (BLI) provides a means for monitoring physiological processes in real time, ranging from cell survival to gene expression to complex molecular processes. In mice and rats, BLI provides unmatched sensitivity because of the absence of endogenous luciferase expression in mammalian cells and the low background luminescence emanating from animals. In the field of stem cell therapy, BLI provides an unprecedented means to monitor the biology of these cells in vivo, giving researchers a greater understanding of their survival, migration, immunogenicity, and potential tumorigenicity in a living animal. In addition to longitudinal monitoring of cell survival, BLI is a useful tool for semiquantitative measurements of gene expression in vivo, allowing a better optimization of drug and gene therapies. Overall, this technology not only enables rapid, reproducible, and quantitative monitoring of physiological processes in vivo but also can measure the influences of therapeutic interventions on the outcome of cardiac injuries.

bioluminescence imaging; heart; gene therapy; stem cell therapy; in vivo cell tracking

Histopathological examination has been the main approach for ex vivo evaluation of the distribution, the engraftment, and the differentiation of injected cells. However, histopathology precludes the evaluation and monitoring of these parameters in real time and in vivo. Molecular and cellular imaging has provided various techniques for identifying and tracking transplanted cells in cardiovascular research (15). Magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and single photon emission computed tomography (SPECT) offer deep tissue penetration and high spatial resolution (64, 70, 94a). However, in small animal studies, these techniques are more costly and time consuming to implement compared with optical imaging. Among the optical imaging tools, bioluminescence imaging (BLI) is a promising technique that is especially useful in small animal models and does not require the use of radionuclides with their associated hazards. BLI is a high throughput technique that can provide unmatched sensitivity because of the absence of endogenous luciferase expression in mammalian cells and the low background luminescence emanating from animals. BLI can be very useful in evaluating the delivery efficiency of therapeutic genes and their expression levels in vivo. By the use of different cellular promoters to drive the expression of a luminescent reporter gene, BLI allows monitoring of the transplanted cells’ differentiation status as well as their location and functional characteristics in vivo (39, 51, 72, 78, 94).

Overall, BLI of reporters cloned into promoter/enhancer sequences or engineered into fusion proteins has demonstrated the modality’s ability to monitor fundamental processes such as transcriptional regulation, signal transduction cascades, pro-

THIS ARTICLE is part of a collection on Assessing Cardiovascular Function in Mice: New Developments and Methods. Other articles appearing in this collection, as well as a full archive of all collections, can be found online at http://ajpheart.physiology.org/.

Cell-based therapies have rapidly emerged as a potential therapeutic approach for heart disease. After the initial work to characterize putative endothelial progenitor cells (1) and their potential to promote cardiac neoangiogenesis and to attenuate ischemic injury, a decade of intense research has examined several novel approaches to promote cardiac repair in adult life. A variety of adult stem and progenitor cells from different sources have been examined for their potential to promote cardiac repair and regeneration: bone marrow-derived cells (55, 72), circulating and mobilized CD133+ and CD34+ stem and progenitor cells (36), mesenchymal stem cells (56, 65, 83), cardiac resident stem cells (43, 62), and skeletal myoblasts (54, 97). However, many questions such as the optimal type and number of progenitor cells to be administered, the route of administration, and the best time to administer cells after injury remain unresolved. This is particularly true in vivo where it is difficult to assess cellular activity in the heart in real time. Therefore, it would be helpful to have practical tools to accurately track cell location and their functional status over time in vivo.

Address for reprint requests and other correspondence: J. C. Wu, Stanford Univ. School of Medicine, Lokey Stem Cell Research Bldg., 265 Campus Dr., Rm. G1120B, Stanford, CA, 94305-5454 (e-mail: joewu@stanford.edu).

http://www.ajpheart.org 0363-6135/11 Copyright © 2011 the American Physiological Society
tein-protein interactions, protein degradation, oncogenic transformation, cell trafficking, and targeted drug action under in vivo spatial registration. In this review, we will discuss recent advances and applications of BLI, specifically as they apply to cardiovascular research.

Principles of Bioluminescence

In nature, numerous luminous species exist in more than 700 genera, of which 80% are marine species (93). Luciferase enzymes have been cloned from both marine (e.g., Renilla luciferase) and terrestrial (e.g., firefly and click beetle luciferase) eukaryotic organisms and are commonly used as reporters for in vitro and in vivo studies. They emit long wavelengths of bioluminescence (\(\lambda > 600\) nm) in the red and near-infrared regions of the spectrum and are efficiently transmitted through mammalian tissues (20, 86). These wavelengths can avoid absorbing and scattering environment of mammalian tissues (69), thus can be efficiently detected outside a small animal’s body using BLI.

BLI is based on the detection of light emitted by cells that express light-generating enzymes such as luciferase. In bioluminescent reactions, luciferase generates visible light through the oxidation of enzyme-specific substrates such as D-luciferin for terrestrial organisms (29, 30) and coelenterazine for marine organisms (31, 53). Luciferases from different organisms can be distinguished by their abbreviations: lux (bacterial), luc (firefly), and lcf (dinoflagellate). To track cells in vivo by BLI, the cells of interest need to be genetically modified to express luciferase. The animal recipient of the cells receives the luciferase substrate either intraperitoneally or intravenously and is placed in a light-tight dark box where luminescence is detected (Fig. 1). In a bioluminescent reaction, the generation of light depends on several factors. Firefly luciferase requires ATP, Mg\(^{2+}\), and oxygen to catalyze the oxidation of its substrate D-luciferin and generates CO\(_2\), AMP, inorganic pyrophosphate, oxyluciferin, and a yellow-green light at a wavelength that peaks at 562 nm (Fig. 2). By comparison, Renilla luciferase catalyzes the oxidative decarboxylation of coelen-
terazine in the presence of dissolved oxygen to yield oxyluci-
ciferin, CO₂, and blue light that peaks at 480 nm (49). Biolu-
minescence generated by the luciferase reporter reaction is
captured by a cooled charge-coupled device camera that can
detect very low levels of visible light emitted from internal
organs (19). Charge-coupled device camera-imaged biolumi-
nescence can then be superimposed on photographic images
of the mouse to detect quantitatively and repetitively the biolu-
mencescent signal from a given location (95). Imaging can be
conducted 10 to 15 min after intraperitoneal injection of
β-luciferin (reporter probe), with relatively stable light emis-
sion levels for 30 to 60 min, depending on the experimental
conditions. The sensitivity of detection depends on the wave-
length of light emission, expression levels of the enzyme in the
target cells, the location of the source of bioluminescence in
the animal, the efficiency of the collection optics, and the
sensitivity of the detector (95).

Reporter genes commonly used for BLI. To track cells in
vivo, reporter genes and reporter probes must be able to reveal
cellular and molecular processes throughout an entire study
period, be highly sensitive to small changes in cell function and
distribution over time, and must not alter the labeled biological
process itself. A variety of reporter genes have been introduced
and validated for different imaging modalities, including vari-
ous luciferases for BLI (5, 67, 74). Luciferase (Luc) is the only
one that produces light without requiring an external excitation
source, and they offer inherently low background signals
because animal tissues do not emit significant amounts of light.
Since the first report of the cDNA encoding Luc in 1985 (23),
DNA sequences have been optimized so that the Luc gene can
be expressed at high levels and its product localized in the
cytoplasm of the cells. For all reporter systems, the intensity of
light is proportional to the amount of luciferase expressed in
each individual cell or the number of cells in which a gene has
been transferred.

Of the many available luciferase enzymes, only a subset
have been developed and used as reporter genes. Luciferase
from the North American firefly Photinus pyralis is the most
common choice, but other luciferases such as the sea pansy
Renilla reniformis, the click beetle Pyrophorus plagiophala-
num, and the copepod Gaussia princeps have also been inves-
tigated (48, 79). Gaussia and Renilla luciferase enzymes emit
in the blue/green region of the UV visible spectrum, where
light is strongly absorbed and scattered by tissues. Conse-
quently, the imaging performance suffers from poor sensitivity
and spatial resolution. On the contrary, Photinus pyralis and
click beetle luciferase emit ~60% of their light at >600 nm,
which enables great tissue penetration. Photinus pyralis has
emission at 620 nm when collected at 37°C, making it among
the longest emitting luciferases at mammalian body tempera-
tures and the most sensitive for in vivo applications (100).

Bacterial luciferases (Lux) such as from Photobacterium lu-
mencescens emit blue light that has been used for BLI of
bacterial infections. They are unique in that their lux operon
cassette codes for the luciferase and also for enzymes that
produce the substrate required for luminescence reaction,
thereby eliminating the need for exogenous substrate. Trans-
ferring this operon to mammalian cells would be advantageous
for in vivo imaging; however, limited research has been done
to determine whether this is possible (16). Mutations in lu-
ciferases can change the wavelength of the luminescence
emitted. A mutation of a single amino acid has been shown to
cause red-shifted luminescence and improve in vivo perform-
ance. Recently, a red-shifted mutant of luciferase from Pho-
tinus pyralis was created and described to have an emission
maximum of 612 nm at pH 7.0, a narrow emission bandwidth,
and to be thermostable (with a half-life 8.8 h at 37°C vs. 0.26
h for the wild-type luciferase). This Photinus pyralis mutant,
called Ppy RE-TS reporter, has been successfully used in small
animals to visualize cancer progression and shown to have
superior in vivo imaging performance compared with the
wild-type Photinus pyralis luciferase (6, 7).

The choice of reporter gene reporter probe pair to be used
should ultimately be based on the specific biological process to
be monitored, the duration and intensity of the signal needed,
and the tissue to be imaged (21, 100) (Fig. 3). As longer
wavelengths of light penetrate mammalian tissues with less
absorbance, luciferase from Photinus pyralis (>600 nm) is
more readily detected (17). Moreover, its substrate β-luciferin
remains in circulation longer than other substrates because it is
poorly catalyzed by mammalian tissues (101). However, Pho-
tinus pyralis luciferase has the disadvantage of having a longer
coding sequence (1,653 bp) compared with marine luciferases
such as Renilla and Gaussia. Renilla and Gaussia luciferases
have coding sequences of 936 and 558 bp, respectively, making
them more suitable for studies that require compact trans-
genome sequences, such as gene transfer and gene expression
studies. Another advantage of Renilla and Gaussia luciferases
over Photinus pyralis is the fact that they do not require ATP
as a cofactor during bioluminescent reaction and thus can be
used for imaging cells independent of their metabolic state (5,
66). One particular characteristic of Gaussia luciferase is that
it is naturally secreted (96) and therefore can be used as a
reporter for quantitative assessment of cells in vivo by mea-
suring its concentration in blood (61). However, Renilla and
Gaussia luciferases produce shorter wavelengths of light
(peak at 480 nm), which are not transmitted through tissues as
effectively as Photinus pyralis (100). Overall, the utility of
Renilla and Gaussia for in vivo BLI can benefit from improve-
ments in sensitivity (46–48).

Codon-optimized humanized Gaussia (2, 79) has improved
its sensitivity in mammalian cells, but the pharmacokinetics
of its reporter probe coelenterazine are somewhat limiting in vivo.
Coelenterazine is prone to quick inactivation, including degra-
dation through autooxidation. This substrate is also costly with
low solubility. Furthermore, coelenterazine binds to serum
proteins, is cleared rapidly from the bloodstream (101), and
decays rapidly with time (5). Therefore, when imaging in vivo
with coelenterazine, the signal needs to be acquired immedi-
ately after substrate administration. On the other hand, this
short half-life can be advantageous in cases in which sequential
imaging of two luciferases, such as Gaussia princeps and
Photinus pyralis, is required to monitor two biological pro-
cesses in tandem in the same animal (5).

In summary, the development of reporter gene variants with
better emission spectra, brightness, and stability can ultimately
improve sensitivity and the overall performance of luciferases
in BLI imaging studies. Moreover, cloning new luciferases
from different organisms, such as those from Luciola italic a
and Cratmorhous distinct us, can certainly improve current
applications and make novel ones possible (8, 88).
Vector-mediated expression of reporter genes. A fundamental requirement for BLI is the expression of a reporter gene (e.g., luciferase) by the cells or tissues to be imaged, which requires the introduction of genetically encoded imaging reporters into cells cultured in vitro (50). Alternatively, luciferase-expressing cells can be obtained from luciferase transgenic mice (Fig. 1). This can be achieved by using a reporter vector that incorporates a luciferase gene driven by a promoter that allows luciferase to be constitutively expressed by all cells in the animal’s body (11, 83).

Delivery of bioluminescent reporter genes to cells has been achieved through several means, but lentiviral-based gene transfer has been the method of choice because of its effective gene delivery and high expression levels of transgenes in mammalian cells in culture as well as in vivo (22). Lentiviruses have the capability to deliver target genes to both dividing and nondividing cells and are capable of inserting genetic information into the host genome, ensuring prolonged gene expression with a more limited host immune response (80). The safety of the lentiviral vectors has been further improved with the generation of self-inactivating vectors and the use of minimal packaging systems. The efficiency of gene expression has been improved by the introduction of a relatively strong internal promoter such as cytomegalovirus. This promoter drives the expression of the reporter gene and guarantees that the reporter expression is always “on” under all conditions, in all tissue types. Moreover, lentiviral vectors can be used to simultaneously induce the expression of multiple genes in a cell. Coupling the expression of a gene with a luciferase reporter gene provides a simple yet effective mechanism for studying the regulation of gene expression and monitoring it by BLI. This provides exciting opportunities for transcriptional targeting, double reporter labeling for BLI monitoring, as well as gene therapy. For example, by linking the expression of luciferase to the cardiac-specific promoter myosin light chain 2v, it is possible to monitor cells undergoing cardiac differentiation over time via the detection of reporter gene expression by BLI (27, 35).

Applications of BLI in Cardiovascular Research

Because physiological processes are dynamic in time and space, end-point assays do not always provide a comprehensive understanding of biology in vivo. In cardiovascular research and many other scientific areas, BLI has been used for noninvasive visualization of a variety of biological processes in real time. In this section, we will review the many applications of BLI in cardiovascular research.

Monitoring expression of therapeutic genes in the heart. Gene therapy is a rapidly evolving field in cardiovascular medicine. This technology allows for the correction of functional gene loss and enables the expression of a therapeutic gene in a target tissue (52). Nevertheless, gene therapy studies continue to be plagued by suboptimal delivery of genes, poor survival of cells carrying the therapeutic gene(s), and the inability to evaluate the levels of gene expression in vivo (28). Thus the information gathered from BLI studies in small animals can be used to design solid clinical trials that will help improve gene therapy for cardiac repair. In cardiovascular research, BLI has been used to address a variety of questions that range from determining the effects of transgene expression (e.g., BCL2) on cardiomyoblast survival and cardiac repair (40) to testing the efficiency of anti-inflammatory drugs on the expression of specific genes (e.g., inducible nitric oxide synthase) (99). Additionally, BLI has been used to optimize vector systems (33) and treatment regimens (71) for delivery of therapeutic genes (e.g., hypoxia-inducible factor-1α) to the heart.

The applications of BLI in research are constantly expanding with the development of new therapeutic modalities. For instance, BLI enabled tracking the activity of a short hairpin RNA plasmid in knocking down inhibitory factors of angio-
BLI has demonstrated the poor survival of bone marrow mononuclear cells (72), cardiac resident stem cells (43), mesenchymal stem cells (83), adipose stromal cells (82), ESC-derived cardiac cells (10), and ESC-derived endothelial cells (45) by 8 wk after cell injection. Conversely, the tumorigenic potential of ESCs can also be uncovered by BLI. Lee et al. (42) demonstrated that a minimum of 100,000 human ESCs are necessary to form teratoma in the heart. Overall, BLI studies have provided significant insights into the biology of stem cells in vivo and how issues such as poor survival and potential tumorigenicity must be overcome before translation into the clinic.

Monitoring immune rejection of heart transplant and cell grafts. Allogeneic heart transplantation is the most commonly used therapy for end-stage heart disease. Transplant loss due to immune rejection remains a significant problem in clinical heart transplantation despite current immunosuppressive therapies (73). Acute rejection of heart transplants is an immune response mediated by the coordinated infiltration and function of host alloantigen-specific T cells in the allograft (57). In this context, BLI has allowed monitoring of cardiac allograft over time and revealed its rejection by day 12 after transplantation. A decrease in the intensity of bioluminescence signals was detected as early as day 4, suggesting acute graft rejection (78). The correlation of bioluminescence intensity to other measures of heart function such as beating score, fractional shortening, and lymphocyte infiltration has provided additional means to assess function and to determine the possible mechanisms responsible for rejection.

Acute rejection can also be a major issue in human ESC-based therapy (25, 84). BLI has provided significant information for the development of therapeutic strategies to prevent the rejection of stem cells. An immunosuppressant cocktail consisting of tacrolimus and sirolimus has been shown by BLI to mitigate the rejection of human ESCs (77). Recently, Pearl et al. (63) used BLI to demonstrate that a cocktail of costimulatory blockade agents (anti-CD40 ligand, cytotoxic T-lymphocyte antigen 4-immunoglobulin, and anti-lymphocyte function-associated antigen-1) induced long-term allogeneic and xenogeneic human ESCs and induced pluripotent stem cell engraftment. Overall, these studies elucidate the importance of in vivo imaging for the development of therapies that may one day enable stem cell therapy to become feasible.

Table 1. Cells types that have been investigated for their potential to promote cardiac repair using BLI

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Information Gained from BLI</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow mononuclear cells</td>
<td>Comparison of different cell types for treatment of MI</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Timing of cell delivery on acute vs. chronic MI</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Systemic homing to injured heart</td>
<td>72</td>
</tr>
<tr>
<td>Adipose tissue-derived stem cells (ASCs)</td>
<td>Long-term survival of cells in injured heart</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Homing and survival of fresh versus cultured cells to injured heart</td>
<td>4</td>
</tr>
<tr>
<td>Mesenchymal stem cells (MSCs)</td>
<td>Comparison of ASCs vs. MSCs for treatment of MI</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Growth factor-treated MSCs for treatment of MI</td>
<td>24</td>
</tr>
<tr>
<td>Human CD34+ cells</td>
<td>Cell fate in the heart using a MI model</td>
<td>89</td>
</tr>
<tr>
<td>Rat cardiomyoblasts</td>
<td>Improvement of engraftment and survival with collagen matrix</td>
<td>38, 39</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Cell survival, proliferation, and migration</td>
<td>9, 42, 77</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>Cell fate in MI model</td>
<td>83</td>
</tr>
<tr>
<td>ESC-derived endothelial cells (ESC-EC)</td>
<td>Cell fate in MI model</td>
<td>44, 45</td>
</tr>
<tr>
<td>Resident cardiac stem cells</td>
<td>Effects of nicotine on the therapeutic effects ESC-ECs</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Cell fate and function in MI model</td>
<td>43</td>
</tr>
</tbody>
</table>

BLI, bioluminescence imaging; MI, myocardial infarction.

Review

IN VIVO BIOLUMINESCENCE FOR TRACKING CELL FATE AND FUNCTION

H667

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.1 on October 23, 2017
Limitations

BLI has been successfully used to obtain semiquantitative measurements of biological processes because of a strong correlation between the number of cells and the bioluminescence signal detected both in vitro (75) and in vivo (68). However, a simple quantification of light emission may not provide a true representation of biological processes. This is because the firefly luciferase reaction is a complex interaction of a variety of molecules (e.g., ATP, Mg$^{2+}$, oxygen, and luciferin) and because the intensity of the bioluminescence signal depends on multiple factors. In particular, the number of metabolically active luciferase-transfected cells, the concentration of luciferin, ATP and oxygen levels, the spectral emission of bioluminescence probes, and the depth and optical properties of tissues are known to alter the intensity of bioluminescence signal (69). Another issue that should be considered during quantitative BLI is the limited and wavelength-dependent transmission of light through animal tissues. Light sources closer to the surface of the animal appear brighter compared with deeper sources because of tissue attenuation properties (91). It is estimated that for every centimeter of depth, there is a 10-fold decrease in bioluminescence signal intensity (18). Mathematical models can be used to predict in vivo imaging signal levels and spatial resolution as a function of depth and to help define the requirements for imaging instrumentation. However, the overall low spatial resolution (3–5 mm range) and limited tissue penetrance restrict the use of BLI to small animal studies (69).

Changes in tissue oxygenation can also alter bioluminescence signal. In rat gliosarcoma for instance, bioluminescence signal has been shown to decrease by $\sim$50% at 0.2% oxygen (58). Thus, for reliable BLI measurements, it is important to understand the effects of local niche in which the luciferase-expressing cells of interest reside. This is especially the case in cardiovascular studies involving hypoxia (e.g., myocardial infarction). Similarly, BLI quantification has to be carefully interpreted in studies that involve surgical procedures. Changes in tissue thickness because of the presence of inflammation, edema, sutures, and animal growth can alter light absorption and scattering as well as the bioluminescence signal.

Conclusions and Future Directions

Many different organisms, ranging from bacteria and fungi to fireflies and fish, are endowed with the ability to emit light. The discovery of new luminescence reporters and the use of genetically modified reporters may further strengthen reporter gene expression in mammalian cells, thus improving the sensitivity and expanding the applications of this imaging

---

**Fig. 4.** Monitoring survival and homing of cells via BLI. A: skeletal myoblasts (SkMb), bone marrow-derived mononuclear cells (MN), mesenchymal stem cells (MSC), and fibroblasts (Fibro) were injected intramyocardially after myocardial infarction. All cell types demonstrated a decrease in bioluminescence signal intensity starting at 4 wk postinjection. Yellow arrows indicate homing of MN to femur, spleen, and liver. Red arrows indicate cells retained in the heart and lungs. Values in y-axis are in photons·s$^{-1}$·cm$^{-2}$·sr$^{-1}$. Abbreviations in x-axis: d, day; w, week. Reprinted with permission (83). B: BLI demonstrating preferential homing of bone marrow mononuclear cells to the ischemic myocardium in a model of ischemic reperfusion injury (I/R). Bioluminescence was also detected in the spleen and bone marrow (yellow and red arrows, respectively). A progressive decrease in bioluminescence signal was observed starting at day 14 postinjection. Reprinted with permission (72). C: cardiac resident stem cells were injected in the heart following myocardial infarction. Robust bioluminescence activity was detected on day 2 but mostly disappeared by 8 wk postinjection. Values in x-axis represent days post-cell injection. Reprinted with permission (43).
modality (7, 14). Additionally, alternative methods for transduction of reporter gene may reduce the risk of anomalous and inappropriate transcription of unwanted sequences in mammalian cells (37).

BLI allows real-time monitoring of survival and homing of various therapeutic cells that are currently being investigated to promote cardiac repair. By the use of cardiac-specific promoters linked to reporter genes, BLI can be used to monitor cell differentiation in vivo. It also provides excellent opportunities to evaluate strategies designed to improve the survival of therapeutic cells or transplanted hearts. The ability to monitor physiological processes in vivo in real time is extremely beneficial to evaluate the success or failure of the various therapies designed to promote cardiac repair. As most basic cardiovascular research is performed in rodent models, BLI can efficiently reveal problems and provide insight into solutions for validating and optimizing novel therapies for the treatment of heart disease. Overall, this technology can furnish great insights that can drive the development of better clinical trials in cardiovascular medicine.

ACKNOWLEDGMENTS

Because of space limitations, we were unable to cite all the important papers relevant to bioluminescence imaging and cardiovascular research. We apologize to investigators not mentioned here who have made significant contributions to this field.

GRANTS

This work was supported in part by National Institutes of Health Grants HL-093172, HL-099117, and EB-009689 (to J. C. Wu) and by the International Society for Heart and Lung Transplantation (to P. E. de Almeida).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


