Potential of Luminescence Based Molecular Animal Imaging in Research Areas Pertaining to Cancer Biology and Therapy

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Animal imaging is getting tremendous importance in biomedical research areas including drug delivery, radiobiology and cancer research. Even though, imaging techniques like CT, PET, SPECT, MRI are available for experimental animals, luminescence-based molecular imaging is still considered as crucial and common tool for biomedical laboratories due to easy handling/maintenance, cost effectiveness and various strategies available to manipulate the molecules/cells employed for imaging purposes. The Molecular Animal Imaging System available in our laboratory is being utilized for various cancer research activities including measurement of tumor growth kinetics, angiogenesis, therapeutic efficacy evaluation and metastasis studies. Moreover, the imaging system is also been used for radio-luminescence imaging based on Cherenkov radiation of radio-pharmaceuticals.

Introduction

Imaging of experimental animals is of great interest for biomedical researchers as well as for pharmaceutical industries for various research activities and pre-clinical drug screening/evaluation. In this direction, a range of imaging systems like PET, CT, PET-CT, SPECT and MRI are available. However, compared to these imaging techniques, luminescence-based imaging is the better choice for the researchers since it does not require handling/application of radiation source (CT) and radio-isotopes (PET, PET-CT and SPECT). Moreover, luminescence-based imaging is easy to handle/maintain and is cost effective. The available options for a range of fluorochromes / bio-luminescence materials for manipulation of cells / biomolecules, further puts the technology at an advantage. Emission of photons is the primary prerequisite for luminescence-based imaging, which can be either bioluminescence or fluorescence or delayed fluorescence. Radio-luminescence imaging based on the Cherenkov radiation from the beta-emitting radio-isotopes has also been reported and used for bio-distribution/organ localization and efficacy studies of radio-pharmaceuticals.[1;2;3]

Despite a considerable technological progress, fluorescence imaging still faces some of the serious limitations including surface reflectance, autofluorescence, absorption and scattering (deviation of photons from the original path). Out of these, autofluorescence and absorption are the two major issues especially while imaging in the wavelengths of visible region (400-650 nm) since biological tissues (hair, skin and blood) also absorb and emit at this wavelength range. Such limitations could be addressed upto some extent by the use of fluorochromes emitting in near infra-red (NIR) region (650-900 nm) and application of bioluminescence material. Bioluminescence has advantages over fluorescence imaging because it does not require excitation of fluorochromes and tissue penetration of excitation light [4;5;6.] Moreover, the limitation of autofluorescence could also be overcomed substantially during bioluminescence imaging [7;8]. This improves the signal-to-noise ratio and makes possible the sensitive measurement of fast kinetics biological processes.

Due to various advantages, luminescence-based animal imaging has been proved as a very useful tool in the research areas pertaining to cancer biology and therapy. Like other in vivo imaging techniques, molecular animal imaging system is also a non-invasive and sensitive technique with the possibility to monitor tumor growth at multiple time points and even at smaller tumor sizes. The conventional tumor growth measurement by vernier caliper poses some serious limitations: (i) it requires at least palpable sizes of tumor which for the most of the tumor types can be achieved only after 7-10 days after transplantation; (ii) the inter-individual variation in the measured values (due to manual adjustment of caliper) always keeps the investigator towards disadvantage; (iii) it provides only gross size of the tumor without any specific information about viable number of tumor cells and contribution of the necrotic/dead tissue regions in the tumor mass. On the other hand, the bioluminescence-based animal imaging is more sensitive and conquers most of the above limitations (Table 1). In this regard, imaging of bioluminescent tumor cells is possible when tumors are not even visible. Results are more consistent since the technique does not involve any manual intervention during tumor size measurement. Additionally, the bioluminescence images provide information about the live tumor cells contributing in the tumor growth.

To study the process/modification of cancer metastasis is another application of the bioluminescence-based animal imaging, in which animals need not be sacrificed at multiple time points and even in the same animal metastasis could be monitored in multiple organs at different time points. These are major advantages of the imaging technique over the conventional metastasis methodology, which involves sacrifice of the animals followed by manual identification of

Table 1: Advantages of Bioluminescence-based Animal Imaging over Conventional Method of Tumor Growth Measurement

Parameters	Conventional system (e.g. Vernier calipers)	Bioluminescence signal based imaging
Basis of measurement	Size	Viable cell number
Sensitivity	Require visible palpable size tumors, poor sensitivity	Even few thousand cells can be detected. Has sensitivity when tumor is physically undetectable / not visible
Distinction of viable tumor cells	Does not distinguish viable and dead cells in tumor mass	Signal only from viable tumor cells in the tumor mass
Requirement of animals	Large number of animals for various time points	Same animal can be used for multiple time points hence require less number of animals
Deep organ tumor imaging	Not possible	Possible for primary and secondary tumors
Variation in tumor measurements	Inter-individual variation possible	More accurate and reproducible results with lesser inter-individual variation

tumor spread in the different organs. Moreover, imaging can reveal some unknown metastatic sites, which otherwise is not possible to be identified following conventional methodologies.

Imaging Set Up

To investigate the localization/bio-distribution of drugs/cells/molecules/radiopharmaceuticals, efficacy of anti-cancer drugs/therapeutic approaches and metastasis/angiogenesis in tumor, luminescence-based animal imaging system (Photon Imager, Biospace Lab, France; Fig. 1A) was purchased and installed in our laboratory. The imaging system is comprised of light source, from which light passes through excitation filter to illuminate the experimental animals (mice/rat). The emitted light from animal body is transmitted through emission filter, which then passes through lens and gets captured in intensified cooled charge-coupled device (CCD) camera. Using this camera, the read-out and thermal noise of the CCD can be minimized through amplification process taking place in the intensifier tube (gain ~10^6) and by cooling down the photocathode to -25 °C, respectively. These features improve the signal to noise ratio substantially suitable for bio-imaging applications. The captured images are processed and transferred to computer

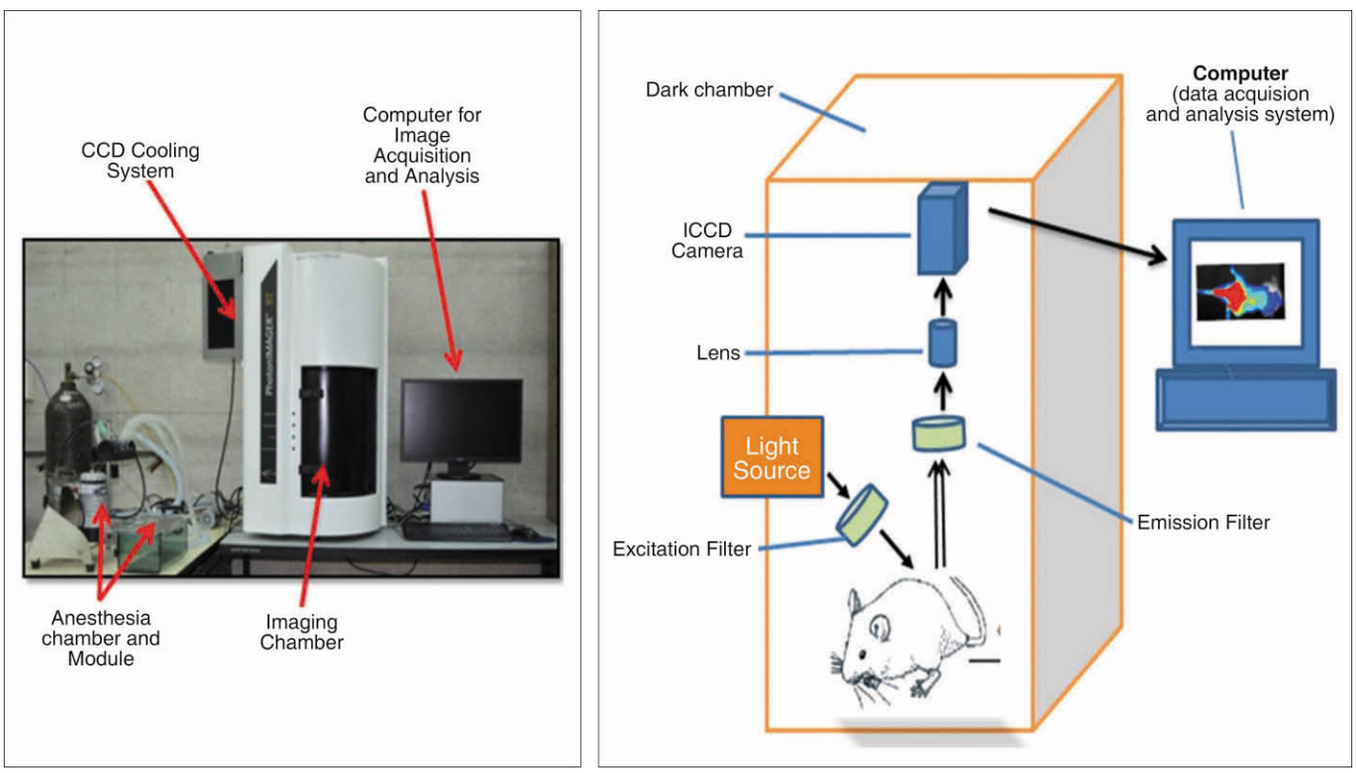

Fig. 1: (A) Picture of the in vivo imaging system and (B) Schematic representation of the in vivo imaging system

for further analysis. The anesthesia module of the unit keeps the animals under anesthesia during imaging which facilitates acquiring the stable images (Fig. 1B). The other modules available with imager make it possible to image the moving animals, the organs dissected out from the animals/small animals (e.g. insect larvae) and to quantify the depth/location of luminescence signal in the animal body.

Tumor Growth Kinetics

For bioluminescence-based imaging, $1x10^6$ mouse fibrosarcoma cells (WEHI-164 stably transfected with luciferase gene) were transplanted intramuscularly in the hind leg of BALB/c mice[9;10]. The luciferase enzyme, produced in the viable tumor cells, converts the substrate (D-luciferin) as bioluminescence signal in the presence of ATP and oxygen.

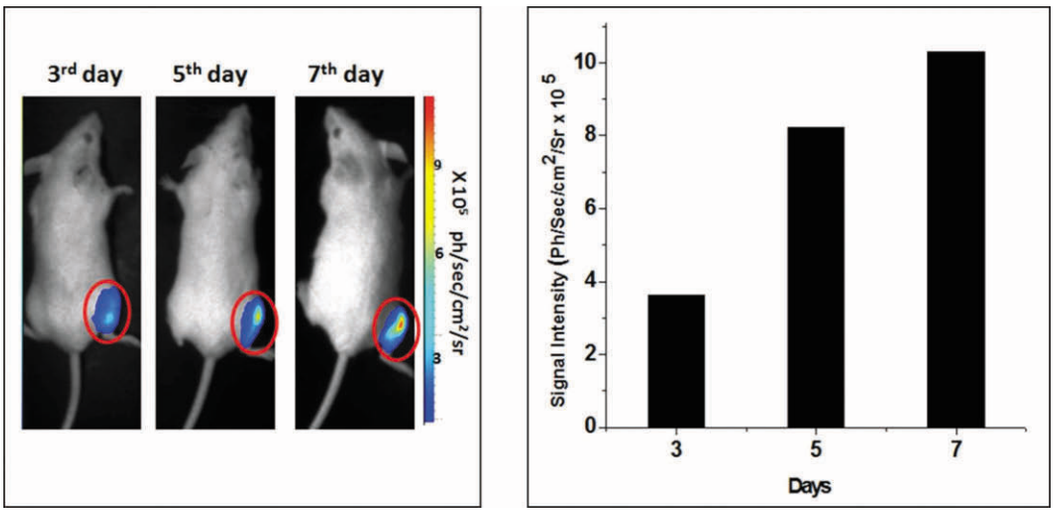

Fig. 2: Tumor growth kinetic measurement using bioluminescence imaging.
(A) Representative bioluminescence images of the animals transplanted with $1x10^6$ WEHI-164 cells stably transfected with luciferase gene. Images were taken after 3rd, 5th and 7th day of transplantation. Circles show tumor regions.
(B) Signal intensities of these images were quantified and plotted.

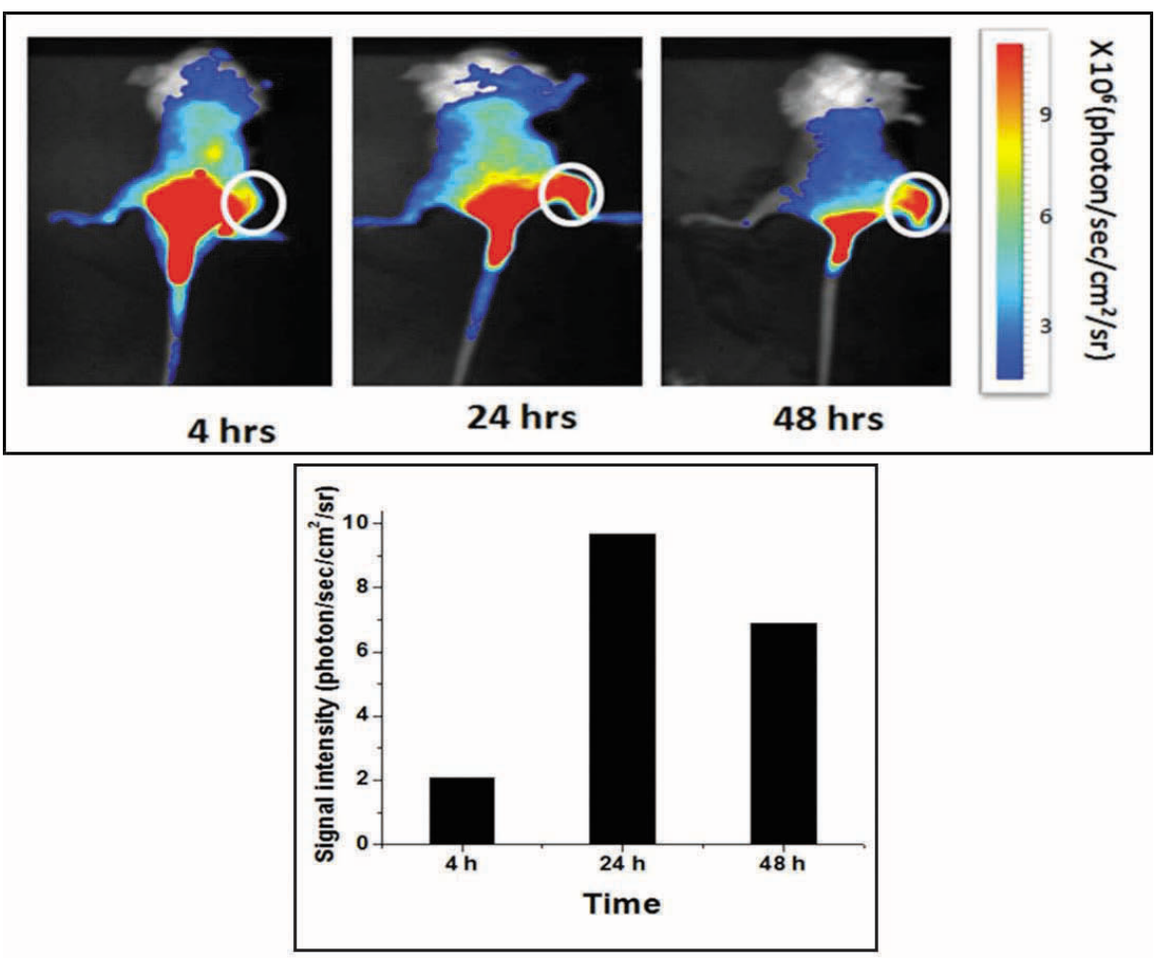

Fig. 3: Measurement of angiogenesis in WEHI-164 fibrosarcoma tumors in mice. Tumor bearing animals were injected (intravenous) with AngioSense dye. Fluorescence imaging was performed after 4, 24 and 48 h of dye injection.
(A) Representative images at various time points. Circles show tumor regions.
(B) Signal intensities of these tumor region images were quantified and plotted.

For this, substrate is intra-peritoneally injected in mice a few minutes before the imaging. Before transplantation, tumor cells were cultured under standard culture conditions followed by harvesting them during exponential growth phase using trypsin-EDTA. Cells were washed and suspended in PBS before transplantation in mice. Animals were imaged after 3rd, 5th and 7th day of transplantation (Fig. 2 A and B). Results showed a time dependent increase in bioluminescence signal intensity. It may be important to mention that in the imaging experiment, tumors even at 3rd and 5th day after transplantation also showed significant bioluminescence signal when no-palpable tumors could be visible, suggesting high sensitivity of the technique to detect the tumor and its growth.

Tumor Angiogenesis

Angiogenesis is a critical feature of tumor growth and progression, which can be monitored using fluorescence-based animal imaging [11;12]. For this, AngioSense 750 EX (Perkin Elmer, USA) dye was used. The dye is known to specifically bind to newly developed blood vasculature. Fluorescence imaging (excitation: 745 nm; emission: 800 nm; high pass filter cut off: 770 nm; illumination: 30 %) was performed in the anesthetized animals at the different time points (4, 24 and 48 h) after intravenous injection of dye through the tail vein (Fig. 3). After 4 h of injection, accumulation of dye was observed in the tumor regions, which however, was increased at 24 h. At longer period of time (48 h), dye concentration was decreased in the tumor areas.

Conclusion

Luminescence based molecular animal imaging has shifted the attention from structures / morphology to the real-time visualization of biological processes and progression of tumor growth. Therefore, in future it will be a promising technology that has translational applications towards developing preclinical diagnosis and to improve the therapeutic efficacy of anti-cancer agents.

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