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Optical spectroscopic imaging for cell therapy and tissue engineering

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Abstract

Cell-based therapies hold great potential to treat a wide range of human diseases, yet the mechanisms responsible for cell migration and homing are not fully understood. Emerging molecular imaging technology enables *in vivo* tracking of transplanted cells and their therapeutic efficacy, which together will improve the clinical outcome of cell-based therapy. Particularly, optical imaging provides highly sensitive, safe (non-radioactive), cost-effective, and fast solutions for real-time cellular trafficking compared to other conventional molecular imaging modalities. This review provides a comprehensive overview of current advances in optical imaging for cell-based therapy and tissue engineering. We discuss different types of fluorescent probes and their labeling methods with a special focus on cardiovascular disease, cancer immunotherapy, and tissue regeneration. In addition, advantages and limitations of optical imaging-based cell tracking strategies along with the future perspectives to translate this imaging technique for a clinical realm are discussed.

Keywords

Cell trafficking; optical imaging; fluorescence imaging; real-time imaging; cell therapy

Introduction

Cell-based therapy involves manipulation and administration of live cells for the treatment of human diseases (1, 2). A better understanding of the cell fate after administration will determine the successful clinical outcome. Advances in molecular imaging and targeted contrast agents have generated new opportunities to visualize and optimize therapeutic

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efficacy of cell-based therapy by allowing longitudinal assessment of engrafted cell survival, integration, and proliferation (Figure 1) (1, 2). In order to visualize the transplanted cells with high specificity and high resolution, tremendous efforts have been made in developing molecular imaging techniques, such as computed tomography, magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) (3). These imaging modalities can provide reconstructed 3D tomographic images with high resolution and quantitative volumetric analysis. Despite providing non-invasive volumetric imaging, they are generally limited by high cost, long acquisition times, and non-continuous operation. In addition, implementing these imaging modalities into intraoperative setup are difficult and time consuming (3). Nuclear imaging is also restrained by the half-life of administered radioisotope as well as radiation exposure. On the other hand, optical spectroscopic imaging is cost-effective, rapid, easy to use, and non-ionizing, and can be readily applied to the clinic and surgical setting (4–6).

The principle of optical fluorescence imaging for cell-based therapy is to provide real-time visualization of the labeled cells through an imaging device. Fluorescence imaging systems are generally consisted of excitation light sources, appropriate excitation and emission filters, and a sensitive image sensor (i.e., integrated CCD [charge-coupled device], EM-CCD [electron multiplying-CCD], or sCMOS [scientific complementary metal oxide semiconductor]) (7). Cells can be fluorescently labeled directly by intracellular delivery of fluorescent molecules (direct labeling) or engineered to express fluorescent proteins to generate detectable intracellular signals (indirect labeling) (8). Compared to visual ranged fluorescence (400– 650 nm), near-infrared (NIR) fluorescence (650–900 nm) generates high penetration depth of excitation and emission light as well as high contrast to the background due to minimal autofluorescence. In addition, NIR fluorophores possess high quantum yield and high resistant to photobleaching and chemical degradation, which together make them optimal for visualization of molecular behaviors in living organisms (5). Thus, optical imaging in the NIR window can play a crucial role in tailoring cell-based therapy by assessing cell localization and viability in real time. In addition, the single cell sensitivity of fluorescence imaging allows comprehensive understandings in elusive biological events. However, the major limitation of optical fluorescence imaging for clinical translation is still in the limited depth penetration for human use and poor spatial resolution due to high scattering (discussed in Future Perspectives) (9).

This article reviews the current progression in optical spectroscopic imaging for *in vivo* cell tracking and highlights the potential clinical application using NIR fluorophores. The optical imaging labeling methods are discussed along with how they can be applied to meet the criteria of highly sensitive and quantitative imaging to determine the fate of administered cells during cell therapy. We specially capitalize recent advances in developing cellular imaging technologies in cardiovascular disease, cancer immunotherapy, and regenerative medicine. Finally, we elaborate the future perspective and potential improvement of optical imaging in the context of using multimodal molecular imaging for quantitative and qualitative trafficking of transplanted cells for clinical translation.

Optical imaging probes

The development of novel optical contrast agents is crucial for successful imaging as they are the key to identifying the host–guest interactions between administered cells and surrounding tissues. Hence, optical imaging probes for cell therapy should have 1) high specificity to track the physiological/biological fate of transplanted cells, 2) high sensitivity of detection in terms of wavelength, quantum yield, and photostability, 3) low cytotoxicity and systemic toxicity during tissue regeneration, and 4) high integrity to keep the original function of cells when administered into the host tissue (4). Currently none of the available optical imaging probes meet all of these criteria. In addition, fluorescent contrast agents must undergo clinical trials and be proved for their safety before human use. Major types of optical probes used for cell-based therapy are summarized in Figure 2 (10, 11).

Fluorescent organic dyes

The wavelength of fluorophores spans from UV (300–400 nm) and visible spectrum (400–650 nm) to the NIR spectrum (650–900 nm) (12). Among these fluorophores, NIR dyes represent the most prominent characteristics for cell tracking in terms of well-disciplined molecular properties, such as size, charges, brightness, and toxicity (10). Consequently, NIR cyanine dyes are most utilized in *in vivo* cell trafficking to specifically visualize the key components of transplanted cells and/or tissues in cell-based therapy. For these reasons, many efforts have been focused on to improve optical and physicochemical properties of indocyanines (6). The optical properties of an NIR dye are defined by the polymethine core and aromatic rings at each end of the structure (6). Cy and Alexa are commercially available lipophilic dyes for short-term monitoring purposes due to reversible endocytosis/exocytosis of the dyes taken up by cells. Chloromethyl fluorescein diacetate dyes (Cell Tracker) and long chain carbocyanine dyes (DiL, DiO, DiD, CM-DiI, ZW) stay longer inside the cell with high labeling efficiency and are used for long-term monitoring (11). Because of the small size (≈ 1 nm), they rarely perturb the functionality of labeled cells and/or biomolecules. However, fluorescent dyes suffer from the loss of emission signals from photobleaching or quenching due to the interactions with solvent molecules or reactive species (e.g., oxygen) as well as association with other dye molecules (π - π stacking or aggregations). The fundamental limitations of using organic dyes for cellular targeting is poor cellular retention and nonspecific binding, where unlabeled excess dyes are washed during incubation and can contaminate the target tissue during imaging procedures.

Fluorescent proteins

Currently, fluorescent proteins are most frequently utilized in cellular imaging due to their stability and robust fluorescence under multiple conditions (13). The most well-known protein is the green fluorescent protein (GFP), which was discovered and extracted from jellyfish *Aequorea victoria* in 1962 (14, 15). After this pioneering discovery, many other fluorescent proteins with emitting longer photons, such as yellow fluorescent protein and infrared fluorescent protein, have been discovered in different species (16). Fluorescent proteins are generally less bright and light-sensitive than most fluorescent dyes. However, specific covalent labeling of recombinant fluorescent proteins inside living cells allow a wide range of tuning of wavelengths and expression levels (see *Labeling Methods*).

Furthermore, fluorescent proteins are stable in the cell during cell proliferation, differentiation, and migration processes. As a result, fluorescent proteins have played a key role in studying the biological function of cellular organelles, cells, and tissues. However, fluorescent proteins are challenged by a variety of optical and biological factors such as stochastic blinking, low quantum yield and sensitivity, photobleaching, quenching, and oligomerization that can cause cytotoxicity (10, 17).

Fluorescent nanoparticles

Nanoparticles for the purpose of optical imaging have been developed in recent years (reviewed in (10)). Nanoparticles label cells with different labeling techniques such as surface chemistry or intracellular localization (by endocytosis or protein transporters) (18). Organic/inorganic hybrid nanoparticles like quantum dots (QDs) hold great promise to label cells by overcoming the low sensitivity of fluorescent proteins with outstanding brightness, high quantum yield, large Stokes' shifts, and minimal photo-bleaching (10). QDs also have a broad absorption, symmetric narrow emission, high photostability, and versatile conjugation capability, allowing long-term tracking of labeled cells or bioconjugates in living organism (19, 20). Cell binding specificity of nanoparticles can be improved by conjugating natural or synthetic ligands to nanoparticles through surface chemistry (21). However, there exists the concern of toxicity to cells and dispersing in the cytosol because most QDs are made of heavy metals (i.e., Pb^{2+} or Cd^{2+}) (22). Furthermore, relatively large sized nanoparticles can be impeded by the cell membrane barrier resulting in low loading efficiency and poor retention, which together limit their practical use for *in vivo* cell tracking.

Labeling methods

There are two main optical cell labeling methods (Figure 3): 1) direct labeling with exogenous organic/inorganic fluorophores inside the cell (diffusion or endocytosis) or on the cellular membrane (bioconjugation) and 2) indirect labeling based on genetic modification of cells using fluorescent proteins (23).

Direct labeling

The first step of direct labeling is isolation of specific cell populations with a suitable fluorophore (organic dye or nanoparticle), then *ex vivo* labeling with one of the following methods: 1) Functional fluorophore conjugation to a certain targeted protein on the cell surface, 2) lipophilic cations or nanoparticles movement into the cell by diffusion (passive transport), or 3) active transport across the cell membrane via ion channels or protein transporters. The advantage of using direct labeling is that optical probes can be taken up specifically (internalization) or non-specifically (accumulation) into various types of cells with high loading efficiency (24). However, this labeling is generally diluted during the process of cell division, resulting in gradual reduction of signals and errors in cell number quantification. Due to these factors, direct labeling only allows short-term tracking of cells, with inability to visualize proliferation, activation, or death of cells in living organism (8). To avoid this limitation, many efforts have been made to label specific cellular organelles, such as the Golgi complex, nucleus, mitochondria, or lysosomes (11, 12). Another caveat of direct labeling is difficulty in distinguishing dead cells from live cells.

Indirect labeling

The fundamental principle of indirect labeling involves genetic modification of cells using a reporter gene by encoding enzymes, receptors, or fluorescent/bioluminescent proteins (8). Cells are generally transfected with fluorescent reporter expression gene constructs either by viral or non-viral delivery system. With the stable integration of reporter gene constructs into the host genome, the reporter gene can be passed on to the daughter cells. This stable cell labeling is retained after many divisions, which permits monitoring of cell proliferation and viability over the entire lifetime of cells (25). Consequently, indirect labeling allows imaging of expanding cell populations and cell viability (8). However, the introduction of foreign genes into a human can lead to an immunogenic risk along with potential toxicity with unknown long-term side effects; therefore, this labeling technique has been limited only to pre-clinical validations (26).

Preclinical and clinical applications

Cell-based therapy has recently been used in numerous biomedical applications. Among many applications for cell-based therapy, here we further discuss the use of optical imaging for cardiovascular disease, cancer immunotherapy, and regenerative medicine. The key to success in cell-based therapy will depend on the successful tracking and monitoring of the transplanted cells in order to elucidate their mode of action (i.e., cell retention, survival, migration, proliferation, differentiation, and integration into the host tissue).

Optical imaging in cardiac stem cell therapy

Heart failure is a major cause of death worldwide for both men and women (27). Unfortunately, current treatment options can only delay or alleviate the symptom until heart transplantation becomes available. In recent years, to cure acute or chronic myocardial infarction, medical therapy with skeletal myoblasts, bone marrow-derived cells, and cardiac resident stem cells has been widely investigated to regenerate the damaged cardiac tissue (28–31). Particularly, there is a growing body of evidence that the treatment by transplantation of stem cells and progenitor cells have direct or indirect therapeutic effects that prevent cell death, promote angiogenesis, and augment myocardial contractility (30). Since such cardiac stem cell therapy largely depends on the survival of transplanted cells, real-time tracking of the transplanted cells with a quantitative and qualitative method is crucial to improve clinical outcomes of patients with cardiac diseases. This could be achieved by developing the most optimal delivery method of stem cells and by monitoring *in vivo* fate of the delivered stem cells, which together could improve the consistency in cardiac stem cell therapy (29). Also, optical imaging spectroscopy will further elucidate the mechanical insight and molecular mechanisms of how stem cells contribute to cardiac tissue recovery.

To track the fate of transplanted cells in the heart, Ly et al. monitored myocardial distribution and retention of multipotent progenitor cells after intracoronary delivery in a swine model of myocardial infarction (32). As shown in Figure 4, living cells labeled with NIR fluorescent IR-786 were found in the uninjured heart after migrating into diagonal artery. Especially, vessel plugging was observed progressively in both healthy and injured

hearts during recirculation of injected cells with poor myocardial retention. In the following paper, Hoshino *et al.* tracked the fate of mesenchymal stem cells (MSCs) using NIR fluorescence imaging to monitor the behavior of injected stem cells within the host tissue (29). MSCs labeled with IR-786 were injected into intracoronary of myocardial infarcted swine and imaged at various tracking time points immediately after intracoronary delivery. The results of both studies suggest that the immediate distribution and retention of injected cells in the infarcted heart varied depending on cell type and population, which could potentially impact the clinical efficacy of cardiac cell therapy (33). However, this could be difficult to perform in humans due to potential cell death after transplantation, which remains a major roadblock to cell-based cardiac therapies.

Optical imaging in cancer immunotherapy

After the initial trial of tumor targeting using monoclonal antibodies in 1975 (34), cancer immunotherapy has focused on harnessing the immune system to fight cancer (35). With the FDA approval of new immunotherapies for prostate cancer and melanoma (36), cell-based immunotherapy has been effective in treating various cancers by stimulating patients' own immune mechanism to create an antitumoral environment (37). However, it has been a challenge to show consistency in treatment results due to lack of quantitative and qualitative imaging data, which limits clinical trials of oncological endpoint (38). To selectively kill tumor cells and obtain efficient therapeutic outcomes, effector cells including cytotoxic T lymphocytes (i.e., CD4⁺ and CD8⁺ T cells), natural killer (NK) cells, and dendritic cells are often modified to present tumor antigens, amplified *ex vivo*, and then transfused back into the host (39). To target T cells toward tumors, for example, Kalos and *et al.* genetically modified T cells directly with lentiviral vector expressing chimeric antigen receptors for a tumor-specific antigen combined with stimulatory signaling domains. These chimeric antigen receptor T cells are then reintroduced to the patients to boost immune response, and chronic lymphoid leukemia and acute lymphoblastic leukemia were successfully treated during clinical trials (40).

The development and discovery of effector cells have advanced cell-based immunotherapy dramatically over the years. However, in order to understand the function of immune stimulators and the underlying mechanisms, real-time monitoring of *in vivo* behavior and localization of these cells should be carefully examined. To track the behavior of immune cells, current focus is geared towards the development of NIR fluorescence imaging due to its superiority against visual wavelength fluorophores (10, 41, 42). Lim *et al.* injected human NK cells labeled with antibody coated QDs intratumorally to monitor the process of selective tumor cell death using the optical fluorescence imaging (43). Recently, Li *et al.* reported that immunotherapeutic efficacy of gastric cancer was enhanced by using allogeneic dendritic cells fused with tumor cells combined with cytokine-induced killer cells (44). The strategic development of fused effector vaccine and labeling cells with PEGylated QDs enhanced immunotherapeutic and prophylactic potential of the vaccine. Similarly, upconversion nano-particles emitting high-energy photons were also used to label dendritic cells and track presenting tumor antigens in the draining lymph nodes is shown in Figure 5 (45). On the other hand, Deguine *et al.* studied the behavior of different types of effector cells with GFP-tagged proteins and discovered distinct dynamics for NK and CD8⁺ T cells

during tumor regression through intravital imaging (46). Overall, molecular imaging plays a key role in tracking immune cells and assessing tumor immunology, and its development will allow clinical application of cancer immunotherapy by providing a real-time therapeutic response.

Optical imaging in regenerative medicine

Regenerative medicine focuses on *in vivo* repair of tissues and/or organs to restore or regenerate the function of damaged organs by using appropriate scaffolds and cell sources (47–49). The potential of stem cells to differentiate into bone, fat, cartilage, and muscle cells has shed light on cell-based tissue regeneration to treat critical conditions such as osteoarthritis, osteoporosis, and spinal injury (50). For successful tissue regeneration, a suitable bio-compatible scaffold should provide an optimal environment for tissue formation (51). Also, the rate of scaffold degradation and tissue formation should generally be equivalent. Therefore, accurate and longitudinal measurements of these changes play key roles in tissue engineering and regenerative medicine. However, current analytical methods, such as histological and microscopic analyses, tend to be invasive and demand multiple samples, which result in batch-to-batch variations and thus inaccurate conclusions (52).

Advanced optical imaging outperformed the traditional imaging methods by providing longitudinal non-invasive information on tissue constructs. Artzi *et al.* studied *in vitro* and *in vivo* tracking of biodegradable hydrogels conjugated with low wavelength fluorophores, such as fluorescein (λ_{em} 512 nm) and Texas Red (λ_{em} 615 nm) (53). They proposed a dual exponential decay model to quantitatively correlate the *in vitro* biodegradation pattern to the obtained *in vivo* animal data. However, this is rather complicated due to endogenous tissue autofluorescence in the visible wavelength range and physiochemical instability in the body, which conflicts the visible dyes with the erosion rate. To overcome these limitations, NIR fluorophores have been used to monitor *in vitro* and *in vivo* scaffold degradation because of their long-term stability, reduced background signals, and higher sensitivity compared to visible fluorophores.

As shown in Figure 6, Kim *et al.* implanted collagen scaffolds labeled with zwitterionic hepta-methine indocyanine ZW800-1 (λ_{em} 780 nm) in living animals (52). By introducing invisible NIR fluorescent light, the long-term scaffold degradation profile was successfully achieved in the same animal over a month and no endogenous interference was observed. Although promising contributions of NIR fluorophores in the detection of scaffold degradation *in vivo* were made, the non-invasive application was limited to imaging superficial tissues and organs such as skin, muscle, cartilage, bone, eye (vasculature), and white/brown adipose tissue due to its intrinsic optical scattering (4–6).

Future perspectives

Optical imaging and novel contrast agents designed for cell-based therapy have made significant contributions and will continue to provide important insights into cell distribution, engraftment, and survival. Whole body imaging of small animals using highly sensitive optical probes in preclinical studies revealed many of the unanswered questions in cell-based therapy for clinical use. However, optical imaging itself has fundamental

limitations regarding deep tissue imaging (up to 5 mm; reflectance-based imaging) and quantification, which are major hurdles for its clinical use.

Development of multimodal imaging probes for both optical and radioisotopic or magnetic imaging to overcome pros and cons of each imaging method can advance the cell therapy for future clinical use. Table 1 details the advantages and limitations of different imaging modalities used for *in vivo* cell tracking (1, 54). The approach of multi-modal imaging for the purpose of cell tracking is powerful in that it can maximize the strength of different imaging modalities which allow comprehensive evaluation of cell behaviors as well as its functionality (55). For these reasons, many efforts have been made to combine optical imaging with MRI, SPECT, or PET in preclinical studies. For example, Li *et al.* used MRI and NIR optical imaging for dual modal tracking of transplanted MSCs after myocardial infarction (56). These two imaging modalities were chosen to explore the effects of different delivery modes of MSCs on cell retention and cardiac function, which are determined by measuring the iron and optical signals in the explanted organs. The combination of MRI and NIR modes enabled to conclude that intramyocardial injection of MSCs increase cell engraftment within infarcted hearts and improve cardiac function (56).

Recent advances in optical imaging instrumentation also employed the improvement of spatial resolution and depth penetration. Fluorescence lifetime imaging microscopy (FLIM) generates contrast by measuring the time dependent intensity decay of NIR fluorophores, which minimizes photon scattering in thick layers of a sample (57). This imaging technique modulates the fluorescence excitation light and has a more specific approach to identify the target (58). FLIM also enables the detection of composite molecular assemblies within a single voxel for studies of cell function and interaction in optically transparent tissues (57).

Photoacoustic imaging, which is also known as optoacoustic imaging, is an imaging modality based on the ultrasonic emission of the absorbed energy that is converted to heat, allowing in-depth optical spectroscopic imaging (59, 60). This imaging modality has the potential to image human organs more efficiently in the long-term with high contrast and spatial resolution. Using a transducer that provides higher central frequency and broader bandwidth, higher axial resolution can be obtained for studying cellular and genetic processes in deep mammalian tissues. Previously, photoacoustic imaging has focused on endogenous vasculature imaging without the use of exogenous fluorophores. Recently, 3D photoacoustic imaging was obtained using tyrosinase-expressing human cells labeled with genetically encoded contrast agent to study gene expression, cell growth, and biological behaviors in xenograft tumor mice (61). This could be imposed by optical scattering to provide high resolution optical contrast information in deep tissues, to depths approaching 10 mm with a spatial resolution below 100 μm (Figure 7). The combination of tomographic imaging modalities (radioisotopic, MR, FLIM, or high-resolution photoacoustic imaging) with the assistance of exogenous NIR fluorophores goes beyond the limits of optical fluorescence imaging and brings a new dimension to the study of cell based therapy.

Conclusions

Optical imaging has provided significant insights into the clinical applications of cell therapy by 1) tracking the progress of delivered cells, 2) confirming cell viability, and 3) monitoring the overall cellular and molecular behaviors during tissue regeneration. Nonetheless, current imaging machinery is unable to meticulously monitor the complex process of cell-based therapy. As it is important to analyze the efficacy of transplanted cells, extensive investigations will hopefully further evaluate the fate of cells and their functional therapeutic effects. As we discussed here, recent advances in optical imaging with the assistance of novel optical contrast agents may allow non-invasive sequential imaging of long-term cellular tracking, which has great potential to bring cell therapy to a new level of treatment options.

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Figure 1.

Stem cell-based therapy: Barriers to clinical translation. Current challenges in cell-based therapy are (1) limited engraftment, survival, and proliferation, (2) poor differentiation, maturation, and integration, (3) immunogenicity with allogeneic transplantation, and (4) potential tumorigenicity with pluripotent stem cell derivatives. Molecular imaging will play a crucial role in overcoming these challenges and promote the clinical translation of cell-based therapy. Reproduced with permission from Ref. (1). Copyright 2014 Cell Press.

Type:	Fluorescent Organic Dyes	Fluorescent Proteins	Fluorescent Nanoparticles
Shape:
Size:	1 nm	3 - 5 nm	5 - 30 nm
Molecular weight:	0.5 - 2.0 kDa	20 - 30 kDa	30 - 100 kDa
Wavelength:	300 - 900 nm	500 - 700 nm	300 - 1200 nm
Optical properties:	Quenching, photobleaching	Quenching, blinking, photobleaching	Minimal photobleaching
Toxicity:	Less toxic	Toxic (gene transfection)	Toxic (heavy metals)

Figure 2.

Comparison of physicochemical and optical properties of functional fluorophores: Fluorescent organic dyes, fluorescent proteins, and fluorescent nanoparticles. Modified with permission from Ref. (10). Copyright 2010 SAGE Publications, Inc.

Figure 3.

Cell labeling and tracking by molecular imaging modalities. Direct cell labeling utilizes exogenous contrast agents, while indirect labeling is based on genetic modification of cells using fluorescent proteins. Methods for tracking labeled cells include optical imaging, MRI, PET, SPECT, CT, or their combination. Reproduced with permission from Ref. (23). Copyright 2014 Ivyspring International Publisher.

Figure 4. Preclinical imaging to track labeled cells in the heart: (a) Real-time intraoperative optical imaging system. (b) NIR fluorophore-labeled bone marrow stem cells were injected into a swine model of myocardial infarction. (c) Myocardial distribution of multipotent progenitor cells labeled with IR-786, showing evidence of progressive vessel plugging after intracoronary delivery (LAD: left anterior descending artery). Modified with permission from Ref. (32). Copyright 2009 Oxford Academic.

Figure 5. In vivo

tracking of upconversion nanoparticle-labeled dendritic cells. (a) Schematic illustration of antigen-loaded UCNP for DC stimulation and tracking. (b) Color-fluorescence merged image of a C56BL/6 mouse subcutaneously injected with various numbers of labeled DCs (≈ 50 to 50,000) and their quantification. (c) Labeled dendritic cells were injected into the right footpad of a mouse and found in the draining lymph nodes (white circles). (d) Immunofluorescence images of the lymph nodes dissected from the mouse. T, T-cell zone; B, B-cell zone; Scale bar = 20 μm . Reproduced with permission from Ref. (45). Copyright 2015 ACS Publications.

Figure 6.

Optical and MR imaging for tracking tissue regeneration. (a) NIR fluorophore-conjugated collagen scaffold. (b) Quantification of *in vivo* scaffold degradation in nude mice by optical fluorescence imaging. (c) Quantification of *in vivo* tissue regeneration in nude mice by MR imaging. (d) NIR fluorescence and (e) H&E histological evaluations of the resected tissue. Reproduced with permission from Ref. (52). Copyright 2015 Nature Publishing Group.

Figure 7.

In vivo photoacoustic imaging of cancer cells and vasculature. (a) Schematic illustrating of photoacoustic scanning. (b) Volume-rendered tumor image in a nude mouse inoculated with tyrosinase-expressing K563 cells. (c–e) Serial longitudinal *in vivo* photoacoustic images (x – y MIPs) (vasculature is color-coded for depth; cells are false-colored yellow) obtained in the same animal at different time points post-inoculation at day 0 (c), day 15 (d), and day 30 (e). Reproduced with permission from Ref. (61). Copyright 2016 Nature Publishing Group.

Table 1
Comparison of imaging modalities used for *in vivo* cell tracking (1, 54)

Imaging modality	Spatial resolution	Sensitivity: cells	Acquisition time	Labeling strategy	Pros	Cons
BLI FLI	5–20 mm	2–20 mm	10 ³ –10 ⁶	msec–sec	Reporter gene, fluorophores, nanoparticles	Cheap, simple, fast, no ionizing radiation 2D imaging, low resolution, limited depth penetration
SPECT	5 mm ³	10 ⁵	min–h	Reporter gene, radiotracer	3D imaging, whole body scanning	Ionizing radiation, limited spatial resolution
PET	3 mm ³	10 ⁴	min–h	Reporter gene, radiotracer	3D imaging, high sensitivity, whole body scanning	Radiotracer required, limited spatial resolution
MRI	1 mm ³	10 ⁴	msec–min	Nanoparticles	3D imaging, high resolution, no ionizing radiation	High cost, contraindications
CT	<1 mm ³	N/D	sec	Nanoparticles	3D imaging, high resolution	Ionizing radiation, limited soft tissue imaging
US	1 mm	N/D	sec–min	Microbubbles	Cheap, relatively simple	Low signal to noise ratio

BLI, bioluminescence imaging; CT, computed tomography; FLI, fluorescence imaging; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computed tomography; US, ultrasound. h, hours; min, minutes; msec, milliseconds; sec, seconds; N/D, not defined.