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- (71) Applicant: MAYO FOUNDATION FOR MEDICAL
EDUCATION AND RESEARCH [US/US]; 200 First
Street SE, Rochester, MN 55905 (US).
- (72) Inventors: BANSAL, Aditya; 4023 Alberta Drive NE,
Rochester, MN 55906-3457 (US). DEGRADO, Timothy,
R.; 2026 Telemark Ct. NW, Rochester, MN 55901-2432
(US). PANDEY, Mukesh, K.; 402 31st Street NE. Apt.
140, Rochester, MN 55906 (US).
- (74) Agent: ROCHE, Richard, T.; Quarles & Brady LLP, 411
E. Wisconsin Avenue, Milwaukee, WI 53202 (US).

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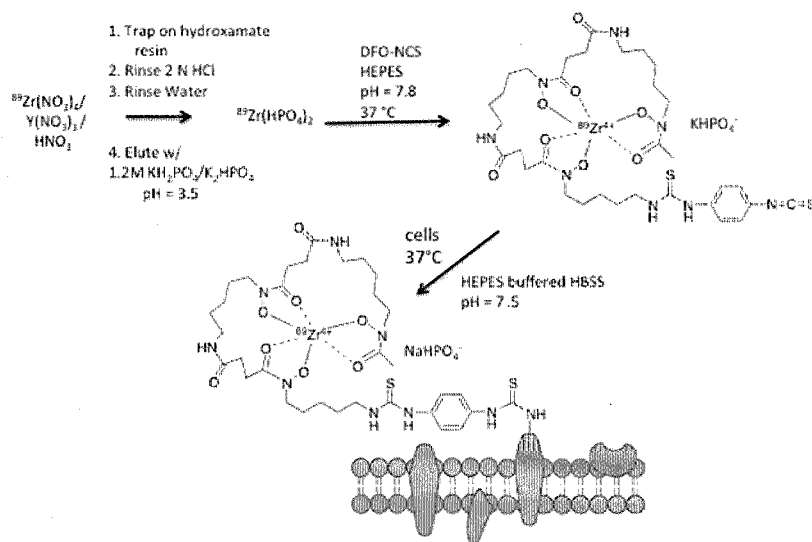


FIG. 1

(57) **Abstract:** Methods of ex vivo labeling of a biological material for in vivo imaging, methods of labeling a biological material in vivo, methods for preparing a labeling agent, and methods for in vivo imaging of a subject using a biological material labeled with a labeling agent are disclosed. In one non-limiting example, the biological material is selected from cells and the labeling agent is a ^{89}Zr -Desferrioxamine-NCS labeling agent.

Methods for Cell Labeling and Medical Imaging

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent Application No. 62/129,406 filed March 6, 2015.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not Applicable.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] This invention relates to methods of *ex vivo* labeling of a biological material for *in vivo* imaging, methods of labeling a biological material *in vivo*, methods for preparing a labeling agent, and methods for *in vivo* imaging of a subject using a biological material labeled with a labeling agent. In one non-limiting example, the biological material is selected from cells.

2. Description of the Related Art

[0004] A number of radioisotopic cell labeling methods have traditionally been used for single-photon emission computerized tomography (SPECT) and positron emission tomography (PET) imaging based cell tracking [Ref. 1]. However, a PET-based approach would offer superior quantification and imaging sensitivity characteristics over a SPECT-based approach, which are critical for tracking of small numbers of administered cells [Ref. 1]. In this regard, ⁸⁹Zr has emerged as an attractive PET radionuclide for cell labeling applications due to its high spatial resolution and 78.4 hour half-life that may allow monitoring of administered cells up to a 2-3 week period.

[0005] Traditionally, cell-labeling strategies have relied on transport of a radiometal (¹¹¹In, ^{99m}Tc, ⁶⁴Cu, ⁸⁹Zr) into cells in conjunction with oxine, hexamethylpropyleneamine oxime (HMPAO), pyruvaldehyde-bis(N4-methylthiosemicarbazone) (PTSM) or protamine sulfate [Ref. 1]. After entry into the cell, the radiometal dissociates and binds to a variety of intracellular biomolecules. The major drawback of this approach is that appreciable efflux of sequestered radioactivity is observed post-labeling (See Table 1).

Table 1. Present Direct Radioisotopic Cell Labeling Methods

Isotope-Compound/ $T_{1/2}$	Cells labeled	Labeling and imaging characteristics	Ref.
^{111}In -oxine/ 67.4 hours	Leukocytes Lymphocytes HPCs*	~80% cell labeling yield in 30 minutes - Significant efflux rate reported in lymphocytes (~70% effluxed in 24 hours) and HPCs (~75% effluxed in 96 hours) - Suboptimal image quality and sensitivity	[2-6]
^{64}Cu -PTSM/ 12.7 hours	C6 glioma cells	70-85% cell labeling yield in 5 hours - Significant efflux rate from cells (~80% effluxed in 24 hours h)	[7]
^{64}Cu -TETA- or ^{89}Zr -DFO- antiCD45/ 12.7 hours (^{64}Cu) 78.4 hours (^{89}Zr)	hPBSCs [#]	- Binds to only CD45 membrane protein expressing cells - Approach was sub-optimal possibly due to insufficient CD45 molecules on the plasma membrane of stem cells	[8]
^{89}Zr -oxine/ 78.4 hours	myeloma cells and natural killer cells	~32% cell labeling yield in 30 minutes - Significant efflux rate reported for myeloma cells (29% effluxed in 24 hours) and natural killer cells (70-80% effluxed in 7 days) - Loss of cell viability possibly due to oxine exposure	[9-10]
^{89}Zr - protamine sulfate/ 78.4 hours	dendritic cells and T lymphocytes	~34% cell labeling yield (dendritic cells) in 30 minutes ~12% cell labeling yield (T lymphocytes) in 30 minutes - Weakly binds to non-specific intracellular biomolecules - Efflux rate not reported	[11]

*HPCs - Hematopoietic Progenitor Cells, [#]hPBSCs - human Peripheral Blood Stem Cells

[0006] The extent of efflux has been as high as 70-80% in 24-96 hours as reported for ^{111}In -oxine labeled lymphocytes [Ref. 5], ^{111}In -oxine labeled hematopoietic progenitor cells [Ref. 4], and ^{64}Cu -PTSM labeled C6 glioma cells [Ref. 7]. Recently, ^{89}Zr -oxine has been reported as a labeling molecule but like ^{111}In -oxine, it also undergoes efflux (10-29% at 24 hours in macrophages, breast cancer cells, and myeloma cells [Ref. 9] and 70-80% at 24 hours in natural killer cells [Ref. 10]). Efflux of radiolabel significantly limits monitoring cell trafficking over longer observational periods. Cells have also been labeled with ^{18}F -FDG [12-16] ($T_{1/2} = 109.8$ minutes), $^{99\text{m}}\text{Tc}$ -HMPAO [17] ($T_{1/2} = 6$ hours) and ^{64}Cu -labeled anti-CD45 [Ref. 8] ($T_{1/2} = 12.7$ hours), but the short half-lives of these radioisotopes limit their utility for cell tracking to shorter observational periods. An alternative antibody based stem cell labeling method employed ^{89}Zr -labeled anti-CD45 for *ex vivo* labeling of stem cells expressing CD45 membrane protein. However, this radiotracer yielded poor *in vivo* imaging characteristics, possibly due to insufficient CD45 molecules on the plasma membrane of stem cells [Ref. 8].

[0007] Therefore, with the growth of interest in cell-based therapies, there is a need to develop more sensitive, robust and quantitative imaging methods for *in vivo* tracking of living cells.

SUMMARY OF THE INVENTION

[0008] In one aspect, the invention provides a method of *ex vivo* labeling of a cell for *in vivo* imaging. The method includes the step of contacting a cell *ex vivo* with a labeling agent such that the cell becomes labeled for *in vivo* imaging, wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound. The labeling agent may include a radionuclide, or a paramagnetic metal ion or compound, or both a radionuclide and a paramagnetic metal ion or compound.

[0009] In another aspect, the invention provides a method of labeling a cell *in vivo*. The method includes the step of administering to a subject a labeling agent thereby labeling a cell *in vivo*, wherein the labeling agent does not include an antibody, and wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound. The

labeling agent may include a radionuclide, or a paramagnetic metal ion or compound, or both a radionuclide and a paramagnetic metal ion or compound.

[0010] In another aspect, the invention provides a method of *ex vivo* labeling of a biological material for *in vivo* imaging. The method includes the step of contacting a biological material *ex vivo* with a labeling agent such that the biological material becomes labeled for *in vivo* imaging, wherein labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound, and wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids. The labeling agent may include a radionuclide, or a paramagnetic metal ion or compound, or both a radionuclide and a paramagnetic metal ion or compound.

[0011] In another aspect, the invention provides a method of labeling a biological material *in vivo*. The method includes the step of administering to a subject a labeling agent thereby labeling a biological material *in vivo*, wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound, and wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids. The labeling agent may include a radionuclide, or a paramagnetic metal ion or compound, or both a radionuclide and a paramagnetic metal ion or compound.

[0012] In the foregoing methods of the invention, the chelating moiety may be a hydroxamic acid group, and preferably, the hydroxamic acid group is a desferrioxamine group. The conjugation moiety may include an isothiocyanate group. The conjugation moiety may include a benzyl group, or a maleimide group, or an NHS ester group, or a combination thereof. The labeling agent may be ⁸⁹Zr-isothiocyanato-benzyl-desferrioxamine.

[0013] In the foregoing methods of the invention, the radionuclide may be selected from the group consisting of ¹¹C, ¹³N, ¹⁵O, ¹⁸F, ^{34m}Cl, ³⁸K, ⁴⁵Ti, ⁵¹Mn, ⁵²Mn, ^{52m}Mn, ⁵²Fe, ⁵⁵Co, ⁶⁰Cu, ⁶¹Cu, ⁶²Cu, ⁶⁴Cu, ⁶⁶Ga, ⁶⁸Ga, ⁷¹As, ⁷²As, ⁷⁴As, ⁷⁵Br, ⁷⁶Br, ⁸²Rb, ⁸⁶Y, ⁸⁹Zr,

⁹⁰Nb, ^{94m}Tc, ^{99m}Tc, ^{110m}In, ¹¹¹In, ¹¹⁸Sb, ¹²⁰I, ¹²¹I, ¹²²I, ¹²³I, and ¹²⁴I. The radionuclide may be a positron emitter, such as ⁸⁹Zr. The paramagnetic metal ion may be selected from Gd⁺³, Fe⁺³, Mn⁺², and Y⁺³. There are a number of chelators that may be used to chelate the paramagnetic metal ion (e.g., Gd⁺³, Fe⁺³, Mn⁺², and Y⁺³).

5 Non-limiting example chelators for the paramagnetic metal ion are diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and 1,4,7-triazacyclononane-triacetic acid (NOTA). The paramagnetic compound may be compounds including ¹⁹F, such as a perfluorocarbon.

10 **[0014]** The biological material may be cells selected from microorganisms; blood-derived cells, cancer cells, stem cells, and dendritic cells. The biological material may be selected from stem cells. The biological material may be selected from viruses.

15 **[0015]** In another aspect, the invention provides a labeling agent comprising a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound, wherein the chelating moiety includes a hydroxamic acid group and the conjugation moiety includes an isothiocyanate group, and wherein the labeling agent is in a reactive form suitable for labeling a biological material selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses,
20 nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids.

25 **[0016]** In the labeling agent, the chelating moiety may be a hydroxamic acid group, and preferably, the hydroxamic acid group is a desferrioxamine group. The conjugation moiety may include an isothiocyanate group. The conjugation moiety may include a benzyl group, or a maleimide group, or an NHS ester group, or a combination thereof. The labeling agent may be ⁸⁹Zr-isothiocyanato-benzyl-desferrioxamine. The radionuclide may be selected from the group consisting of ¹¹C, ¹³N, ¹⁵O, ¹⁸F, ^{34m}Cl, ³⁸K, ⁴⁵Ti, ⁵¹Mn, ⁵²Mn, ^{52m}Mn, ⁵²Fe, ⁵⁵Co, ⁶⁰Cu, ⁶¹Cu, ⁶²Cu, ⁶⁴Cu, ⁶⁶Ga, ⁶⁸Ga, ⁷¹As, ⁷²As, ⁷⁴As, ⁷⁵Br, ⁷⁶Br, ⁸²Rb, ⁸⁶Y, ⁸⁹Zr, ⁹⁰Nb, ^{94m}Tc, ^{99m}Tc, ^{110m}In, ¹¹¹In, ¹¹⁸Sb, ¹²⁰I, ¹²¹I, ¹²²I, ¹²³I, and ¹²⁴I. The radionuclide may be a positron emitter,
30 such as ⁸⁹Zr. The paramagnetic metal ion may be selected from Gd⁺³, Fe⁺³, Mn⁺²,

and Y^{+3} . The paramagnetic compound may be compounds including ^{19}F , such as a perfluorocarbon. The biological material may be cells that are selected from microorganisms, blood-derived cells, cancer cells, stem cells, and dendritic cells. The biological material may be selected from stem cells. The biological material may be selected from viruses.

[0017] In another aspect, the invention provides a method for preparing a labeling agent. The method includes the steps of: (a) providing a compound including a chelating moiety and a conjugation moiety; and (b) mixing the compound with at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound to create the labeling agent before reacting the compound with an additional moiety, wherein the chelating moiety includes a hydroxamic acid group and the conjugation moiety includes an isothiocyanate group.

[0018] In the method for preparing a labeling agent, the additional moiety may be a lysine residue. The additional moiety may be a functional group of at least one of a liposome, DNA aptamer, RNA aptamer, a virus, nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids. The additional moiety may be a functional group of a stem cell. The additional moiety may be a functional group of a virus. The chelating moiety may be a desferrioxamine group. The conjugation moiety may include a benzyl group. The labeling agent can be a ^{89}Zr -isothiocyanato-benzyl-desferrioxamine.

[0019] In another aspect, the invention provides a method for *in vivo* imaging of a subject. The method includes the steps of: (a) administering to the subject a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound; (b) waiting a time sufficient to allow the labeled cells to accumulate at a tissue site to be imaged; and (c) imaging the tissues with a non-invasive imaging technique such as positron emission tomography imaging, wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.

[0020] In another aspect, the invention provides a method of imaging a subject by emission tomography. The method comprises: (a) administering to the subject a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound; (b) using a plurality of detectors to detect gamma rays emitted from the subject and to communicate signals corresponding to the detected gamma rays; and (c) reconstructing from the signals a series of medical images of a region of interest of the subject, wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.

[0021] In another aspect, the invention provides an imaging method comprising acquiring an image of a subject to whom a detectable amount of a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound has been administered, wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids. In the method, an image is acquired using positron emission tomography imaging, positron emission tomography with computed tomography imaging, or positron emission tomography with magnetic resonance imaging.

[0022] In another aspect, the invention provides an emission tomography system for acquiring a series of medical images of a subject during an imaging process using a radiotracer. The system comprises: a plurality of detectors configured to be arranged about the subject to acquire gamma rays emitted from the subject over a time period relative to an administration of the radiotracer to the subject and communicate signals corresponding to acquired gamma rays; a data processing system configured to receive the signals from the plurality of detectors; and a reconstruction system configured to receive the signals from the data processing system and reconstruct therefrom a series of medical images of the subject. The radiotracer comprises a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a

paramagnetic metal ion or compound. The biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.

[0023] In the imaging methods and systems of the invention, the image may be acquired using positron emission tomography imaging, positron emission tomography with computed tomography imaging, or positron emission tomography with magnetic resonance imaging. The biological material may be selected from cells. The chelating moiety may be a hydroxamic acid group. The hydroxamic acid group may be a desferrioxamine group. The conjugation moiety may include an isothiocyanate group, a benzyl group, or a maleimide group, or an NHS ester group, or a combination thereof. The cells may be selected from microorganisms, blood-derived cells, cancer cells, stem cells, and dendritic cells. The biological material may be selected from stem cells. The biological material may be selected from viruses.

[0024] In the imaging methods and systems of the invention, the radionuclide may be selected from the group consisting of ^{11}C , ^{13}N , ^{15}O , ^{18}F , $^{34\text{m}}\text{Cl}$, ^{38}K , ^{45}Ti , ^{51}Mn , ^{52}Mn , $^{52\text{m}}\text{Mn}$, ^{52}Fe , ^{55}Co , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{66}Ga , ^{68}Ga , ^{71}As , ^{72}As , ^{74}As , ^{75}Br , ^{76}Br , ^{82}Rb , ^{86}Y , ^{89}Zr , ^{90}Nb , $^{94\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$, $^{110\text{m}}\text{In}$, ^{111}In , ^{118}Sb , ^{120}I , ^{121}I , ^{122}I , ^{123}I , and ^{124}I . The radionuclide may be a positron emitter, such as ^{89}Zr . The paramagnetic metal ion may be selected Gd^{+3} , Fe^{+3} , Mn^{+2} , and Y^{+3} . The labeling agent may include a metal ion that is both a positron emitter and paramagnetic. For example, ^{52}Mn is a positron emitter which has a half-life similar to ^{89}Zr (Half-life: 3.26 days). Also, ^{52}Mn is paramagnetic which allows for a dual modality cell labeling reagent (e.g., positron emission tomography with magnetic resonance imaging). The paramagnetic compound may be compounds including ^{19}F , such as a perfluorocarbon.

[0025] The imaging methods and systems and labeling agents of the invention can provide for detection of the radiolabeled biological material *in vivo* for a time period of one or more days. For example, a detection time period of the radiolabeled biological material *in vivo* can be beyond 24 hours, or beyond 2 days, or beyond 3 days, or beyond 4 days, or beyond 5 days, or beyond 7 days, or beyond 9 days, or beyond 13 days, or beyond 21 days.

[0026] The invention also provides a novel cell labeling strategy that covalently binds a ^{89}Zr -Desferrioxamine-labeling agent to cell surface proteins independent of cell type.

[0027] It is therefore an advantage of the invention to provide methods of *ex vivo* labeling of a cell for *in vivo* imaging, methods of labeling a cell *in vivo*, methods for preparing a labeling agent, and methods for *in vivo* imaging of a subject using a biological material labeled with a labeling agent.

[0028] It is another advantage of the method that Zr-89 DBN may act as a general Zr-89 labeling synthon. It can be produced at a centralized facility and shipped to various sites and labs that need to perform labeling of biological materials such as, without limitation, cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids.

[0029] These and other features, aspects, and advantages of the present invention will become better understood upon consideration of the following detailed description, drawings and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1 is a scheme for synthesis of ^{89}Zr -DBN and cell labeling.

[0031] FIG. 2 is a schematic of a positron emission tomography (PET) system.

[0032] FIG. 3 is a comparison of cell population doubling times for ^{89}Zr -labeled and unlabeled mMCs, hMSCs and mDCs.

[0033] FIG. 4A is a graphical representation of the viability of ^{89}Zr -labeled and unlabeled cells.

[0034] FIG. 4B is a graphical representation of the cytotoxicity of ^{89}Zr -labeled and unlabeled cells.

[0035] FIG. 4C is a graphical representation of the apoptosis of ^{89}Zr -labeled and unlabeled cells.

[0036] FIG. 5 shows the retention of ^{89}Zr in ^{89}Zr -labeled cells expressed as radioactivity in MBq in the cell population.

[0037] FIG. 6A is representative PET images labeled hMSCs and $^{89}\text{Zr}(\text{HPO}_4)_2$ following intravenous injection.

[0038] FIG. 6B is biodistribution data of ^{89}Zr -labeled hMSCs and $^{89}\text{Zr}(\text{HPO}_4)_2$ following intravenous injection.

[0039] FIG. 6C is biodistribution data of $^{89}\text{Zr}(\text{HPO}_4)_2$ labeled hMSCs and $^{89}\text{Zr}(\text{HPO}_4)_2$ following intravenous injection.

5 [0040] FIG. 7A shows representative PET images of ^{89}Zr -labeled hMSCs following myocardial delivery.

[0041] FIG. 7B is representative biodistribution data of ^{89}Zr -labeled hMSCs following myocardial delivery.

10 [0042] FIG. 8 shows a cell infection assay using ^{89}Zr -labeled AAV2-GFP and 293T cells.

[0043] FIG. 9 shows a representative elution profile of labeled AAVs after size exclusion PD-10 column purification.

15 [0044] FIG. 10 shows representative serial PET images and bioluminescence images showing biodistribution and transfection profile of ^{89}Zr -DBN-AAV1-Cre with $^{89}\text{Zr}(\text{HPO}_4)_2$ and AAV1 as control.

[0045] FIG. 11 shows representative fluorescence images of DAPI (4',6-diamidino-2-phenylindole) counter-stained 10μ liver sections confirming transfection. DAPI signal (blue) is showing stained nucleus, GFP signal (green) is showing AAV1 transfected cells and RFP signal (red) is showing untransfected cells.

20 [0046] Figure 12A shows photomicrographs (original magnification, x20) showing the localization of human adipose tissue-derived mesenchymal stem cells (MSCs). GFP was used to stably transfect 2.5×10^5 MSCs, which were injected into the adventitia of the outflow vein of the arteriovenous fistula (AVF) of mice, at the time of creation. GFP-labeled human adipose tissue-derived MSCs are present on day 7 in vessels with transplanted MSCs compared with outflow vein vessels removed from control animals after AVF placement. There are GFP-positive cells (arrows) in the vessel wall of the outflow vein on day 7. * = lumen.

25 [0047] Figure 12B shows serial PET images of ^{89}Zr distribution in mice after adventitial delivery of ^{89}Zr -labeled MSCs or $^{89}\text{Zr}(\text{HPO}_4)_2$. The anatomic reference skeleton images are formed by using the mouse atlas registration system algorithm

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with information obtained from the stationary top-view planar x-ray projector and side-view optical camera. In Figure 12B, SUV = standardized uptake value.

[0048] Figure 13 shows PET images of ^{89}Zr -labeled AAVs following intravenous delivery in mice.

5 **[0049]** Figure 14 shows representative serial PET images and bioluminescence images showing biodistribution and transfection profile of ^{89}Zr -labeled AAVs.

DETAILED DESCRIPTION OF THE INVENTION

[0050] For cell-based therapies, early engraftment period of 2-5 weeks post cell delivery is the most critical time period. Therefore, imaging based methods should
10 be robust over this time frame to allow evaluation of various interventions for improving cell engraftment. The ability to monitor cells *in vivo* beyond 24 hours is also of high importance for evaluation of infection using radiolabeled leukocytes. Conventional infection imaging protocols perform imaging at 1 hour, 4 hours and 24 hours post-injection to differentiate between inflammatory, acute infection and chronic
15 infection loci, however in some patients, 48 hours are necessary for reliable detection of infected lesions.

[0051] In one non-limiting example embodiment, a method of this disclosure may employ a two-step method (see Figure 1): (1) preparation of ^{89}Zr -labeled p-isothiocyanato-benzyl-desferrioxamine (^{89}Zr -DBN) and (2) random labeling of primary
20 amines of cell surface proteins with ^{89}Zr -DBN. This labeling strategy has been evaluated in cell types such as mouse melanoma cells (mMCs), human mesenchymal stem cells (hMSCs) and mouse dendritic cells (mDCs). The labeled cells were evaluated for 7 days post-labeling for label retention and probable changes in cell proliferation, cell viability and degree of apoptosis in radiolabeled
25 cells as compared to their unlabeled counterparts. Out of these, labeled hMSCs were further tested for imaging characteristics and stability of radiolabel in an *in vivo* mouse model.

[0052] Improvement to the stability of the ^{89}Zr radiolabel on cells can be seen when using ^{89}Zr -desferrioxamine-NCS (^{89}Zr -DBN) as a labeling entity capable of
30 forming covalent bonds with primary amines of cell surface protein as described herein. Because all cells express cell surface protein with exposed lysine residues

and other primary amines, labeling with ^{89}Zr -DBN also provides a general labeling method to label a broad array of cells.

[0053] ^{89}Zr -DBN cellular-labeling exploits both the strength of chelation of ^{89}Zr by desferrioxamine (DFO) with three hydroxamate groups (qualitative Zr-binding constant = ~ 1031) as well as the inherent biostability of the thiourea bond that conjugates $-\text{NCS}$ group in ^{89}Zr -DBN to primary amines of a protein. One could also replace the conjugation moiety from the NCS group of Zr-89 DBN to a maleimide group that would then conjugate thiols on cell surface protein. Alternatively, an NHS ester group could be employed to target primary amines, just like the NCS group of ^{89}Zr -desferrioxamine-NCS. Generally, an activated group with amine, thiol or hydroxyl moiety could react with the NCS group.

[0054] The generality of the labeling target, along with the multiplicity of primary amines available on the cell surface, also avoids the specific targeting of highly sensitive processes that might affect cellular function or viability.

[0055] A method for labeling cells with ^{89}Zr -DBN can be seen in FIG. 1. The method employs two steps: (i) preparation of ^{89}Zr -labeled p-isothiocyanato-benzyl-desferrioxamine (^{89}Zr -DBN) and (ii) random labeling of primary amines of cell surface proteins with ^{89}Zr -DBN.

[0056] In another non-limiting example embodiment, a method of this disclosure may employ a two-step method (see Figure 1): (1) preparation of ^{89}Zr -labeled p-isothiocyanato-benzyl-desferrioxamine (^{89}Zr -DBN) and (2) random labeling of viruses with ^{89}Zr -DBN. This labeling strategy has been evaluated in viruses such as AAV1, AAV2, AAV8 and AAV9.

[0057] Referring now to FIG. 2, a PET system 100 that can be used with the labeled cells of the present invention comprises an imaging hardware system 110 that includes a detector ring assembly 112 about a central axis, or bore 114. An operator work station 116 including a commercially-available processor running a commercially-available operating system communicates through a communications link 118 with a gantry controller 120 to control operation of the imaging hardware system 110.

[0058] The detector ring assembly 112 is formed of a multitude of radiation detector units 122 that produce a signal responsive to detection of a photon on communications line 124 when an event occurs. A set of acquisition circuits 126 receive the signals and produce signals indicating the event coordinates (x, y) and the total energy associated with the photons that caused the event. These signals are sent through a cable 128 to an event locator circuit 130. Each acquisition circuit 126 also produces an event detection pulse that indicates the exact moment the interaction took place. Other systems utilize sophisticated digital electronics that can also obtain this information regarding the precise instant in which the event occurred from the same signals used to obtain energy and event coordinates.

[0059] The event locator circuits 130 in some implementations, form part of a data acquisition processing system 132 that periodically samples the signals produced by the acquisition circuits 126. The data acquisition processing system 132 includes a general controller 134 that controls communications on a backplane bus 136 and on the general communications network 118. The event locator circuits 130 assemble the information regarding each valid event into a set of numbers that indicate precisely when the event took place and the position in which the event was detected. This event data packet is conveyed to a coincidence detector 138 that is also part of the data acquisition processing system 132.

[0060] The coincidence detector 138 accepts the event data packets from the event locator circuit 130 and determines if any two of them are in coincidence. Coincidence is determined by a number of factors. First, the time markers in each event data packet must be within a predetermined time window, for example, 0.5 nanoseconds or even down to picoseconds. Second, the locations indicated by the two event data packets must lie on a straight line that passes through the field of view in the scanner bore 114. Events that cannot be paired are discarded from consideration by the coincidence detector 138, but coincident event pairs are located and recorded as a coincidence data packet. These coincidence data packets are provided to a sorter 140. The function of the sorter in many traditional PET imaging systems is to receive the coincidence data packets and generate memory addresses from the coincidence data packets for the efficient storage of the coincidence data.

In that context, the set of all projection rays that point in the same direction (θ) and pass through the scanner's field of view (FOV) is a complete projection, or "view". The distance (R) between a particular projection ray and the center of the FOV locates that projection ray within the FOV. The sorter 140 counts all of the events that occur on a given projection ray (R, θ) during the scan by sorting out the coincidence data packets that indicate an event at the two detectors lying on this projection ray. The coincidence counts are organized, for example, as a set of two-dimensional arrays, one for each axial image plane, and each having as one of its dimensions the projection angle θ and the other dimension the distance R . This θ by R map of the measured events is call a histogram or, more commonly, a sinogram array. It is these sinograms that are processed to reconstruct images that indicate the number of events that took place at each image pixel location during the scan. The sorter 140 counts all events occurring along each projection ray (R, θ) and organizes them into an image data array.

[0061] The sorter 140 provides image datasets to an image processing / reconstruction system 142, for example, by way of a communications link 144 to be stored in an image array 146. The image arrays 146 hold the respective datasets for access by an image processor 148 that reconstructs images. The image processing/reconstruction system 142 may communicate with and/or be integrated with the work station 116 or other remote work stations.

[0062] The PET system 100 provides an example emission tomography system for acquiring a series of medical images of a subject during an imaging process after administering a pharmaceutically acceptable composition including labeled cells as described herein. The system includes a plurality of detectors configured to be arranged about the subject to acquire gamma rays emitted from the subject over a time period relative to an administration of the composition to the subject and communicate signals corresponding to acquired gamma rays. The system also includes a reconstruction system configured to receive the signals and reconstruct therefrom a series of medical images of the subject. In one version of the system, a second series of medical images is concurrently acquired using an x-ray computed

tomography imaging device. In one version of the system, a second series of medical images is concurrently acquired using a magnetic resonance imaging device.

[0063] Administration to the subject of a pharmaceutical composition including radiolabeled cells for *in vivo* detection of the accumulated cells in a target region of the subject can be accomplished intravenously, intraarterially, intrathecally, intramuscularly, intradermally, subcutaneously, or intracavitary. A "subject" is a mammal, preferably a human. In the method of the invention, sufficient time is allowed after administration of a detectable amount of the radiolabeled cells such that the radiolabeled cells can accumulate in a target region of the subject. A "detectable amount" means that the amount of the detectable radiolabeled cells that is administered is sufficient to enable detection of accumulation of the radiolabeled cells in a subject by a medical imaging technique.

[0064] One non-limiting example method of imaging according to the invention involves the use of an intravenous injectable composition including radiolabeled cells or viruses. A positron emitting atom of the radiolabeled cells or viruses gives off a positron, which subsequently annihilates and gives off coincident gamma radiation. This high energy gamma radiation is detectable outside the body through the use of positron emission tomography imaging, or positron emission tomography concurrent with computed tomography imaging (PET/CT). With PET/CT, the location of the injected and subsequently accumulated labeled cells within the body can be identified.

EXAMPLES

[0065] The following Examples are provided in order to demonstrate and further illustrate certain embodiments and aspects of the present invention and are not to be construed as limiting the scope of the invention.

Example 1

Overview of Example 1

[0066] Background: With the recent growth of interest in cell-based therapies and radiolabeled cell products, there is a need to develop more robust cell labeling and imaging methods for *in vivo* tracking of living cells. This study in Example 1 describes evaluation of a novel cell labeling approach with the PET isotope ^{89}Zr ($T_{1/2}$

=78.4 hours). Zr-89 may allow PET imaging measurements for several weeks and take advantage of the high sensitivity of PET imaging.

[0067] Methods: A novel cell-labeling agent, ^{89}Zr -desferrioxamine-NCS (^{89}Zr -DBN), was synthesized. Mouse derived melanoma cells (mMCs), dendritic cells (mDCs) and human mesenchymal stem cells (hMSCs) were covalently labeled with ^{89}Zr -DBN via the reaction between the NCS group on ^{89}Zr -DBN and primary amine groups present on cell surface membrane protein. The stability of the label on the cell was tested by cell efflux studies for 7 days. The effect of labeling on cellular viability was tested by proliferation, trypan blue and cytotoxicity/apoptosis assays. The stability of label was also studied in *in vivo* mouse models by serial PET scans and *ex vivo* biodistribution following intravenous and intramyocardial injection of ^{89}Zr -labeled hMSCs. For comparison, imaging experiments were performed after intravenous injections of ^{89}Zr hydrogen phosphate ($^{89}\text{Zr}(\text{HPO}_4)_2$).

[0068] Results: The labeling agent, ^{89}Zr -DBN, was prepared in $55\pm 5\%$ decay-corrected radiochemical yield measured by silica gel iTLC. The cell labeling efficiency was 30-50% after 30 minutes labeling depending on cell type. Radioactivity concentrations of labeled cells of up to $0.5\text{MBq}/1\times 10^6$ cells were achieved without a negative effect on cellular viability. Cell efflux studies showed high stability of the radiolabel out to 7 days. Myocardially delivered ^{89}Zr -labeled hMSCs showed retention in the myocardium, as well as redistribution to lung, liver and bone, whereas intravenous delivery of ^{89}Zr -labeled hMSCs distributed primarily to lung, liver and bone which are expected distribution sites for human MSCs in mouse model. $^{89}\text{Zr}(\text{HPO}_4)_2$ distributed to liver and bone with no activity in lung. Thus, the *in vivo* stability of the radiolabel on the hMSCs was evidenced.

[0069] Conclusions: We have developed a robust, general, and biostable ^{89}Zr -DBN based cell-labeling strategy with promise for wide applications of PET-based non-invasive *in vivo* cell trafficking.

Methods

Cell culture

[0070] B16-F10 Mouse melanoma cells (mMCs) from ATCC, Manassas, Virginia, USA, human mesenchymal stem cells (hMSCs) from patients and JAWSII mouse

dendritic cells (mDCs) from ATCC, Manassas, Virginia, USA, were used for evaluating the ^{89}Zr -DBN based labeling method. The mMCs and hMSCs were cultured in complete DMEM (DMEM + 10% FBS) and mDCs were cultured in complete alphaMEM (alpha MEM + 4 mM L-glutamine + 1 mM sodium pyruvate + 5 ng/mL murine GM-CSF + 20% FBS). The cultures were maintained in a humidified cell culture chamber (21% O_2 , 74% N_2 , 5% CO_2) at 37°C.

Production and isolation of ^{89}Zr

[0071] $^{89}\text{Zr}^{4+}$ was produced in aqueous solution through the $^{89}\text{Y}(p,n)^{89}\text{Zr}$ nuclear reaction using a solution target containing yttrium nitrate and dilute nitric acid. The $^{89}\text{Zr}^{4+}$ was isolated from $^{89}\text{Y}^{3+}$ using a hydroxamate resin based purification method with the exception that the final elution of $^{89}\text{Zr}^{4+}$ off the hydroxamate resin was performed with an appropriate volume of 1.2M $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer (pH 3.5). The $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer was allowed to sit on the column for 30 minutes before elution to promote release of ^{89}Zr as Zirconium hydrogen phosphate, $^{89}\text{Zr}(\text{HPO}_4)_2$ from the column. The elution percentage of ^{89}Zr from the column was ~ 89% collected in four fractions of 0.5mL each.

Synthesis of ^{89}Zr -DBN

[0072] The eluted $^{89}\text{Zr}(\text{HPO}_4)_2$ solution (120 μl) was neutralized to pH 7.8 with ~100 μl 1M HEPES-KOH buffer (pH 7.5) and ~65 μl 1M K_2CO_3 . To this, 4 μl 5mM DFO-Bz-NCS in DMSO (Macrocyclics, Dallas, Texas, USA) was added and chelation of $^{89}\text{Zr}^{4+}$ proceeded at 37°C for 1 hour in a thermomixer at 550 rpm. Chelation efficiency was determined by silica gel iTLC (Agilent Technologies, Santa Clara, California, USA) with 50 mM DTPA pH 7 as the mobile phase. ^{89}Zr -DBN showed an $R_f=0$, whereas $^{89}\text{Zr}(\text{HPO}_4)_2$ had an $R_f=0.9$.

Labeling of cells with $^{89}\text{Zr}(\text{HPO}_4)_2$ and ^{89}Zr -DBN

[0073] The adherent cells were trypsinized and washed once with PBS and twice with HEPES buffered GIBCO Hanks Balanced Salt solution buffered (ThermoFisher, Atlanta, Georgia, USA) (H-HBSS), pH 7.5. The cell labeling reaction was performed with ~ 6×10^6 cells in 500 μl H-HBSS at pH 7.5. To this, either 100 μl $^{89}\text{Zr}(\text{HPO}_4)_2$ (~ 6MBq) or 100 μl ^{89}Zr -DBN (~ 6MBq) was added and was allowed to incubate at 37°C for 30 minutes on a shaker for cell labeling. After incubation, the cells were washed 4

times with appropriate volume of complete medium. The final labeling efficiency was calculated from the radioactivity bound to cells after all the washes.

Incorporation of ^{89}Zr -DBN in protein fraction

[0074] To understand the sub-cellular localization of the label, incorporation of ^{89}Zr -DBN into different protein fractions in mMCs, hMSCs and mDCs was evaluated using a subcellular protein fractionation kit (Piercenet Thermo Scientific) at day 1, 4 and 7 post-labeling. The cytosolic proteins, hydrophobic membrane proteins, nuclear proteins and cytoskeletal proteins were isolated and each protein fraction was counted for radioactivity using a 2480 Wizard² automatic gamma counter (Perkin Elmer, Waltham, Massachusetts, USA).

Efflux of ^{89}Zr -DBN from labeled cells

[0075] To determine cellular efflux, 0.3×10^6 ^{89}Zr -labeled cells were plated into each well of a 6-well culture plate. The medium was replaced with fresh medium daily for 7 days and radioactivity in the replaced medium was counted. For mDCs with mix of adherent and suspension cells, the plate was centrifuged at 1000 rpm for 10 minutes before replacing the medium to avoid loss of unattached ^{89}Zr -labeled cells.

CyQUANT cellular proliferation assay

[0076] The effect of radiolabeling on cellular proliferation was assessed by the CyQUANT DNA content assay (ThermoFisher, Atlanta, Georgia, USA). A known number of unlabeled and ^{89}Zr -labeled cells ($\sim 10^4$ cells/well) were plated in 21 wells of a 96-well culture plate and maintained at 37°C in a CO₂ incubator. The amount of DNA in each well was quantified from absorbance values as a surrogate marker of the number of cells present. The culture medium was replaced daily. The CyQUANT assay was performed for 3 wells per day over 5 days.

Trypan-Blue exclusion assay cellular viability test

[0077] The effect of labeling on cellular viability was assessed using Trypan-Blue exclusion assay test within 1 hour of labeling, 3rd and 7th day post-labeling. The culture medium was replaced daily and maintained at 37°C in a CO₂ incubator. Unlabeled cells served as control.

ApoTox-Glo viability/cytotoxicity and apoptosis assay

[0078] The effect of radiolabeling on cellular viability was also assessed using the ApoTox-Glo viability, cytotoxicity and caspase 3/7 apoptosis assay (Promega Corporation, Madison, Wisconsin, USA). Unlabeled cells served as control. A known number of unlabeled and ^{89}Zr -labeled cells ($\sim 10^4$ /well) were plated in a 96-well culture plate. The culture medium was replaced daily and maintained at 37°C in a CO_2 incubator. At day 7, cell viability, cytotoxicity and apoptosis was quantified in triplicate using the ApoTox-Glo assay. As positive controls, cells were incubated with $30\mu\text{g/mL}$ digitonin for 30 minutes for the viability and cytotoxicity assays, while $2\mu\text{M}$ staurosporine was added for 16 hours for the caspase 3/7 dependent apoptosis assay.

PET imaging and ex-vivo biodistribution of ^{89}Zr -labeled cells and $^{89}\text{Zr}(\text{HPO}_4)_2$

[0079] Experiments were performed with 2-month old athymic nude *Foxn1nu* mice (Harlan Laboratories, Inc., Indianapolis, Indiana, USA). $^{89}\text{Zr}(\text{HPO}_4)_2$ ($\sim 0.074\text{ MBq}$) or ^{89}Zr -labeled cells (2×10^5 cells with radioactivity concentration $\sim 0.37\text{ MBq}/1 \times 10^6$ cells) were injected intravenously through a tail vein. On days 2, 4, and 7, the mice were anesthetized under 1-2% isoflurane and underwent PET imaging using a small animal PET/X-Ray system (Sofie BioSystems Genesys4, Culver City, California, USA). At day 7, the mice were sacrificed and tissues were extracted and radioactivity counted using a gamma counter to evaluate the biodistribution of ^{89}Zr -radioactivity. Images were presented as a whole-body maximal images projection (MIPs) to display sites of uptake.

In vivo tracking of stem cell engraftment in ischemia/reperfusion mouse model

[0080] Athymic nude *Foxn1nu* mice (2 months old) were anesthetized under 1-2% isoflurane and placed on a heating pad. Respiratory and heart rates were monitoring continually. After intubation, mechanical ventilation and intercostal block of bupivacaine and lidocaine, an incision was made in through the 4th or 5th intercostal space for access into the thoracic space, the heart was exposed and the pericardium was incised anterior and parallel to the phrenic nerve. With visualization of the coronary vasculature, the left coronary artery was ligated to induce myocardial ischemia at the anterior wall of the left ventricle. One hour after the coronary ligation,

the suture was untied for reperfusion. Myocardial reperfusion was confirmed by color change of the left ventricle and electrocardiographic changes. During reperfusion, ^{89}Zr -labeled cells (2×10^5 cells with radioactivity concentration $\sim 0.37 \text{ MBq}/10^6$ cells) were injected at 4 sites within the ischemic region. After myocardial injection, the intercostal space, the chest musculature and the skin were closed with a 7-0 Ethilon suture. The animals were imaged at day 2, day 5 and day 7 using small animal PET/X-Ray system (Sofie BioSystems Genesys4, Culver City, California, USA). At day 7, the mice were sacrificed and tissues were extracted and radioactivity counted using gamma counter to evaluate cell trafficking.

Results

Synthesis of ^{89}Zr -DBN and cell labeling studies

[0081] ^{89}Zr hydrogen phosphate was readily chelated by DFO-NCS to form ^{89}Zr -DBN in a 1.2 M $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ and HEPES buffer system (pH 7.8), with radiolabeling efficiency of $55 \pm 5\%$ after 30 minutes. This reaction mixture was then used directly for labeling of cells. The cell labeling efficiency using ^{89}Zr -DBN was $\sim 30\text{-}50\%$ as determined by cell-bound radioactivity. Radioactivity concentrations of 0.50 ± 0.10 , 0.47 ± 0.10 , and $0.39 \pm 0.20 \text{ MBq}/10^6$ cells were achieved when 6×10^6 cells were incubated for 30 minutes with $\sim 6 \text{ MBq}$ ^{89}Zr -DBN with mMCs, hMSCs and mDCs, respectively. In contrast, no cell labeling was observed using $^{89}\text{Zr}(\text{HPO}_4)_2$.

Cellular proliferation and viability studies

[0082] The CyQUANT proliferation assay showed no difference in proliferation rate between unlabeled and ^{89}Zr -labeled cells. See Figure 3 for a comparison of cell population doubling times for ^{89}Zr -labeled and unlabeled mMCs, hMSCs and mDCs. The cells were plated at appropriate cell number at day 3 and CyQUANT assay was performed at day 7 post-labeling. No significant differences were observed between radiolabeled and unlabeled cells. Values in Figure 3 are shown as mean $\pm$ standard deviation, $n=3$.

[0083] Trypan Blue cell viability tests were performed on radiolabeled cells immediately after labeling and up to 7 days post-labeling and compared with unlabeled cells. No change was observed in number of dead cells (blue stained

cells) over live cells (unstained cells) in both ^{89}Zr -labeled and unlabeled cells, with percentage of dead cells <5% in all days tested.

ApoTox-Glo viability/cytotoxicity and apoptosis assay

[0084] The ApoTox-Glo assay showed no loss in cellular viability and no increase in cytotoxicity or apoptosis in radiolabeled cells as illustrated in Figures 4A-4C showing an assessment of (Fig. 4A) viability, (Fig. 4B) cytotoxicity and (Fig. 4C) apoptosis in ^{89}Zr -labeled and unlabeled cells. No statistically significant differences were observed between ^{89}Zr -labeled and unlabeled cells after 7 days of culture with regard to viability, cytotoxicity or apoptosis. As positive controls, 30 mg/mL digitonin was used for assays (Fig. 4A) and (Fig. 4B), and 2mM staurosporine for (Fig. 4C). * $p < 0.05$ versus assessments in ^{89}Zr -labeled and unlabeled cells using unpaired t-test. Values are shown as mean $\pm$ standard deviation, $n=3$. Viability was lost and cytotoxicity enhanced when 30 $\mu\text{g/mL}$ digitonin was added to cells and apoptosis was increased with the addition of 2 μM staurosporine.

Subcellular distribution of ^{89}Zr -radioactivity

[0085] At days 1, 4 and 7 after ^{89}Zr -labeling of mMCs, hMSCs and mDCs, subcellular protein fractionation of the cells was performed. ^{89}Zr -radioactivity was incorporated predominantly (~99%) in hydrophobic membrane protein fraction of all cell types studied, strongly supporting the proposed mechanism of reaction of ^{89}Zr -DBN with cell surface membrane protein to form a stable covalent bond.

Efflux of ^{89}Zr -radioactivity from labeled cells

[0086] Retention of ^{89}Zr -radioactivity by ^{89}Zr -DBN labeled cells was found to be stable in all the cells studied with negligible efflux observed over 7 days post-labeling. See Figure 5 showing retention of ^{89}Zr in ^{89}Zr -labeled cells expressed as radioactivity in MBq in the cell population. The retention value is representing total radioactivity/ 10^6 cells in the proliferating cell population. No significant change was observed in retention of ^{89}Zr in radiolabeled cells. Values are shown as mean $\pm$ standard deviation, $n=3$.

PET imaging and biodistribution studies in mice with intravenous injections

[0087] PET images and biodistribution data of intravenously administered ^{89}Zr -labeled hMSCs and $^{89}\text{Zr}(\text{HPO}_4)_2$ in healthy mice are in Figures 6A-6C which show

representative PET images and biodistribution data of ^{89}Zr -labeled hMSCs and $^{89}\text{Zr}(\text{HPO}_4)_2$ following intravenous injection. ^{89}Zr -labeled human MSCs (2×10^5 cells with radioactivity concentration $\sim 0.37 \text{ Mbq}/10^6$ cells) and $^{89}\text{Zr}(\text{HPO}_4)_2$ ($\sim 0.074 \text{ Mbq}$ radioactivity) were intravenously injected in athymic mice. Most of the radioactivity was distributed in lung, liver and bones following injection of ^{89}Zr -labeled hMSCs whereas most of the radioactivity was distributed in liver and bones following injection of $^{89}\text{Zr}(\text{HPO}_4)_2$. Values in graphs are shown as mean $\pm$ standard deviation, $n=3$. The ^{89}Zr -labeled hMSCs were concentrated primarily in lung and liver, followed by bone. On the other hand, $^{89}\text{Zr}(\text{HPO}_4)_2$ accumulated in bone and liver and did not distribute to lung.

In vivo tracking of stem cell engraftment in ischemia/reperfusion mouse model

[0088] Following myocardial delivery, ^{89}Zr -labeled hMSCs ($\sim 19.5 \pm 9.5\%$) were retained for 7 days in heart. See Figures 7A-7B for representative PET images and biodistribution data of ^{89}Zr -labeled hMSCs following myocardial delivery. ^{89}Zr -labeled hMSCs (2×10^5 cells with radioactivity concentration $\sim 0.37 \text{ Mbq}/10^6$ cells) were delivered to myocardium of an ischemia/reperfusion mouse model. Most of the radioactivity was distributed in heart (arrow), lung, liver and bones following myocardial delivery of ^{89}Zr -labeled hMSCs. Values in graph are shown as mean $\pm$ standard deviation, $n=5$. The remaining cells were concentrated in lung, followed by bones and liver. The higher uptake in lung relative to liver is consistent with the biodistribution of ^{89}Zr -labeled hMSCs released into the circulation (Figures 6A-6C).

Discussion

[0089] Various strategies have been employed in the past to label cells with imaging isotopes for non-invasive *in vivo* cell tracking for cell based therapies and infection imaging. Among them, ^{18}F -FDG (for PET) [Ref. 12-16] and ^{111}In -oxine (for SPECT) [Ref. 2-6] are the most widely used. Although ^{18}F -FDG is useful for assessment of immediate delivery of cells and early fate of cells ($\sim$ first few hours), it is not suited for *in vivo* cell tracking after 24 hours post-injection due to its short half-life and poor retention in cells. Inability of ^{18}F -FDG to allow cell tracking after 24 hours limits its utility in cell-based therapies. For cell-based therapies, early engraftment period of 2-5 weeks post cell delivery is the most critical time period

[Ref. 20]. Therefore, imaging based methods should be robust over this time frame to allow evaluation of various interventions for improving cell engraftment. The ability to monitor cells *in vivo* beyond 24 hours is also of high importance for evaluation of infection using radiolabeled leukocytes. Conventional infection imaging protocols perform imaging at 1 hours, 4 hours and 24 hours post-injection to differentiate between inflammatory, acute infection and chronic infection loci, however in some patients, 48 hours was necessary for reliable detection of infected lesions [Ref. 21].

[0090] The use of ^{111}In ($T_{1/2} = 2.8$ days) as a radiolabel for cell labeling allows longer observation periods for cell tracking but with lower spatial resolution of SPECT imaging. Cell labeling with ^{111}In typically requires a lipophilic carrier molecule (e.g. oxine) for transporting the radiometal into cells [Ref. 2-6]. After entering the cells, the radiometal then dissociates and gets trapped in the cell by binding to non-specific intracellular metal-binding proteins. The two major disadvantages of this approach are chemotoxicity of the lipophilic carrier molecule [Ref. 22] and efflux of radiolabel from cells [Ref. 2-6].

[0091] Recently, two groups (Charoenphun *et al.* [Ref. 9] and Davidson-Moncada *et al.* [Ref. 10]) reported synthesis of ^{89}Zr -oxinate or ^{89}Zr -oxine as a cell-labeling reagent for PET based cell tracking. As expected, both groups faced the problem of chemotoxicity and significant efflux of radioactivity from the cells post-labeling commonly associated with oxine based labeling. Charoenphun *et al.* [Ref. 9] showed reduced viability of ^{89}Zr -oxine labeled 5T33 myeloma cells (from 93% to $76.3 \pm 3.2\%$ in first 24 hours) and significant efflux of radioactivity post-labeling (29% effluxed in 24 hours). Davidson-Moncada *et al.* [Ref. 10] also reported similar results with ^{89}Zr -oxine labeled human and rhesus macaques' natural killer cells. They observed a broad range of viability of 60-100% in the radiolabeled cells over the first 24 hours, which declined to 20-30% after 6 days. A significant efflux of radioactivity was also observed in these viable ^{89}Zr -oxine labeled cells, ~ 20-25% effluxed in the first 24 hours and 70-80% of radioactivity was effluxed after 7 days of culture. These drawbacks associated with the ^{89}Zr -oxine labeling method compromises its utility for PET-based monitoring of *in vivo* cell trafficking.

[0092] To improve the stability of the ^{89}Zr radiolabel on cells, this Example proposed ^{89}Zr -DFO-NCS (^{89}Zr -DBN) as a labeling entity capable of forming covalent bonds with primary amines of cell surface protein (see Figure 1). Since all cells express cell surface protein with exposed lysine residues and other primary amines, this strategy also provides a general labeling method to label a broad array of cells. The new strategy exploits both the strength of chelation of ^{89}Zr by DFO with three hydroxamate groups (qualitative Zr-binding constant = $\sim 10^{31}$) [Ref. 23-26] as well as the inherent biostability of the thiourea bond that conjugates -NCS group in ^{89}Zr -DBN to primary amines of protein [Ref. 27,28]. Furthermore, the labeling agent, ^{89}Zr -DBN, is also expected to be well-tolerated by cells as opposed to toxic lipophilic carrier molecules like oxine, relying on the fact that DFO-NCS has been routinely used to conjugate DFO to IgG and IgM antibodies with no loss of antibody protein function [Ref. 23, 26-29]. The generality of the labeling target, along with the multiplicity of primary amines available on the cell surface, also avoids the specific targeting of highly sensitive processes that might affect cellular function or viability. In this study, 30-50% labeling efficiencies were achieved with ^{89}Zr -DBN in several cell types and the cells showed no evidence of chemotoxicity or radiotoxicity. Furthermore, in contrast with the previously noted ^{89}Zr -oxine results [Ref. 9,10], no efflux of radiolabel was observed from cultured cells labeled with ^{89}Zr -DBN out to 7 days. All ^{89}Zr -radioactivity was found to be incorporated into the membrane bound protein fraction of the cells confirming the anticipated targeting of membrane protein.

[0093] After encouraging *in vitro* validation tests, *in vivo* validations were performed by investigating the biodistribution of ^{89}Zr -labeled hMSCs after intravenous injection in athymic nude mice for 7 days post-injection. Trapping of MSCs in lungs following intravenous injection is well documented [Ref. 30,31]. Therefore, major accumulation of ^{89}Zr -labeled human MSCs in mouse lungs was expected following intravenous injection with slow clearance. With time, cells were expected to dislodge from this physical pulmonary entrapment and distribute to other organs. As expected, the majority of intravenously injected ^{89}Zr -labeled hMSCs were trapped in lung ($50\pm 27\%$) and the remainder was found in liver ($27\pm 19\%$) and bones ($16\pm 5\%$) after 7 days post-injection, which are expected homing sites for injected

mesenchymal stem cells after dislodging from lung [Ref. 32,33]. This was in contrast to the biodistribution of $^{89}\text{Zr}(\text{HPO}_4)_2$, which distributed primarily in bone ($59\pm 13\%$) and liver ($32\pm 15\%$) but did not accumulate in lungs. The distinct biodistributions of ^{89}Zr -labeled hMSCs and $^{89}\text{Zr}(\text{HPO}_4)_2$, together with the stability of radiolabel and lack of cytotoxicity strongly supports the robustness of the ^{89}Zr -DBN based cell labeling approach.

[0094] To further test the application of ^{89}Zr -labeled hMSCs, a stem cell engraftment study was performed using a myocardial acute ischemia/reperfusion mouse model. ^{89}Zr -labeled hMSCs were delivered to the myocardium of athymic mice following an acute myocardial ischemia/reperfusion insult. After 7 days post-delivery, ^{89}Zr -labeled hMSCs were found in heart ($20\pm 7\%$), lung ($40\pm 16\%$), bone ($29\pm 11\%$) and liver ($7\pm 5\%$). The observed retention in heart is in accordance with previously published work on hMSC engraftment estimated by invasive quantitative PCR method in a similar rodent model [Ref. 34].

[0095] Low levels of *in vitro* demetalation of ^{89}Zr -DFO complexes (2-3% / wk) in serum at 37°C have been reported [Ref. 23, 29] and clinical studies using ^{89}Zr -DFO labeled antibodies out to 7 days have yet to show significant bone uptake of ^{89}Zr indicative of demetalation [Ref. 35-39]. The initial Example 1 findings of distribution of ^{89}Zr -labeled hMSCs in mouse models confirm the biostability of the radiolabel bound to the DFO moiety supporting further exploration of the ^{89}Zr -DBN labeling method for monitoring stem cell engraftment and cell trafficking.

Conclusions

[0096] The ^{89}Zr -DBN labeling agent is shown to be a robust, general, and biostable cell labeling strategy for PET-based non-invasive *in vivo* cell or virus tracking.

Example 2

[0097] In this Example, we report the synthesis and evaluation a novel virus labeling agent, ^{89}Zr -desferrioxamine-NCS (^{89}Zr -DBN) for non-invasive PET based *in vivo* tracking of administered virus.

Background

[0098] There is a growing interest in using viral based gene therapy for treating wide range of diseases. Viral based gene delivery agents for gene therapy hold tremendous promise but like drugs, a better understanding of their pharmacokinetics is critical to their advancement. This Example 2 study describes a novel viral labeling approach with ^{89}Zr ($T_{1/2} = 78.4$ h) for potential PET imaging of viral biodistribution in the body.

Methods

[0099] A new viral labeling agent, ^{89}Zr -desferrioxamine-NCS (^{89}Zr -DBN), was synthesized. The adeno-associated viruses (AAVs) were covalently labeled with ^{89}Zr -DBN via the reaction between the NCS group on ^{89}Zr -DBN and primary amine groups present on capsid/envelope protein. The viral radiolabeling yield was quantified, following which cell binding and cell-infection assays were performed using 293T cells to evaluate the transfection potential of purified ^{89}Zr -DBN-AAV2-GFP. In addition to this, *in vivo* biodistribution of intravenous delivered ^{89}Zr -DBN-AAV1-Cre was assessed by PET imaging and correlated with transfection profile by bio-luminescence imaging (Lum) in a mouse model system.

Results

[00100] The scAAV1-Cre and AAV2-GFP were labeled with ^{89}Zr -DBN with viral labeling yield of $\sim 20\text{mCi}/10^{14}$ viral particles after 30 minutes of labeling. A cell-binding assay was performed at 4°C with multiplicity of infection (MOI) = 10,000. Following 2 hours of co-incubation of 2.5×10^9 ^{89}Zr -AAV2-GFP particles and 2.5×10^5 293T cells at 4°C , 7% of the ^{89}Zr -AAV2-GFP were found to be bound to 293T cells as compared to 0.6% in control group including cells and ^{89}Zr -hydrogen phosphate ($^{89}\text{Zr}(\text{HPO}_4)_2$). In the cell infection assay, ^{89}Zr -AAV2-GFP was incubated with 293T cells at 37°C for 5 days, at MOI = 2500 and 10,000. The fraction of 293T cells expressing GFP correlated with MOI of ^{89}Zr -AAV2-GFP. The biodistribution of intravenously injected ^{89}Zr -AAV1-Cre assessed by PET imaging correlated with the tissue transfection profile assessed by bi-luminescence imaging and fluorescence microscopy.

Conclusions

[00101] AAVs were successfully labeled with ^{89}Zr -DBN. The Zr labeling of AAVs did not affect their viral properties like infection potential and tissue tropism. In principle, ^{89}Zr -DBN is a promising tool for labeling a wide variety of viruses. Some advantages of this labeling method are that the viral labeling conditions are mild and there is no need for modification of the virus before labeling.

Methods

[00102] $^{89}\text{Zr}^{4+}$ was produced in aqueous solution through the $^{89}\text{Y}(p,n)^{89}\text{Zr}$ nuclear reaction using a solution target containing yttrium nitrate and dilute nitric acid. The $^{89}\text{Zr}^{4+}$ was isolated from $^{89}\text{Y}^{3+}$ using a hydroxamate resin-based purification method in the form of $^{89}\text{Zr}(\text{HPO}_4)_2$.

[00103] ^{89}Zr -DBN was synthesized and scAAV1-Cre produced at the Mayo Clinic and AAV2-GFP was purchased from Cell Biolabs, Inc. The scAAV1-Cre and the AAV2-GFP were labeled with ^{89}Zr -DBN.

[00104] Post-labeling, the ^{89}Zr -DBN-AAVs were purified from free label and reaction mixture using size exclusion PD-10 desalting columns. The eluted virus in fractions were quantified using protein assay and radioactivity elution pattern was recorded.

[00105] Purified fractions were tested for infection potential using 293T (for AAV2-GFP) and biodistribution or transfection profile was assessed in *in vivo* mouse model (for AAV1-Cre) strain FVB.129S6(B6)-Gt(ROSA)26Sortm1(Luc)Kael/J from the Jackson Laboratory by PET using Genesys4 PET imaging system (Perkin Elmer) and bioluminescence imaging using Xenogen spectrum imaging system (Perkin Elmer). This mouse strain has ubiquitous expression of red fluorescent protein (RFP) which after transfection with AAV1-Cre changes to green fluorescent protein (GFP) expression. This enabled confirmation of transfection at cellular level using a fluorescence microscope.

[00106] FIG. 8 shows representative data showing cell infection assay. Cell infection assay was performed with 2.5×10^5 293T cells with radiolabeled viral particles ($\sim 20\text{mCi}/10^{14}$ viral particles) at two multiplicity of infection (MOI) ~ 2500 and

10,000 in DMEM culture medium + 1% FBS in CO₂ incubator. The GFP signal after 5 day incubation correlated with MOI.

[00107] FIG. 9 shows a representative elution profile of labeled AAVs after size exclusion PD-10 column purification.

5 [00108] FIG. 10 shows representative serial PET images and bioluminescence images showing biodistribution and transfection profile of ⁸⁹Zr-DBN-AAV1-Cre with ⁸⁹Zr(HPO₄)₂ and AAV1 as control.

[00109] FIG. 11 shows representative fluorescence images of DAPI counter-stained 10μ liver sections confirming transfection.

10 Summary

[00110] ⁸⁹Zr(HPO₄)₂ was readily chelated by DFO-NCS to form ⁸⁹Zr-DBN, with radiolabeling efficiency of 55% ± 5% after 1 hour of reaction.

[00111] AAVs were labeled with ⁸⁹Zr-DBN and purified using size exclusion PD-10 column.

15 [00112] Radioactivity concentrations of ~20mCi/10¹⁴ ⁸⁹Zr-DBN-AAVs were achieved after 30 minutes incubation (N=3).

[00113] The ⁸⁹Zr labeling of AAVs did not affect its viral properties in terms of biodistribution and tissue tropism in a mouse model.

[00114] Thus, Example 2 illustrates a novel ⁸⁹Zr based virus-labeling method for
20 PET imaging of viral trafficking.

Example 3

[00115] In this Example, we report the tracking of transplanted Zirconium-89 (⁸⁹Zr)-labeled mesenchymal stem cells (MSCs) serially with positron emission tomography for 21 days.

25 [00116] Human MSC Preparation: Human MSCs from healthy donors were obtained from the Human Cellular Therapy Laboratory, Mayo Clinic, Rochester, Minnesota, USA. These cells have been characterized with respect to surface markers. Briefly, they are CD73(+), CD90(+), CD105(+), CD44(+), and HLA-ABC(+), and they are being used in several clinical trials.

30 [00117] Green fluorescent protein (GFP) Transfection: MSCs were transfected with GFP lentivirus from Mayo Clinic Rochester Labs, Rochester, Minnesota, USA.

MSCs were grown overnight in media containing the GFP lentivirus. The medium was changed to complete growth medium the next day, and cells were checked for fluorescence after 48 hours. Once fluorescence was confirmed, the cells were cultured in complete media that contained 1 µg puromycin per milliliter. Cells containing the plasmid were expanded in complete growth media.

[00118] ⁸⁹Zr Labeling and in Vivo Tracking of Stem Cells: Noninvasive PET imaging was used to evaluate the biodistribution of MSCs delivered to the adventitia outside the arteriovenous fistula (AVF) in CD1-*Foxn1nu* mice. For this, MSCs were labeled with ⁸⁹Zr-DBN. After delivery of 2 x 10⁵ ⁸⁹Zr-labeled MSCs (at a radioactivity concentration of approximately 0.55 MBq per 10⁶ cells) into the adventitia, the ⁸⁹Zr-labeled MSCs were tracked for 3 weeks by using a small-animal PET/ radiography system (Genesys4; Sofie BioSystems, Culver City, California, USA). In the control group, 0.28 MBq of ⁸⁹Zr(HPO₄)₂ was delivered into the adventitia. PET images were normalized to units of standardized uptake value, which was calculated as follows: tissue radioactivity concentration/(injected dose/body weight in grams).

[00119] PET images of mice after adventitial delivery of ⁸⁹Zr-labeled MSCs showed that more than 90% of administered ⁸⁹Zr radioactivity was retained at the delivery site on day 4 (see Fig. 12A). Adventitial retention of ⁸⁹Zr radioactivity cleared slowly from day 4 to day 21, losing approximately 20% over this period (see Fig. 12B). Most ⁸⁹Zr radioactivity that was cleared from the adventitia appeared to translocate to bones. This result confirmed results obtained by using confocal microscopy with GFP-labeled cells on day 7. PET imaging of ⁸⁹Zr-labeled MSCs allowed tracking of cells beyond 7 days, which was not possible with GFP-labeled cells. The retention of most of the delivered stem cells at the delivery site on day 21 demonstrates that the effect is longer than what was visualized by using GFP labeling. In the case of the control group in which ⁸⁹Zr(HPO₄)₂ was administered, a biodistribution similar to that of ⁸⁹Zr-labeled MSCs was seen, with most of the radioactivity (approximately 80%) retained at the delivery site and the rest redistributing to bones.

[00120] PET imaging of ⁸⁹Zr-labeled MSCs showed most (approximately 80%) of the radiolabel to be retained at the adventitial delivery site, even 3 weeks after

delivery. ^{89}Zr -labeled MSCs delivered intravenously home to the lung, followed by the liver and bones, and free $^{89}\text{Zr}(\text{HPO}_4)_2$ is mainly localized in the liver and bones. The use of ^{89}Zr -labeled MSCs was more sensitive than GFP labeling for detection of cellular location after delivery. Example 3 highlights the fact that MSCs can be tracked after delivery by using PET imaging of ^{89}Zr -labeled MSCs.

Example 4

Labeling of viruses with ^{89}Zr -DBN

[00121] A viral suspension in HEPES buffered Hanks Balanced Salt Solution (H-HBSS), pH 7.5-9 (ThermoFisher, Atlanta, Georgia, USA) was labeled with ^{89}Zr -DBN. The AAV labeling reaction was performed with 10^{10} - 10^{15} viral particles in 500ul H-HBSS. To this, 100ul ^{89}Zr -DBN (~ 6MBq) was added and incubated at 37°C for 10-40 minutes on a shaker. After incubation, the Zr-89 labeled virus was purified from free $^{89}\text{Zr}^{+4}$ on a PD-10 Sephadex G-25 column.

[00122] Adeno-associated virus serotype 8 (AAV8) and adeno-associated virus serotype 9 (AAV9) were labeled with ^{89}Zr -DBN as above. The *in vivo* biodistribution of intravenous delivered ^{89}Zr -DBN-AAV8 and ^{89}Zr -DBN-AAV9 was assessed by PET imaging and correlated with transfection profile by bio-luminescence imaging in a mouse model system. See Figures 13 and 14. Thus, Example 4 illustrates other ^{89}Zr based virus-labeling methods for PET imaging of viral trafficking.

[00123] List of abbreviations used herein:

$^{89}\text{Zr}(\text{HPO}_4)_2$: Zirconium hydrogen phosphate

AAV: adeno-associated virus

Alpha MEM: Alpha Modified Eagle's Medium

Anti-CD45: antibody against cluster of differentiation-45 antigen

DBN: desferrioxamine-NCS

DFO-Bz-NCS: Desferrioxamine-Benzyl-Sodium thiocyanate

DMEM: Dulbecco's Modified Eagle's Medium

DTPA: Diethylene triamine pentaacetic acid

FBS: Fetal Bovine Serum

FDG: ^{18}F -2-fluoro-2-deoxy-D-glucose

GFP: Green fluorescent protein

GM-CSF: Granulocyte-macrophage colony-stimulating factor

HBSS: Hank's Balanced Salt Solution

HEPES-KOH: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)-Potassium
5 hydroxide

HMPAO: hexamethylpropyleneamine oxime

hMSCs: human mesenchymal stem cells

IgG: immunoglobulin G

IgM: immunoglobulin M

10 iTLC: instant thin layer chromatography

K₂CO₃: Potassium carbonate

K₂HPO₄/KH₂PO₄: dipotassium hydrogen phosphate/potassium hydrogen phosphate

MBq: Mega Becquerel

mDCs: mouse derived dendritic cells

15 MIPs: maximal images projection

mMCs: mouse derived melanoma cells

PET: Positron Emission Tomography

PTSM: Pyruvaldehyde-bis(N4-methylthiosemicarbazone)

Rf: Retention factor

20 SPECT: single-photon emission computerized tomography

T_{1/2}: Half-life of radioisotope

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The citation of any document or reference is not to be construed as an admission that it is prior art with respect to the present invention.

[00125] Thus, the invention provides methods of *ex vivo* labeling of a biological material for *in vivo* imaging, methods of labeling a biological material *in vivo*, methods for preparing a labeling agent, and methods for *in vivo* imaging of a subject using a biological material labeled with a labeling agent. In one non-limiting example, the
5 biological material is selected from cells.

[00126] Although the invention has been described in considerable detail with reference to certain embodiments, one skilled in the art will appreciate that the present invention can be practiced by other than the described embodiments, which have been presented for purposes of illustration and not of limitation. Therefore, the
10 scope of the appended claims should not be limited to the description of the embodiments contained herein.

CLAIMS

What Is Claimed Is:

1. A method of *ex vivo* labeling of a cell for *in vivo* imaging, the method comprising:

contacting a cell *ex vivo* with a labeling agent such that the cell becomes labeled for *in vivo* imaging,

wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound.

2. A method of labeling a cell *in vivo*, the method comprising:
administering to a subject a labeling agent thereby labeling a cell *in vivo*,
wherein the labeling agent does not include an antibody, and
wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound.

3. A method of *ex vivo* labeling of a biological material for *in vivo* imaging, the method comprising:

contacting a biological material *ex vivo* with a labeling agent such that the biological material becomes labeled for *in vivo* imaging,

wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound, and

wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids.

4. A method of labeling a biological material *in vivo*, the method comprising:
administering to a subject a labeling agent thereby labeling a biological material *in vivo*,
wherein the labeling agent comprises a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound, and
wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.
5. The method of any of claims 1 to 4 wherein:
the chelating moiety is a hydroxamic acid group.
6. The method of claim 6 wherein:
the hydroxamic acid group is a desferrioxamine group.
7. The method of any of claims 1 to 4 wherein:
the conjugation moiety includes an isothiocyanate group.
8. The method of any of claims 1 to 4 wherein:
the conjugation moiety includes a benzyl group.
9. The method of any of claims 1 to 4 wherein:
the conjugation moiety includes a maleimide group.
10. The method of any of claims 1 to 4 wherein:
the conjugation moiety includes an NHS ester group.

11. The method of any of claims 1 to 4 wherein:
the labeling agent comprises a radionuclide, and
the radionuclide is selected from the group consisting of ^{11}C , ^{13}N , ^{15}O , ^{18}F , $^{34\text{m}}\text{Cl}$, ^{38}K , ^{45}Ti , ^{51}Mn , ^{52}Mn , $^{52\text{m}}\text{Mn}$, ^{52}Fe , ^{55}Co , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{66}Ga , ^{68}Ga , ^{71}As , ^{72}As , ^{74}As , ^{75}Br , ^{76}Br , ^{82}Rb , ^{86}Y , ^{89}Zr , ^{90}Nb , $^{94\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$, $^{110\text{m}}\text{In}$, ^{111}In , ^{118}Sb , ^{120}I , ^{121}I , ^{122}I , ^{123}I , and ^{124}I .
12. The method of any of claims 1 to 4 wherein:
the labeling agent comprises a radionuclide, and
the radionuclide is a positron emitter.
13. The method of claim 12 wherein:
the positron emitter is ^{89}Zr .
14. The method of any of claims 1 to 4 wherein:
the cells are selected from microorganisms, blood-derived cells, cancer cells, stem cells, and dendritic cells.
15. The method of any of claims 1 to 4 wherein:
the labeling agent is a ^{89}Zr -isothiocyanato-benzyl-desferrioxamine.
16. The method of any of claims 1 to 4 wherein:
the biological material is selected from stem cells.
17. The method of any of claims 1 to 4 wherein:
the biological material is selected from viruses.
18. The method of any of claims 1 to 4 wherein:
the labeling agent comprises a paramagnetic metal ion.

19. The method of claim 18 wherein:
the paramagnetic metal ion is selected from the group consisting of Gd^{+3} , Fe^{+3} , Mn^{+2} , and Y^{+3} .
20. The method of claim 18 wherein:
the chelating moiety is selected from the group consisting of diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and 1,4,7-triazacyclononane-triacetic acid (NOTA).
21. The method of any of claims 1 to 4 wherein:
the labeling agent comprises a paramagnetic compound, and
the paramagnetic compound includes one or more ^{19}F atoms.

22. A labeling agent comprising:
 a chelating moiety, a conjugation moiety, and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound,
 wherein the chelating moiety includes a hydroxamic acid group and the conjugation moiety includes an isothiocyanate group, and
 wherein the labeling agent is in a reactive form suitable for labeling a biological material selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids.

23. The labeling agent of claim 22 wherein:
 the hydroxamic acid group is a desferrioxamine group.

24. The labeling agent of claim 22 wherein:
 the conjugation moiety includes a benzyl group.

25. The labeling agent of claim 22 wherein:
 the labeling agent comprises a radionuclide, and
 the radionuclide is selected from the group consisting of ^{11}C , ^{13}N , ^{15}O , ^{18}F , $^{34\text{m}}\text{Cl}$, ^{38}K , ^{45}Ti , ^{51}Mn , ^{52}Mn , $^{52\text{m}}\text{Mn}$, ^{52}Fe , ^{55}Co , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{66}Ga , ^{68}Ga , ^{71}As , ^{72}As , ^{74}As , ^{75}Br , ^{76}Br , ^{82}Rb , ^{86}Y , ^{89}Zr , ^{90}Nb , $^{94\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$, $^{110\text{m}}\text{In}$, ^{111}In , ^{118}Sb , ^{120}I , ^{121}I , ^{122}I , ^{123}I , and ^{124}I .

26. The labeling agent of claim 22 wherein:
 the labeling agent comprises a radionuclide, and
 the radionuclide is a positron emitter.

27. The labeling agent of claim 22 wherein:
 the positron emitter is ^{89}Zr .

28. The labeling agent of claim 22 wherein:
the labeling agent comprises a paramagnetic metal ion.

29. The labeling agent of claim 28 wherein:
the paramagnetic metal ion is selected from the group consisting of Gd^{+3} , Fe^{+3} , Mn^{+2} , and Y^{+3} .

30. The labeling agent of claim 22 wherein:
the labeling agent comprises a paramagnetic compound, and
the paramagnetic compound includes one or more ^{19}F atoms.

31. The labeling agent of claim 22 wherein:
the biological material is selected from stem cells.

32. The labeling agent of claim 22 wherein:
the biological material is selected from viruses.

33. A method for preparing a labeling agent, the method comprising:
- (a) providing a compound including a chelating moiety and a conjugation moiety; and
 - (b) mixing the compound with a radionuclide to create the labeling agent before reacting the compound with an additional moiety,
- wherein the chelating moiety includes a hydroxamic acid group and the conjugation moiety includes an isothiocyanate group.
34. The method of claim 33, wherein the additional moiety is a lysine residue.
35. The method of claim 33, wherein the additional moiety is at least one of a liposome, DNA aptamer, RNA aptamer, a virus, nanoparticles, microorganisms, antibodies, proteins, peptides, scaffolds, polymers, and nucleic acids.
36. The method of claim 33, wherein the chelating moiety is desferrioxamine group.
37. The method of claim 36, wherein the conjugation moiety includes a benzyl group.
38. The method of claim 33, wherein the labeling agent is a ^{89}Zr -isothiocyanato-benzyl-desferrioxamine.
39. The method of claim 33, wherein the additional moiety is a stem cell.
40. The method of claim 33 wherein the additional moiety is a virus.

41. A method for *in vivo* imaging of a subject, the method comprising:

- (a) administering to the subject a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound;
- (b) waiting a time sufficient to allow the labeled cells to accumulate at a tissue site to be imaged; and
- (c) imaging the tissues with a non-invasive imaging technique, wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.

42. The method of claim 41 wherein:
the non-invasive imaging technique is selected from positron emission tomography imaging, positron emission tomography with computed tomography imaging, or positron emission tomography with magnetic resonance imaging.

43. A method of imaging a subject by emission tomography, the method comprising:

- (a) administering to the subject a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound;
- (b) using a plurality of detectors to detect gamma rays emitted from the subject and to communicate signals corresponding to the detected gamma rays; and
- (c) reconstructing from the signals a series of medical images of a region of interest of the subject, wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.

44. An imaging method comprising acquiring an image of a subject to whom a detectable amount of a biological material labeled with a labeling agent comprising a chelating moiety, a conjugation moiety and at least one of (i) a radionuclide and (ii) a paramagnetic metal ion or compound has been administered, wherein the biological material is selected from cells, liposomes, DNA aptamers, RNA aptamers, viruses, nanoparticles, microorganisms, proteins, peptides, scaffolds, polymers, and nucleic acids.

45. The method of claim 44, which comprises acquiring the image using positron emission tomography imaging, positron emission tomography with computed tomography imaging, or positron emission tomography with magnetic resonance imaging.

46. The method of any of claims 41-45 wherein:
the biological material is selected from cells.

47. The method of any of claims 41-45 wherein:
the chelating moiety is a hydroxamic acid group.

48. The method of any of claims 41-45 wherein:
the hydroxamic acid group is desferrioxamine group.

49. The method of any of claims 41-45 wherein:
the conjugation moiety includes an isothiocyanate group.

50. The method of any of claims 41-45 wherein:
the conjugation moiety includes a benzyl group.

51. The method of any of claims 41-45 wherein:
the conjugation moiety includes a maleimide group.

52. The method of any of claims 41-45 wherein:
the labeling agent comprises a radionuclide, and
the radionuclide is selected from the group consisting of ^{11}C , ^{13}N , ^{15}O , ^{18}F , $^{34\text{m}}\text{Cl}$, ^{38}K , ^{45}Ti , ^{51}Mn , ^{52}Mn , $^{52\text{m}}\text{Mn}$, ^{52}Fe , ^{55}Co , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{66}Ga , ^{68}Ga , ^{71}As , ^{72}As , ^{74}As , ^{75}Br , ^{76}Br , ^{82}Rb , ^{86}Y , ^{89}Zr , ^{90}Nb , $^{94\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$, $^{110\text{m}}\text{In}$, ^{111}In , ^{118}Sb , ^{120}I , ^{121}I , ^{122}I , ^{123}I , and ^{124}I .

53. The method of any of claims 41-45 wherein:
the labeling agent comprises a radionuclide, and
the radionuclide is a positron emitter.

54. The method of claim 53 wherein:
the positron emitter is ^{89}Zr .

55. The method of any of claims 41-45 wherein:
the labeling agent comprises a paramagnetic metal ion.

56. The method of claim 55 wherein:
the paramagnetic metal ion is selected from the group consisting of Gd^{+3} , Fe^{+3} , Mn^{+2} , and Y^{+3} .

57. The method of claim 55 wherein:
the chelating moiety is selected from the group consisting of
diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA),
1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and 1,4,7-triazacyclononane-triacetic acid (NOTA).

58. The method of any of claims 41-45 wherein:
the labeling agent comprises a paramagnetic compound, and
the paramagnetic compound includes one or more ^{19}F atoms.

60. The method of any of claims 41-45 wherein:
the cells are selected from microorganisms, blood-derived cells, cancer cells,
stem cells, and dendritic cells.

61. The method of any of claims 41-45 wherein:
the biological material is selected from stem cells.

62. The method of any of claims 41-45 wherein:
the biological material is selected from viruses.

63. The method of any of claims 41-45 wherein:
the labeling agent comprises a paramagnetic metal ion.

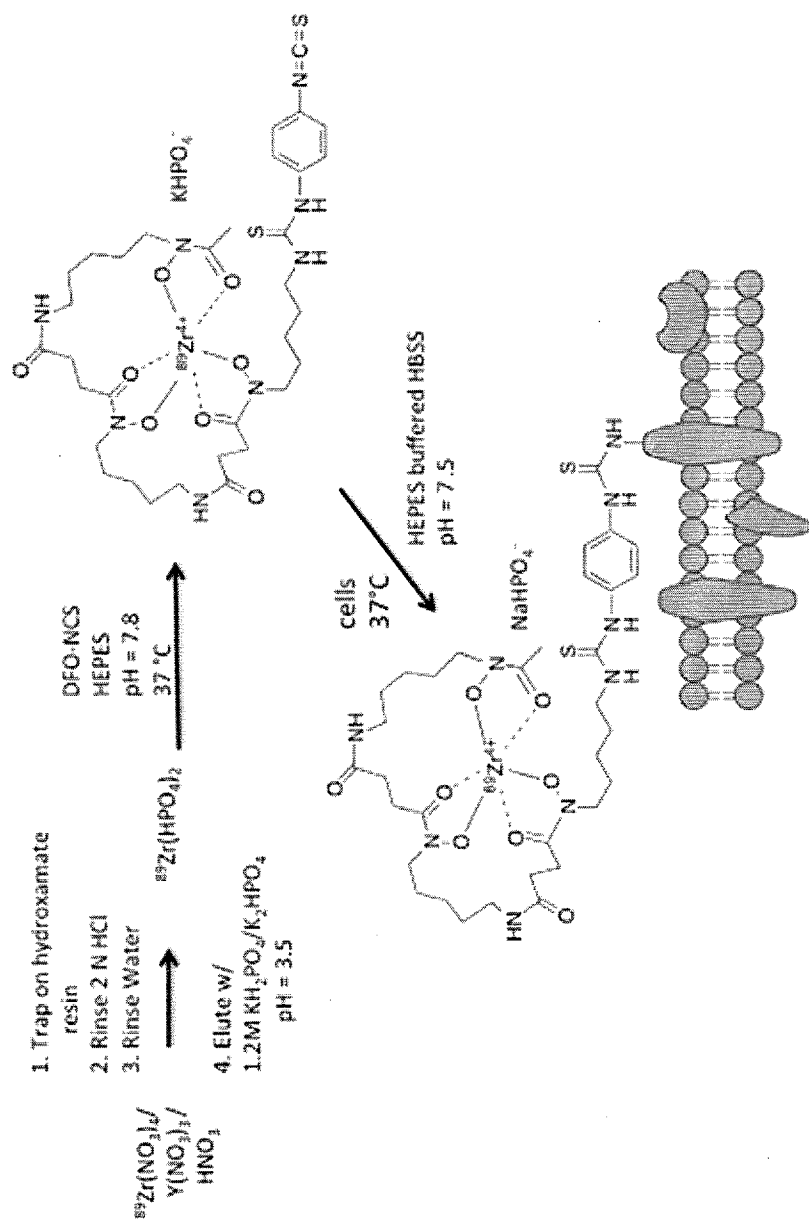


FIG. 1

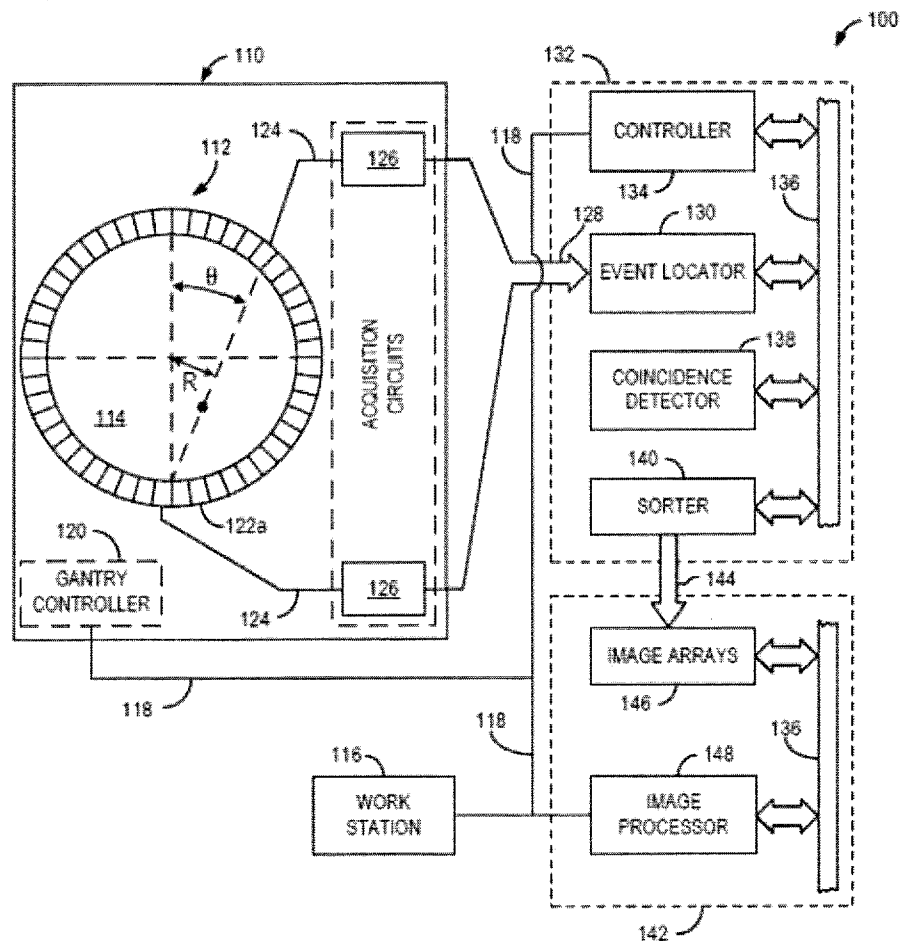


FIG. 2

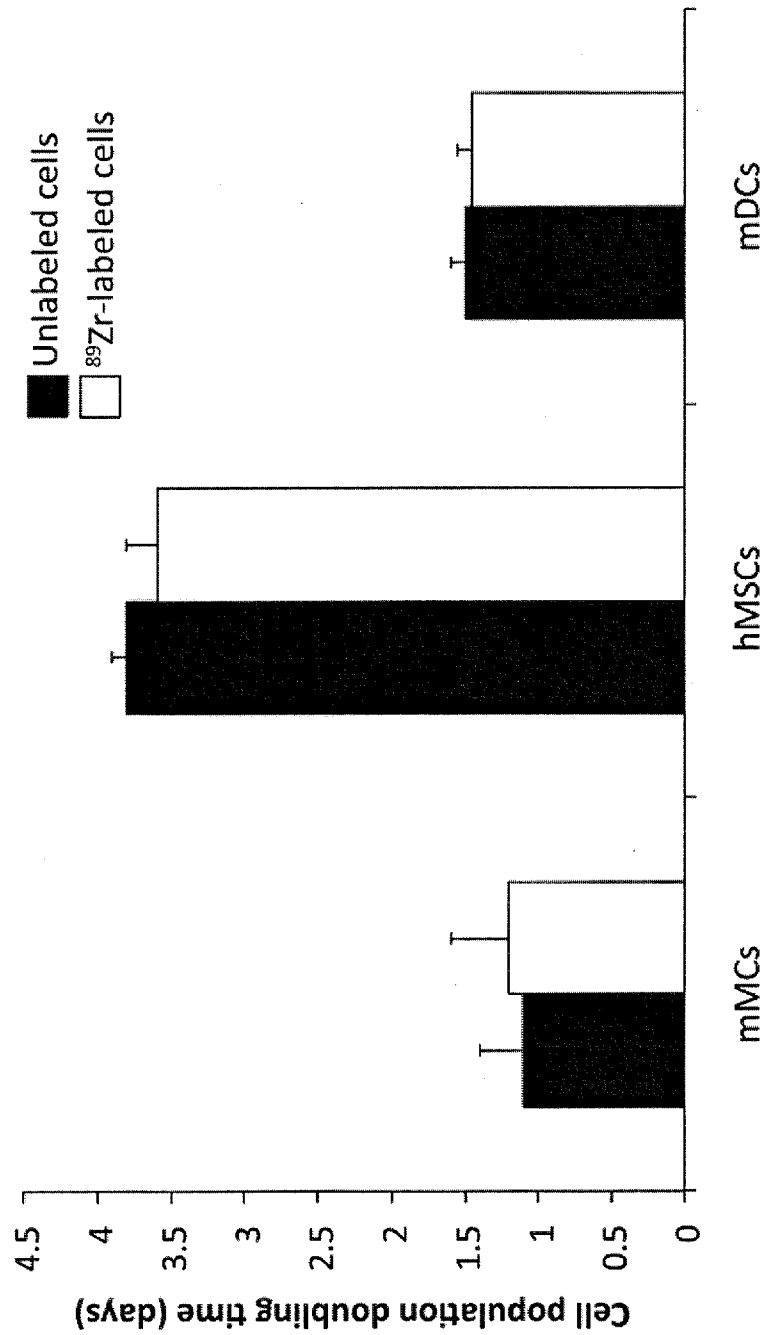


FIG. 3

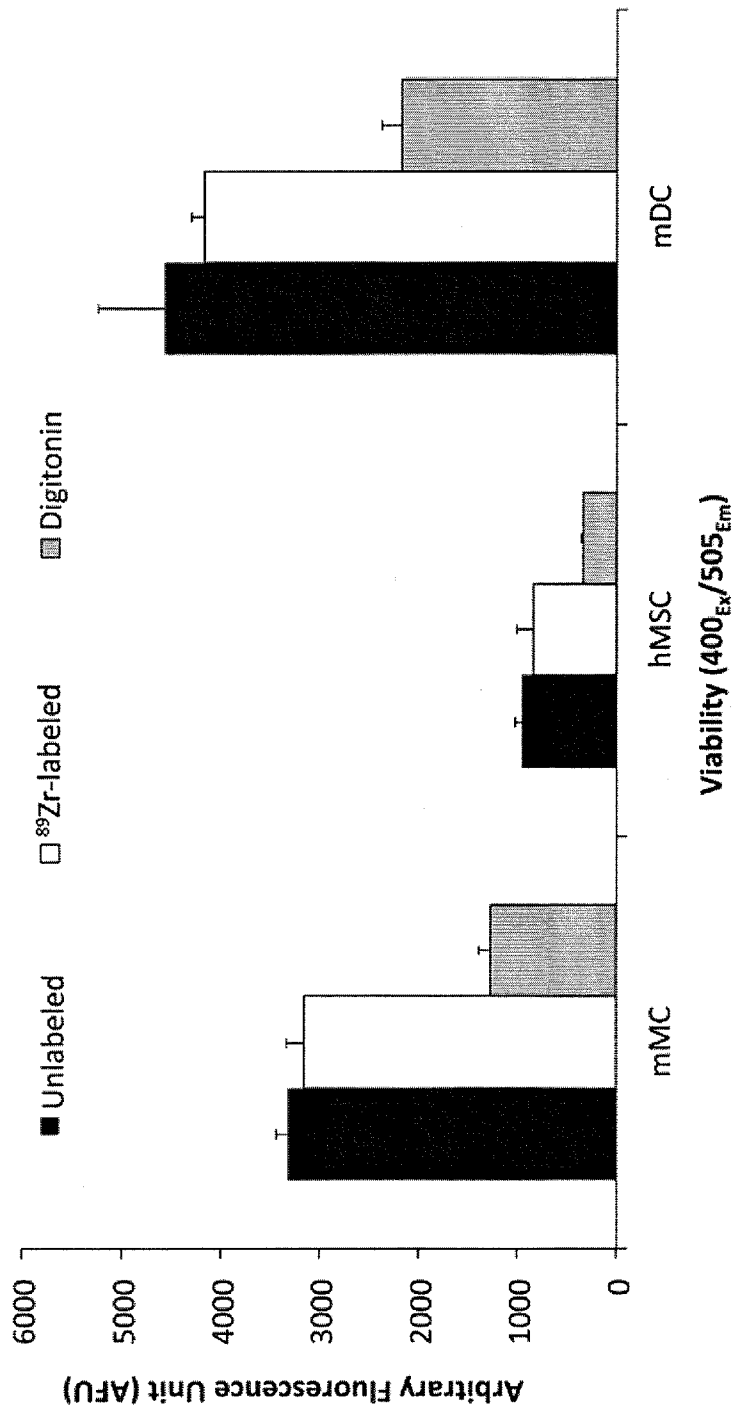


FIG. 4A

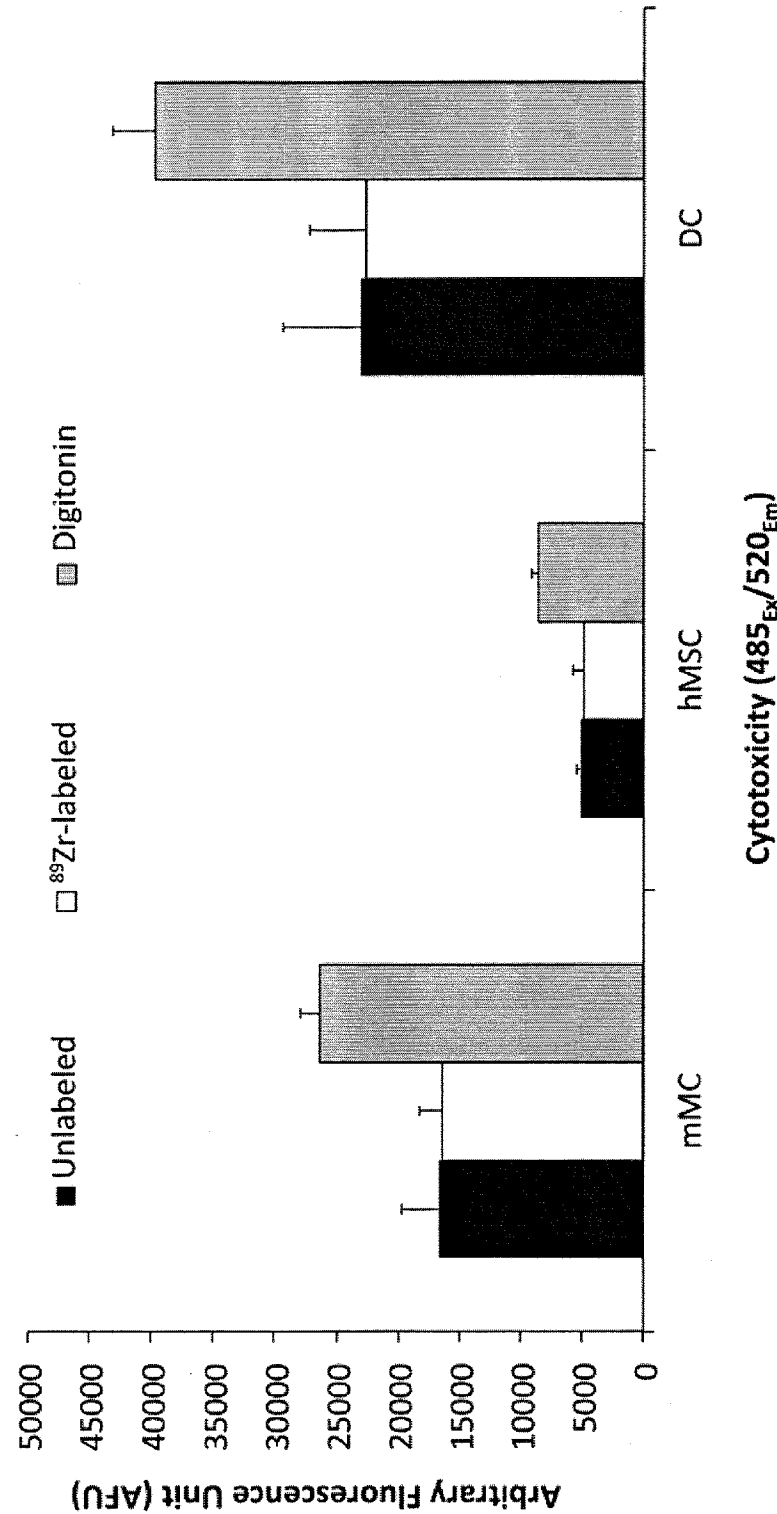


FIG. 4B

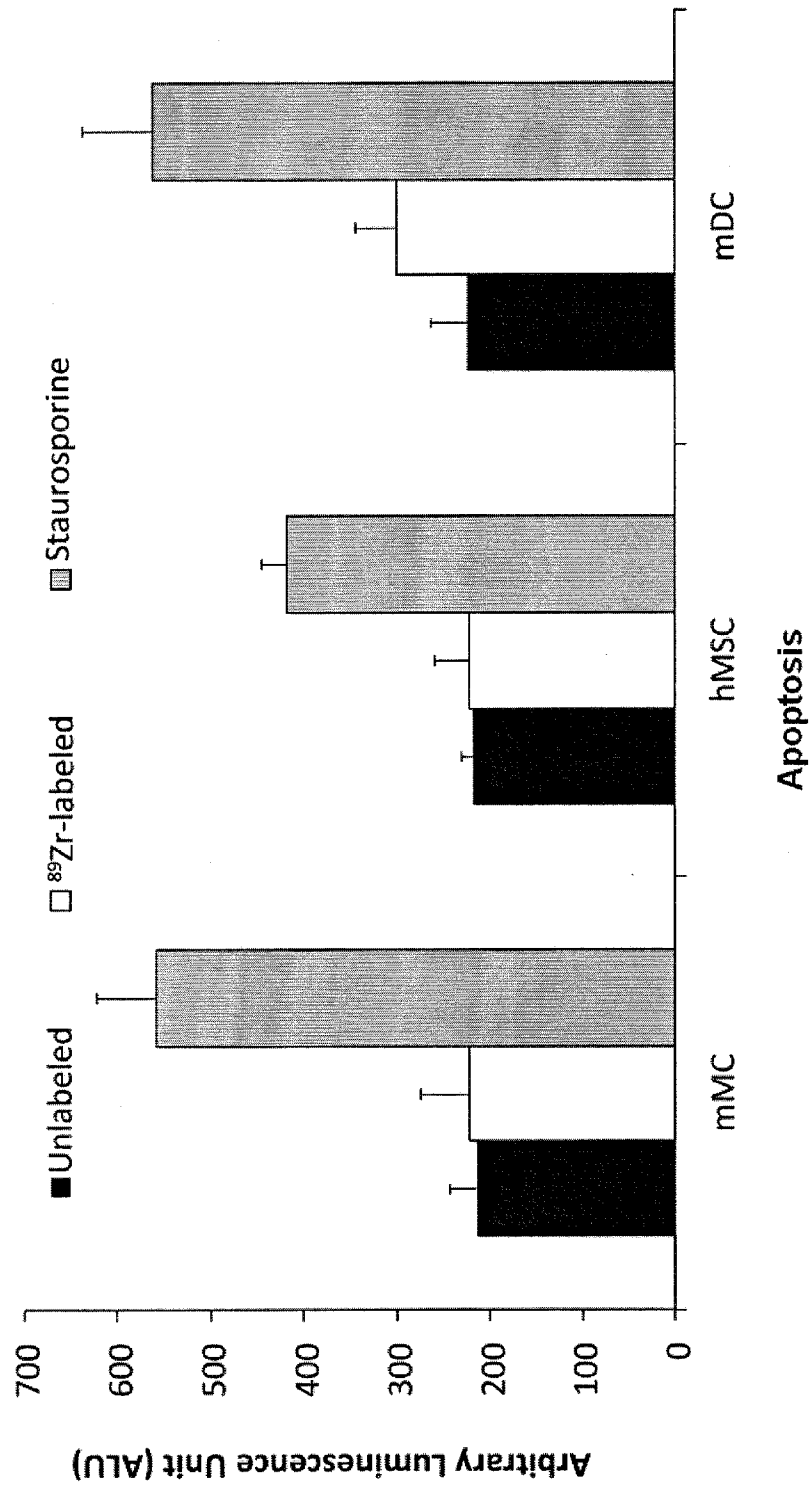


FIG. 4C

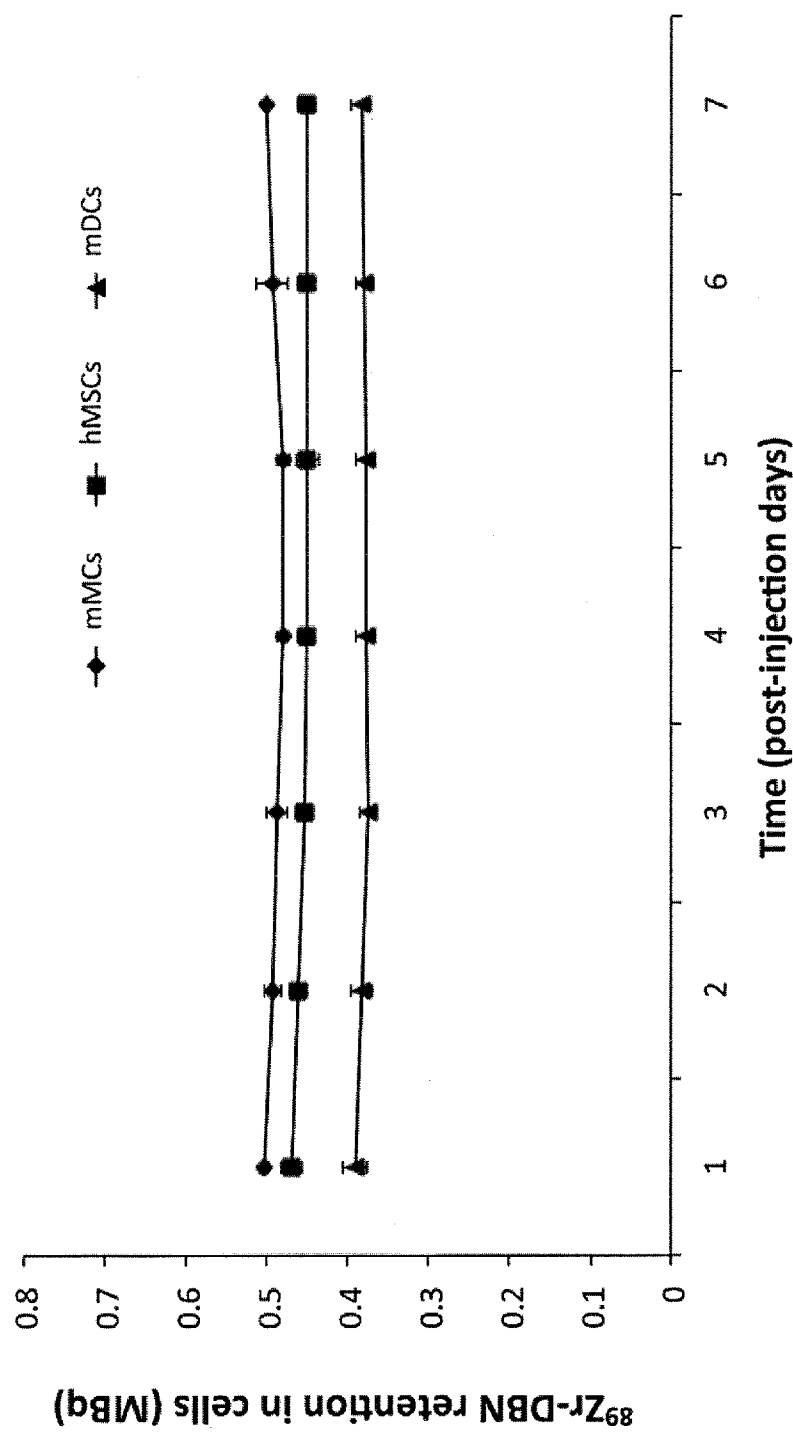


FIG. 5

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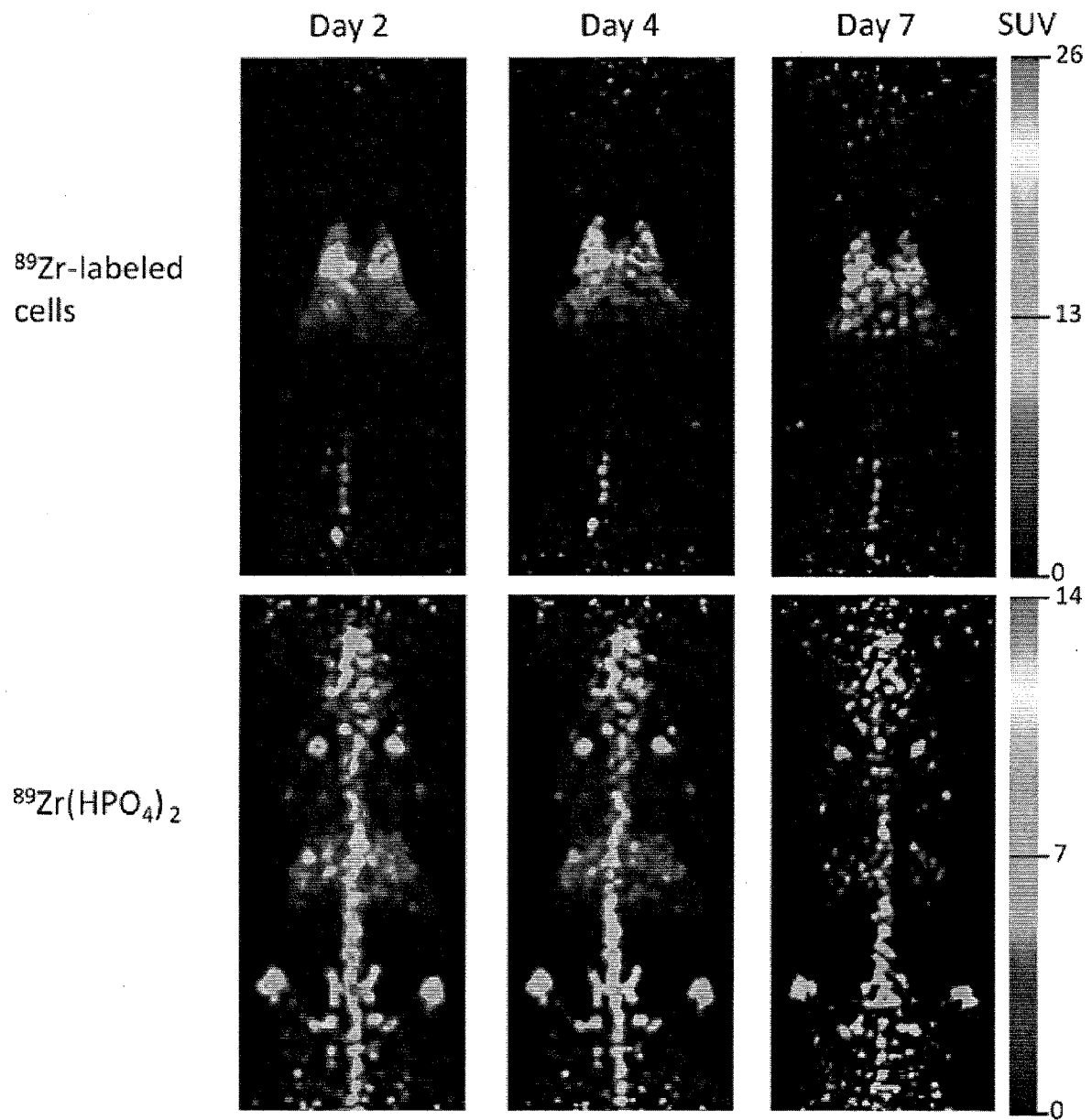


FIG. 6A

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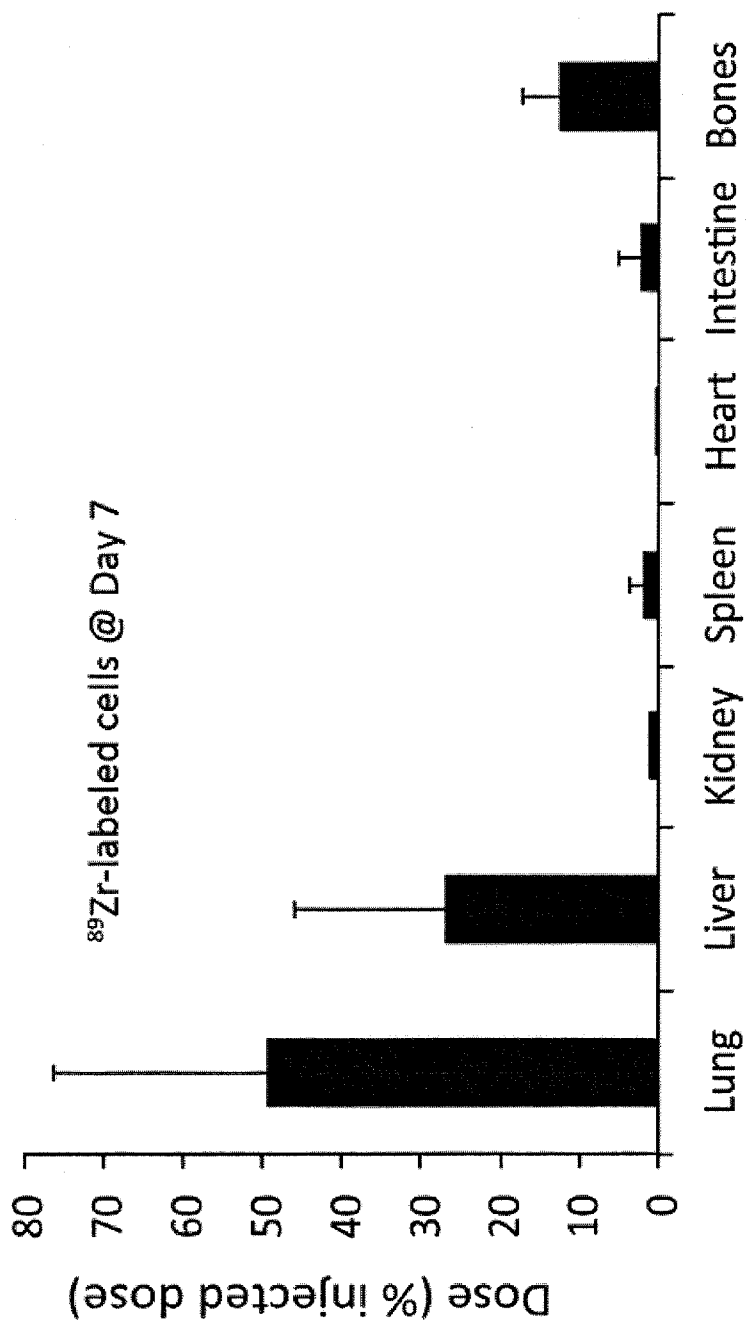


FIG.6B

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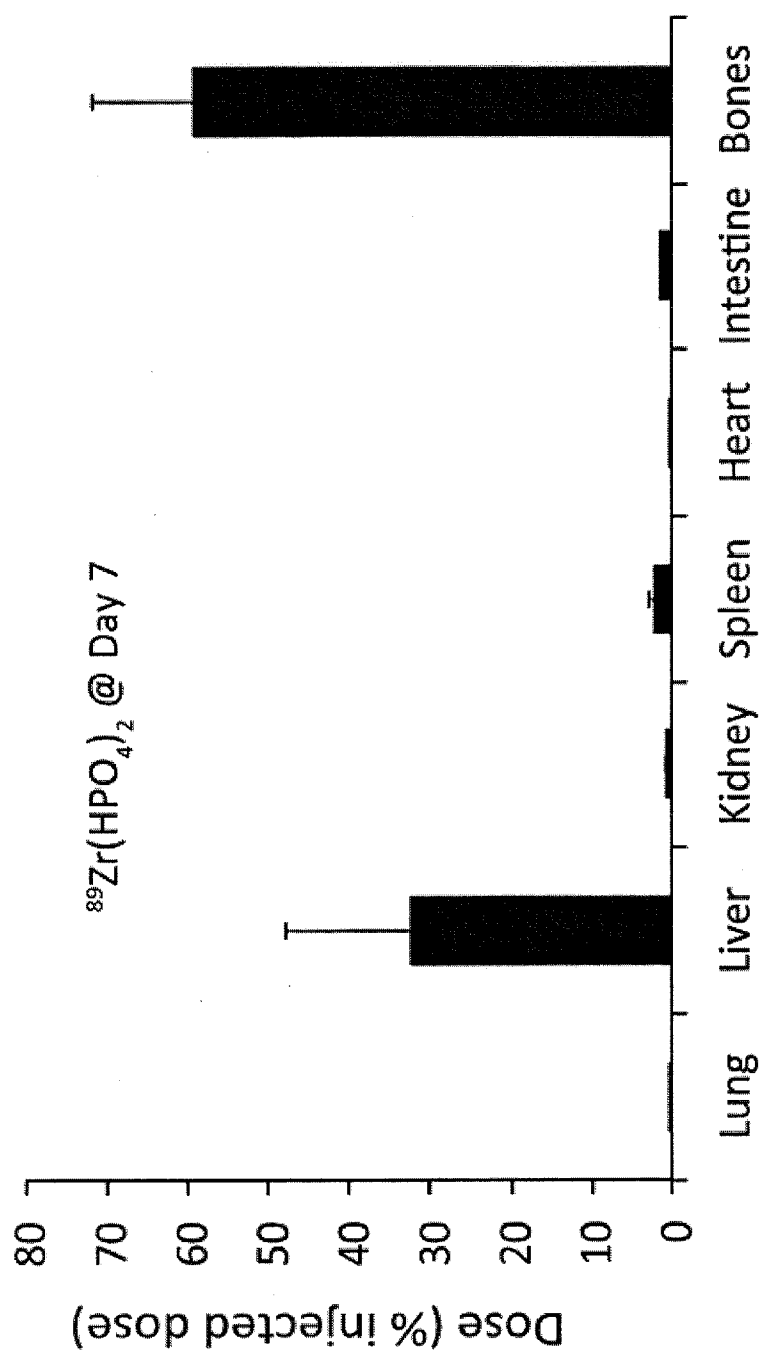


FIG. 6C

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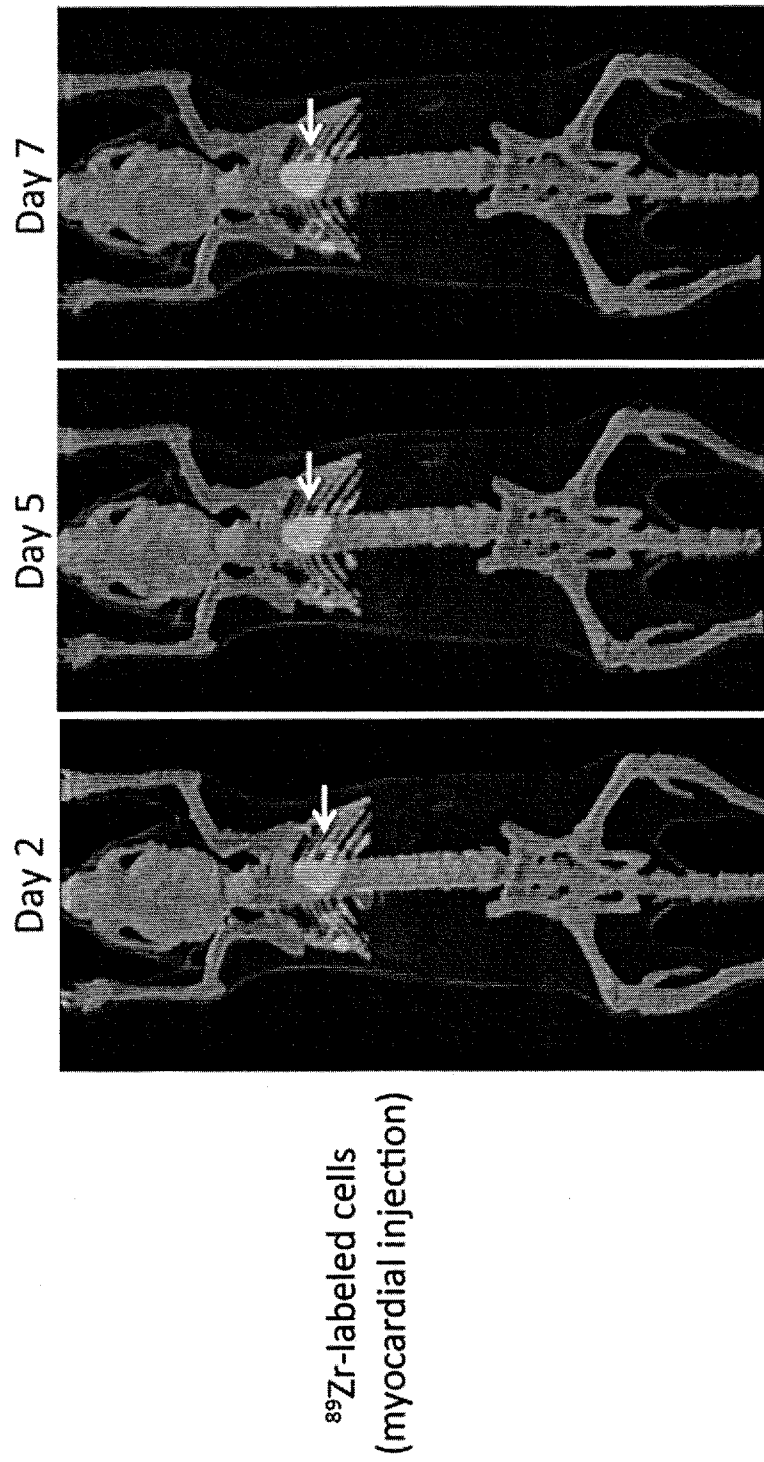


FIG. 7A

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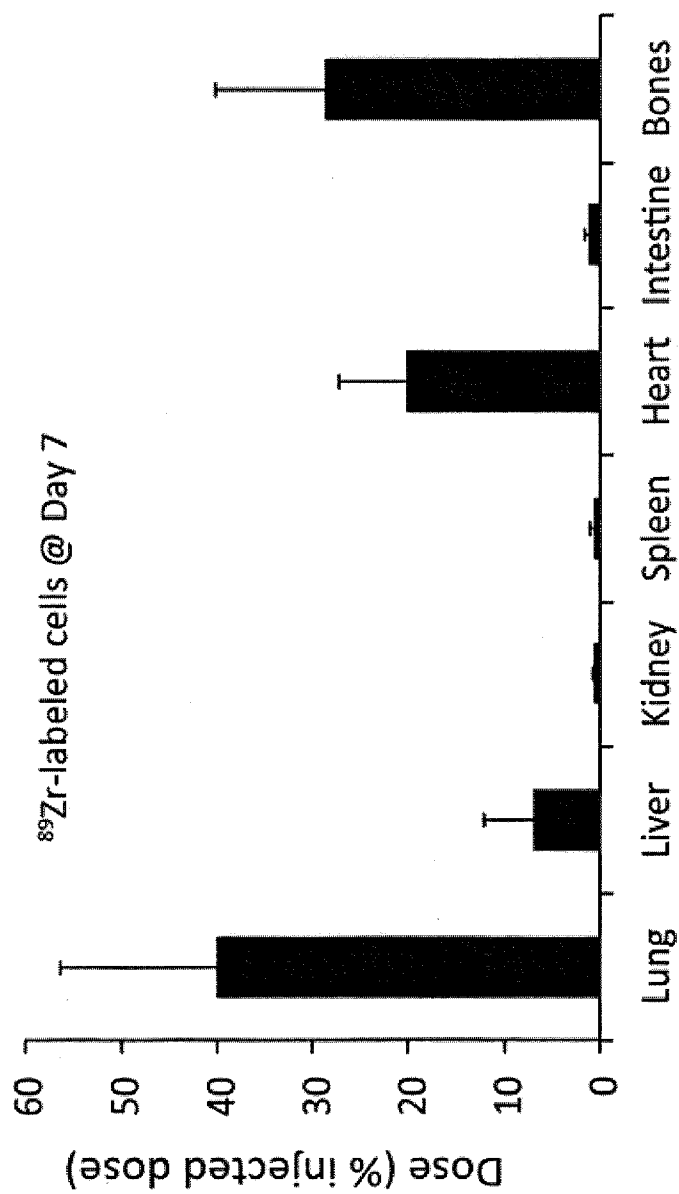


FIG. 7B

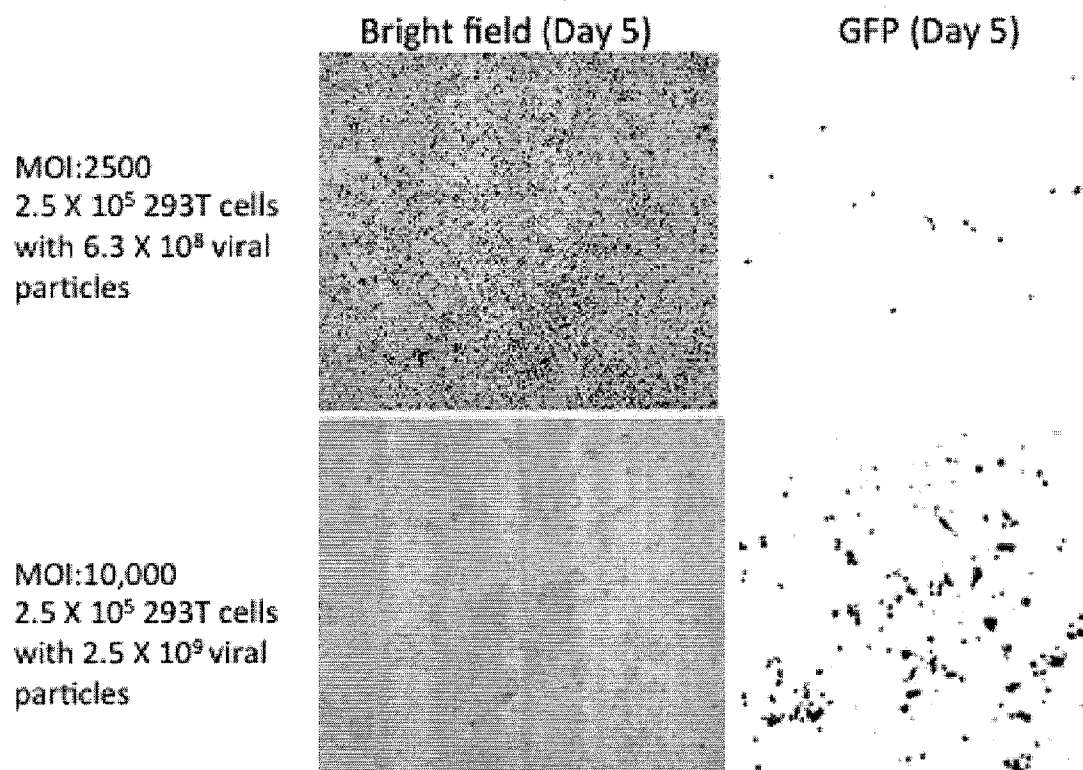


FIG. 8

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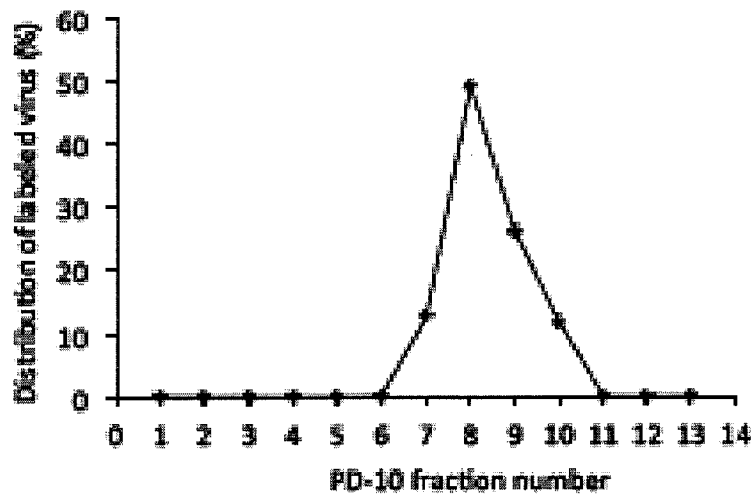


FIG. 9

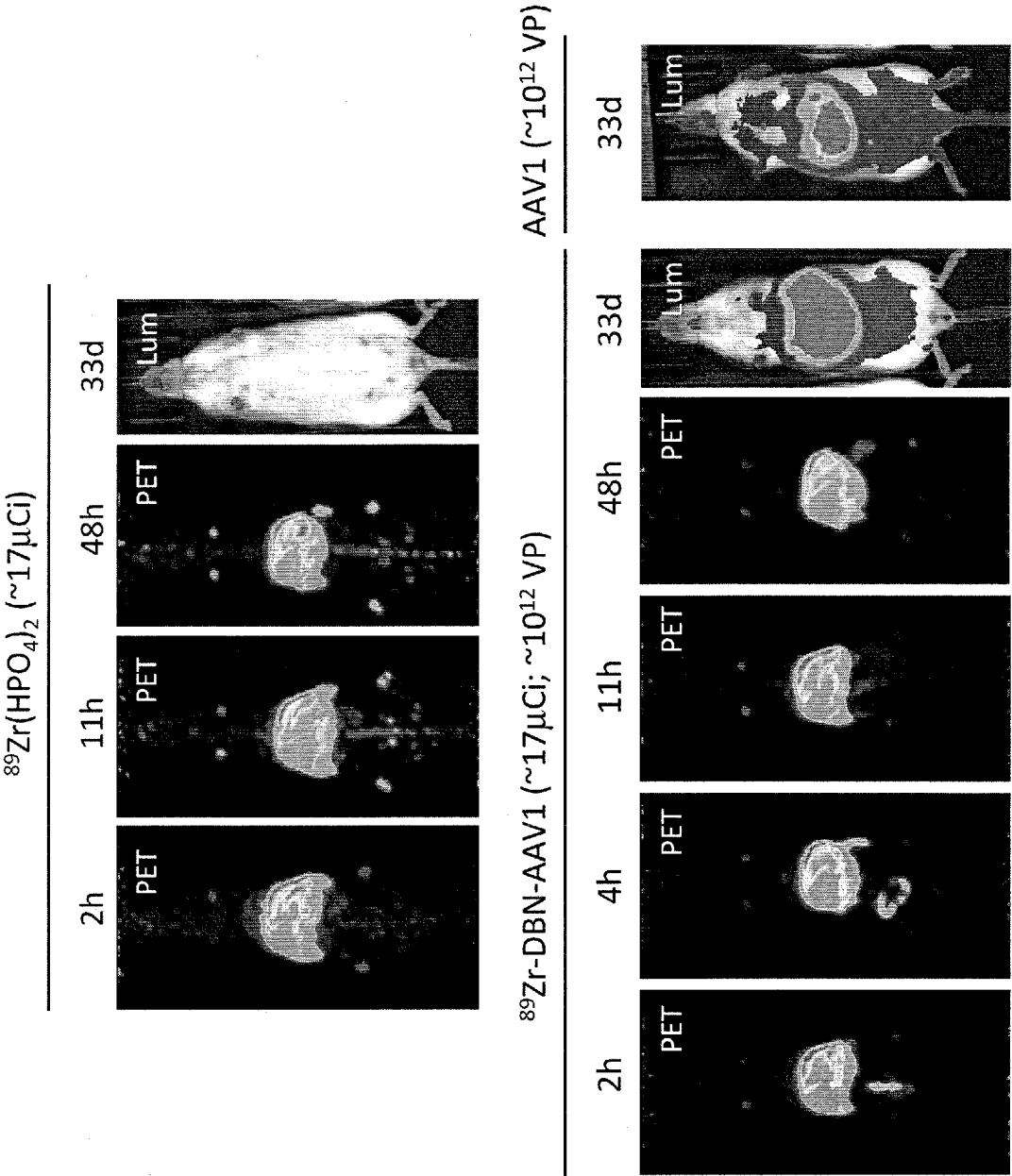


FIG. 10

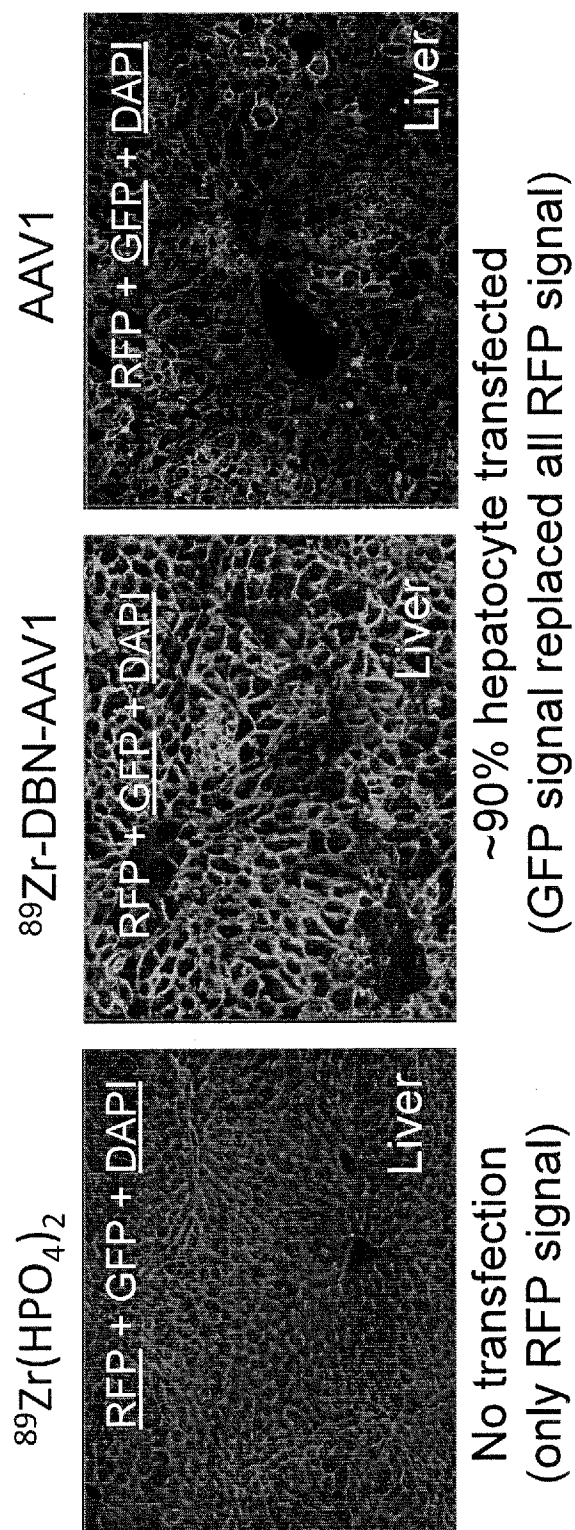


FIG. 11

FIG. 12A

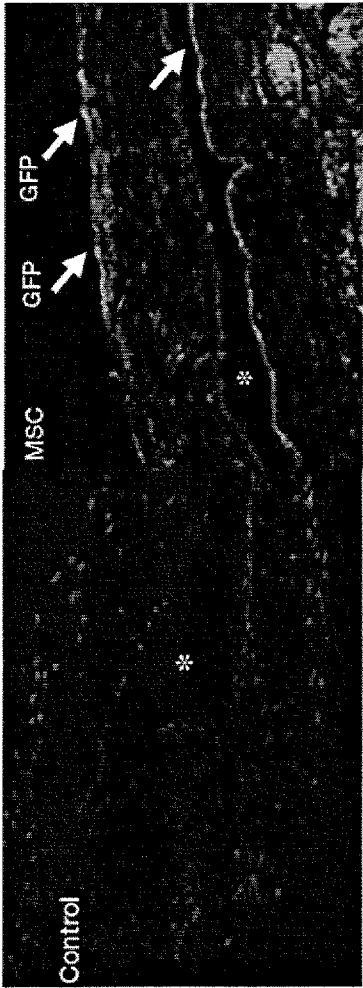
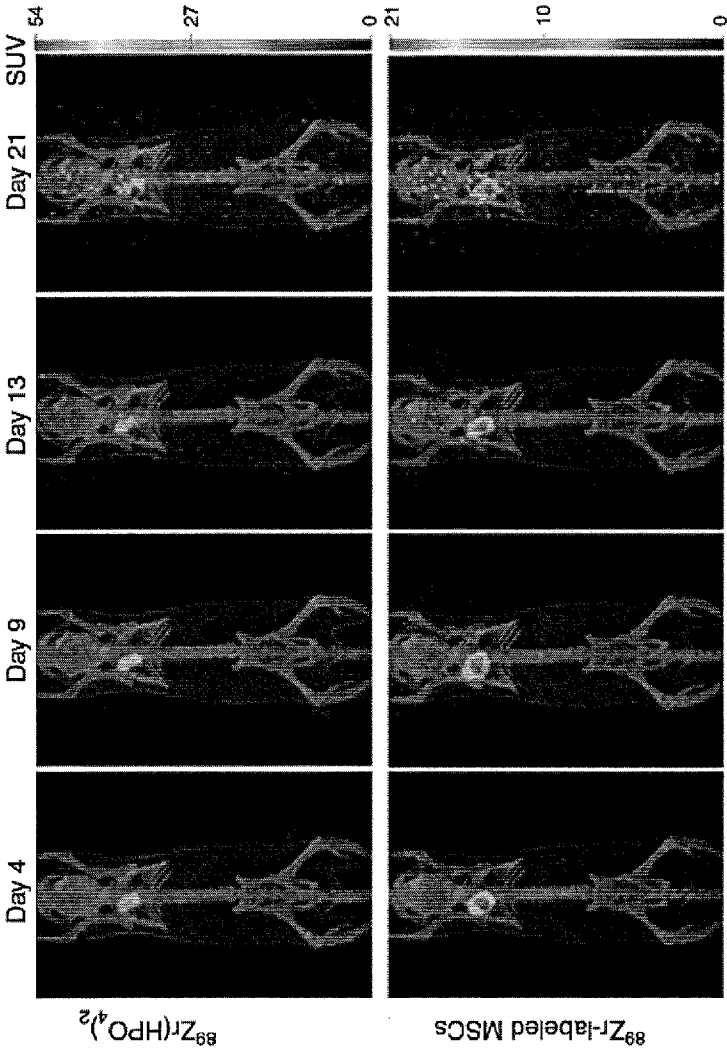


FIG. 12B



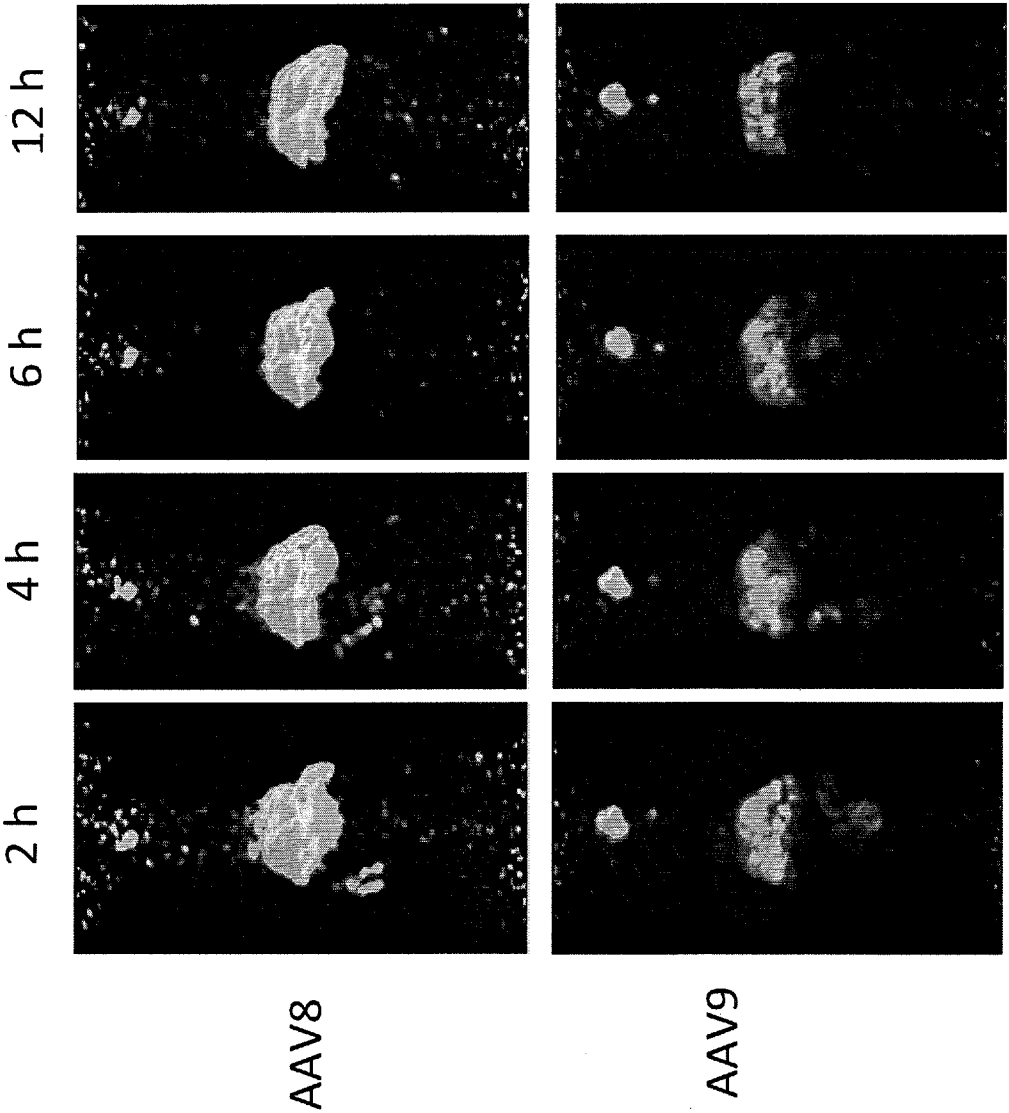


FIG. 13

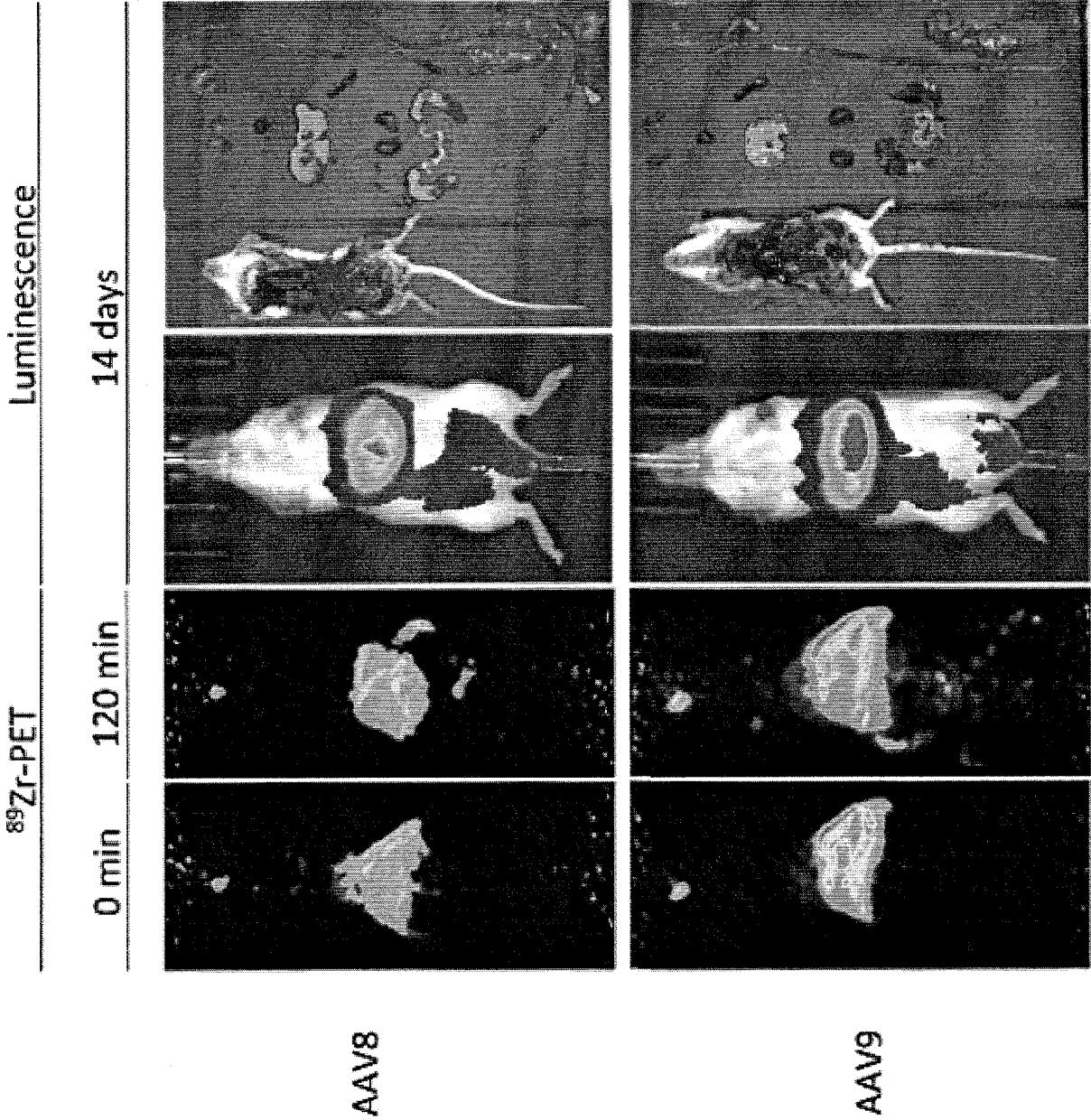


FIG. 14