Advanced Review

Cell tracking using gold nanoparticles and computed tomography imaging

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Cell-based therapies utilize transplantation of living cells with therapeutic traits to alleviate numerous diseases and disorders. The use of such biological agents is an attractive alternative for diseases that existing medicine cannot effectively treat. Although very promising, translating cell therapy to the clinic has proven to be challenging, due to inconsistent results in preclinical and clinical studies. To examine the underlying cause for these inconsistencies, it is crucial to noninvasively monitor the accuracy of cell injection, and cell survival and migration patterns. The combination of classical imaging techniques with cellular contrast agents—mainly nanotechnological-based—has enabled significant developments in cell-tracking methodologies. One novel methodology, based on computed tomography (CT) as an imaging modality and gold nanoparticles (AuNPs) as contrast agents, has recently gained interest for its clinical applicability and cost-effectiveness. Studies have shown that AuNPs can be used to efficiently label a variety of cell types, including stem cells and immune cells, without damaging their therapeutic efficacy. Successful *in vivo* experiments have demonstrated noninvasive, quantitative and longitudinal cell tracking with high sensitivity. This concept has the potential to be used not only as a research tool, but in clinical settings as well.

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INTRODUCTION

Cell-based therapies harness living cells as nature-made medicines for many diseases, and thus offer an attractive alternative to traditional therapeutics. Two main types of cells used as therapeutic agents are stem cells and immune cells. Stem cells, with their ability to home to sites of injury and inflammation and secrete therapeutic factors, have shown potential for use in the fields of regenerative medicine, cardiology, neurology, oncology and muscular regeneration.[1,2] Immune cells, such as T-cells and natural killer cells, serve as 'troops' of the immune system, and are usually genetically modified to target malignant cells[3,4] for cancer immunotherapy.

As promising as it may seem, translating cell therapy to the clinic has proven to be very challenging. The main obstacle to achieving a breakthrough in clinical translation is the inconsistency of outcomes seen in preclinical and clinical studies; while some patients exhibit major improvement, others exhibit minimal to no improvement.[5,6] These inconsistencies remain a puzzle, due to the lack of data on the fate of the injected cells. Currently, the only means for assessing the success or failure of treatment is by evaluating symptom improvement, which can only be done weeks after treatment. To address this challenge, the fate of the injected cells must be assessed in real time. This requires a reliable, noninvasive cell tracking and imaging technique, which can provide data on the functionality, viability and trafficking of the cells post injection.

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To answer this need, a range of imaging modalities combined with contrast or reporter agents have recently become available for imaging and tracking of cells. Each of these modalities has its advantages and limitations with respect to clinical safety, temporal and spatial resolution, anatomical details, and functional information.[7–12] Using optical imaging techniques, target cells are transfected with a reporter gene encoding the synthesis of either a fluorescent protein detectable with fluorescence imaging,[13,14] or a luciferase enzyme, detectable with bioluminescence imaging. Optical techniques have the advantage of being reliable, inexpensive and fast, but are limited to preclinical studies.[15] Additional modalities are positron-emission tomography (PET)[16] and single-photon emission tomography (SPECT),[17] which, with the use of radiolabeling, are clinically relevant and have excellent sensitivity, but are unsuitable for long-term cell tracking because of radioisotope decay.[18] In addition, radionuclide-based techniques are unable to provide anatomical imaging alone, and must be combined with anatomical imaging methods [i.e., computed tomography (CT) or magnetic resonance imaging (MRI)], which is highly complex and costly.

Nanoparticle-based imaging is a rapidly growing field in molecular imaging.[19] Nanoparticles, which serve as contrast agents, are used to label cells before injection into the body, and enable cell visualization within the body with various imaging modalities. MRI of nanoparticle-labeled cells has been broadly studied over the past decade.[8,20] More recently, the concept of CT imaging of nanoparticle-labeled cells has been introduced, and is being increasingly used in research.[21–26]

The use of biocompatible nanoparticles, combined with top imaging modalities with excellent resolution, has the potential to be broadly used in the clinic, enabling tracking of labeled cells over long periods of time, with simultaneous anatomical imaging.

CHALLENGES OF NANOPARTICLE-BASED CELL TRACKING

Nanoparticle-based cell tracking involves two steps: (a) *In vitro* labeling of therapeutic cells with nanoparticles, and (b) *in vivo* injection of the cells, followed by noninvasive imaging with the appropriate imaging modality.

Efficient cell labeling is the first key for successful imaging, and in the case of cell therapy, there are two competing requirements that need to be addressed. On the one hand, to achieve loading of a maximum amount of particles into each cell, and thereby attain maximum contrast, because signal intensity is usually proportional to the concentration of the contrast agent. On the other hand, the cells must maintain their biological function and viability, for maximal therapeutic effect.

The next challenge for reliable cell tracking appears after cells are injected and imaged in the body: namely, accurate analysis of the obtained images, to identify small clusters of cells, and differentiate the cells from the surrounding soft tissue. The ability to quantify the number of cells detected is another requirement for detailed evaluation of the fate of the transplanted cells. Finally, when performing longitudinal cell tracking studies, with images being taken days and weeks after treatment, a new question arises: do the nanoparticles being tracked remain inside the cells over time, or have they already been secreted from the cells, possibly due to cell death, or through other various mechanisms? It is further conceivable that released nanoparticles may be uptaken by macrophages or other endogenous cells, meaning that the endogenous cells are being tracked instead of the therapeutic cells. In this review, we will discuss these challenges using an emerging nanoparticle-based cell tracking method, in which gold-nanoparticles (AuNPs) serve as labeling agents and are tracked with CT as an imaging modality.

THE CONCEPT OF CELL TRACKING WITH AuNPs AND CT

CT is one of the most widely used imaging techniques in the clinic, due to its cost effectiveness, high spatial resolution, short scan time, and ease of imaging procedures (see Box 1). This makes CT a very important imaging modality and a potential interest for cell tracking, and induces the motivation to develop nanoparticle-based cell tracking using CT. However, the CT image contrast is derived from differences in x-ray attenuation by tissues, and the ability to distinguish between neighboring tissues can be problematic due to subtle differences in X-ray attenuation of many soft tissues. This leads to low sensitivity and limited soft tissue image contrast of CT,[27,28] and therefore, without an appropriate contrast agent, transplanted cells cannot be imaged with this modality.

The key factor for an effective CT contrast agent is use of materials with high atomic numbers—the higher the atomic number of the contrast agent,

BOX 1

COMPUTED TOMOGRAPHY IMAGING

Wilhelm Roentgen discovered X-ray radiation in 1895. Since then, X-ray imaging has become an important clinical diagnostic tool.[28] Decades later, in 1972, CT was invented by the British engineer Godfrey Hounsfield and the physicist Allan Cormack. 'Tomography' originates from the Greek word '*tomos*,' meaning slice or section, as the CT scan allows imaging of virtual 'slices' of specific areas of a scanned object. The term 'computed tomography' usually refers to the computation of tomography from two-dimensional X-ray images.

Using three-dimensional CT, X-ray projection images are obtained at many angles of view around an axis, through an object. A tomographic reconstruction algorithm is then applied to generate a stack of thin tomographic images of contiguous trans-axial slices through the object, thus providing a representation of the object's structure, including inner geometries. CT has become widely utilized in clinical diagnostic imaging, with an estimated 70 million CT scans performed annually in the USA.[29] CT has been progressively improved in speed, patient comfort, and resolution. CT scan times have considerably decreased, enabling scanning of more anatomical structures in less time and with fewer artifacts. Tremendous research and development has been conducted to provide excellent imaging quality for diagnostic confidence at the lowest possible X-ray dose.

Microscopic computed tomography (micro-CT) was first developed in the early 1980s and is widely used in preclinical studies. Live animals are usually positioned horizontally and scanned by rotating the X-ray source and its imaging array around a horizontal axis through the animal.[30]

BOX 2

APPLICATIONS OF AuNPs AS CONTRAST AGENTS FOR CT IMAGING

The introduction of AuNPs as contrast agents has expanded the role of CT imaging beyond that of mere structural imaging, to that of molecular and functional imaging as well. Several potential clinical applications have been recently demonstrated[27]:

Imaging cardiovascular conditions: Compared to the current clinically used iodine compounds, AuNPs achieve better CT contrast and extend blood circulation time, which permits longer imaging times. This is beneficial for vasculature and microvasculature imaging, in which the role of AuNPs is to enable sharp blood vessel delineation.[39–41] CT imaging with AuNPs has also been preclinically applied for staging of atherosclerotic plaques, which are critical factors in determining the risk of myocardial infarction and acute ischemic events.[42]

Cancer diagnostics: Appropriately sized nanoparticles passively accumulate in tumor tissues more readily than in normal surrounding tissues, due to the enhanced permeability and retention effect in tumors.[28] AuNPs can also be actively targeted to cancer cells and tumors by conjugation of antibodies, peptides, or other ligands onto the particle surface. This enables cancer diagnostics and early detection of small tumors with CT imaging.[43–48] In addition, glucose-coated AuNPs were effectively used for metabolic-based tumor imaging and diagnosis with CT.[49]

Neurodegenerative disorders: AuNPs have been suggested for imaging and therapy of neurodegenerative disorders, due to particle transport through the restrictive blood–brain barrier.[50]

Imaging lymphoid tissue: Targeted AuNPs that specifically bind to receptors on macrophages, T cells or other scavenger cells, can be transported by these cells to lymphoid tissue such as peripheral lymph nodes, and thus increase local CT contrast enhancement.[51]

the better the resultant CT contrast. This makes AuNPs ideal candidates for CT contrast agents, as the high atomic number of gold ($Z = 79$) can induce strong X-ray attenuation.[27] AuNPs have been widely researched in the past few years, for varied applications and potential clinical implementations. Substantial research has been conducted on their *in vivo* chemical stability, pharmacokinetics, biodistribution, and biotoxicity.[31–35] AuNPs are well-known for their biosafety,[36,37] along with their high density and high degree of flexibility in terms of particle size, shape and functional groups for coating and targeting. Altogether, these properties indicate AuNPs as the next generation of contrast agents for CT imaging, with a wide range of biological and clinical

applications (see Box 2). AuNPs are also appealing for both optical imaging and photothermal therapy, due to their unique optical properties.[27,38]

The novel concept of labeling cells with AuNPs, followed by *in vivo* injection and CT imaging, enables noninvasive tracking and visualizing of the therapeutic cells as they migrate and accumulate at sites of injury or malignancy (Figure 1). This concept was first introduced by Astolfo et al. several years ago,[52–57] and recently several other groups have applied this concept for tracking cells using different particle coatings, labeling techniques and *in vivo* cell tracking applications.[21–26]

HOW MUCH GOLD IS NEEDED TO ENABLE CT IMAGING OF CELLS?

As the signal obtained by CT is proportional to the AuNP concentration, a maximum amount of particles is needed within each cell to achieve maximum contrast. The effective dose for CT contrast agents is currently in the millimolar range, as compared to micromolar range sensitivity for MRI.[58,59] To overcome this lower sensitivity, a large amount of gold is needed for sufficient CT contrast.[60] AuNPs enable CT visualization with amounts of Au ranging from tens to hundreds of picogram Au per cell (Table 1). In fact, labeling cells with only 34 pg Au per cell enables CT imaging of malignant cells. It is important to note that CT enables imaging of clusters of cells rather than single cells, and in order to increase the sensitivity, in terms of the number of cells detected, a higher uptake of gold per cell is needed. This explains why in another study, stem cells were labeled with as much as 380 pg Au per cell (see Table 1). Another element that influences the amount of Au per cell is that different cell lines differ considerably in size.

AuNPs are internalized into cells by co-incubation. The process of gold uptake can be controlled by various factors. First, by AuNP properties, including size, shape, and surface functionality (coating). These properties can be controlled during chemical synthesis of the AuNPs, to tailor the particles for both cell uptake and imaging requirements. Chhour et al. performed a detailed study on the effect of size and chemical functionality on AuNP uptake by monocytes.[62] They synthesized a library of particles with various sizes and coatings, totaling 44 unique formulations and demonstrating how cell uptake can be significantly improved (Figure 2). They showed that 15 nm AuNP coated with short carboxylic acid ligands were taken up extensively as well as intermediate sizes of 50 and 75 nm AuNP that are coated with PCOOH. These results demonstrated that gold uptake can be controlled and is dependent on both size and surface functionality.

The labeling process can also be controlled by incubation time and particle concentration. A recent study found that the amount of AuNPs taken up by cells stabilizes after a short period of only 1 h.[22] However, many cell labeling protocols consist of longer incubation times (12–24 h; Table 1), perhaps due to differences in particle types and cell lines. Reducing the incubation time may have a positive impact on cell viability and function. Increasing AuNP incubation concentration, on the other hand, has a significant impact and can increase cellular uptake, with cell-dependent differences. Validation of successful uptake is performed by microscopy imaging (Figure 3) and analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS).

DO CELLS MAINTAIN THEIR FUNCTIONALITY POST LABELING?

In order to safely use AuNPs for cell tracking, it is necessary to validate that cell labeling is performed with minimal impairment of viability and functionality of cells. This was thoroughly investigated by Betzer et al., by loading AuNPs in three different cell lines, namely, mesenchymal stem cells, T-cells, and a squamous carcinoma cell line.[22] In all three cell types, minimal impairment of cell viability and functionality was found up to 3 days after loading, confirming that AuNPs can be safely used with therapeutic cells (Figure 4). Biocompatibility assays conducted in other studies also show minimal damage to cell viability and function after AuNP uptake (Table 1).

Another important indication of cell functionality is demonstration of a therapeutic effect *in vivo*. In a study on mice bearing human melanoma xenografts, AuNP-labeled T-cells were shown to accumulate at the tumor site, and to cause significant tumor regression.[21] In addition, treatment with AuNP-labeled stem cells was effective in alleviating symptoms in a genetic rat model for depression up to 21 days,[24] and up to 2 weeks in a mouse model of Duchenne muscular dystrophy.[23,24] These findings indicate that AuNP labeling can be effectively and safely used for immunotherapy and stem cell therapy.

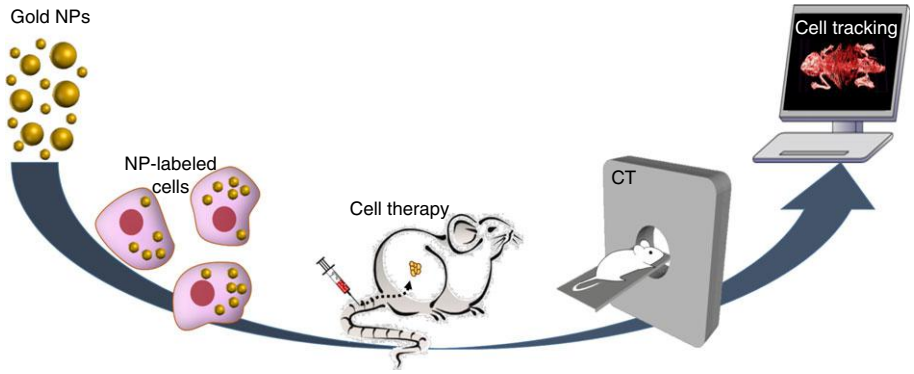

FIGURE 1 | Noninvasive cell tracking by gold nanoparticle (AuNP) labeling. The cells are first labeled with AuNPs *in vitro*, then intravenously injected to the subject. *In vivo* computed tomography (CT) imaging enables real time, noninvasive cell tracking.

TABLE 1 | Summary of Cell Labeling Studies with Different AuNPs

Size (nm)	Coating	Cell Type	Incubation Time (h)	Concentration (mg/mL)	Au uptake (pg/cell)	Biocompatibility Assays
20[21]	Glucose	T cells	1	0.75	195	Viability, proliferation and function (cytokine release)
15[25]	11-Mercapto-undecanoic acid	Monocytes	24	0.5	127	Viability and function (cytokine production)
20[23,24]	Glucose	hMSCs	3	0.3	89	Viability, metabolism and proliferation
40[26]	Poly-L-lysine	hMSCs	12	0.1	380	Viability and differentiation
50[61]	Horse serum	Malignant	22	0.05	34	Proliferation

hMSCs, human mesenchymal stem cells.

IT'S ALL ABOUT BALANCE: FINDING AN OPTIMAL LABELING METHOD

Altogether, the above findings indicate that the key to efficient cell labeling is by achieving a balance between high AuNP uptake for maximum contrast, and minimal interference with cell functionality. This balance is controlled by several crucial parameters, which need to be optimized in each individual experimental system and for each cell type. As summarized

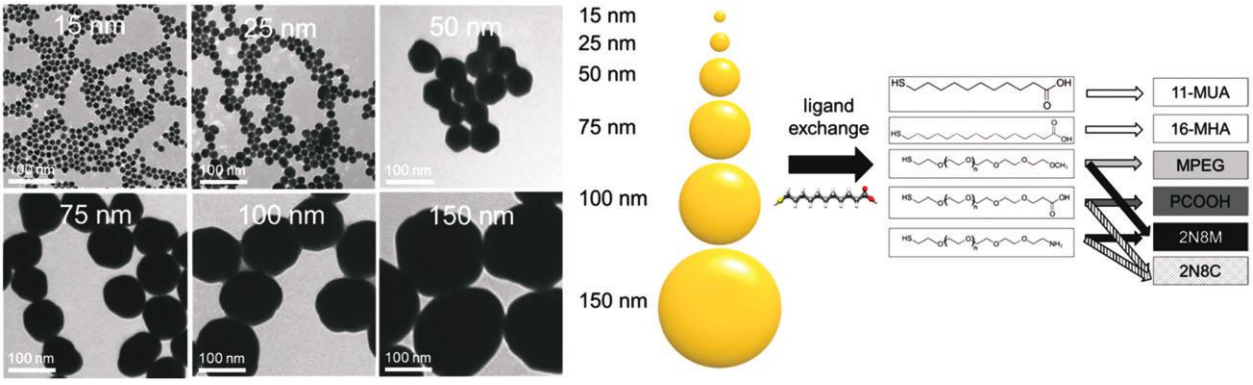

FIGURE 2 | Designing of gold nanoparticles of different sizes and coatings for cell labeling. Size, as well as coating of gold nanoparticles (AuNPs), affects their cell labeling abilities. Left: Transmission electron microscopy (TEM) images of spherical AuNPs of increasing size, from 15 to 150 nm. Right: Schematic depiction of the range of AuNP sizes used in the study, and the chemical structures of the ligands used as coatings. Ligands examined represent different functionalities and charges.[62] (Reprinted with permission from Ref 62. Copyright 2017 American Chemical Society)

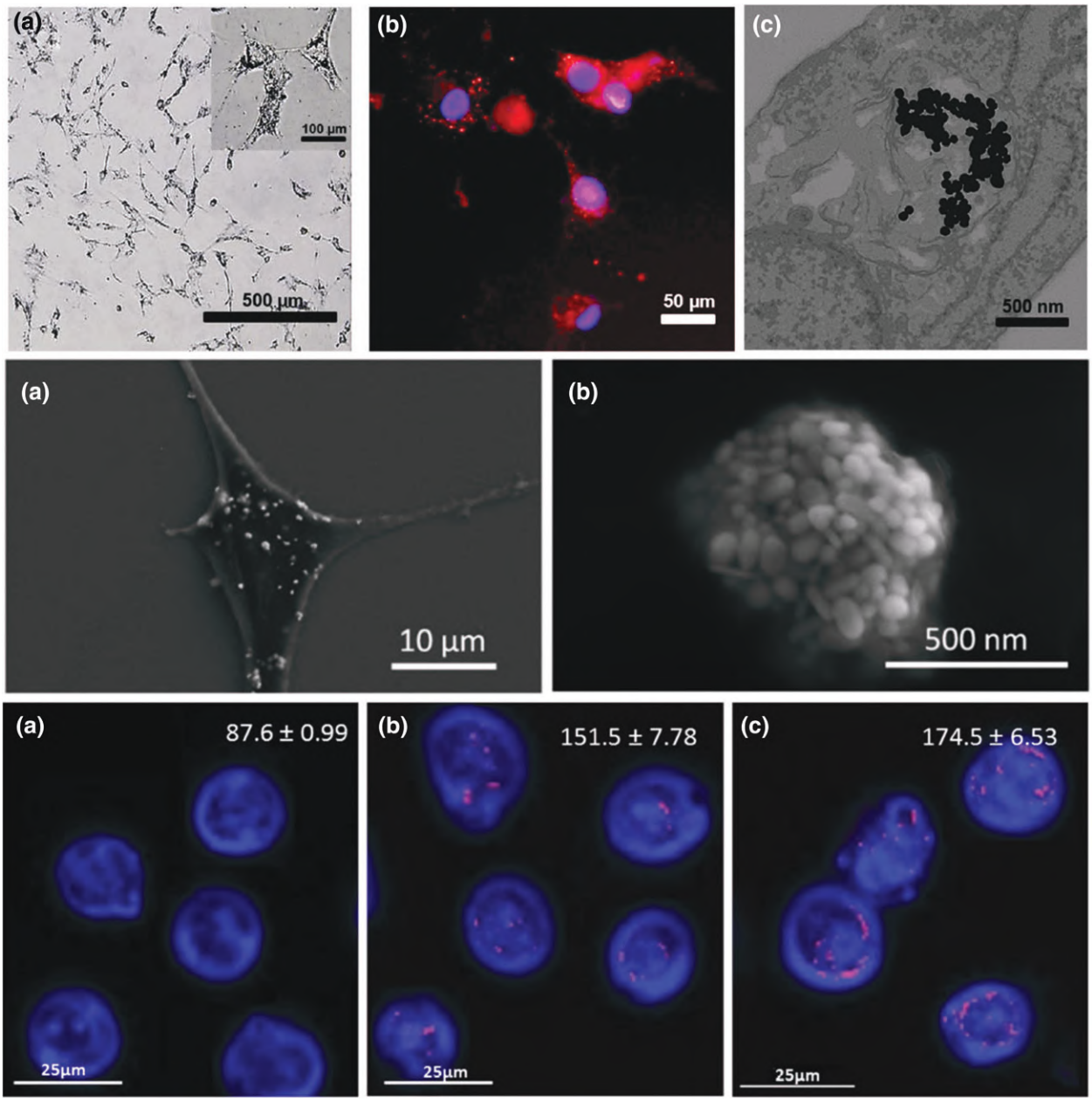

FIGURE 3 | Microscopy images of cells post labeling with gold nanoparticles (AuNPs). Top: Intracellular uptake of AuNP complexed with poly-l-lysine and rhodamine B isothiocyanate (RITC) in human mesenchymal stem cells (MSCs). Shown are (a) bright field, (b) fluorescence (blue = DAPI, red = RITC), and (c) TEM images.[26] Center: (a) SEM image of a labeled C6 cancer cell, and b) zoom in of a cluster of AuNPs inside the cell.[61] Bottom: (a)–(c) Dark field microscopy of A-431 cancer cell line (blue) labeled with increasing concentrations of AuNPs.[22] (Top: Reprinted with permission from Ref 26. Copyright 2017 John Wiley and Sons. Center: Reprinted with permission from Ref 61. Copyright 2013 Royal Society of Chemistry. Bottom: Reprinted with permission from Ref 22. Copyright 2015 Nature Publishing Group)

in Figure 5, these parameters include particle design in terms of size, shape, and coating, and the labeling protocol in terms of incubation time and concentration. Controlling the various parameters generates an optimal labeling system that combines maximum CT contrast with sustained cell viability and function.

CAN SMALL CLUSTERS OF CELLS BE DETECTED AND QUANTIFIED *IN VIVO*?

A major advantage of CT cell tracking is the ability to quantify the number of cells imaged, because the

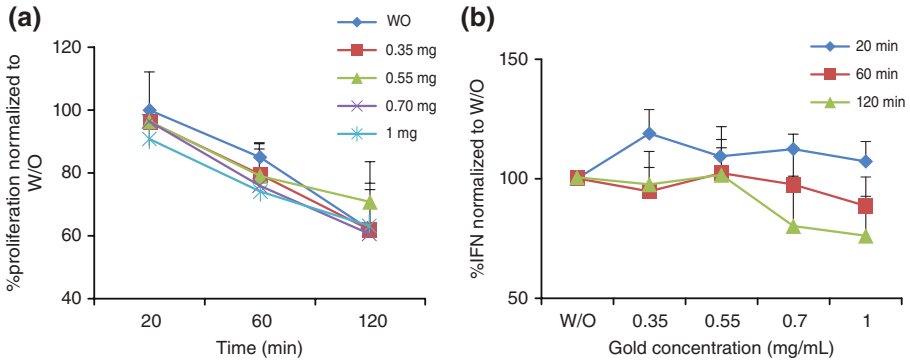

FIGURE 4 | Cell proliferation and functionality assays for T-cells. (a) Proliferation assay with carboxyfluorescein succinimidyl ester (CFSE). CFSE-labeled T-cells loaded with increasing AuNP concentrations (0.35, 0.55, 0.70, and 1 mg/mL), stimulated for 3 days and analyzed for CFSE dilution. Data shown as average percentage of proliferative cells, normalized to control [cells without AuNPs (W/O)] ± SEM. No significant differences were observed between cells loaded with the different amounts of AuNPs and controls ($p > 0.05$). (b) Functionality assay. T-cells loaded with increasing AuNP concentrations (0.35, 0.55, 0.70, and 1 mg/mL), co-cultured with a positive target tumor cell line (888-A2) for 120 min. IFN-γ secretion (measured by ELISA) was normalized to control [cells w/o AuNPs (W/O)] ± SEM ($n = 3$) ($p < 0.05$, Student's paired t-test). Cell-function was impaired only for high gold concentrations (0.75, 1 mg/mL), after 120 min of incubation. (Reprinted with permission from Ref 22. Copyright 2015 Nature Publishing Group)

attenuated CT contrast is linear to the total amount of Au.[26] The potential of combining AuNPs and CT for imaging small cell clusters was first demonstrated *in vivo* in mouse brain, for approximately 1700 cells.[52,57] Several additional studies have been carried out over recent years in animal models, showing that small clusters of AuNP-labeled cells can be clearly visualized *in vivo*, with high CT contrast. Sensitivity in terms of the number of detected cells varies between studies, due to different labeling protocols, administration modes, and imaging parameters, as presented in Table 2. Nonetheless, these studies clearly show that AuNPs function as appropriate contrast agents with a low detection limit of several hundreds of cells.

Kim et al. were able to image stem cells in a rat brain using CT, with a detection limit of 2×10^4 cells per μL *in vivo*,[26] and showed a linear correlation between the number of cells and the signal intensity obtained. In another study, a CT quantitative ruler was established, which extrapolates the exact number of cells within each brain region in a

FIGURE 5 | The key to efficient cell labeling. Achieving a balance between high nanoparticle uptake for maximum contrast while concurrently maintaining cell viability and function. This balance is controlled by five different factors: Particle size, shape and coating, loading time and concentration.

TABLE 2 | Overview of AuNP-labeled Cell Tracking Experiments *In Vivo*

Cell type	Administration Mode	Model	# of Cells Injected	Time Points	Main Results	Sensitivity	Validation Method
T cells[21]	Intravenous	Melanoma-bearing mouse	$15*10^6$	Days 1, 2, 3, and 5	Distribution, migration, and kinetics of T-cells. T-cells accumulated at tumor site. Tumor regression was observed.	NR	Fluorescence imaging
Monocytes[25]	Intravenous	Atherosclerosis mouse model	$1*10^6$	Days 3, 4, and 5	Visualizing labeled monocyte recruitment into atherosclerotic plaque, as seen by progressive CT signal attenuation in the aorta.	15 cells per voxel	*Ex vivo* TEM
MSCs[23]	Intramuscular	Muscular dystrophy mouse model	$5*10^2$–$1*10^6$	3 days to 4 weeks	Cells imaged over 4 weeks. Small numbers of cells were detected and quantified. Therapeutic effect was observed.	500 cells	NR
MSCs[26]	Intracerebral (striatum)	Sprague–Dawley rat	$2*10^4$–$5*10^5$	30 min	Cells were detected with micro-CT. Attenuated CT contrast was linear to the total amount of Au aggregated inside cells or free in solution.	20,000 cells per μL	Immune-fluorescence microscopy
MSCs[24]	Intracerebro-ventricular	Genetic rat model for depression	$2*10^5$	1 day to 1 month	Cell migration to distinct brain regions detected at 24 h and up to 1 month. Number of cells residing in specific brain regions was quantified. Long-term therapeutic effect was observed.	13 cells per voxel	Immunohistochemistry
Glioblastoma-like[57]	Intracerebral	Athymic nude mouse	$2*10^5$	8 days	Cells were localized and tracked with synchrotron CT.	1700 cells	MRI imaging

CT, computed tomography; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; NR, nonreported.

nondestructive manner.[24] This capability is important for cell tracking within the brain and periphery, because it allows quantitative tracing of small numbers of cells, their migration to sites of injury, and whole-body biodistribution. Although such quantification can also be achieved with methods such as fluorine nanoparticles in ^{19}F MRI,[63] it cannot be performed with standard MRI cell tracking using iron-oxide contrast agents, because MRI quantification of iron is unreliable, and there is no clear correlation between the iron-oxide signal and the number of cells.[64]

DOES THE ROUTE OF ADMINISTRATION AFFECT CELL TRACKING ABILITIES?

The route of administration affects cell biodistribution, and consequently it is a major experimental factor affecting cell imaging and tracking capabilities. Stem cells can be implanted *in vivo* via either systemic or local administration, depending on the therapeutic goal. Systemic administration, including intravenous (IV; the most common approach) or intraperitoneal (IP) delivery, can mimic the course of endogenous stem cells in the circulation, with final homing to target sites. Local administration by intra-organ infusion is the most efficient route for cell homing, and can produce immediate local action in the injured tissue.[65] It is unclear whether systemically injected stem cells can actively cross the blood–brain barrier to home to injured regions[66]; therefore, for treatment of brain disorders, stem cells can be intracerebroventricularly injected and migrate to the damaged brain region, or injected directly into the injured brain area.[67] Immune cells are most commonly administrated by IV injection. Following local administration, the cells remain in the injected cavity, yielding a strong signal from small clusters of cells; however, following IV injection, the cells are diluted in the blood, which weakens the obtained signal and makes cell tracking more challenging. Nonetheless, we have previously demonstrated that immune cell migration can be successfully tracked *in vivo* after IV injection.[25] T cells transduced to express a melanoma-specific T-cell receptor and labeled with AuNPs were injected IV into mice bearing human melanoma xenografts.[21] Whole-body CT imaging allowed examination of the distribution, migration, and kinetics of these T-cells, as well as T-cell migration to the tumor site (Figure 6).

CAN CELLS BE IMAGED OVER LONG PERIODS OF TIME?

An important aspect of cell tracking is the longitudinal visualization of cell migration and homing to sites of injury, as this provides important data for determining the long-term fate of therapeutic cells. Indeed, longitudinal CT studies have shown that cells can be tracked for up to 1 month post injection (Table 2). For example, stem cells transplanted in the brain of a rat model for depression were found to migrate to distinct depression-related brain regions, and were detected as early as 24 h, and up to 1 month post-transplantation (Figure 7).[24] In another study, local migration of stem cells injected into injured muscle could be imaged for over a period of 4 weeks.[23] The ability to image cells over long periods is one of the major advantages of CT, as there is no loss of signal over time, as opposed to other, more sensitive techniques that image radiolabeled cells, but are suitable only for short periods due to radioisotope decay. However, a limitation of longitudinal studies is that many types of cells, including stem cells, continue to divide after transplantation, and consequently, the nanoparticles are distributed into daughter cells. Thus, while the number of nanoparticles imaged remains unchanged, the number of cells grow over time. This leads to less accurate quantification of cell numbers at later time points.

CELL TRACKING OR MERELY NANOPARTICLE TRACKING?

One of the main concerns using the AuNP-based CT cell tracking technique is whether or not the signal represents live cells. This concern is less relevant when imaging the cells immediately post-transplantation, but when imaging over longer periods, questions arise regarding the reliability of this technique, as there is no indication as to the viability of the cells *in vivo*, and whether the nanoparticles remain within the cells. However, it is notable that this question is inherent to any form of nanoparticle labeling, such as iron-oxide particles in MRI. Only use of reporter genes can produce more reliable longitudinal cell tracking, because the signal obtained from these genes represents live cells only, but reporter genes have their own limitation such as difficulties in performing a stable transfections and concerns regarding altering the cell biology and immunogenicity.

Therefore, to address the concern of cell viability, CT cell tracking results must be validated. This is generally achieved by one of two means. First, by

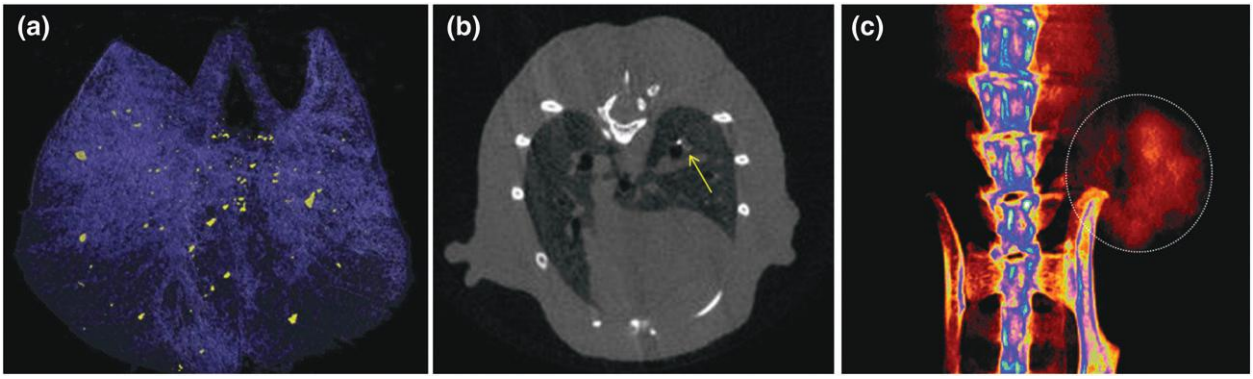

FIGURE 6 | Computed tomography (CT) scans demonstrating migration of gold nanoparticle (AuNP)-labeled T-cells and their whole-body biodistribution. (a) 3D volume rendering CT image of T-cells that accumulated in the lungs 48 h post injection. Yellow areas represent AuNP-labeled T-cells. (b) Representative 2D CT image of lungs. Arrow indicates gold-labeled cells. (c) Maximum intensity projection of micro-CT scans 48 h post injection. Circles demarcate T-cell accumulation in the tumor area. (Reprinted with permission from Ref 21. Copyright 2015 American Chemical Society)

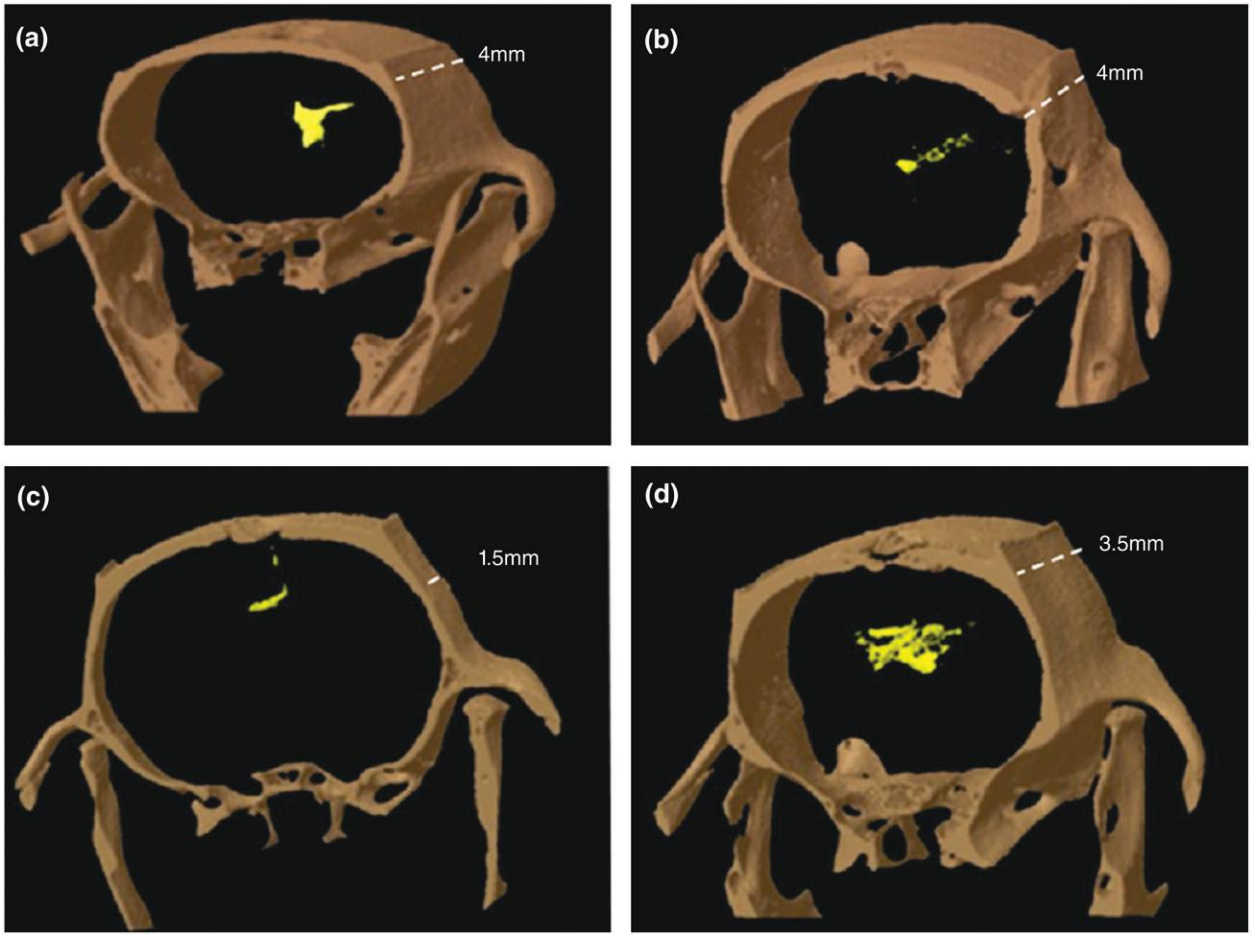

FIGURE 7 | 3D *in vivo* volume rendering micro-computed tomography (CT) scans of brain post injection of AuNP-labeled hMSC into the left ventricle. (a) One-hour post injection; (b) 24 h post injection; (c) 1 month post injection; (d) 1 month post free AuNP injection (control rat). (Reprinted with permission from Ref 24. Copyright 2014 American Chemical Society)

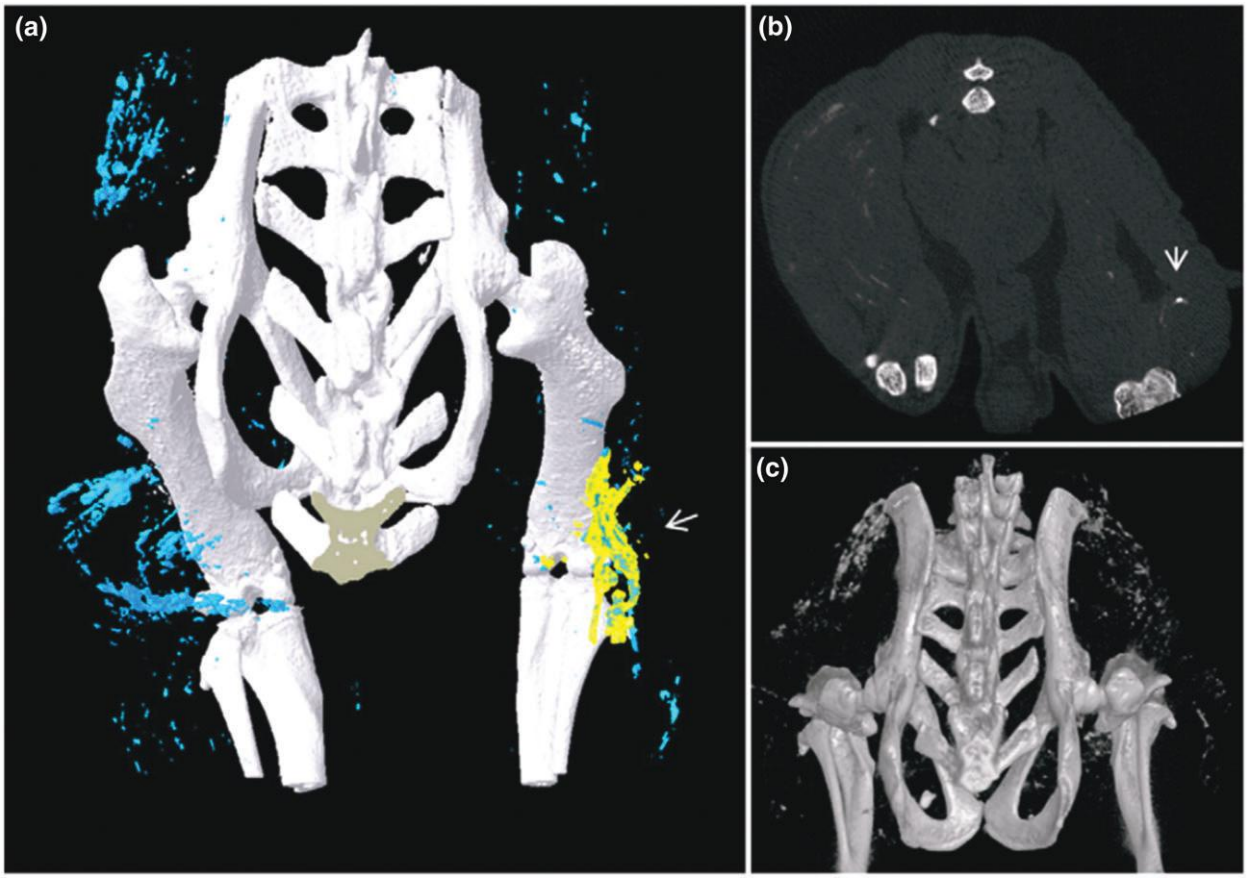

FIGURE 8 | Cell tracking with simultaneous monitoring of muscle recovery. Imaging cell treatment of muscle in a mouse model for Duchenne muscular dystrophy (MDX). (a) 3D volume-rendered computed tomography (CT) scan of mouse 2 weeks posttransplantation of AuNP-loaded mesenchymal stem cells in the right limb (arrow indicates injection site). Yellow: AuNPs located at the injection site, blue: calcification. Calcification is considerably more pronounced in the untreated left limb as compared to the treated limb. (b) 2D cross-sectional slice. (c) Untreated MDX mouse; calcification signal is observed in both limbs. (Reprinted with permission from Ref 23. Copyright 2017 Elsevier)

dual-labeling with a reporter gene and a contrast agent. Using this method in T-cells, labeled with a green fluorescent protein for fluorescence imaging and AuNPs for CT imaging, a good correlation in signal intensity was found at the tumor site, over a period of 5 days.[21] A second method is *ex vivo* immunostaining analysis of the desired tissue, to validate co-location of nanoparticles and cells (see Table 2). Both methods have provided evidence for the accuracy of CT cell tracking abilities.

CAN CELL TRACKING AND ANATOMICAL IMAGING BE PERFORMED SIMULTANEOUSLY?

Another key benefit of AuNP-based cell tracking with CT is that cell imaging can be performed simultaneously with monitoring of anatomical pathologies, whereas using MRI, the contrast generated by nanoparticles can interfere with imaging of the surrounding tissue. For instance, CT is one of the best and most frequently used modalities to image the coronary arteries noninvasively in patients.[68] This means that the feasibility of using AuNPs as effective contrast agents for noninvasive imaging of monocyte accumulation within plaques with CT is of great relevance. Chhour et al. demonstrated the use of AuNP-labeled monocytes, injected IV in a mouse model of atherosclerosis, for noninvasive tracking of monocyte recruitment into atherosclerotic plaques using CT.[25] In addition, the use of CT for simultaneous anatomical imaging and stem cell tracking has been demonstrated in a mouse model for Duchenne muscular dystrophy.[23] Intramuscularly-injected stem cells were tracked longitudinally, with no loss of signal over a period of 4 weeks, concurrently with monitoring of the muscle condition. Two weeks post-stem cell injection, CT scanning showed clear muscle recovery at the area of injection (Figure 8).

This dual ability is an attractive advantage for use of CT imaging with AuNPs, and can likely be applied to many other future clinical applications.

CONCLUSIONS AND FUTURE PROSPECTS

The CT imaging modality is clinically applicable, and enables good tissue penetration, high resolution, and three-dimensional anatomical imaging. Accumulating evidence demonstrates the feasibility of CT cell tracking with AuNPs *in vivo*. This concept has several advantages, including the ability to quantify even relatively small cell numbers and perform longitudinal studies, simultaneously with anatomical imaging. The main limitation of CT cell tracking is the uncertainty regarding retention of particles in cells over time, and long-term viability of cells. CT can provide reliable information on the fate of the injected cells at relatively short time periods, while further technological developments are required for obtaining reliable data regarding longer periods. However, long-term therapeutic effects were seen in various animal models treated with AuNP-loaded cells, supporting the long-term viability, and functionality of these cells.

As compared to other imaging methods, CT has the disadvantage of requiring a relatively high dose of radiation. However, as compared to MRI, PET, and SPECT, CT is lower in cost and more widely found in clinical centers, making it more accessible, as the number of centers that could perform real-time CT-guided injections is high. In addition, CT enables accurate quantification of the number of AuNP-labeled cells imaged, while quantification using MRI can only be achieved by labeling with fluorine containing compounds, but these compounds are not easy to stabilize *in vivo*.[8,69] As multimodal imaging systems are a well-known strategy for overcoming imaging limitations,[70] developing nanoparticles that can serve as contrast agents for several imaging modalities at once can combine the strengths and versatility of several modalities within one formulation, and provide a solution for the inherent shortcomings of each modality. The goal of such multimodal imaging would be to achieve signal cell tracking with an ability to report on cell functionality.

In conclusion, while numerous studies and advances have been achieved using cell tracking with CT, more studies are needed to further establish CT cell tracking as a reliable imaging concept. More preclinical studies should be conducted with a variety of therapeutic cell types. Further research can optimize and unify cell labeling protocols, and further clarify the reliability of the AuNP-labeled cell tracking method with CT, thus paving the way to clinical trials.

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REFERENCES

1. Park JS, Suryaprakash S, Lao Y-H, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. *Methods* 2015, 84:3–16.
2. Kang SK, Shin IS, Ko MS, Jo JY, Ra JC. Journey of mesenchymal stem cells for homing: strategies to enhance efficacy and safety of stem cell therapy. *Stem Cells Int* 2012, 2012:342968.
3. Ankri C, Shamalov K, Horovitz-Fried M, Mauer S, Cohen CJ. Human T cells engineered to express a programmed death 1/28 costimulatory retargeting molecule display enhanced antitumor activity. *J Immunol* 2013, 191:4121–4129.
4. Daniel-Meshulam I, Ya'akobi S, Ankri C, Cohen CJ. How (specific) would like your T-cells today? Generating T-cell therapeutic function through TCR-gene transfer. *Front Immunol* 2012, 3:186.
5. Bongso A, Fong C-Y, Gauthaman K. Taking stem cells to the clinic: major challenges. *J Cell Biochem* 2008, 105:1352–1360.
6. Nguyen PK, Nag D, Wu JC. Methods to assess stem cell lineage, fate and function. *Adv Drug Deliv Rev* 2010, 62:1175–1186.
7. Srinivas M, Aarntzen EHJG, Bulte JWM, Oyen WJ, Heerschap A, de Vries IJM, Figdor CG. Imaging of cellular therapies. *Adv Drug Deliv Rev* 2010, 62:1080–1093.
8. Ahrens ET, Bulte JWM. Tracking immune cells in vivo using magnetic resonance imaging. *Nat Rev Immunol* 2013, 13:755–763.

9. Jokerst JV, Khademi C, Gambhir SS. Intracellular aggregation of multimodal silica nanoparticles for ultrasound-guided stem cell implantation. *Sci Transl Med* 2013, 5:177ra35.
10. Cui W, Tavri S, Benchimol MJ, Itani M, Olson ES, Zhang H, Decyk M, Ramirez RG, Barback CV, Kono Y, et al. Neural progenitor cells labeling with microbubble contrast agent for ultrasound imaging in vivo. *Biomaterials* 2013, 34:4926–4935.
11. Bulte JWM, Walczak P, Janowski M, Krishnan KM, Arami H, Halkola A, Gleich B, Rahmer J. Quantitative "Hot Spot" imaging of transplanted stem cells using superparamagnetic tracers and magnetic particle imaging (MPI). *Tomography* 2015, 1:91–97.
12. Zheng B, Vazin T, Goodwill PW, Conway A, Verma A, Ulku Saritas E, Schaffer D, Conolly SM, Bjorklund LM, De Vries IJM, et al. Magnetic particle imaging tracks the long-term fate of in vivo neural cell implants with high image contrast. *Sci Rep* 2015, 5:14055.
13. Turgeman L, Fixler D. Photon efficiency optimization in time-correlated single photon counting technique for fluorescence lifetime imaging systems. *IEEE Trans Biomed Eng* 2013, 60:1571–1579.
14. Fixler D, Garcia J, Zalevsky Z, Weiss A, Deutsch M. Speckle random coding for 2D super resolving fluorescent microscopic imaging. *Micron* 2007, 38:121–128.
15. Jha P, Golovko D, Bains S, Hostetter D, Meier R, Wendland MF, Daldrup-link HE. Monitoring of NK-cell immunotherapy using non-invasive imaging modalities. *Cancer Res* 2011, 70:6109–6113.
16. Yaghoubi SS, Jensen MC, Satyamurthy N, Budhiraja S, Paik D, Czernin J, Gambhir SS. Noninvasive detection of therapeutic cytolytic T cells with 18F–FHBG PET in a patient with glioma. *Nat Clin Pract Oncol* 2009, 6:53–58.
17. Chin BB, Nakamoto Y, Bulte JWM, Pittenger MF, Wahl R, Kraitchman DL. 111In oxine labelled mesenchymal stem cell SPECT after intravenous administration in myocardial infarction. *Nucl Med Commun* 2003, 24:1149–1154.
18. Zhang SJ, Wu JC. Comparison of imaging techniques for tracking cardiac stem cell therapy. *J Nucl Med* 2007, 48:1916–1919.
19. Fixler D, Nayhoz T, Ray K. Diffusion reflection and fluorescence lifetime imaging microscopy study of fluorophore-conjugated gold nanoparticles or nanorods in solid phantoms. *ACS Photonics* 2014, 1:900–905.
20. Srivastava AK, Kadayakkara DK, Bar-Shir A, Gilad AA, McMahon MT, Bulte JWM. Advances in using MRI probes and sensors for in vivo cell tracking as applied to regenerative medicine. *Dis Model Mech* 2015, 8:323–336.
21. Meir R, Shamalov K, Betzer O, Motiei M, Horovitz-Fried M, Yehuda R, Popovtzer A, Popovtzer R, Cohen CJ. Nanomedicine for cancer immunotherapy: tracking cancer-specific T-cells *in vivo* with gold nanoparticles and CT imaging. *ACS Nano* 2015, 9:6363–6372.
22. Betzer O, Meir R, Dreifuss T, Shamalov K, Motiei M, Shwartz A, Baranes K, Cohen CJ, Shraga-Heled N, Ofir R, et al. In-vitro optimization of nanoparticle-cell labeling protocols for in-vivo cell tracking applications. *Sci Rep* 2015, 5:15400.
23. Meir R, Betzer O, Motiei M, Kronfeld N, Brodie C, Popovtzer R. Design principles for noninvasive, longitudinal and quantitative cell tracking with nanoparticle-based CT imaging. *Nanomed Nanotechnol Biol Med* 2017, 13:421–429.
24. Betzer O, Shwartz A, Motiei M, Kazimirsky G, Gispan I, Damti E, Brodie C, Yadid G, Popovtzer R. Nanoparticle-based CT imaging technique for longitudinal and quantitative stem cell tracking within the brain: application in neuropsychiatric disorders. *ACS Nano* 2014, 8:9274–9285.
25. Chhour P, Naha PC, O'neill SM, Litt HI, Reilly MP, Ferrari VA, Cormode DP. Labeling monocytes with gold nanoparticles to track their recruitment in atherosclerosis with computed tomography. *Biomaterials* 2016, 87:93–103.
26. Kim T, Lee N, Arifin DR, Shats I, Janowski M, Walczak P, Hyeon T, Bulte JWM. In vivo micro-CT imaging of human mesenchymal stem cells labeled with gold-poly-L-lysine nanocomplexes. *Adv Funct Mater* 2017, 27:1604213.
27. Shilo M, Reuveni T, Motiei M, Popovtzer R. Nanoparticles as computed tomography contrast agents: current status and future perspectives. *Nanomedicine (Lond)* 2012, 7:257–269.
28. Cole LE, Ross RD, Tilley JM, Vargo-Gogola T, Roeder RK. Gold nanoparticles as contrast agents in X-ray imaging and computed tomography. *Nanomedicine (Lond)* 2015, 10:321–341.
29. Berrington de González A, Mahesh M, Kim K-P, Bhargavan M, Lewis R, Mettler F, Land C. Projected cancer risks from computed tomographic scans performed in the United States in 2007. *Arch Intern Med* 2009, 169:2071.
30. Ritman EL. Current status of developments and applications of micro-CT. *Annu Rev Biomed Eng* 2011, 13:531–552.
31. Ghosh P, Han G, De M, Kim CK. Gold nanoparticles in delivery applications. *Adv Drug Deliv Rev* 2008, 60:1307–1315.
32. Arvizo R, Bhattacharya R, Mukherjee P. Gold nanoparticles: opportunities and challenges in nanomedicine. *Expert Opin Drug Deliv* 2010, 7:753–763.
33. Zhang G, Yang Z, Lu W, Zhang R, Huang Q, Tian M, Li L, Liang D, Li C. Influence of anchoring ligands and particle size on the colloidal stability and in vivo biodistribution of polyethylene glycol-coated

gold nanoparticles in tumor-xenografted mice. *Biomaterials* 2009, 30:1928–1936.

34. Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Sadagopa Ramanujam VM, Urayama A, Vergara L, Kogan MJ, Soto C. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. *Biochem Biophys Res Commun* 2010, 393:649–655.
35. Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V. A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. *Crit Rev Toxicol* 2010, 40:328–346.
36. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 2005, 1:325–327.
37. Sherman AI, Ter-Pogossian M. Lymph-node concentration of radioactive colloidal gold following interstitial injection. *Cancer* 1953, 6:1238–1240.
38. Polak P, Shefi O. Nanometric agents in the service of neuroscience: manipulation of neuronal growth and activity using nanoparticles. *Nanomed Nanotechnol Biol Med* 2015, 11:1467–1479.
39. Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. *Br J Radiol* 2006, 79:248–253.
40. Cai Q-Y, Kim SH, Choi KS, Kim SY, Byun SJ, Kim KW, Park SH, Juhng SK, Yoon K-H. Colloidal gold nanoparticles as a blood-pool contrast agent for X-ray computed tomography in mice. *Invest Radiol* 2007, 42:797–806.
41. Peng C, Wang H, Guo R, Shen M, Cao X, Zhu M, Zhang G, Shi X. Acetylation of dendrimer-entrapped gold nanoparticles: synthesis, stability, and X-ray attenuation properties. *J Appl Polym Sci* 2011, 119:1673–1682.
42. Cormode DP, Roessl E, Thran A, Skajaa T, Gordon RE, Schlomka J-P, Fuster V, Fisher EA, Mulder WJM, Proksa R, et al. Atherosclerotic plaque composition: analysis with multicolor CT and targeted gold nanoparticles. *Radiology* 2010, 256:774–782.
43. Kim D, Park S, Lee JH, Jeong YY, Jon S. Antibiofouling polymer-coated gold nanoparticles as a contrast agent for in vivo X-ray computed tomography imaging. *J Am Chem Soc* 2007, 129:7661–7665.
44. Wang H, Zheng L, Peng C, Guo R, Shen M, Shi X, Zhang G. Computed tomography imaging of cancer cells using acetylated dendrimer-entrapped gold nanoparticles. *Biomaterials* 2011, 32:2979–2988.
45. Popovtzer R, Agrawal A, Kotov NA, Popovtzer A, Balter J, Carey TE, Kopelman R. Targeted gold nanoparticles enable molecular CT imaging of cancer. *Nano Lett* 2008, 8:4593–4596.
46. Chanda N, Kattumuri V, Shukla R, Zambre A, Katti K, Upendran A, Kulkarni RR, Kan P, Fent GM, Casteel SW, et al. Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. *Proc Natl Acad Sci USA* 2010, 107:8760–8765.
47. Reuveni T, Motiei M, Romman Z, Popovtzer A, Popovtzer R. Targeted gold nanoparticles enable molecular CT imaging of cancer: an in vivo study. *Int J Nanomedicine* 2011, 6:2859–2864.
48. Hainfeld JF, O'Connor MJ, Dilmanian FA, Slatkin DN, Adams DJ, Smilowitz HM. Micro-CT enables microlocalisation and quantification of Her2-targeted gold nanoparticles within tumour regions. *Br J Radiol* 2011, 84:526–533.
49. Motiei M, Dreifuss T, Betzer O, Panet H, Popovtzer A, Santana J, Abourbeh G, Mishani E, Popovtzer R. Differentiating between cancer and inflammation: A metabolic-based method for functional computed tomography imaging. *ACS Nano* 2016, 10:3469–3477.
50. Shilo M, Motiei M, Hana P, Popovtzer R. Transport of nanoparticles through the blood–brain barrier for imaging and therapeutic applications. *Nanoscale* 2014, 6:2146–2152.
51. Eck W, Nicholson AI, Zentgraf H, Semmler W, Bartling S. Anti-CD4-targeted gold nanoparticles induce specific contrast enhancement of peripheral lymph nodes in X-ray computed tomography of live mice. *Nano Lett* 2010, 10:2318–2322.
52. Schültke E, Menk R, Pinzer B, Astolfo A, Stampanoni M, Arfelli F, Harsan L-A, Nikkhah G. Single-cell resolution in high-resolution synchrotron x-ray CT imaging with gold nanoparticles. *J Synchrotron Radiat* 2014, 21:242–250.
53. Menk RH, Schültke E, Hall C, Arfelli F, Astolfo A, Rigon L, Round A, Ataelmannan K, MacDonald SR, Juurlink BHJ. Gold nanoparticle labeling of cells is a sensitive method to investigate cell distribution and migration in animal models of human disease. *Nanomedicine* 2011, 7:647–654.
54. Schültke E, Menk R-H, Stampanoni M, Arfelli F, Astolfo A, Hall C, Pinzer B, Tromba G. Can we develop an early warning system for patients after cell transplantation therapy using X-ray imaging? *J Instrum* 2013, 8:C07008.
55. Astolfo A, Qie F, Kibleur A, Hao X, Menk RH, Arfelli F, Rigon L, Hinton TM, Wickramaratna M, Tan T, et al. A simple way to track single gold-loaded alginate microcapsules using X-ray CT in small animal longitudinal studies. *Nanomedicine* 2014, 10:1821–1828.
56. Astolfo A, Schültke E, Menk R-H, Hall C, Juurlink B, Arfelli F. X-ray cell tracking: from ex-vivo to in-vivo experiments. *J Instrum* 2013, 8:C06010.

57. Astolfo A, Schültke E, Menk RH, Kirch RD, Juurlink BHJ, Hall C, Harsan L-A, Stebel M, Barbetta D, Tromba G, et al. In vivo visualization of gold-loaded cells in mice using x-ray computed tomography. *Nanomedicine* 2013, 9:284–292.

58. Wolf GL. Magnetic resonance imaging and the future of cardiac imaging. *Am J Cardiol* 1989, 64:E60–E63.

59. Skotland T, Iversen T-G, Sandvig K. New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. *Nanomedicine* 2010, 6:730–737.

60. Dreifuss T, Barnoy E, Motiei M, Popovtzer R. Theranostic gold nanoparticles for CT imaging. In: *Design and Applications of Nanoparticles in Biomedical Imaging*. Cham: Springer International Publishing; 2017, 403–427.

61. Astolfo A, Arfelli F, Schültke E, James S, Mancini L, Menk R-H. A detailed study of gold-nanoparticle loaded cells using X-ray based techniques for cell-tracking applications with single-cell sensitivity. *Nanoscale* 2013, 5:3337–3345.

62. Chhour P, Kim J, Benardo B, Tovar A, Mian S, Litt HI, Ferrari VA, Cormode DP. Effect of gold nanoparticle size and coating on labeling monocytes for CT tracking. *Bioconjug Chem* 2017, 28:260–269.

63. Srinivas M, Morel PA, Ernst LA, Laidlaw DH, Ahrens ET. Fluorine-19 MRI for visualization and quantification of cell migration in a diabetes model. *Magn Reson Med* 2007, 58:725–734.

64. Wang PC, Shan L. Essential elements to consider for MRI cell tracking studies with iron oxide-based labeling agents. *J Basic Clin Med* 2012, 1:1–6.

65. Liu S, Zhou J, Zhang X, Liu Y, Chen J, Hu B, Song J, Zhang Y. Strategies to optimize adult stem cell therapy for tissue regeneration. *Int J Mol Sci* 2016, 17:982.

66. Liu L, Eckert MA, Riazifar H, Kang D-K, Agalliu D, Zhao W. From blood to the brain: can systemically transplanted mesenchymal stem cells cross the blood–brain barrier? *Stem Cells Int* 2013, 2013:435093.

67. Willing AE, Garbuzova-Davis S, Sanberg PR, Saporta S. Routes of stem cell administration in the adult rodent. *Methods Mol Biol (Clifton, NJ)* 2008, 438:383–401.

68. Mowatt G, Cummins E, Waugh N, Walker S, Cook J, Jia X, Hillis GS, Fraser C. Systematic review of the clinical effectiveness and cost-effectiveness of 64-slice or higher computed tomography angiography as an alternative to invasive coronary angiography in the investigation of coronary artery disease. *Health Technol Assess* 2008, 12:iii–iv, ix–143.

69. Srinivas M, Heerschap A, Ahrens ETT, Figdor CG, de Vries IJM. (19)F MRI for quantitative in vivo cell tracking. *Trends Biotechnol* 2010, 28:363–370.

70. Arifin DR, Long CM, Gilad AA, Alric C, Roux S, Tillement O, Link TW, Arepally A, Bulte JWM. Trimodal gadolinium-gold microcapsules containing pancreatic islet cells restore normoglycemia in diabetic mice and can be tracked by using US, CT, and positive-contrast MR imaging. *Radiology* 2011, 260:790–798.

