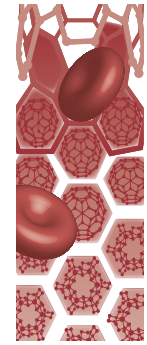
Review

Nanomedicine



Gold nanoparticles for *in vivo* cell tracking

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Cell-based therapy offers a promising solution for the treatment of diseases and injuries that conventional medicines and therapies cannot cure effectively, and thus comprises an encouraging arena for future medical breakthroughs. The development of an accurate and quantitative noninvasive cell tracking technique is a highly challenging task that could help in evaluating the effectiveness of treatments. Moreover, cell tracking could provide essential knowledge regarding the fundamental trafficking patterns and poorly understood mechanisms underlying the success or failure of cell therapy. This article focuses on gold nanoparticles, which provide cells with 'visibility' in a variety of imaging modalities for stem cell therapy, immune cell therapy and cancer treatment. Current challenges and future prospects relating to the use of gold nanoparticles in such roles are discussed.

Keywords: cancer • cell therapy • cell tracking • computed tomography • gold nanoparticles • immune cells • *in vivo* imaging, noninvasive • photoacoustic • stem cells

Cell therapy: the future of medicine

Despite tremendous advances in medicine and pharmacology, many diseases and medical conditions remain incurable. In an attempt to find treatments for many such ailments, among other approaches, researchers have turned to the use of living cells as therapeutic agents [1]. As opposed to chemical compounds, cells are naturally capable of performing physiologic and metabolic tasks, such as: exhibiting homing abilities to sites of lesion, injury and inflammation; producing neurotrophic factors; and exerting anti-inflammatory effects. Thus, cell-based therapy provides a promising approach for diseases and injuries that conventional medicines and therapies cannot cure effectively [2]. Cell therapy research is comprised of two main fields. First, therapy based on stem cells is used in regenerative medicine, enabled by the inherent biological properties of stem cells, such as the ability to differentiate into multiple cell types, plasticity, self-renewal and migration [3]. Second, immune cell therapy manifests great promise in treating autoimmune diseases and cancer [4].

The need for cell tracking & conventional cell tracking techniques

The idea of cell therapy has existed for decades, with recent advances in technology allowing research in the field to progress from preclinical to advanced clinical trials [5]. However, as exciting as it may seem, applying cell-based therapy in routine clinical practice has proven to be very challenging [1]. Clinical trials have begun to test cell transplantation in human patients, but the results of these trials are highly mixed – some patients exhibit major improvements, while others experience modest (if any) clinical benefit. This variability in therapeutic outcome, which exists not only between different trials and centers, but also within groups of patients transplanted at the same center [6], prevents this field from reaching its full potential. It remains unclear whether the different outcomes are driven by individual diversity in inherent physiological reactions, or rather relate to factors of the cell transplantation procedure itself, such as suboptimal injection, poor cell survival or

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variations in differentiation, biodistribution and final fate [7].

At present, the symptoms of illnesses are instrumental in determining a response to cell-based treatment. For example, the size of a tumor is measured weeks or months after commencing the treatment, and internal disorders such as diabetes, liver failure or myocardial infarction can be evaluated with comparatively objective measures and markers [8,9]. However, important questions remain unanswered regarding the cell dosage, optimal route of delivery, engraftment, viability, biology and safety of transplanted cells, as well as their interactions with the microenvironment [10,11].

The development of a reliable, noninvasive, real-time means to image and trace these cells post-transplantation and evaluate their biodistribution, final fate and functionality is critical to answering these questions. Small groups of cells must be tracked continuously *in vivo* within large tissue volumes over long periods of time in order to assess the fate of the implanted cells within the human body.

Several conservative approaches exist for tracking cells *in vivo*. The most commonly used method – optical-based imaging – enables the detection of light emitted by either fluorescent or bioluminescent reporter genes [3,10]. For the purposes of cell tracking, such a genetic alteration, when stably expressed, exhibits certain advantages, since the reporter genes can be expressed as long as the cells are alive and without dilution after cell division [10]. This characteristic enables cell tracking that truthfully represents the locations and fates of the target cells without any loss of signal and using a radiation-free modality. However, optical imaging involves several limitations, such as the low tissue penetration of light that prevents imaging of deep body structures, the lack of quantification and it being a 2D projection technique [12]. These crucial limitations, in addition to the method's inability to be applied clinically, are quite restraining. Radionuclides have short half-lives and thus are not ideal for long-term imaging studies. Consequently, a need has arisen for the development of the next generation of cell-labeling techniques.

Nanoparticles for cell labeling

Nanoparticles introduce an alternative method for cell labeling. This novel approach bears great promise for becoming the next generation in biomedical engineering. Nanoparticle-based contrast agents are highly versatile and can be used in dual or even triple modalities by using ultrasound (US), computed tomography (CT) and MRI, as demonstrated by Arifin *et al.* [13]. In the future, nanoparticles might be able to sense and signal cell-to-cell communication, intercellular enzyme activity and pH *in vivo* in real time. Moreover, nanoparticles that can be traced by MRI and CT will potentially be clinically applicable. Clearly, any progress in the field of nanoparticle-based labeling and imaging will be critical, with many future applications yet to be developed.

The main drawback of this method relates to the possible uncertainty regarding the fate of the nanoparticles incorporated in the cells once they have been injected *in vivo*. Several scenarios exist in which the nanoparticles can be freed from the cells, including cells that undergo apoptosis, and the possible process of exocytosis of the particles from the cells. In such cases, free nanoparticles may be phagocytized by macrophages, which means that rather than tracking the cells of interest, the free nanoparticles and macrophages are being imaged instead. Another limitation of nanoparticles relates to cell proliferation and the probable decrease in the amount of particles in daughter cells, resulting in reduced signaling. These obstacles must be seriously tested both *in vitro* and *in vivo* by using control groups for the labeled cells. Some of the studies presented in this article describe experiments that compare imaging of nanoparticle-labeled cells with reporter gene-labeled cells (e.g., see 'Immune cell tracking' section). Only such extensive experiments will result in the establishment of methods for nanoparticle cell labeling and achieving greater accuracy via these methods.

Advantages of gold nanoparticles as labeling agents

Recently, gold has gained broad attention and wide-scale research is being conducted regarding its medical applicability due to its inert and nontoxic nature. Gold nanoparticles (GNPs) have unique physical, chemical and biological properties, making them attractive contrast agents for several imaging modalities. For example, the high atomic number of gold can induce strong x-ray attenuation, as the higher the atomic number of the contrast agent, the better the resultant CT contrast. In addition, GNPs are easy to synthesize, their surface coating can be easily modified and their size and shape can be precisely controlled (Figure 1) in order to influence cellular uptake [14–16]. Furthermore, gold is known to be an inert metal, which makes the GNPs biocompatible and suitable for biological applications. Surface modifications of GNPs expand their utility by enabling them to target specific sites on the cell surface, organelles, the nucleus or the extracellular matrix. The use of GNPs enables the imaging of particles that are deep inside the body tissue by means of various modalities [17]. Moreover, GNPs can be effectively loaded into cells pretransplantation, without affecting cell viability or differentiation.

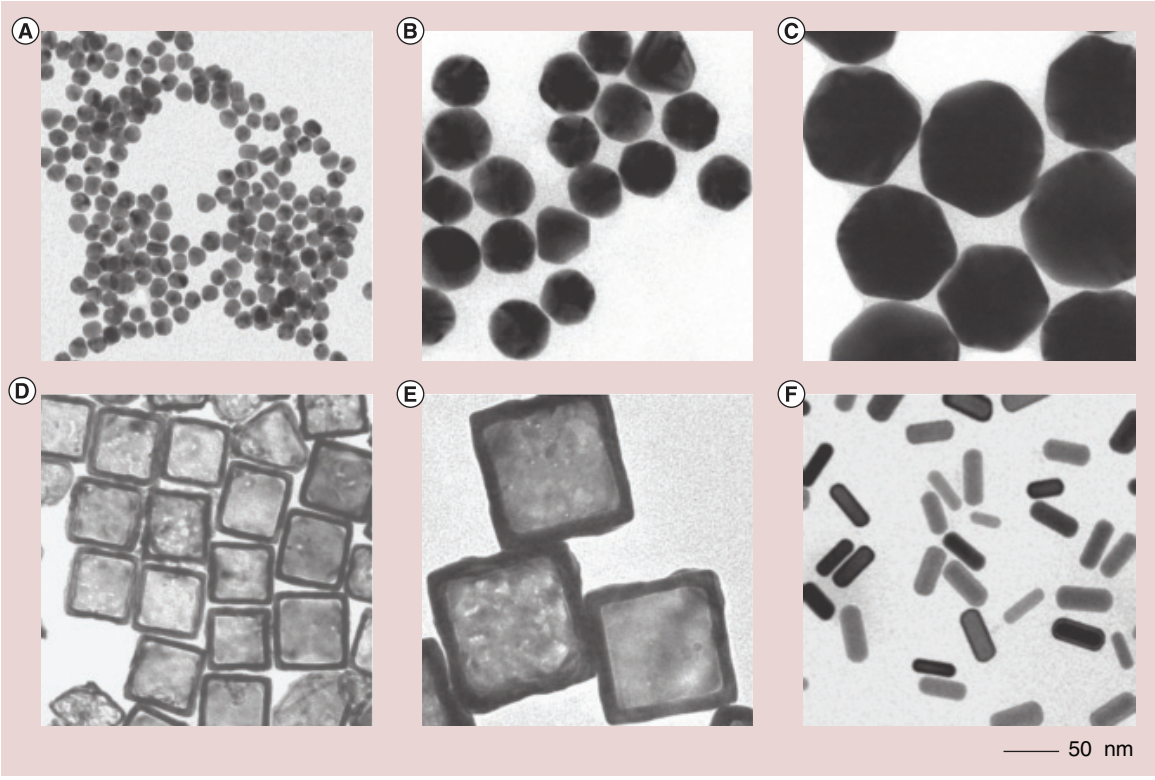

Figure 1. Transmission electron microscopy images showing six types of gold nanoparticles. (A) 15-nm (diameter) nanospheres; **(B)** 54-nm nanospheres; **(C)** 100-nm nanospheres; **(D)** 62-nm (outer edge length) nanocages; **(E)** 118-nm nanocages; and **(G)** nanorods (16 × 40 nm diameter by length). The 50-nm scale bar applies to all images.
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Applications of cell tracking using GNPs

Ensuring efficient and reliable tracking is quite a challenge. Two central aspects that are necessary for performing cell tracking with GNPs must be addressed: cell labeling and *in vivo* imaging (Figure 2).

Stage 1: labeling the cells with nanoparticles

In order to allow sensitive imaging, the cells must first be labeled *in vitro* with contrast agents. An acute need exists to assess the efficiency of the cell labeling, as well as the effect of the nanoparticles on cell functioning and viability. For cell-based therapies, it is crucial that particles have no (or at least minimal) effects on cell function.

The following questions must be explored when developing an efficient cell labeling protocol:

- Type of nanoparticles – what is the ideal size and coating of the particle?
- Quantity – how many particles enter a single cell?
- By which mechanism do the particles enter the cell and what is the ideal incubation time of cells and particles?
- What is the gold distribution within the cell and do the particles partition during mitosis?
- Do the particles undergo exocytosis or does retention take place over time?
- Equally important is the effect of the particles on the cells:
- Is the cell viability affected?
- Are the proliferation and differentiation abilities of the cell retained?
- Can the therapeutic abilities of the cells be maintained?

Stage 2: *in vivo* cell tracking

Once the cells are labeled, they are injected into the animal and an imaging modality is used. Each imaging modality has its advantages and disadvantages, and many parameters can affect the cell tracking ability. Several fundamental questions need to be addressed:

- What is the minimum amount of cells that can be detected?

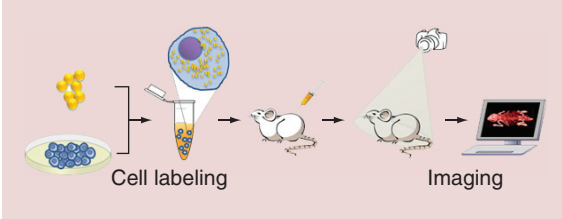

Figure 2. Process of tracking cells with gold nanoparticles. The first stage is *in vitro* cell labeling with nanoparticles; the second stage is cell tracking using a variety of *in vivo* imaging modalities.

- Is long-term imaging feasible?
- Moreover, the technical procedure by which the cells are injected can differ significantly:
- How many cells are injected?
- What is the site of injection?

To date, three main applications of cell tracking using GNPs exist: imaging malignant cells as a general proof-of-concept for *in vivo* cell tracking; tracking stem cells in regenerative medicine; and tracking immune cells for cancer immunotherapy. We present the current state of the field in the following sections. We describe prominent publications followed by a detailed comparison between different *in vitro* cell-labeling methods and *in vivo* imaging modalities (Table 1).

Tracking malignant cells

Imaging of malignant cells is explored both as a means for preclinical studies of tumor model progression and as a general proof of concept for *in vivo* cell tracking techniques. Research regarding tracking malignant cells is performed with GNPs combined with x-ray CT.

X-ray CT is one of the leading radiological technologies applied in the field of biomedical imaging [23]. CT is among the most convenient imaging tools used in hospitals to date in terms of availability, efficiency and cost, and it is characterized by high temporal and spatial resolutions. Therefore, it is a leading candidate modality for cell tracking and imaging. The ability of CT to distinguish between different tissues is based on

Table 1. Summary of *in vivo* cell tracking experiments using gold nanoparticles.

Feature	Malignant cells [18,19]	Mesenchymal stem cells [20]	Mesenchymal stem cells [21]	Immune cells [22]
Cell labeling with GNPs				
Size of nanoparticles	50 nm	42/14-nm gold core Final size: 83/64 nm (nanorods)	20 nm	7-nm gold core Final size: 35 nm
Coating of nanoparticles	Horse serum	Silica	Citrate-stabilized	GNP–^{64}Cu/PEG 2000
Time of incubation	22 h	3 h	24 h	Electrotransfer (instant)
Number of particles per cell	26,000	102,000	453,000	NR
Biocompatibility: effect of particles on cells	No significant effect on proliferation	No toxicity or proliferation changes were observed, pluripotency was retained	Viability, proliferation and differentiation were not significantly affected	Activity was not affected
***In vivo* imaging of labeled cells**				
Imaging modality	Synchrotron x-ray CT	Photoacoustic	Ultrasound/ photoacoustic	μPET/CT
Number of cells injected	200,000	800,000	30,000 cells in pegylated fibrin gel	20,000,000
Limit of detection	1700 cells	90,000 cells *in vivo*, 50,000 *ex vivo*	200 cells (demonstrated *in vitro*)	NR
Time of imaging	8 days	4 days	10 days	18 h
Place of injection	Striatum	Subcutaneous (intramuscular)	Subcutaneous (intramuscular)	Intravenous

GNP: Gold nanoparticle; NR: Not reported.

the fact that different tissues provide different degrees of x-ray attenuation. Thus, CT provides superior visualization of bone structures due to the inherent contrast between electron-dense bones and the more permeable surrounding soft tissues. However, the method is limited in terms of distinguishing between different soft tissues that have similar densities [24]. In order to enable better delineation of soft-tissue structures with similar or identical contrast properties, GNPs have been introduced. Indeed, GNPs comprise an ideal candidate for CT imaging due to their high atomic number and electron density [25].

Visualizing small clusters of malignant cells with CT *in vivo*

A study by Astolfo and colleagues demonstrated the ability of performing cell tracking using x-ray CT for the *in vivo* assessment of a tumor in a small-animal model [18]. The researchers achieved cell tracking by labeling the malignant cells with GNPs and injecting the labeled cells for *in vivo* imaging. This study provided the first examples of the potential of this technique *in vivo* [18,26].

Astolfo *et al.*'s experiment shows that small clusters of approximately 1700 cells could be detected in the brain of a mouse by synchrotron CT [18]. The scanning was performed 8 days after stereotactically injecting 100,000 malignant cells into a mouse brain that mimic the human glioblastoma multiforme. The results were verified by scanning the heads *ex vivo* at post-mortem (Figure 3) with a higher radiation dose. The work demonstrates the potential for monitoring the fate of the injected cells, as well as the ability to study the dynamics of a growing tumor in detail by measuring its volume and shape and calculating cell doubling times.

This efficient tracking capability is achieved as a consequence of an efficient gold labeling protocol (Figure 4) [27]. Malignant cells were labeled *in vitro* by 22-h exposure to GNPs of approximately 50 nm in diameter [19]. According to the authors, approximately 80% of cells internalized enough gold to generate a sufficient x-ray CT contrast.

Longitudinal tracking of cancer cells is more challenging than in other cell lines such as stem cells, in the sense that cancer cells grow and divide very rapidly. This key challenge requires an understanding of the fate of the GNPs after the cancer cell undergoes proliferation. In addition, rapid tumor growth results in the development of a necrotic core [28], and necrotic cells release their GNPs. The authors mention that it is quite likely that the GNPs from the necrotic tumor cells are phagocytized by activated resident microglia and macrophages, and although they most probably remain within the site of activation, they do interfere with accurate calculations of tumor cell doubling times. In the future, in order to explore the effectiveness of this tracking modality, a longitudinal cell visualization study with repeated CT on the same animal should be conducted.

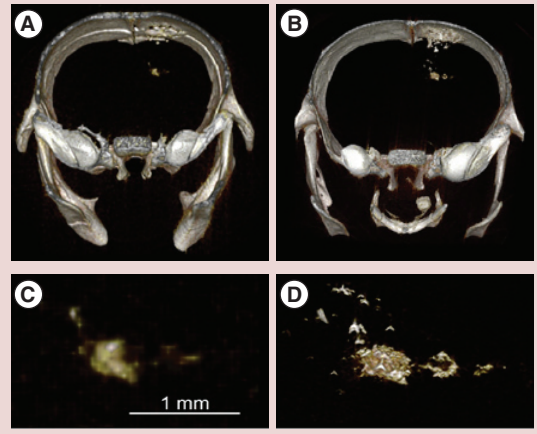

Figure 3. Comparison of two 3D renderings of a CT scan of a mouse injected with 100,000 gold nanoparticle-loaded F98 cells. **(A & C)** The low x-ray dose *in vivo* data and **(B & D)** the high x-ray dose *ex vivo* data are shown. The images in **(C)** and **(D)** are enlargements at the full system resolution of the developed tumors depicted in **(A)** and **(B)**, respectively. Reproduced with permission from [18].

Stem cell tracking

Studies regarding the transplantation of stem cells of various origins, particularly mesenchymal stem cells (MSCs) – which are able to enhance regeneration and repair [29] – have been conducted for years, initially in animal models and then in patients, offering hope for effective treatment.

The innovative work of Nam *et al.* presents an alternative imaging method for stem cell labeling and *in vivo* tracking using a photoacoustic (PA) imaging-technique [30]. This study demonstrated the feasibil-

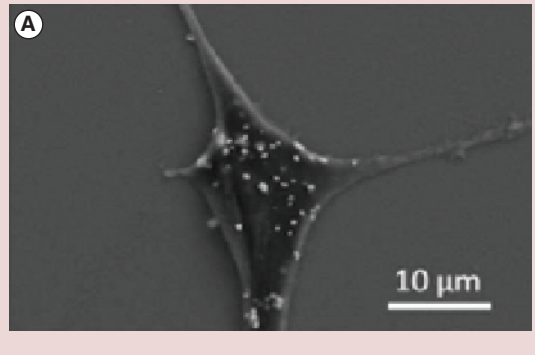

Figure 4. Scanning electron microscopy image of a gold-loaded C6 cancer cell.
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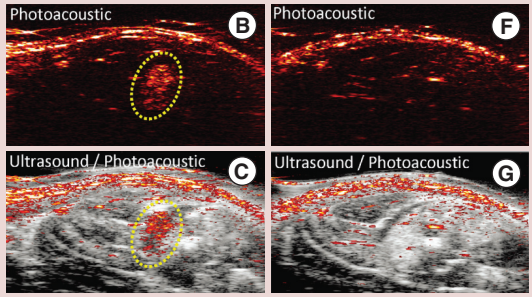

Figure 5. *In vivo* monitoring of gold nanotracer-labeled mesenchymal stem cells using combined ultrasound and photoacoustic imaging. *In vivo* ultrasound/photoacoustic images of the lateral gastrocnemius into which pegylated fibrin gel containing gold nanotracer-loaded mesenchymal stem cells was injected. The pegylated fibrin gel location is outlined with a yellow dotted circle. The injection depth was approximately 5 mm under the skin. For color images please see online http://www.futuremedicine.com/doi/full/10.2217/nnm.14.129. Adapted with permission from [21].

ity of longitudinal *in vivo* monitoring of MSCs labeled with GNPs.

PA imaging is an alternative biomedical imaging technique that can be used in combination with US imaging. In PA imaging, an ultrasonic emission is detected by an US transducer in response to short, nonionizing laser pulses that are being absorbed within biological tissue. PA imaging can image more deeply that optical imaging modalities, since it detects sound rather than light. Similar to US, PA imaging provides a penetration depth of several centimeters and submillimeter spatial resolutions [30]. The PA signal is specific to the optical absorption properties of contrast agents, which makes GNPs excellent candidates for this methodology [31,32], as they have high sensitivity, penetration depth and potential for quantification [33,34]. US combined with PA imaging can map a cell–scaffold construct along with neighboring tissues and provide both morphological and functional information [31]. This method enables visualization of morphological, functional and molecular properties in a noninvasive manner [35].

A recent study demonstrated that a combination of PA and US imaging allowed for MSCs to be noninvasively detected after being injected into tissue [21]. Previously, the same group found that pegylated fibrin gels can promote MSC differentiation towards a vascular cell type, thus contributing to regeneration [31]. Therefore, the MSCs were labeled with GNPs (20 nm in diameter) and later incorporated into a pegylated fibrin gel system. The fibrin gel was then injected intramuscularly into the lower limb of a Lewis rat, as represented in Figure 5. The MSC distribution could be monitored using US/PA imaging of cells loaded with nanotracers.

In the aforementioned experiment, while the MSCs without GNPs did not produce any PA signal, gold-labeled MSCs were imaged over a 1-week time period, which implies the possibility of longitudinal cell tracking using PA imaging. This modality could be an efficient imaging method for monitoring the stem cell distribution and in order to better understand the process of neovascularization.

The fact that the cells were incorporated into a gel allowed for imaging over an extensive period of time. Future studies should include *in vivo* monitoring of long-term MSC behaviors following an injury in order to assess and monitor the effects of MSCs in the process of neovascularization.

An earlier *in vitro* study performed by the same group demonstrated that GNPs can be safe and effective nanotracers for labeling MSCs [36]. The effect of GNP loading on cell viability and cytotoxicity was analyzed using a LIVE/DEAD stain and an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition, the ability of MSCs to differentiate after nanoparticle loading was examined. The study showed that the labeling protocol did not alter cell function; therefore, long-term imaging and tracking of MSCs could be feasible. In particular, neither cell viability nor cell proliferation nor cell differentiation were significantly affected by nanotracer uptake. The research was performed using GNPs of various sizes and surface coatings, showing that as opposed to citrate-stabilized GNPs, particles coated with polyethylene glycol did not allow for proper labeling of the cells. The results of this study also demonstrate that nanoparticle loading decreased exponentially over a 2-week period. This finding could be attributed mainly to cell division, as well as other mechanisms, such as exocytosis of the nanoparticles by the cells.

A study by Jokerst *et al.* presented real-time monitoring and quantification of the implantation of MSCs into the musculature of living mice [20]. The researchers managed to accomplish this feat by using silica-coated gold nanorods as a PA contrast agent in order to label the MSCs. The authors were able to produce real-time videos showing implantation of 800,000 cells (Figure 6), and the technique enabled a detection limit of as little as 100,000 cells *in vivo*. The cell bolus could be monitored for 4 days after injection. The resolution of the PA imaging technique could offer real-time information regarding cell location and number [20].

The silica coat of the gold nanorods played two important roles: it enhanced the PA signal of the GNRs and it increased the uptake of the gold nanorods

into the cell. The SiGNRs were found to have no effect on MSC viability, proliferation, differentiation, or cytokine expression *in vitro*, suggesting that the therapeutic benefit of the MSCs will be retained despite the presence of a contrast agent.

In order to determine whether labeled MSCs can be imaged, the injection of 800,000 MSCs labeled with nanorods was imaged. So as to verify the results, two important controls were tested in the *in vivo* experiment: the positive control only consisted of nanorods (without cells) and the negative control only consisted of phosphate-buffered saline. The imaging data were also validated with histological analysis and by fluorescent imaging with GFP of the muscle tissue that was removed after injection, confirming that the increase in imaging signal was due to the cells themselves.

Immune cell tracking

Immunotherapy is an emerging area of cancer research that involves the use of a patient's own immune system in order to combat cancer [37]. A novel immunotherapy approach involves the use of injected immune cells – such as T cells [38], natural killer cells

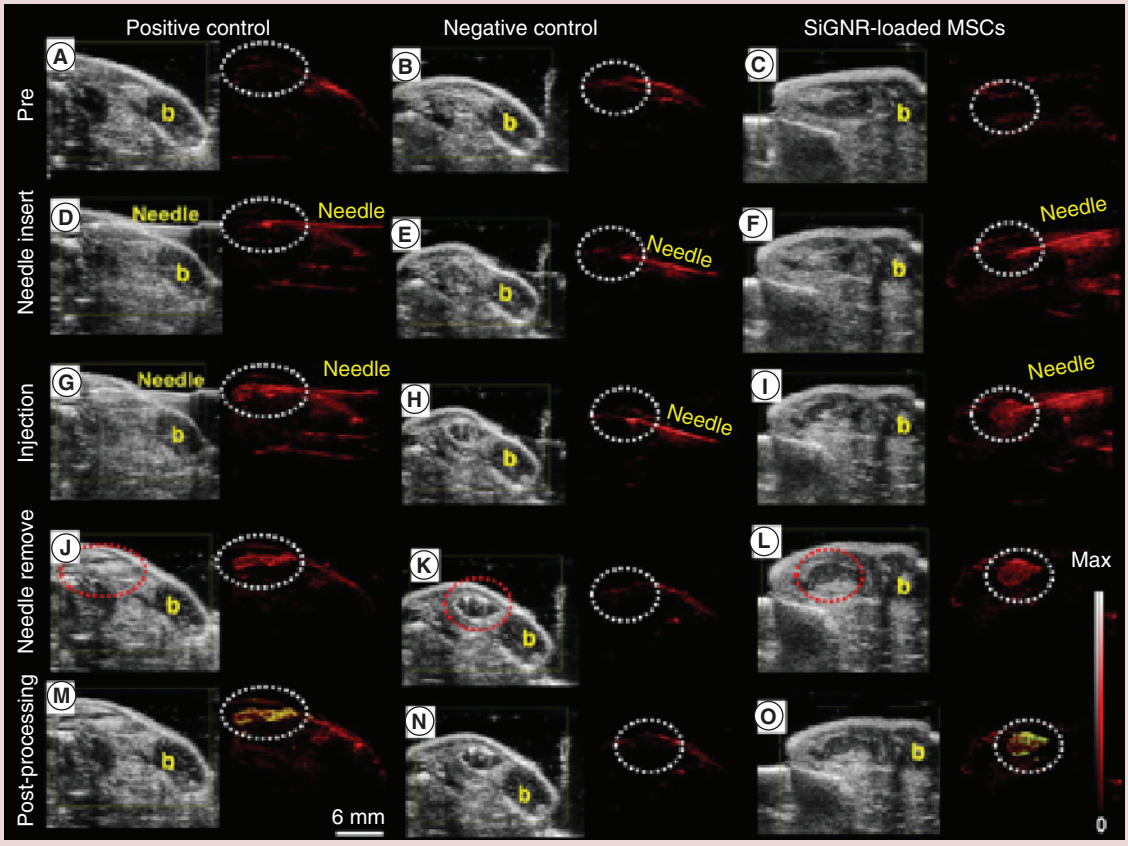

Figure 6. *In vivo* positive and negative controls and labeled mesenchymal stem cell intramuscular injection into the hind limb muscle of an athymic mouse. This figure presents both brightness-mode ultrasound (grayscale) and photoacoustic (red) images of the intramuscular injection of a positive control (0.7 nM SiGNRs; left), negative control (0 nM SiGNRs [no cells]; middle) and 800 000 SiGNR-labeled MSCs (right), all injected in 50% Matrigel™ (Sigma-Aldrich, MO, USA)/phosphate-buffered saline into the hind limb muscle of an athymic mouse. The imaging sequence is as follows: **(A–C)** preinjection; **(D–F)** needle insertion and positioning; **(G–I)** postinjection; **(J–L)** needle removal and final imaging; and **(M–O)** contrast enhancement in order to illustrate increased signaling. Pixels that were increased relative to the preinjection image are coded in yellow. Note the significant signal increase in **(M)** and **(O)** at the injection site, relative to **(A)** and **(C)** (dashed circles highlight the injection site). Also note the low signal in the negative control **(N)**. The scale bar in **(M)** and the intensity scale in **(L)** and **(O)** apply to all images. The red dashed circles in **(J–L)** indicate that the injection bolus can also be seen with brightness-mode ultrasound. Real-time injection imaging of the cells between **(F)** and **(I)** may be seen in the supporting information of [20], with video 1 at 8× speed and video 2 in real-time.
For color images please see online http://www.futuremedicine.com/doi/full/10.2217/nnm.14.129.
b: Bone; MSC: Mesenchymal stem cell; SiGNR: Silica-coated gold nanorod.
Reproduced with permission from [20].

[12] and dendritic cells [37] – in order to fight tumors and induce additional immune responses. One of the major obstacles in developing immunotherapeutic approaches is the absence of noninvasive imaging tools for the *in vivo* monitoring of immune cells [39].

Clinical-grade primary T cells provide an important example of the immune cells that are used in this approach. *Ex vivo* manipulation of T cells improves *in vivo* effector functions. An acute need exists for a clinically appealing approach in order to assess the biodistribution of T cells.

In a recent study, GNPs were developed by coupling gold to radioactive ^{64}Cu, and these were electrotransferred into genetically modified T cells for imaging with PET. The T cells were found to be capable of reporting their distribution *in vivo* by PET, which represents a step towards developing GNPs as radiolabels for cell-based therapies [22].

Although endocytosis is the most common mechanism for the internalization of GNPs, is not practical with radioactive materials that have short half-lives, as it requires extended periods of time. Therefore, electroporation was used as a method to label T cells.

In addition to being labeled with nanoparticles, the T cells were genetically modified in order to enforce expression of a reporter gene – *ffLuc* – for bioluminescent imaging. The *ffLuc* activity is a measure of T-cell viability, as it is optically active only in live cells. The researchers showed that the presence of nanoparticles in the cells did not detract from the cells' ability to affect *ffLuc* activity. The PET signals colocalized with the bioluminescent imaging signal (Figure 7). This supports the hypothesis that viable genetically modified T cells can be tracked using a positron emitter – ^{64}Cu – and imaged by a μPET/CT scanner.

An advantage of using GNPs is that the conjugation to ^{64}Cu, as well as the electrotransfer into T cells, can be undertaken using methods that are in compliance with current good manufacturing practices for Phase I/II trials. However, improvements are still necessary in this field, as electroporation can induce T-cell death, followed by a release of the nanoparticles from the necrotic cells.

The study mentioned above is the first to have demonstrated the use of GNPs for immune cell tracking. Other imaging modalities and types of GNPs ought to be investigated, as well as suitable techniques for imaging T cells and other immune system cells used for cell-based immunotherapies.

Overview of *in vitro* cell-labeling methods & *in vivo* imaging modalities

The aforementioned GNP-based *in vivo* cell tracking techniques highlight this field's potential. Table 1 provides a detailed summary comparing the different *in vitro* parameters in each type of cell and the different *in vivo* imaging studies that have been undertaken. As demonstrated, the GNPs used for cell labeling are unanimously proven to be biocompatible, with no significant effects on the cells. Importantly, research has demonstrated that the size, shape and surface charge of the nanoparticles, as well as the incubation time, have a crucial impact on the rate and quantity of the *in vitro* labeling process. Moreover, variability between

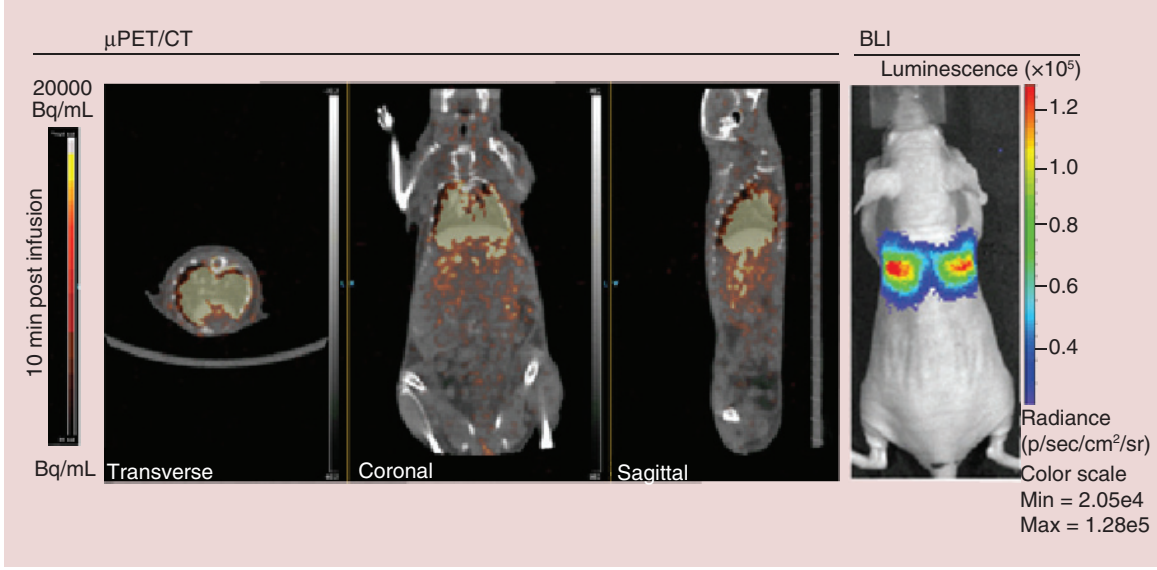

Figure 7. PET images (transverse, coronal and sagittal planes) from T cells labeled with particles infused in a mouse, correlated with a bioluminescent imaging signal.
BLI: Bioluminescence imaging ;CT: Computed tomography.
Reproduced with permission from [22].

cell origin and type, number of cells injected, injection site and divergent animal models make it difficult to draw definite design principles from across these studies. Further research is essential in order to establish basic design principles regarding the detection limit and the maximum possible time for cell tracking for each imaging modality.

Conclusion & future perspective

In this article, we have summarized recent GNP-based cell tracking approaches that are applied in a variety of biological applications. Although the studies reviewed above seem highly promising, with feasible clinical applications, various factors complicate any conclusions regarding definite design principles from across these studies. Such difficulties stem from the differences in size and chemical coating of the nanoparticles, different labeling strategies, variability among animal models and diverse imaging techniques. Further research is essential in order to establish basic design principles that will determine the optimal *in vitro* labeling procedure and highlight the technical abilities and constraints of each imaging modality. Moreover, since no single imaging modality meets all of the cell tracking requirements, multimodal imaging is also necessary.

In this context, nanoparticle-based cell-labeling strategies, which can be used in combination with clinically established anatomical imaging methods (such as CT, MRI and US), will provide additive value, as they can enable visualization not only of the cells that have been injected, but also of the pathology of the illness at the same time. In addition, nanoparticles can also serve as a platform for gene and drug delivery and even as nanobiosensors within cells. Clearly, progress in this field will crucially impact cellular therapy research avenues and clinical applications.

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Executive summary

Gold nanoparticles for the future of cell-based therapy

- Cell therapy is the transplantation of living cells for the treatment of diseases and injuries. A clinically applicable approach for noninvasive *in vivo* cell tracking is necessary for further the development of such cell-based therapies.
- Gold nanoparticles (GNPs), engineered to label transplanted cells and serve as contrast agents for several imaging modalities, have proven to be a promising novel tool for biomedical researchers.
- GNPs are biocompatible and do not significantly affect cell viability, proliferation and differentiation.

Applications of GNP cell labeling

- As a proof of concept for this *in vivo* cell tracking technique, imaging of malignant cells could be efficiently performed by combining GNPs with x-ray computed tomography.
- Stem cells labeled with GNPs could be tracked over long periods of time with photoacoustic imaging post-subcutaneous injection. The combination of photoacoustic imaging with ultrasound also allows for real-time monitoring of the implantation of the cells.
- Immune cells could report their biodistribution *in vivo* by PET by labeling T cells with GNPs coupled to a radiolabel. These cells have the potential to prevent and treat cancer.

Future perspective

- Challenges remain regarding establishing basic design principles in order to determine the ideal size, shape and coating of GNPs, as well as the optimal cell-labeling procedure.
- As no single imaging modality fulfills all of the cell tracking needs, multimodal imaging is required. This will improve the limit of detection, allow for long-term imaging, and provide relevant anatomical knowledge.

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