Nanoparticles for Stem Cell Tracking and the Potential Treatment of Cardiovascular Diseases

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Stem cell-based therapies have been shown potential in regenerative medicine. In these cells, mesenchymal stem cells (MSCs) have the ability of self-renewal and being differentiated into different types of cells, such as cardiovascular cells. Moreover, MSCs have low immunogenicity and immunomodulatory properties, and can protect the myocardium, which are ideal qualities for cardiovascular repair. Transplanting mesenchymal stem cells has demonstrated improved outcomes for treating cardiovascular diseases in preclinical trials. However, there still are some challenges, such as their low rate of migration to the ischemic myocardium, low tissue retention, and low survival rate after the transplantation. To solve these problems, an ideal method should be developed to precisely and quantitatively monitor the viability of the transplanted cells in vivo for providing the guidance of clinical translation. Cell imaging is an ideal method, but requires a suitable contrast agent to label and track the cells. This article reviews the uses of nanoparticles as contrast agents for tracking MSCs and the challenges of clinical use of MSCs in the potential treatment of cardiovascular diseases.

Keywords: nanoparticles, stem cell therapy, mesenchymal stem cell, cardiovascular diseases, imaging technique

INTRODUCTION

In the medical world, stem cell-based therapies have emerged and expanded as a potentially promising therapeutic treatment (Heslop et al., 2015). In such a treatment process, stem cells, possessing properties of self-renewal and potency, play a significant role, and under appropriate conditions, these cells can be differentiated into a variety of cells (Srivastava and Ivey, 2006; Maumus et al., 2011; Hao et al., 2014; Aly, 2020). Compared with direct transplantation of tissue or organ for patients, stem cell-based therapies are more promising and potential because these cells can be feasibly and readily obtained avoiding donor shortage and immune incompatibility (Dickman and Guilak, 2013; Lilly et al., 2016). In general, stem cells can be divided into two major types, embryonic stem cells (ESCs) and adult stem cells (ASCs) (Lv et al., 2014). The transplantation of ESCs derived from the early embryo has been demonstrated to be effective in the treatment of many diseases, due to their being highly undifferentiated cells and having the ability of evolving into all tissues and organs (Krebsbach and Robey, 2002; Hao et al., 2009). However, the research for ESCs is controversial, being difficult to accepted by common people (Li et al., 2010). Compared with ESCs, ASCs, which originated from tissues, are easily accepted and have been widely applied...
in preclinical and clinical trials, but leads to low capacity of differentiation (Young and Black, 2004; Ye et al., 2006; Alison and Islam, 2009). For example, the hematopoietic stem cells (bone marrow), originated from ASCs in the blood, can differentiate into various types of mature blood cells, and their transplantation has been used to treat all kinds of blood diseases (Carella et al., 2000; Schmitz et al., 2002; Mikkola and Orkin, 2006; DiPersio et al., 2009; Jaiswal et al., 2009). Neural stem cells, possessing the ability of differentiating into neurons and astroglia, are also derived from ASCs, and their transplantation may treat diseases of the nervous systems (Temple, 2001; Lee et al., 2005, 2009; Andres et al., 2008; Stenudd et al., 2015). In 1970, Friedenstein discovered a rare type of stromal cells in human bone marrow that are now known as mesenchymal stem cells (MSCs) (Friedenstein et al., 1970). Although MSCs have similar abilities to ASCs, there is still a lot of debate about their origin. Moreover, some researchers claimed that the name of MSCs should be changed to medicinal signaling cells in order to accurately reflect the fact that these cells play the role of constructing new tissues (Caplan, 2017). At present, MSCs are found in many tissues beyond the bone marrow, including adipose tissue, lung tissue, synovial membrane, endometrium, and peripheral blood. In different tissues, MSCs exhibit differences in immunophenotype, differentiation potential, and immunomodulatory. The researches show that the transplantation of MSCs is an effectively promising and potentially therapeutic treatment for various diseases including wound healing, ischemic encephalopathy, and ischemic heart disease (Grauss et al., 2007; Wu et al., 2007; Uccelli et al., 2008; Kim et al., 2015a; Xie et al., 2016).

To date, stem cell-based therapies have been intensively employed in the treatment of wounds, blood and cardiovascular diseases, cartilage defect, diabetes, etc. (Goradel et al., 2018; Vagnozzi et al., 2020). However, there are still much to be further explored for stem cell-based therapies, in order to identify therapeutic efficacy and further dosage (Yi et al., 2017). For these purposes, it is necessary to track stem cells in vitro and in vivo with high temporal and spatial resolution in a real-time manner during a prolonged period, which involves monitoring stem cells’ behavior in vivo, identifying if they are capable of surviving, integrating with the host tissue, undertaking the desired cellular differentiation, and finally unveiling the responses and behaviors of transplanted cells upon exposure to various diseased environments.

THE MEANS OF TRACKING STEM CELLS AND THE IMPORTANCE OF NANOPARTICLES

For tracking stem cells, non-invasive cell imaging is currently a very hot field of research (Kircher et al., 2011). Compared with traditional histopathological techniques, non-invasive cell imaging can monitor the behaviors of the transplanted cells over time in vivo for further treatment offering detailed information, thus, showing more efficient therapeutic effects (Cahill et al., 2004; Walczak and Bulte, 2007; Bulte, 2017; Meir and Popovtzer, 2018; Peng et al., 2018). Only by using non-invasive means can the viability of the transplanted cells be estimated in the clinical setting, showing potential prospects (Shang et al., 2017; Tkaczyk, 2017). Non-invasive cell tracking can be divided into direct and indirect labeling techniques. Compared with direct labeling, which can directly label cells, possessing easy operation but being unable to track daughter cells due to fast signal decay, indirect labeling obtains the ability of labeling cells by implanting an indicator into cells (Li et al., 2008; Kraitchman and Bulte, 2009; Kim et al., 2016a,b). Although indirect labeling is complex and expensive, it is more effective and stable for tracking the differentiation of the transplanted cells (Davis, 1972; Akins and Dubey, 2008; Hong et al., 2010). In general, indirect labeling can be divided into two major classes, gene modification and external label. Gene modification is suitable for long-term tracking because the labeled genes can be steadily expressed in the next generation (Chan et al., 2017; Su et al., 2019). However, the imaging technique of gene modification is single and only being detected by a tracking fluorophore leading to the limitation of tissue penetration (Herschman, 2004; Loewen et al., 2004). In addition, the labeled genes are derived from exosomes, leading to immune incompatibility (Zheng et al., 2017). Another indirect labeling method, external label, can overcome the obstacle of the foreign gene by introducing additional contrast agents, but it still has some limitations (Crabbe et al., 2010; Li et al., 2013; Hachani et al., 2017). Because the introduction of contrast agents brings some new problems, including the reduction effect of photo-signal over time, optical interference is induced by tissue autofluorescence, incompatibility, cytotoxicity, and low labeling efficiency (Bellin, 2006; Cosgrove, 2006; Lusic and Grinstaff, 2013). Thus, an appropriate contrast agent should be developed and utilized to break through these limitations.

Nanoparticles (NPs), the diameter of which ranges from 1 to 100 nm, cover a diverse range of chemical composition with an equally diverse number of applications (Khan et al., 2019). Due to their nanoscale dimensions, NPs can easily transport across cell membrane and reach the crucial organelles including the endoplasmic reticulum, mitochondria, and nucleus (McNamara and Tofail, 2017). Moreover, a high surface area-over-volume ratio augments their interaction with cellular components (Bhirde et al., 2011; Edmundson et al., 2013). Furthermore, the morphology and size of NPs determine their physicochemical properties leading to the differences in cellular uptake and interaction with biological tissues, which could not be achieved by bulk materials (Barua and Mitragotri, 2014). As a result, NPs have been regarded as a promising contrast agent. Nowadays, nanotechnology in medical science has been regarded as a great breakthrough in this century (Zhang et al., 2008; Lin, 2015). Importantly, some special NPs have unique magnetic and/or optical properties, which can offer real-time imaging in vivo for tracking the transplanted stem cells, showing a strong potential in breaking through the obstacle of external label (Rhyner et al., 2006; Hahn et al., 2011). These NPs for tracking stem cells include organic, inorganic, and composite NPs, and the representative examples have magnetic NPs and photoacoustic NPs (Van Den Bos et al., 2003; Ferreira, 2009; Wang et al., 2012b; Gao et al., 2013; Accomasso et al., 2016; Riera et al., 2019).
The transplanted stem cells labeled by these NPs can be detected by multiple imaging methods, such as magnetic resonance imaging (MRI), nuclear imaging (including single-photon emission computed tomography imaging (SPECT) and positron emission tomography-computed tomography (PET-CT)), and photoacoustic imaging (Sutton et al., 2008; Andreas et al., 2012; Cheng et al., 2016; Guo et al., 2019b; Patrick et al., 2020). However, there are still many problems to be addressed in the actual application process for NPs. First, NPs for tracking stem cells in vivo always require good cellular uptake and efficient internalization. Therefore, the surface chemistry of NPs should be importantly considered (Accomasso et al., 2016). In most cases, NPs with positive charge have the improvement of cellular uptake due to electrostatic interaction, leading to an accelerated internalization (Saha et al., 2013; Behzadi et al., 2017). Certainly, NPs with positive charge also may delay their internalization process, due to the influence on the adsorption of some specific proteins (Jambhrunkar et al., 2014). Second, importantly, before use, NPs should be comprehensively characterized for their composition and purity under different conditions, in order to evaluate their toxic effects on cells and ensure safety in vivo (Chandran et al., 2017). The reasons are that their toxic effects on cells may highly depend on the unique characteristics of NPs themselves. What is more, compared with bulk material, NPs have higher surface area-to-volume ratio and surface reactivity, which are more susceptible to the environment, so they should be modestly employed (Alkilany and Murphy, 2010; Lankoff et al., 2012). In addition, close attention should be paid to the agglomeration of NPs, which may lead to bad cellular uptake and further induced cytotoxicity (Gliga et al., 2014).

The internalization of NPs is influenced by their charge, size, shape, and the ability of cellular uptake, due to the force of their internalization driven by endocytosis. After internalization by endocytosis, NPs will pass environments of lysosomes and peroxisomes where the acidic material and oxidative substance could entirely degrade them. Possibly, a part of them may undergo exocytosis or escape to other intracellular locations (Chen et al., 2013; Calero et al., 2014).

**NANOPARTICLES FOR TRACKING MSCS AND THE POTENTIAL TREATMENT OF CARDIOVASCULAR DISEASES**

Cardiovascular diseases (CVDs), with high incident, are one of the major causes of human diseases worldwide and responsible for more than 17.7 million deaths in 2015, according to the World Health Organization (Zampelas and Magriplis, 2020). Therefore, developing an effective means for treating CVDs is of paramount urgency (Behlke et al., 2020). Currently, the means for treating CVDs include, but are not limited to, surgical treatment, delivering the targeted drug, and the repair and regeneration of the cardiac tissues (Godin et al., 2010; Cui et al., 2016; Deng et al., 2020). However, surgical treatment and targeted drug delivery only can delay and relieve the progression of CVDs but are unable to solve the inherent problems due to the deficiency of the regeneration for dead heart cells. In regenerative medicine, adult stem cell-based therapies have been shown as a promising potential for treating cardiovascular diseases in preclinical trials because the repair and regeneration of the cardiac tissues can be expected to solve the differentiation of stem cells into heart cells, thereby, offering improved efficacy (Kastrup, 2011). In these cells, MSCs play an important role in the therapy of CVDs, due to their ability of self-renewal and differentiating into various types of cells, including cardiovascular cells (Caplan, 2009; Ye et al., 2014). Moreover, after transplantation of MSCs, they can generate a structure resembling cardiac myocytes and exert anti-inflammatory effects, which can protect the myocardium, further improving the repair of the cardiovascular system. Notably, MSCs have been demonstrated by preclinical studies to have a strong ability to generate blood vessels with minimal adverse effects on the tissues near the injected region. Therefore, the transplantation of MSCs is potentially promising to improve the treatment of CVDs (Trivedi et al., 2010; Goradel et al., 2018; Guo et al., 2020). Despite its great potential for CVD treatment, MSC-based therapies still face some challenges, for example, the real-time tracking for MSC fate after their transplantation, which can provide information such as cell differentiation, the role that MSCs play in vascular repair, and mechanisms of cardiovascular regeneration to guide therapy process and improve therapeutic effect.

Cell imaging is an ideal method for real-time tracking of MSCs, but requires a suitable contrast agent to label MSCs. NPs are a group of contrast agent, which have been widely applied in MSC tracking (Zhang and Wu, 2007; Fu et al., 2011; Liang et al., 2013; Santoso and Yang, 2016; Yi et al., 2017; Guo et al., 2019a). Especially, MSCs labeled by magnetic and/or optical NPs can directly be imaged to offer a real-time tracking in vivo (Jing et al., 2008; Setzer et al., 2014; Wu et al., 2014; Perez et al., 2017). These NPs include superparamagnetic iron oxide (SPIO) NPs, gadolinium (Gd)-based NPs, gold (Au)-based NPs, quantum dots (QDs)-based NPs, upconversion (UC)-based NPs, silicon-based NPs, and several other classes of NPs. Here, we will summarize and discuss in detail NPs for labeling and tracking MSCs in vivo, shown in Table 1.

**Superparamagnetic Iron Oxide Nanoparticles**

Generally, SPIO NPs are a kind of NPs with a core–shell structure, in which the core is composed of an iron oxide and then is covered by a coating layer as the shell. The coatings layer alters the surface properties of SPIO NPs, and the iron oxide core provides its magnetic properties (Singh et al., 2010; Mahmoudi et al., 2011). Due to unique magnetic properties, SPIO NPs have the ability to generate a strong inhomogeneous field leading to the contraction of T2 relaxation time, thereby, generating obvious contrast in T2-weighted MRI, and are generally used as a T2-contrast agent (Andreas et al., 2012; Li et al., 2013; Bull et al., 2014; Santoso and Yang, 2016; Jiang et al., 2019). So far, SPIOs are the only commercial NPs, several formulations of which have already been approved by the Food and Drug Administration and have been regulated for clinical applications (Wang et al., 2013). MSCs labeled by SPIO NPs can be tracked by MRI, providing a
### TABLE 1 | Comparison between nanoparticles for tracking mesenchymal stem cells (MSCs).

| Example | Types of nanoparticles (NPs) | Preparation | Imaging modality | Types of MSCs | Advantages | Disadvantages | Main results |
|---------|-----------------------------|-------------|-----------------|---------------|------------|--------------|-------------|
| Lu et al., 2007; Andreas et al., 2012 | Superparamagnetic iron oxide (SPIO) NPs | Easy | T2-weighted magnetic resonance imaging (MRI) | Human MSCs | Obvious contrast | Low cell-labeling efficiency for MSCs; necessary to functionalize SPIO NPs | No apparent influences on viability of MSCs |
| Babic et al., 2008; Kim et al., 2011; Szpak et al., 2013; Lewandowska-Lancucka et al., 2014; Park et al., 2014; Liao et al., 2016; Rawat et al., 2019; Gu et al., 2018; Lee et al., 2020 | SPIO NPs | Complex | T2-weighted MRI | Human MSCs; mouse MSCs | Obvious contrast; enhancement of cellular internalization | Induce precipitation of NPs; perturb the cell membrane | No apparent influences on viability of MSCs |
| Zhang et al., 2010 | SPIO NPs | Easy | Proton ($^1$H) MRI images | Human MSCs | High sensitivity of cell detection | Difficult to accurately quantify cell population | No apparent influences on viability of MSCs |
| Sehl et al., 2019 | SPIO NPs | Complex | Proton ($^1$H) MRI images | Mouse MSCs | High sensitivity of cell detection; quantify the persistence of transplanted MSCs | Low cellular uptake for MSCs | No apparent influences on viability of MSCs |
| Sherry et al., 1988; Ratzinger et al., 2010 | Gd-based NPs | Easy | T1-weighted MRI | Human MSCs | Distinguish some similar low signal | No apparent influences on viability of MSCs |
| Kim et al., 2015b; Santelli et al., 2018 | Gd-based NPs | Easy | T1-weighted MRI | Human MSCs | Distinguish some similar low signal; long-term tracking | No apparent influences on viability of MSCs |
| Huang et al., 2020 | Au-based NPs | Easy | Photoacoustic imaging and CT imaging | Mouse MSCs | Detected in deep tissue at a high resolution; directly labeled; excellent biocompatibility | No apparent influences on viability of MSCs |
| Chen G. et al., 2015; Li et al., 2016 | QD-based NPs | Complex | Fluorescence imaging | Human MSCs | Longer lifetime than traditional fluorescence dye; photochemical stability | Unsatisfied cytotoxicity and stochastic blinking; the contradiction of sensitivity and definition | No apparent influences on viability of MSCs |
| Idris et al., 2009; Ang et al., 2011; Zhao et al., 2013; Chen X. et al., 2015; Ma et al., 2016 | UC-based NPs | Complex | UC luminescence imaging | Human MSCs | Detected in deeper tissue; more stable and higher definition | Poor biocompatibility; low uptake for cellular | No apparent influences on viability of MSCs |
| Huang et al., 2005; Chen and Jokerst, 2020; Yao et al., 2020 | Silicon-based NPs | Complex | Fluorescence imaging | Human MSCs | Excellent biocompatibility and chemical inertness; easily modified by bioconjugation; good cellular uptake | No apparent influences on viability of MSCs |
| Chen et al., 2019 | Silicon-based NPs | Complex | Photoacoustic imaging | Human MSCs | Good biocompatible and cells tracking capacity; long time | No apparent influences on viability of MSCs |
| Gao et al., 2016 | Other-based NPs | Complex | Aggregation-induced emission imaging | Mouse bone marrow-derived MSCs | Possessing long-term tracking and strong anti-photobleaching ability | No apparent influences on viability of MSCs |
| Yin et al., 2018 | Other-based NPs | Complex | Photoacoustic imaging | Human MSCs | High signal-to-noise; deeper tissue imaging | No apparent influences on viability of MSCs |
non-invasive method to monitor the fate of transplanted MSCs in vivo (Li et al., 2013; Bull et al., 2014; Guldris et al., 2017; Xie et al., 2019; Elkhenany et al., 2020).

However, MSCs lack phagocytic capacity, and the surface charge of SPIO NPs is negative, which is a similar charge to that of the cellular membrane. Therefore, the internalization of SPIO NPs is limited, leading to a low efficiency of cell labeling. In order to solve these problems, it is necessary to functionalize SPIO NPs by introducing positive charges or other cell membrane-penetrating moieties on the surface (Lu et al., 2007; Andreas et al., 2012). Moreover, these unique functionalized surfaces also increase the stability of SPIO NPs in hydrophilic conditions, as well as are used as a coating layer for delaying the degradation of the iron oxide core (Barrow et al., 2015). For example, the uses of cationic polymers such as poly-L-lysine (PLL) and chitosan have commonly been utilized to improve the cellular internalization of SPIO NPs and protect them from being easily degraded in vivo (Babic et al., 2008; Szpak et al., 2013; Lewandowska-Łańcucka et al., 2014; Park et al., 2014). As an MRI probe for tracking MSCs, PLL-SPIO NPs were more efficient and safer than naked iron oxide NPs, which was in accordance with the fact that their $R_2$ value was higher than that of uncoated nanoparticles. However, the highly positive charge of PLL induced the precipitation of NPs and perturbed the cellular membrane. Liu et al. (2011) used polyethylenimine with a low molecular weight (2 kDa) to wrap SPIO NPs and obtain PEI–SPIO NPs, which could be readily internalized by MSCs for long-term tracking (19 days). Compared with single SPIO NPs, the modified SPIO NPs hold a controlled clustering structure, leading to much higher T2 relaxivity. Moreover, Lewin et al. (2000) used a short HIV-Tat peptide to functionalize SPIO NPs, which could be effectively engulfed by hematopoietic and neural progenitor cells. The results suggested that the cellular uptake of iron can reach an extremely high level (10–30 pg per cell) showing effective cell labeling and MRI tracking. However, NPs functionalized by cationic polymer or molecules may cause a certain level of toxicity, although it would drastically enhance cellular uptake of NPs. Therefore, some researchers (Kim et al., 2011; Liao et al., 2016) utilized 2-aminoethyl-trimethyl ammonium (TMA) to modified SPIO NPs. It was found that TMA–SPIO NPs could easily and efficiently label MSCs to monitor their fate in vivo, and the labeled MSCs showed low cytotoxicity. In another work, Gu et al. (2018) synthesized PA–SPIO NPs using a self-assembled lipopeptide amphiphile (PA) to modify the surfaces of SPIO NPs for labeling mouse MSCs. The modified NPs showed the enhancement of labeling efficiencies and the improvement of their contrast by shortening the relaxation time.

In addition, these NPs exhibited excellent dispersibility and stability in water. More importantly, MSCs labeled by the PA–SPIO NPs were transplanted into mouse, showing no adverse effects on the osteogenic and adipogenic differentiation. Rawat et al. (2019) synthesized SPIO NPs and then used them to label MSCs. The labeled MSCs could be tracked by MRI to understand their fate in vivo. Moreover, these labeled MSCs still maintained differentiation potential. Lee et al. (2020) used poly lactic-co-glycolic acid (PLGA) to modify SPIO NPs, and then utilized fluorescent dye Cy5.5 to functionalize the prepared NPs for labeling and tracking MSCs to investigate the interactions between PLGA–SPIO NPs and MSCs. The results showed that the prepared NPs had no apparent influences on the survival and differentiation of MSCs when the concentration was at 40 µg/ml for more than 96 h, demonstrating that the internalization pathway of MSCs is via endocytosis.

In proton ($^1$H) MRI images, SPIO NP-labeled cells appear as signal void regions (Miguel et al., 2007). This effect gives rise to enhance the sensitivity of cell detection, but poses challenges for accurate quantification of the cell population as well. Another limitation for SPIO NP-based cell tracking is the lack of specificity in some tissues. It is ambiguous to identify these cells in vivo, as other regions in anatomic MRI appear as dark as well (Zhang et al., 2010). To overcome these limitations, Sehl et al. (2019) utilized the combination of iron-based MRI, $^{19}$F MRI, and MPI cellular imaging technologies to monitor and quantify the persistence of the transfected MSCs and infiltrate macrophages in vivo, shown in Figure 1. First, MSCs were labeled with iron oxide NPs (ferumoxytol) and then implanted into the hind limb muscle of 10 C578/6 mice. Finally, a perfluorocarbon agent was administered intravenously for uptake by phagocytic macrophages in situ. The ferumoxytol-labeled MSCs were detected by proton ($^1$H) MRI and magnetic particle imaging (MPI). Perfluorocarbon-labeled macrophages were detected by fluorine-19 ($^{19}$F) MRI. These three modalities are complementary and provide integrated information (specificity, sensitivity, and quantification of cell number). They proposed that these cellular imaging techniques could be used to monitor MSC engraftment over time and detect the infiltration of macrophages at transplant sites.

**Gadolinium-Based Nanoparticles**

T1-weighted sequences are often collected before and after infusion of T1-shortening MR contrast agents, so as to reduce the T1 relaxation times of $^1$H atoms in water molecules and produce hyperintensities in T1-weighted images appearing white (Na and Hyeon, 2009). Currently, Gd-based NPs are the most widely used T1-contrast agent for labeling and tracking stem cells (Ni and Chen, 2015). Compared with T2-contrast agent of SPIO NPs, T1-contrast agent of Gd-based NPs can distinguish some similar low signal resulting from intrinsic signal in tissue or hemorrhage, due to their capability of generating a bright positive signal. Therefore, a T1-contrast agent generally is predominately employed as a probe in detecting the transplanted stem cells in a low-signal region (Na and Hyeon, 2009; Zhu et al., 2013; Robert et al., 2015).

Gd-based NPs usually are complex NPs, being composed of Gd$^{3+}$ and their chelating ligand of which diethyltriamine pentaacetic acid (DTPA) is the most common (Sherry et al., 1988; Ratzinger et al., 2010). Modified by DTPA, GD-based NPs reduced cytotoxicity due to the enhancement of hydrophilicity, but also weakened the interaction with the cell membrane leading to low cellular uptake for MSCs. Furthermore, their labeling efficiency was low resulting from the poor targeting ability of MSCs. Considering these problems, Tseng et al. (2010) prepared Gd-based NPs used as an MRI contrast agent to label and track human MSCs. Due to the modification of hexaneidine, the
FIGURE 1 | (A) Histological validation showing the presence of MSCs surrounded in connective tissue (CT) and muscle (M) in hematoxylin and eosin at ×10 magnification (scale bar 500 µm). (B) In vivo proton (\(^1\)H)/fluorine 19 (\(^19\)F) magnetic resonance imaging (MRI) and magnetic particle imaging (MPI) (adapted Sehl et al., 2019).

biocompatibility of the prepared Gd-based NPs was improved. Moreover, compared with Gd-based NPs modified by DPTA, the prepared Gd-based NPs showed more excellent cellular uptake for MSCs and could be easily detected in vivo due to the shortening of T1 relaxation times. In another work, Kim et al. (2015b) reported a Gd-based NP to label MSCs, in which Gd\(^{3+}\) was chelated with a pullulan derivative ligand. It was found that the prepared Gd-based NPs could dramatically enhance cellular uptake for MSCs, leading to the enhancement of internalization efficiency from 32 ± 2 to 98 ± 4 pg Gd/cell. Moreover, the labeled MSCs achieved a long-term tracking of 21 days in vivo, due low signal attenuation. Cai et al. (2017) fabricated Gd-based NPs of 7 nm for tracking MSCs by utilizing melanin to modify Gd\(^{3+}\). The results showed that Gd-based NPs modified by melanin increased the stability of MRI contrast agent and shorten T1 relaxation time, compared with traditional Gd-based NPs modified by DTPA. The labeled MSCs achieved long-term tracking for more than 4 weeks, due to the enhancement of labeling efficiency. Importantly, the labeled MSCs had no apparent cytotoxicity at a proper concentration of 800 µg/ml. However, preclinical studies suggested that for tracking MSCs in vitro and in vivo, the single-imaging technique was difficult to complete, and the integration of multiple labeling means often was required. Consequently, Santelli et al. (2018) developed multimodality imaging to track MSCs labeled by Gd-based NPs, shown in Figure 2. The GD-based NPs composed of spherical europium-doped gadolinium oxysulphide (Gd\(_2\)O\(_2\)S:Eu\(^{3+}\)) could be detected by MRI, X-ray imaging, and photoluminescence imaging. In the in vitro test, the number of MSCs labeled by Gd\(_2\)O\(_2\)S:Eu\(^{3+}\) was up to 2,500, showing feasible cell tracking.
Moreover, the results suggested that the effects of the NPs on viability, proliferation, migration, and differentiation of the transplanted MSCs were innocuousness.

**Gold-Based Nanoparticles**

Due to their cytocompatibility and strong optical absorption in the near-infrared region, Au-based NPs are potential contrast agents of photoacoustic imaging. In addition, Au-based NPs can be detected in deep tissue at 2 cm at a high resolution of 100 µm, so they are emerging as an alternative method for tracking cells *in vivo* (Murphy et al., 2008; Giljohann et al., 2010).

More importantly, MSCs can be directly labeled by Au-based NPs, so their differentiation after transplantation *in vivo* can be detected using photoacoustic imaging *in vivo* (Turjeman et al., 2015). Donnelly et al. (2018) utilized Au-based NPs as a photoacoustic contrast agent for real-time tracking MSCs to guide their delivery. It was found that the labeled MSCs could be detected by ultrasound/photoacoustic imaging in the spinal cord. In another work, Dhada et al. (2019) prepared Au-based NPs to label and track MSCs by photoacoustic imaging, which was composed of gold nanorods and IR775. The results suggested that cell death also could be detected because IR775 was sensitive...
FIGURE 3 | (A) Schematic illustration of the synthesis of AA@ICG@PLL gold (Au)-based nanoparticles (NPs). (B) Experimental design for tracking AA@ICG@PLL-labeled MSCs in a silica-induced PF mouse model. (C) Intracellular Au content measured by ICP-MS. (D) Relative viability of BMSCs labeled with AA@ICG@PLL NPs at various Au concentrations. (E) In vivo computed tomography (CT) images of AA@ICG@PLL-labeled BMSCs at 7, 14, and 21 days after transplantation. (F) Bright-field images of Oil Red O staining and ALP staining of NP-loaded and unloaded BMSCs. Scale bar = 20 mm (adapted by Huang et al., 2020). *p < 0.05 compared with the unlabeled group.

to reactive oxygen species (ROS). Therefore, the viability of the transplanted MSCs in vivo was quantificationally measured. Moreover, Laffey et al. (2020) studied the viability of MSCs labeled by Au-based NPs in a frozen environment. The results showed that the labeled MSCs undergoing the process of freezing, storing, and thawing for 2 months still could be detected by photoacoustic imaging, and their differential ability also had no apparent difference with the unlabeled MSCs.

Certainly, Au-based NPs also can be detected by computed tomography (CT), due to the high X-ray absorption. Thus, they are used as a CT maker to label and track MSCs. For example, Betzer et al. (2017) used Au-based NPs as labeling agents to
longitudinally and quantitatively track MSCs in vivo. It was found that the labeled MSCs were detected for up to 24 h by CT, showing a long-term tracking. Nafiujjaman and Kim (2020) reported a biocompatible Au-based NPs used as a CT maker, in which Au NPs were modified by poly-L-lysine (PLL) to change the charge properties of surface enhancing the cellular uptake. The MSCs labeled by Au-based NPs could be detected by CT and showed high labeling efficiency. Huang et al. (2020) synthesized Au-based NPs (AA@ICG@PLL) with dual-modal imaging (CT and near-infrared fluorescence) to label and track MSCs of mice in vivo, shown in Figure 3. Due to modification by indocyanine green (ICG) and poly-L-lysine (PLL), AA@ICG@PLL showed excellent cellular uptake for MSCs and biocompatibility. It was found that the labeled MSCs could be tracked via dual-modal imaging for more than 21 days, and importantly, the prepared NPs had anti-inflammatory properties.

Quantum Dot-Based Nanoparticles

QD-based NPs are semiconductor NPs with II–VI or III–V elements, which can emit particular frequency light after being stimulated (Jaiswal et al., 2004). The emitted light frequency depends on the size and shape of QD-based NPs. Therefore, QD-based NPs show different colors (Seleverstov et al., 2006). In general, the fluorescence lifetime of QD-based NPs is longer than traditional fluorescence dye (Bailey et al., 2004). In addition, compared with fluorescent dye, QD-based NPs are easier to be detected due to the non-overlapping of absorption and emission (Mazumder et al., 2009). Specifically, QD-based NPs have broad absorption of continuous distribution, but show narrow and symmetrical properties (Yukawa and Baba, 2017). More importantly, they have the advantage of photochemical stability. As a result, QD-based NPs have been used as contrast agents for long-term tracking of MSCs in vivo. However, QD-based NPs also have some disadvantages, such as unsatisfied cytotoxicity and stochastic blinking. Moreover, the augmentation of QD-based NPs in dosage increases the sensitivity of cell imaging, but may decrease their definition (Shah et al., 2007; Liang et al., 2013).

Hsieh et al. (2006) reported a QD-based NP to label human MSCs, in which CdSe was used as the core, and the shell was covered by ZnS. In addition, the commercial QD-based NPs also were developed and utilized as contrast
agents to label and track the cell distributions. However, these QD-based NPs showed unsatisfied biocompatibility and cytotoxicity, so they had to be modified before use. In recent years, Li et al. (2016) prepared QDs-based NPs (RGD-β-CD-QDs) to label and track human MSCs, which were composed of QDs, β-cyclodextrin (β-CD), and Cys-Lys-Lys-ArgGly-Asp (CKKRGD) peptide. The QDs modified by β-CD enhanced the cellular uptake and facilitated the differentiation of MSCs, due to the small molecule dexamethasone and siRNA carried by β-CD. More importantly, the labeled MSCs could be detected for up to 3 weeks. Chen G. et al. (2015) reported a AgS₂ QD-based NP for tracking human MSCs in vivo by utilizing fluorescence imaging, shown in Figure 4. It was found that the prepared NPs with good biocompatibility could be detected in the second near infrared, and the stability of fluorescence reached up to 30 days. Furthermore, the labeled MSCs was dynamically visualized at high resolution of 100 ms for more than 14 days. Chetty et al. (2019) prepared CuInS₂-ZnS QD-based NPs to label umbilical cord-derived MSCs, and the labeling efficiency reached 98% due to the prepared QD-based NPs displaying high photoluminescence quantum yield of 88%. In addition, the labeled MSCs showed no apparent influences on stemness and had the ability of long-term tracking.

**Upconversion-Based Nanoparticles**

UC-based NPs, usually being lanthanide-doped nanocrystals, can undergo a photon UC process in which the sequential absorption of two or more photons leads to anti-Stokes type emission of a single higher-energy photon (Wang et al., 2010). Due to the unique properties of being sensitive to the near-infrared light, UC-based NPs can be detected in deeper tissue compared with traditional contrast agents with fluorescence. More importantly, the imaging of UC-based NPs is more stable and has higher definition (Ang et al., 2011; Chen X. et al., 2015).

Idris et al. (2009) reported a contrast agent of UC-based NPs to label and track MSCs, which was composed of NaYF₄:Yb, Er NPs, and silicon. The labeled MSCs could directly be imaged in vivo to dynamically visualize their behaviors, but the region imaged by confocal microscopy was small. Wang et al. (2012a) fabricated UC-based NPs to label MSCs, which was modified by polyethylene glycol (PEG) and oligo-arginine. MSCs labeled by the UC-based NPs were detected by fluorescence imaging in vivo, and the introduction of oligo-arginine improved the cellular uptake of the NPs, showing excellent labeling efficiency and high sensitivity of 10 cells. In another work, Cheng et al. (2013) developed a multifunctional UC-based NP, integrating magnetic properties into UC luminescence NPs. Utilizing the
prepared NPs, the labeled MSCs can be tracked via fluorescence imaging and MRI, possessing highly effective sensitivity of 10 cells in mouse in vivo. Zhao et al. (2013) prepared a UC-based NP for labeling mouse MSCs, which was composed of polyethyleneimine (PEI) and (α-NaYbF₄:Tm³⁺)/CaF₂, enhancing the detective depth in vivo due to absorption and emission for near-infrared light.

In recent years, Ma et al. (2016) reported a UC-based NP, which was composed of NaYF₄:Yb³⁺, Er³⁺ NPs, poly (acrylic acid) (PAA), and poly (allylamine hydrochloride) (PAH), as a fluorescence maker for tracking bone marrow MSCs in vitro, shown in Figure 5. The biocompatibility and cellular uptake of NaYF₄:Yb³⁺, Er³⁺ NPs were highly enhanced, due to electrostatic interaction. Moreover, it was found that the effects of cellular uptake of UC-based NPs (≤50 µg/ml) on the osteogenic differentiation had no apparent difference by tracking the MSCs. Kang et al. (2018) prepared a UC-based NP with NIR-controllable properties to label MSCs. By a remote-controllable way, the stem cell differentiation was regulated. Furthermore, Ren et al. (2020) used ligand-free NaYF₄:Yb/Er UC-based NPs to label and track mouse bone MSCs, demonstrating that the UC-based NPs at a proper concentration could enhance osteogenic differentiation.

**Silicon-Based Nanoparticles**

Recently, silicon-based NPs have been demonstrated as a good contrast agent to label cells because they can be easily modified by bioconjugation (Shin et al., 2019). Furthermore, it has excellent biocompatibility and chemical inertness, thus, being able to serve as a probe for tracking cells in vivo (Huang et al., 2005; Hsiao et al., 2008). Silicon-based NPs, being simply divided into
silica NPs and silicon carbide NPs, not only contain fluorescent properties but also are used as ultrasound agents (Chen and Jokerst, 2020; Yao et al., 2020).

Huang et al. (2005) prepared a mesoporous silica NP modified by fluorescein isothiocyanate, and the labeled MSCs could be detected by imaging to track their viability in vivo. Due to clathrin-mediated endocytosis, the NPs could be internalized into MSCs and showed good cellular uptake. In addition, the highly efficient labeling had no apparent influences on the viability of MSCs. In another work, Chen and Jokerst (2020) used silica NPs to label MSCs and then track the MSCs in vivo by ultrasound imaging. The results showed that silica NPs could significantly increase the ultrasound signal of MSCs in vivo. Yao et al. (2020) reported a unique core–shell NP in which the core is composed of cobalt protoporphyrin IX (CoPP)-loaded mesoporous silica NPs, and the shell is a 125I-conjugated/spermine-modified dextran polymer, to label and guide the transplantation of MSCs by PA imaging and SPCT nuclear imaging, shown in Figure 6. The obtained NPs not only could instantly image the transplantation of MSCs but also quantitatively tracked their migrations for a long time. Significantly, the NPs steadily released CoPP to increase the survival of MSCs in ischemic mice.

Another type of silicon-based NPs is silicon carbide NPs, which are commonly used as protecting layer due to their unique properties of being durable and chemically inert. Moreover, typically, silicon carbide NPs (<10 nm) are utilized as contrast agents to label and track cells in vivo because of their photoluminescence resulting from quantum confinement effects. Chen et al. (2019) used three sizes of silicon carbide NPs to label MSCs, showing dual modality imaging (photoluminescence and photoacoustic imaging). When the size of the NPs is 620 nm, the labeled MSCs could be detected in vitro for more than 20 days. Moreover, the NPs also exhibited good biocompatibility and the capacity of cell tracking because the differentiated cells also could be imaged.

Other Nanoparticles

Gao et al. (2016) prepared an aggregation-induced emission NP, the surface of which is modified by Tat peptide. The obtained NPs had highly efficient ability of labeling mouse bone marrow-derived MSCs, and the red emission could be detected for more than 12 passages, which were not achieved by traditional fluorescence, possessing long-term tracking and strong anti-photobleaching ability. More importantly, MSCs labeled by the NPs showed low cytotoxicity, and their viability and differentiation had no apparent influences. Lim et al. (2019) developed bicyclo nonyne (BCN)-conjugated glycol chitosan NPs (BCN-NPs) as a delivery system of dual-modal stem cell imaging probes. Yin et al. (2018) reported an organic semiconducting polymer NP (OSPN\textsuperscript{+}) as a PA contrast agent for tracking MSCs, utilizing a second near-infrared (NIR-II) adsorption to broaden
the limitation of conventional inorganic PA contrast agents and the narrow range of the wavelength in the first near-infrared (NIR-I) window, shown in Figure 7. The prepared cationic NPs showed the deeper tissue imaging due to the significantly higher signal-to-noise (SNR) and enhanced the cellular uptake for human MSCs because of their good biocompatibility, appropriate size, and optimized surface property.

**PROBLEMS AND PROSPECTS**

Mesenchymal stem cell transplantation has been shown to be a strong potential for the treatment of CVDs. Many NPs have been fabricated and used as contrast agents by non-invasive imaging to track and monitor the transplanted MSCs, for providing more information to guide further therapy. However, one big issue associated with NPs for labeled MSCs is that NPs released from dead cells will be uptaken by macrophages, which give false-positive information. Moreover, the single-imaging technique can be inefficient to meet all needs for tracking, and each of those has its own disadvantages. Although there are some multimodal imaging techniques, more multimodal labeling agent-based NPs should be developed in the future to optimize MSC dose and delivery route for the treatment of CVDs. Importantly, the poor targeted migration and low survival rate of MSCs transplanted into the cardiovascular should be solved.

**AUTHOR CONTRIBUTIONS**

HH completed the first draft of the manuscript. XD, ZH, and ZY offered the excellent advices and perfected the first draft. WH improved the first draft and finally finished the manuscript and provided the support of funds. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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