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REVIEW

A brief review of cytotoxicity of nanoparticles on mesenchymal stem cells in regenerative medicine

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Abstract: Multipotent mesenchymal stem cells have shown great promise for application in regenerative medicine owing to their particular therapeutic effects, such as significant self-renewability, low immunogenicity, and ability to differentiate into a variety of specialized cells. However, there remain certain complicated and unavoidable problems that limit their further development and application. One of the challenges is to noninvasively monitor the delivery and biodistribution of transplanted stem cells during treatment without relying on behavioral endpoints or tissue histology, and it is important to explore the potential mechanisms to clarify how stem cells work in vivo. To solve these problems, various nanoparticles (NPs) and their corresponding imaging methods have been developed recently and have made great progress. In this review, we mainly discuss NPs used to label stem cells and their toxic effects on the latter, the imaging techniques to detect such NPs, and the current existing challenges in this field.

Keywords: mesenchymal stem cells, nanoparticles, toxic effects, imaging methods

Introduction

Mesenchymal stem cells (MSCs), which are multipotent and can be readily obtained, have shown great promise for treating arthritis, cartilage defects, tissue wounds, stroke, graft versus host disease, myocardial infarction, traumatic brain injury, and even cancer[1–3] owing to their particular therapeutic effects such as significant self-renewability; low immunogenicity; and ability to differentiate into a variety of specialized cells, control inflammation, and modify the proliferation of, and cytokine production by, immune cells.[4] Intravenous injection is a common method for transplanting MSCs in both animal models and clinical trials.[3,5] However, certain barriers significantly limit their long-term efficacy in clinical trials. One of the challenges is to noninvasively monitor the delivery and biodistribution of administered cells during treatment without relying on behavioral endpoints or tissue histology.[3,6,7]

To solve the above problem, reliable and non-invasive tracking of stem cells is urgently needed to understand the long-term fate, migration, and regenerative capability of stem cells, and to evaluate treatment efficacy.[8] To date, there are three main strategies for cell labeling: direct labeling, indirect labeling, and multimodal labeling. The first strategy is to label stem cells with nanoparticles (NPs), including gold NPs,[9] iron oxide NPs,[10,11] organic dyes, and quantum dots (QDs),[12,13] followed by various imaging techniques, such as photoacoustic imaging, fluorescence imaging, magnetic resonance imaging (MRI), and optical imaging, which are used to detect these materials. For the indirect-labeling method,

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a reporter gene is introduced into cells and then translated into enzymes, receptors, fluorescent or bioluminescent proteins.[14–17] Among these, green fluorescent protein or luciferase is used frequently for cell labeling so as to provide precise and quantitative information on the fate and distribution of administered stem cells.[18,19] Multimodal imaging, which combines direct and indirect labeling, can be achieved by using a single label or tracer that is visible using different imaging modalities, or a combination of imaging labels. It is particularly effective in that the strengths of different imaging modalities can be maximized.

At present, various NPs and their corresponding imaging methods have been developed and have shown a promising prospect (Figure 1A-F). In the following review, we will discuss NPs used to label stem cells and their toxic effects on the latter, the imaging techniques to detect such NPs, as well as the currently existing challenges in this field.

NPs and their toxic effects

Currently, the general definition of NPs are materials with 1–100 nm diameter and surface area >60 m^2/cm^3.[20,21] Morphology and size are important in determining the physicochemical properties of the NPs, as they not only lead to different rates of cellular uptake, but also interact with biological tissues which cannot be done with other bulk materials.[22] New synthesis techniques have produced not only spherical NPs, but also NPs of other shapes, such as cubes,[23,24] prisms,[25,26] hexagons,[24] octahedrons,[27] rods, and tubes.[28]

To date, several engineered NPs, such as QDs, silica NPs, and persistent luminescence NPs, have been developed and employed in medical fields owing to their unique magnetic and/or optical properties as well as their capability to offer real-time methods of tracking intracellular processes at a biomolecular level.[8,29,30]

Besides tracking living transplanted therapeutic stem cells,[31] synthetic NPs have also being exploited for many other applications, such as manufacturing industrial products, drug and gene delivery,[32–34] and nanotheranostics.[35] In particular, some NPs are even used for cancer thermal therapy in clinical trials.[36–38]

Although NPs have afforded significant progress in stem cells tracking and allow sensitive detection and long-term localization under non-invasive conditions in vitro, their toxic side effects on cells still limit their clinical applications.[39] In general, toxic effects on cells induced by NPs uptake are mainly due to the following reasons. First, most types of NPs are endocytosed by cells and accumulate in cytoplasmic vesicles, particularly lysosomes or late endosomes.[40,41] However, some NPs may undergo degradation or solubilization due to their sensitivity to the oxidative environment, and thus result in the leaching of free ions or increased abundance of reactive surface groups.[39] In this case, large amounts of reactive oxygen species will also be generated, which can damage labeled cells when the elevated level of reactive oxygen species persists over prolonged time.[42] Second, the physical characteristics of NPs can disrupt the cytoskeletal network during NPs internalization into cells.[43] However, it is the cytoskeleton that plays a major role in the fundamental physiological functions of cells such as shape, motility, division, adhesion, and interaction with the surrounding environment.[44] When the cytoskeleton changes, its biological function would be affected at the same time, thus resulting in biological toxicity. Third, the internalized NPs can interfere with intracellular signaling pathways such as direct NPs-induced DNA damage,[45–47] modulation of intracellular signaling cascades, or membrane damage,[45] and interaction of NPs with cellular transcription/translation machinery.[47,48] As shown in Figure 2, the disruption of these transduction pathways not only affects the basic physiological functions of cells, but also diminishes their proliferation and differentiation ability. For different NPs which are widely used to label stem cells, their toxic effects are listed in Table 1.

Quantum dots

QDs are semiconductor nanocrystals with a diameter of approximately 2–10 nm,[49] possessing excellent optical properties such as narrow, symmetric, and size-tunable emission spectra due to the quantum-confinement effect,[50] and their adjustable spacing by controlling crystal size. In addition, compared with fluorescent proteins or organic fluorophores, QDs exhibit 10–100 times brighter fluorescence, and 100–1,000 times higher fluorescence stability against photobleaching, which not only enables multicolor fluorescent applications, but also facilitates long-term monitoring of intermolecular and intramolecular interactions in living cells and tissues. Based on these special properties, QDs have been used for bioimaging applications since 1998,[51,52] particularly for labeling different cell lines in in vitro and in vivo studies.[53,54]

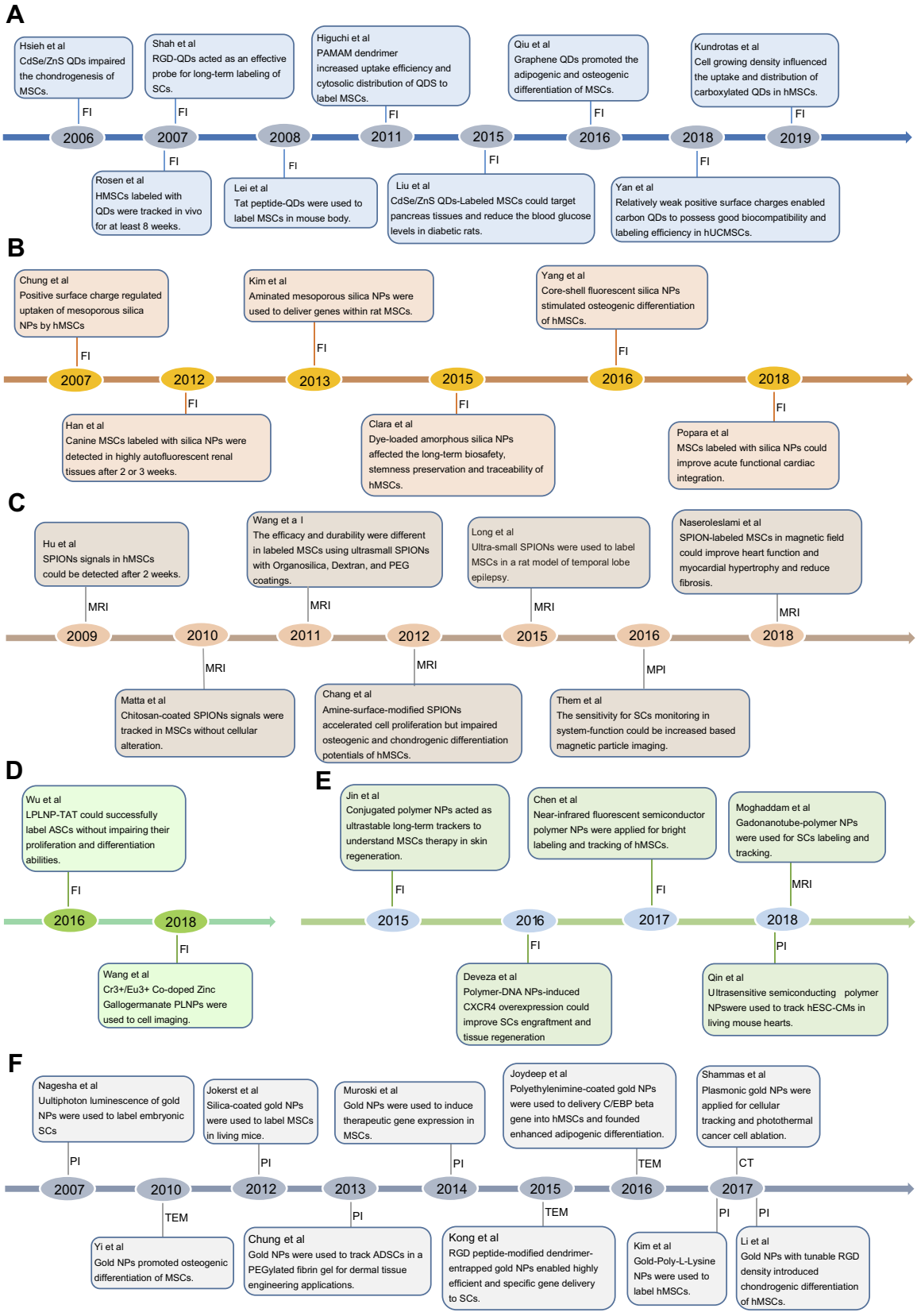

Figure 1 The timeline of the development of different nanoparticles and the related imaging methods (representative articles). Timeline of **(A)** QDs, **(B)** silica NPs, **(C)** SPIONs, **(D)** PLNPs, **(E)** polymer NPs, **(F)** gold NPs.

Abbreviations: QDs, quantum dots; PAMAM, polyamidoamine; NPs, nanoparticles; SPIONs, superparamagnetic iron oxide nanoparticles; RGD, arginine-glycine-aspartic; LPLNP-TAT, TAT penetrating peptide-bioconjugated long-persistent luminescence nanoparticles; FI, fluorescent imaging; MRI, magnetic resonance imaging; MPI, magnetic particle imaging PI, photoacoustic imaging; TEM, transmission electron microscope; CT, computed tomography.

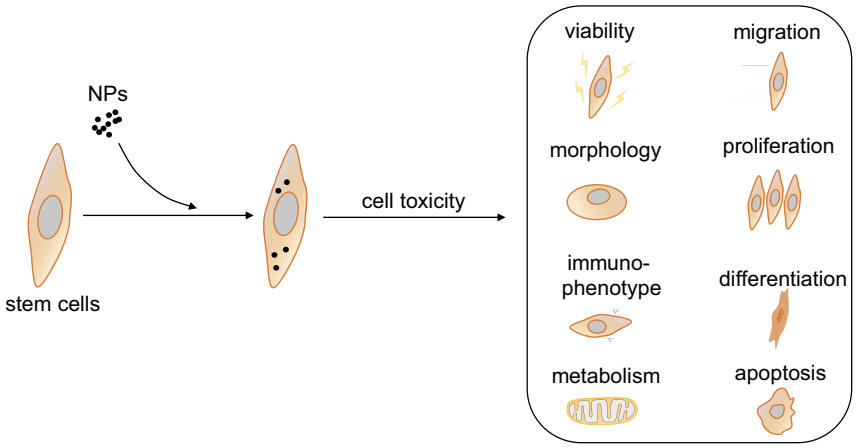

Figure 2 The toxic effects of NPs on cell viability, morphology, immunophenotype, metabolism, migration, proliferation, differentiation, and apoptosis of stem cells.
Abbreviation: NPs, nanoparticles.

Two methods are generally considered for labeling stem cells with QDs: the combination of QDs with stem cells surface[55] and the transduction of QDs into stem cells.[56,57] However, the former would lead to a decrease in the accumulation rate of stem cells in tissues/organs, and separation of QDs from the stem cells surface during in vivo circulation, while the latter results in high cytotoxicity and low transduction efficiency when using physical stimulus methods, such as ultrasonic transduction[58] and electroporation.[59,60]

In contrast, chemical modification methods, such as cationic liposomes, cell penetrating peptides, and high molecular weight nano-carriers (polymer micelles) were reported as feasible in the labeling of stem cells with QDs.[61–63] In this regard, Shah and Mao provided a detailed protocol for the labeling of selected integrins on human mesenchymal stem cells (hMSCs) membranes with bioconjugated QDs by optimizing precise concentrations and incubation times.[64] In addition, stem cells labeled with bioconjugated QDs, whether differentiated or not, could be readily imaged by fluorescence microscopy.

At present, some studies have shown adverse effects while others have shown opposite results. In 2007, Chakraborty et al found no adverse effects on stem cells morphology, viability, proliferation, or differentiation over the duration of their experiments at an optimized labeling efficiency.[65] In another study, no adverse effects were detected as hMSCs differentiated into osteogenic, chondrogenic, and adipogenic cell lineages, even though hMSCs had been labeled with a concentration of 20–50 nM QDs for at least 22 days, while retaining QDs in their cytoplasm.[66] In addition, Wang et al labeled MSCs derived from the human amniotic membrane with different doses of QDs and studied their effects over 1–4 days. Their results showed that cells still maintained viability >80%.[67]

However, others have noticed alterations to stem cells[68,69] and abnormalities during embryo development when labeled with QDs.[70] Researchers used QDs to label hMSCs and reported that, even though cellular proliferation and cell-cycle distribution were not affected, the chondrogenic and osteogenic differentiation potential of cells was disrupted.[68,69]

Therefore, QDs are not completely toxic, but there is likely to be a confined protocol within which they can be applied without major interference in the processes under study.[70]

Silica NPs

Silica NPs are nano-scale sized, possessing good stability, biocompatibility and morphological tunability, and have been widely applied in chemical, agricultural, and cosmetic fields. In addition, they are being continuously developed for medical purposes, such as diagnosis, treatment,[71–73] controlled-release drug delivery, and gene transfection.[74,75] Silica NPs have been approved as reliable ultrasound contrast agents.[76] When used as a tracking method, silica NPs are generally labeled with fluorescein.[77,78] The advantage of this combination is that silicon dioxide not only acts as a matrix to chemically and physically confine the fluorescent dyes, but also protects the dye molecules from external quenchers, thereby increasing their light stability. Furthermore, this combination can also provide a biocompatible and easily functionalized surface.[79]

Among them, exosome-like silica (ELS), which has a unique curvature and cup shape, is more advantageous. This shape provides a double scattering/reflection interface to increase the echo so that it allows lower NPs doses to

Table 1 Toxic effects of different nanoparticles used to label stem cells

Stem cells	Species	Nanoparticles	Analysis	Concentration	Main results	References
MSC	Human	Cholera toxin subunit B QDs	Cell viability, morphology, proliferation and differentiation capacity	250 pm–16 nM	No adverse effects	65
MSC	Human	RGD-conjugated QDs	Proliferation and differentiation capacity	20–50 nM	No adverse effects	66
MSC	Human	CdSe/ZnS QDs	Cell viability, immunophenotypic profiles	0.75–3 μg/mL	No adverse effects	67
MSC	Human	CdSe/ZnS QDs	Cell viability, proliferation and differentiation capacity	1.625 μg	Chondrogenic differentiation impairment	68
MSC	Human	CdSe/ZnS QDs	Cell viability, proliferation and differentiation capacity	1.625 μg	osteogenic differentiation impairment	69
MSC	Rat	CdSe/ZnS QDs	Cell viability, differentiation capacity	16 μg/mL	No adverse effects	185
MSC	Human	Carbon QDs	Cell viability, differentiation capacity Single cell sphere formation capacity	50 μg/mL	No adverse effects	186
ADSC	Human	Graphene QDs	Cell viability, metabolic activity	0.5, 1.0, and 2.0 mg/mL	No adverse effects	187
MSC	Rat	Graphene QDs	Cell viability, proliferation and differentiation capacity	50 μg/mL	Enhanced osteogenic, adipogenic differentiation	188
MSC	Human	Mesoporous silica NPs	Cell adhesion capacity, immunophenotypic profiles	50 μg/mL	Enhanced adhesion capacity and Connexin-43 expression	73
MSC	Human	Spherical core-shell fluorescent silica NPs	Cell viability, adipogenic differentiation capacity	100 μg/mL	Adipogenic differentiation impairment	189
MSC	Human	Core-shell fluorescent silica NPs	Cell viability, osteogenic differentiation capacity	10 μg/mL	Osteogenic differentiation enhancement	190
MSC	Human	Mesoporous silica NPs	Cell viability, migration capacity	100 and 200 μg/mL	No adverse effects	191
MSC	Human	Dye-loaded amorphous silica NPs	Cell viability, proliferation and differentiation capacity	50 μg/mL	No adverse effects	192
MSC	Human	Mesoporous silica NPs	Cell viability, proliferation and differentiation capacity	20 μg/mL	No adverse effects	86
MSC	Human	Mesoporous silica NPs	Cell viability, differentiation capacity	3–10 μg/mL	No adverse effects	87
MSC	Human	Mesoporous silica NPs	Cell viability, morphology, immunophenotypic profiles, proliferation and differentiation capacity	20 μg/mL	No adverse effects	88
MSC	Human	Mesoporous silica NPs	Cell viability, immunophenotypic profiles, proliferation and differentiation capacity	20 μg/mL	No adverse effects	89
MSC	Rat	SPIONs	Cell viability, differentiation capacity	1, 5 μg/mL	Chondrogenic differentiation enhancement	193
MSC	Rat	ASP-SPIONs	Cell viability, apoptosis rate, intracellular ROS level, mitochondrial transmembrane potential, and differentiation capacity	30 μg/mL	No adverse effects	194

(*Continued*)

Table 1 (Continued).

Stem cells	Species	Nanoparticles	Analysis	Concentration	Main results	References
ADSC	Rat	PEG/PVP-SPIONs and PEG/PEI-SPIONs	Cell viability, morphology,	12, 25, and 50 μg/mL	No adverse effects	195
ADSC	Rat	SPIONs	Cell viability, morphology, proliferation capacity	50 μg/mL	No adverse effects	196
MSC	Human	SPIONs	Cell viability, differentiation capacity	25 μg/mL	No adverse effects	99
MSC	Rat	HEDP coated SPIONs	Cell viability, morphology, differentiation capacity	25 μg/mL	No adverse effects	100
MSC	Human	SPIONs	Cell viability, morphology, differentiation capacity	1, 10, and 100 μg Fe/ml	No adverse effects	101
MSC	Human	SPIONs	Cell viability, proliferation and differentiation capacity	13–16 pg Fe/cell	chondrogenic differentiation impairment	102
ADSC	Mouse	Penetrating peptide-bioconjugate-PLNPs	Cell viability, differentiation capacity	50 μg/ml	No adverse effects	109
MSC	Human	Purified polymer NPs	Cell viability, proliferation capacity	0, 5, 10, 20, 40 μg/mL	No adverse effects	119
MSC	Human	R8-Polymer NPs	Cell viability, proliferation and differentiation capacity, tumorigenicity, immunophenotypic profiles	10 μg/mL	No adverse effects	120
MSC	Porcine	GNT-Polymer NPs	Cell viability	10^{14} $Gd3^{+}$ ions/cell	No adverse effects	197
hESC-CM	Human	Polymer NPs	Cell viability, and immunophenotypic profiles	0, 2, 4, 8×10-9M	No adverse effects	198
MSC	Human	Gold NPs	Cell viability, proliferation and differentiation capacity	10^{12} NPs/mL	No adverse effects	135
MSC	Human	Silica-coated gold NPs	Cell viability, proliferation and differentiation capacity	0.0–0.14nM	No adverse effects	139
MSC	Rat	Silica-coated gold NPs	Cell viability, proliferation capacity	10^{12} NPs/mL	No adverse effects	140
MSC	Mouse	PEGylated gold NPs	Cell viability, migration, proliferation, differentiation and capacity to colonize scaffolds	100μg/mL	migration capacity enhancement, osteoblasts differentiation enhancement, capacity to colonize scaffolds enhancement	142
MSC	Human	TEMPO-Conjugated gold NPs	Cell viability, proliferation and differentiation capacity	0.05–1.00mM	Chondrogenic differentiation enhancement, adipogenic differentiation impairment	145
ADSC	Human	NAC modified gold NPs	Cell viability, ALP activity	20 μM	cell viability enhancement	199

produce the same ultrasound contrast, thereby increasing biocompatibility. Meanwhile, compared with most other negatively charged silica NPs due to the presence of hydroxyl groups, ELS NPs are positively charged with amine groups, which greatly increases their affinity for stem cells, thus improving not only the cellular uptake rate but also the ultrasound contrast.

ELS also increases the echogenicity and ultrasound sensitivity of hMSCs. For example, Fang et al injected ELS-labeled hMSCs and Matrigel vectors subcutaneously into nude mouse, PBS and unlabeled cells were also injected as controls, the in vivo ultrasound image results showed a significant increase in echogenicity of transplanted ELS-labeled stem cells compared to controls.[80]

It is worth noting that for medical therapy, injected stem cells tend to die because of the harsh environment.[81,82] To overcome this challenge, growth-promoting survivors could be conjugated with ELS NPs[83–85] so that the cell viability could be increased. Besides, ELS NPs can be easily chemically modified. In addition to labeling with fluorescein, as described by Fang et al, they can also be coated with helium ions or radionuclides for multimodal imaging.[80]

There are few reports regarding toxic effects on stem cells when labeled with silica NPs, which is of great promise in applications involving regenerative medicine.

In 2008, Huang et al reported that the uptake of mesoporous silica NPs into hMSCs failed to influence their osteogenic differentiation and that silica NPs actin polymerization in MSCs could be enhanced.[86] Liu et al demonstrated that silica-coated core-shell superparamagnetic iron oxide NPs co-condensed with fluorescein isothiocyanate-incorporated mesoporous silica were able to facilitate uptake by hMSCs without affecting their viability and differentiation potential at the same time.[87] More meaningfully, cyanine dye-doped silica NPs were reported to label hMSCs without affecting viability, proliferation, stemness surface marker expression, and the ability to differentiate into osteocytes.[88] Also, after the internalization of mesoporous silica NPs conjugated with fluorescein isothiocyanate by hMSCs, Huang et al observed that there were no obvious differences in cell viability, proliferation, and surface markers, compared to unlabeled cells.[89] In addition, their capability to differentiate into adipocytes, chondrocytes, and osteocytes was also not disrupted.

Superparamagnetic iron oxide nanoparticles (SPIONs)

SPIONs, a kind of tiny variant of iron fluorophore particles, have been widely used as contrast agents for stem cells tracking markers owing to their biocompatibility, superparamagnetism, nanoscale control, and tailor-made surface coatings.[90–93] Furthermore, when tracking stem cells, SPIONs can enhance the contrast between different tissues by inducing darker areas (negative contrast).[77,94,95]

However, it should be mentioned that there is still no clear optimal parameter to guide the use of SPIONs in stem cells tracing. One reason is that different doses of SPIONs have been used to label different cell types by containing different types of iron oxide cores and different coatings. Another issue is that they would lose the magnetization vector caused by the applied magnetic field and become highly dispersed when the magnetic field is switched.[96] Despite this, some researchers have still applied it to stem cells tracking and have obtained satisfactory results, for example, Hua et al used SPIONs to label bone marrow MSCs and successfully tracked the transplanted cells with MRI over different time courses.[97] Long et al successfully tracked MSCs labeled with ultra-small SPIONs in a rat body.[98] Naseroleslami et al found SPIONs-labeled MSCs in the presence of magnetic field could markedly improve heart function and myocardial hypertrophy and reduce fibrosis.[95]

There is still controversy concerning the toxicity of SPIONs to labeled cells; some researchers believe that they are toxic, while others believe the opposite. In 2007, Song et al confirmed that internalization of SPIONs (ferumoxides) by hMSCs using lipofectin transfection agents did not affect their cartilage formation, adipogenesis, or osteogenic differentiation.[99] In 2009, Delcroix et al used SPIONs coated with 1-hydroxyethyl-l, l-bisphosphonic acid to label rat MSCs. The cells were incubated with NPs for 48 hrs, and the authors confirmed that more than 90% of the cells contained enough iron to allow their detection with no significant alteration to cell viability. In addition, cellular ultrastructure was conserved, and compared to unlabeled rat MSCs, the differential potential for osteogenic and neuronal lineages of rat MSCs did not show significant differences.[100] In another study, hMSCs were incubated with ferucarbotran for 24 hrs and then labeled with SPIONs. Researchers found that there were no microscopic morphological changes observed up to 12 days after SPION labeling.[101] However, other researchers holding the opposite view also have their own reasons. Bulte et al reported in 2004 that uptake of SPIONs (ferumoxides) by hMSCs with incubation of the transfection agent poly-L-lysine (intracellular iron incorporation of 13–16 pg Fe/cell) would impair their chondrogenic differentiation, and they confirmed that their inhibitory effect is mediated by Fe itself rather than the transfection agents.[102] Chang et al found that amine-surface-modified SPIONs could accelerate cell proliferation but impair osteogenic and chondrogenic differentiation potentials of hMSCs.[103]

Perhaps, more comprehensive and detailed research is still needed to confirm whether SPIONs have toxic effects on cells and thus arrive at credible results.

Persistent luminescent nanoparticles

PLNPs are a group of emerging luminescent materials that have been developed and used in biomedical fields over the past decade. PLNPs exhibit unprecedented advantages

in stem cells tracking due to the following unique features.[104] The most important is that PLNPs can remain glowing for hours or even days after excitation is stopped,[105–107] which makes long-term imaging possible without in situ excitation. The other is that PLNPs have excellent signal-to-noise ratios, and can be distinguished from normal tissue autofluorescence. As a consequence, their detectable sensitivity can be greatly improved. For example, Chermont et al pre-charged $Ca_{0.2}Zn_{0.9}Mg_{0.9}Si_2O_6:Eu^{2+}Dy^{3+}Mn^{2+}$ PLNPs with UV light, and then injected them into the mouse. They found that as low as 20 ng PLNPs could generate detectable signals without in situ excitation, and could eliminate autofluorescence from in situ excitation.[108]

To evaluate PLNPs in vivo toxicity, Wu et al employed penetrating peptide-bioconjugated long persistent nanophosphors to track adipose-derived stem cells during wound healing and they confirmed that the labeled adipose-derived stem cells could effectively promote wound healing without the proliferation and differentiation ability impaired.[109]

However, considering the available data, it is not easy to judge the toxic effects of PLNPs on stem cells. Before being applied on a large scale, more stem cells experiments should be conducted to establish more comprehensive and credible conclusions.

Polymer NPs

Polymer NPs are generally prepared through either dispersion of preformed polymers or polymerization of monomers,[110] and have been applied for medical purposes widely.

New polymer NPs, such as microgels, dendrimers, and modified polysaccharide NPs, are mostly being used as anticancer drugs, drug and gene delivery carriers, and MRI contrast agents.[111–115] For stem cells tracking, fluorescent organic dyes are commonly used, not only because they can be physically entrapped in the polymer interior during the preparation of NPs, but also covalently bound to the polymer chain before the preparation of NPs. At present, the most common fluorescent polymer NPs are polystyrene NPs prepared mainly through the emulsion polymerization method.

For example, Jiang et al investigated the uptake of polystyrene NPs by MSCs using confocal fluorescence microscopy and flow cytometry.[116] In addition to PS, fluorescent polymer NPs can also be prepared with conjugated fluorescent polymers such as poly(arylenediethynlenes),[117] poly(3,4-ethylenedioxythiophene), poly(thiophene-3-yl-acetic acid) and polyacetylene. Polymer NPs prepared by this method can exhibit an amplified fluorescence effect.

Recently, a general strategy has been developed to enhance the photostability of organic fluorophores in biomedical imaging. Using this method, bright and robust fluorescence could be easily observed.[118]

However, despite their wide application, disadvantages such as low incorporation, and inadequate protection of dye molecules leading to consequent leaching, quenching, and photobleaching of the fluorophores, have presented difficult challenges in long-term stem cells tracking.

As for their toxic effects on stem cells, Chen et al found no significant differences between polymer dot-labeled MSCs and unlabeled MSCs in their respective abilities of cell proliferation, differentiation, and phenotypic expression.[119] Also, the polymer dot-labeled MSCs retained robust self-renewal capacity and multi-lineage differentiation potential. In another study, researchers found that, compared to unlabeled MSCs, cell viability, proliferation and differentiation capacity, tumorigenicity, and immunophenotypic profiles of polymer dot-labeled MSCs also showed no differences.[120]

Furthermore, the existing data still do not fully demonstrate that polymer NPs are safe or have no toxic effects on stem cells. Thus, studies on their toxicity still need to be continued to achieve a more comprehensive understanding.

Gold NPs

Gold NPs can be designed into different structures and shapes depending on the intended application.[121,122] These include gold nanospheres, gold nanoshells, and gold nanorods. Moreover, due to the continuous development of gold NPs and the continuous optimization of their properties, more and more gold NPs have been used in the biomedical field, such as gene transfection,[123–125] drug delivery,[126,127] antisense gene control,[128,129] intracellular detection and imaging,[130–132] and stem cells labeling.[133–138]

Since gold NPs have promising applications in biomedicine, more attention has been paid to the assessment of their risk potential, so as to prolong the life cycle and minimize toxicity in vitro and in vivo. To study the capacity of Silica-Coated Gold Nanorod-labeled murine MSCs, authors conducted MTT cell toxicity assays and the results indicated that Silica-Coated Gold Nanorods with 3 hrs of incubation time lead to no statistically significant change in MSCs metabolic activity compared to unlabeled MSCs. Also, there were no significant

differences in terms of normal proliferation of the two cell populations. Furthermore, Silica-Coated Gold Nanorod-loaded cells were still easily transformed into osteogenic and adipogenic cell lines, and fivefold greater osteogenic signals could be detected than in control groups, while the adipogenic signal showed nearly the same characteristics.[139] In another study, it was demonstrated that loading MSCs with gold nanotracers did not alter cell function, and the ability of MSCs to differentiate into adipocytes and osteocytes was maintained.[135] Later, Ricles et al labeled rat MSCs with Silica-Coated Gold Nanorods and found that compared to control MSCs, the labeled cells were viable for up to 5 days and continued to proliferate, with no significant reduction in cell proliferation.[140] Li et al found gold NPs with tunable RGD density could introduce chondrogenic differentiation of hMSCs.[141] In addition, a study revealed that using pegylated hollow gold NPs to label MSCs caused a clear increase in the cellular migration rate compared to untreated cells. Furthermore, no significant differences were found for the cell-cycle phase percentages for MSCs when treated with pegylated hollow gold NPs compared to control cells.[142]

However, reports on the influence of gold NPs on the differentiation process are scarce, and their mechanism of action is still under debate. Some studies have elucidated that gold NPs could enhance osteogenic differentiation while inhibiting adipogenic differentiation at the same time.[143,144] For example, in contrast to the suppressive effect of free 2,2,6,6-tetramethylpiperidine-N-oxyl on stem cells differentiation, Li et al confirmed that TEMPO-conjugated gold NPs showed a promoting influence on osteogenic differentiation, with a suppressive influence on adipogenic differentiation of hMSCs.[145]

Based on this, we still cannot conclude whether gold NPs are toxic to stem cells; thus, more studies should be designed and conducted to elucidate their toxic effects on stem cells.

Imaging modalities

Although stem cells therapy has been applied in many fields, and has achieved some certain efficacy, comprehensive understanding of in vivo behaviors of stem cells is still lacking, which leads to our confusion regarding the contradictory results from current clinical trials.[21,23,24] Hence, it is of great significance to evaluate the migration, differentiation, and survival of transplanted stem cells, and identify the mechanisms behind these behaviors. Traditional methods to track the fate of implanted stem cells mainly involve sacrifice of animals or tissue biopsies. However, an invasive and harmful technique is not acceptable for long term and continuous analyses.[146,147]

Recent developments in stem cells therapy require more accurate and noninvasive methods for qualitatively and quantitatively monitoring transplanted cells inside the host, so as to improve the understanding of treatment outcomes and the fate of the stem cells.[148] To overcome these problems, several tracking modalities, such as fluorescence, magnetic resonance, and photoacoustic imaging combined with engineered NPs possessing unique magnetic and/or optical properties, have been developed and employed in biomedicine to offer real-time imaging of transplanted cells. Although each imaging modality has its own unique advantages, along with intrinsic limitations (as shown in Table 2) which need to be further improved and perfected, their role in the field of regenerative medicine is important and irreplaceable.[149]

Magnetic resonance imaging

MRI is a basic noninvasive method for human in vivo imaging that uses a powerful magnetic field to detect the nuclear magnetization of hydrogen atoms inside the body,[150,151] hence, the signal intensity and distribution are closely correlated with water content and the anatomy of the body.[152] In clinical medicine, MRI is often used to diagnose different diseases and identify cancer metastasis and inflammation sites.[153]

For stem cells tracking, commonly used MRI contrast agents include two major categories based on gadolinium and manganese NPs, such as gadolinium chelating agents[154] and manganese chloride[155] and based on iron oxide NPs, such as paramagnetic/superparamagnetic contrast agents.[156,157] Actually, these agents are ideal in combination with MRI for stem cells tracking because they are able to maintain a strong signal, offer direct and clear cell labeling, and allow non-invasive in vivo scanning.

For example, Blocki et al successfully used MRI to track SPIONs-labeled MSCs, which were encapsulated in collagen-based microcapsules, and then injected intramyocardially. They found that MSCs were progressively released from the microcapsules, and that the signals of labeled MSCs could be monitored for several weeks.[158] Yao et al also used MRI to monitor SPIONs-labeled endothelial progenitor cells injected into rat myocardium, and detected the signal from iron-positive cells at the injection site 10 days later.[159]

Table 2 Evaluation of different imaging modalities

Imaging modality	Advantages	Disadvantages
Magnetic resonance imaging	High spatial resolution[31,200] No tissue penetration limit[200] Allowing quantitative measurements	Relatively low sensitivity[31,200] Long imaging time[201] High cost
Fluorescence imaging	High spatial resolution[202,203] High sensitivity[200]	Poor tissue penetration[204] Interfered by autofluorescence[204] Low spatial resolution[205]
Photoacoustic imaging	Easy expression of functional fluorescent proteins[206,207] Multicolor imaging Activatable	Photobleaching, blinking and cross-linking[208,209] Poor tissue penetration High scattering
Magnetic particle imaging	Allowing quantitative and vertical detection[210] High sensitivity[210]	Low spatial resolution[211,212] Time consuming[213,214]
Multimodality imaging	Obtain more information[176] Strengths of different imaging modalities can be maximized[176]	Time consuming[176] Require precise and thorough planning[176]

Fluorescence imaging

Fluorescent imaging, a tracking method with properties such as low cost, high spatial and temporal sensitivity, and absence of radiation, has been widely used in biomedical applications.

Recently, near-infrared fluorescence (NIRF) molecular imaging, as one kind of fluorescent imaging, has shown great potential.[160] NIRF is based on a fluorescence optical imaging method that uses excitation light from the near-infrared spectrum (700–900 nm) to stimulate fluorescent molecules (fluorophores, contrast agents for NIRF) from the ground state (S0) to an excited (S1, S2) state, and when this molecule moves from the excited state to the ground state, it emits detectable fluorescence at longer wavelengths of light.

After reaching the ground state, the fluorescent molecular is available again for new excitation. The advanced properties of near-infrared light, such as low absorption and high scattering characteristics, make deep tissue penetration (to several centimeters) and diffuse expansion possible. In addition, excitation with near-infrared light in the region of >750 nm can largely reduce tissue autofluorescence[161] and thus improve sensitivity. For example, Huang et al injected infrared fluorescent protein-labeled MSCs into mouse and successfully detected the signal by using NIRF imaging.[162] In another fluorescent imaging method, Rosen et al demonstrated that single QDs-labeled hMSCs could be easily identified with fluorescent imaging in histologic sections, and were able to signal their location for at least 8 weeks.[163] Another study investigated the dynamic cell behavior of green fluorescent protein-transduced myoblasts in tibialis anterior muscles of immunocompetent mdx mouse and immuno-compromised nude mouse over a period of three months using a fluorescent imaging system.[164]

Photoacoustic imaging

Photoacoustic imaging is currently one of the fastest developing non-invasive hybrid imaging methods.[165,166] It combines the high temporal and spatial resolution of ultrasound with the excellent contrast and multiplexing capabilities of optical imaging,[166] so photoacoustic imaging is capable of high spatial resolution mapping in deep tissues (≈6 cm) while maintaining high contrast optical imaging.[167,168]

Contrast agents used in photoacoustic imaging mainly include endogenous agents, small molecule dyes, and NPs.[169] Among them, gold NPs are used widely and have shown great potential.[170,171] For example, Jokerst et al used a photoacoustic imaging system to follow MSCs labeled with silica-coated gold nanorods, and successfully obtained a cell detection limit in vivo of 100,000 cells, which was well below the clinically relevant numbers.[139] Nam et al prepared a PEGylated fibrin gel containing gold NPs-labeled MSCs which were injected into the lateral gastrocnemius of anesthetized Lewis rats, and signals from the labeled MSCs were detected using photoacoustic imaging.[172] Furthermore, they also performed longitudinal in vivo monitoring of the spatial distribution of labeled MSCs at days 3, 7, and 10 after injection, and successfully obtained signals.

Magnetic particle imaging (MPI)

MPI, an imaging modality distinct from MRI, takes advantage of nonlinear SPIONs magnetization behavior, and allows for direct imaging of SPION distribution with positive contrast, as well as high temporal and spatial resolution,[173] and can longitudinally monitor and quantify MSCs administration in vivo. Compared to existing stem cells tracking techniques, MPI offers near-ideal image contrast, depth penetration, and robustness, these properties make MPI both ultra-sensitive and linearly quantitative.

Importantly, since no biological tissue generates similar superparamagnetic signals that would otherwise interfere, MPI is highly sensitive, with low background noise.[173] Theoretical prediction by Knopp and Buzug indicates that MPI may be sufficiently sensitive for 1 pg Fe imaging, which implies a sensitivity able to detect single stem cells.

In 2013, Saritas et al first detected images of labeled stem cells using the MPI system, with a detection limit of approximately 10^4 cells.[174] Another study performed by Zheng et al in 2015 showed that signals from SPION-labeled stem cells in rat can also be detected with the MPI system, where a detection limit of approximately 200 cells with 27.0 3±0.3 pg (Fe)/cell was reported.[175] More importantly, Kolja Them et al used a matching calibration method to detect SPIONs-labeled stem cells, and their results showed that this method can not only reduce artifacts, leading to increased sensitivity, but also can locate and identify a smaller number of labeled stem cells.[173]

Multimodality imaging

The ideal cell labeling agent in vivo should provide accurate information regarding cell behavior, with high sensitivity and resolution, so that it can be better used in biomedical fields. However, since each method of labeling cells or the method used to detect signals has its own defects, it will still be a challenge to use any single modality to meet the above requirements.

Fortunately, multimodal imaging, which is particularly powerful in comprehensive monitoring of labeled cells, may solve these problems, for it can be performed using a single label or tracer that is visible using different imaging modalities, or a combination of imaging modalities. Thus, the strengths of the different imaging modalities can be maximized.[176] For example, Rieffel et al have confirmed that the combination of photoacoustic imaging with other modalities can utilize the advantages of both methods in image acquisition and reconstruction.[177] In addition, Guenoun et al transfected MSCs with a luciferase vector and superparamagnetic iron oxide (SPIO) and found that SPIO signals persisted even after complete loss of fluorescence signals.[178] In clinical applications, multimodal imaging has been reported to successfully track dendritic cells in melanoma patients by detecting cells labeled with both SPIO and radioactive indium isotopes.[179]

Challenges

In the field of combining NPs and stem cells applied to regenerative medicine, efficient cell labeling is of great significance for successful imaging. In order to obtain the maximum contrast, it is essential to load a maximal amount of NPs into each cell, because signal intensity is usually proportional to the concentration of the contrast agent.[180] However, the content of intracellular NPs is also directly proportional to its toxicity.

Although many studies have confirmed that the toxicity of NPs to stem cells is negligible, we still know very little about how abiotic factors might change the time-dependent alteration of NPs properties, and how they would influence its toxicity.[181] Besides, to assess the potential toxicity of NPs, a better understanding of the relationship between biokinetic parameters and NP properties, such as size, size distribution, charge, shape, agglomeration, and surface characteristics is clearly required.[182] Further, the influence of biopersistence on the toxicity of NPs and what criteria should be used to assess biopersistence, together with detecting and understanding their kinetics and transformation so as to assess their potential hazards and risks for humans and the environment should also be taken into account.[183] The next challenge is to perform longitudinal (days or weeks later) cell tracking studies since it was confirmed that the toxicity of NPs is closely related to the extent and mechanism of their uptake, localization and distribution in cells and organs.[184] We cannot confirm whether NPs being tracked remain inside cells over time, if they have already been secreted from the cells, or taken up by macrophages or other endogenous cells, because previous studies have shown that signal intensity would be decreased with the division and differentiation of stem cells,[180] also, researchers have detected signals from NPs in macrophages. Besides, challenges also exist in how to select appropriate NPs and the best matched imaging

modality to generate accurate location information involving the labeled stem cells, so that the obtained images can be accurately analyzed and differentiated from the surrounding tissues.

Conclusions

Combining NPs with stem cells and applying them to regenerative medicine are major directions for future development. As revealed by extensive research, this application has promising prospects. However, due to the cytotoxic effects of various NPs, systematic and comprehensive in vitro and in vivo studies are urgently needed to assess the toxicological profiles of the chosen NPs and to evaluate their potential influence on self-renewal and differentiation properties of stem cells before their wider application as contrast agents for stem cells tracking. Specifically, we need to consider the design strategies of NPs, such as size, shape, coating, incubation time and concentration, synthesis method, and the way NPs enter cells, and clarify which kinds of particle properties may cause influences on labeled cells, and by which mechanisms. In addition, while developing new technologies, we should also contemplate whether existing toxicology testing and risk assessment strategies are reliably applicable and sufficiently suited for the variety and complexity of NPs.

With the development of biomedicine, the criteria of low toxicity of NPs and the high accuracy and clarity of imaging modalities to non-invasively detect labeled cells in vitro will be increased as well. Although the combination of hybrid NPs and multimodal imaging has presented some advantages and promising results, developing better biocompatibility and higher-recognition rate NPs combined with high-resolution imaging modalities to track stem cells in vitro will still be extremely important.

Consent for publication

We confirm the tables in the manuscript are original for this article.

Abbreviation list

MSCs, mesenchymal stem cells; NPs, nanoparticles; QDs, quantum dots; hMSCs, human mesenchymal stem cells; ELS, exosome-like silica; SPIONs, superparamagnetic iron oxide nanoparticles; PLNPs, persistent luminescent nanoparticles; ASP-SPIONs, SPIONs-complexed amylose nanoparticles cationized with spermine; PEI, poly ethylene imine; PEG, poly ethylene glycol; PVP, poly vinyl pyrrolidone; HEDP, 1-hydroxyethylidene-1.1-bisphosphonic acid; R8, octa-arginine; GNT, gadonanotubes; TEMPO, 2,2,6,6-tetramethylpiperidine-N-oxyl; NAC, N-acetyl cysteine; MRI, magnetic resonance imaging; NIRF, near-infrared fluorescence; MPI, magnetic particle imaging.

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Author contributions

All authors contributed to date analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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