Review

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Particle Systems for Stem Cell Applications

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Stem cells have been widely investigated for regeneration of aged, injured, or diseased tissues. Although they have remarkable potential in clinical applications, some critical issues must be addressed, one of which being the poor control of their fates *in vivo*. A variety of particles, typically the organic and inorganic materials, liposomes, and polyplexes, provides multiple functionalities of labeling and tracking of the transplanted stem cells, and versatile capabilities of intracellular delivery of biomolecules for stem cell control *in vivo*. In this report, major applications of different particle systems are reviewed on the topics of tracking transplanted stem cells and labeling of endogenous stem cells *in vivo*. Detailed discussions are provided on recent advances of the particle-assisted intracellular delivery of biomolecules to stem cells for *in vivo* applications. Some novel particle-carriers are reported on stem cell transplantation and drug delivery for cancer therapy. Also discussed are critical challenges and future directions in the development of particle carriers for stem cell applications.

KEYWORDS: *Microparticles, Nanoparticles, Stem Cells, Tracking, Differentiation, Gene Delivery, Drug Delivery.*

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INTRODUCTION

Stem cells are identified with the ability to self-renew and generate specific progeny. They have been extensively used for regeneration of aged, injured, or diseased tissues. They can also be used as *in vitro* models for developmental biology, screening and testing of drugs, and understanding of the underlying mechanisms in human diseases. Stem cells can be categorized to three types: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells. ESCs, derived from blastocysts, are the first source of pluripotent cells while iPSCs are produced by introducing genes encoding multiple transcription factors into somatic cells.[1–3] These stem cells can generate almost any cell type in the body.[4] ESCs and iPSCs are exceptional for the study of developmental biology and the mechanisms of human diseases due to their unlimited self-renewal. Induced pluripotent stem cells may overcome the problem of immune rejection and the ethical issues of ESCs.[1–3] However, there is a lack of robust and reproducible methods that can direct iPSCs and ESCs into specific lineages in high purity and without the risk of tumor formation. These are the major challenges in clinical translations.[5, 6] Adult stem cells are multipotent cell populations in a differentiated tissue after birth. They are found in almost any tissue in the body including bone marrow, blood, brain, spinal cord, skeletal muscle, adipose tissue, dental pulp, skin, the cornea and retina of the eye, the lining of the gastrointestinal tract, liver, pancreas, and

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so on. They can yield specific cell types of the tissue from which they originate. Adult stem cells are normally not tumorigenic, but they lack the potent plasticity of ESCs and iPSCs. And it is a challenging task to generating large quantity that is enough for clinical use. In addition, it is difficult to modify the stem cells through virus-free genetic engineering approaches. The fate of the stem cells after transplantation is difficult to monitor. It is critical, therefore, to develop viable methods that can overcome these hurdles in clinical applications.

To overcome some of the aforementioned limitations currently experienced in stem cell research, materials in the particle form have been used in many stem cell applications. Materials in the particle form are distinctively characterized by their geometry, distribution, and size, ranging from nanometers to micrometers, compared to their bulk counterparts. Representative particles of different sizes, typically used in the stem cell applications, include organic and inorganic particles, liposomes, and polyplexes.[7,8] These particles exhibit unique characteristics such as variable size down to the nanoscale, fluorescence in the visible and near-infrared range, drug loading capacity, biocompatibility with controlled degradation profiles. As such, the potential applications of these particles in stem cell research include, but not limited to,

(1) noninvasive tracking of transplanted stem cells and labeling of endogenous stem cells;

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Donglu Shi received his Ph.D. in Engineering in 1986 from the University of Massachusetts at Amherst. After graduation, he took a Staff Scientist position at the Materials Science Division of Argonne National Laboratory in 1987. At Argonne, he was a principal investigator of a major Department of Energy program on High-Tc superconductors. In 1995, Donglu Shi joined the faculty as an Associate Professor in the Department of Materials Science and Engineering at University of Cincinnati. He was promoted to the full professor position, with tenure, in 2001 at University of Cincinnati. He is currently the Chair of the Materials Science and Engineering Program at the University of Cincinnati. He is currently the Editor-in-Chief of Nano LIFE, and Associate Editor of Materials Science and Engineering: C, and J. of Nanomaterials. He has won the SIGMA XI Research Recognition Award, Honor Roll Professor Award, and Neil Wandmecher Teaching Award. Donglu Shi's main interests include nanostructured materials, nano-biomedicine, and superconductors. The most recent works on nano-biomedicine pioneer some novel approaches in developing multifunctional nano carrier systems for early cancer diagnosis and therapy. Based on new designs of nanostructures, these methods have enabled successful cell targeting for tumor therapy, optical imaging by quantum dots, photothermal ablation of cancer cells, and drug delivery by intelligent triggering mechanisms.

Xuejun Wen is currently the Alice T. and William H. Goodwin, Jr. Endowed Chair and tenured full professor in the Institute for Engineering and Medicine and Department of Chemical and Life Science and Engineering at the Virginia Commonwealth University (VCU). Before moved to VCU, he was a tenured full professor at the Department of Bioengineering, Clemson University. He was also the State of South Carolina Named Hansjörg Wyss Endowed Chair Professor in Regenerative Medicine. He joined Clemson Bioengineering as a tenure-track faculty in 2003, and resided in Charleston, SC to initiate joint bioengineering program between the Clemson University and Medical University of South Carolina (CU-MUSC) with the mission to foster translational research, and promote interinstitutional, multidisciplinary collaborations. He is also being appointed as adjunct faculty in a couple of clinical and basic science departments at MUSC, fields including neuroscience, orthopaedic surgery, dental medicine, and cell biology and anatomy. Through the past 11 years of his independent academic career, he has established a strong independent extramurally funded research lab in the field of biomaterials, tissue engineering, stem cells, and regenerative medicine. His research focuses on regenerating functional and safe human tissues by combining the principles of biomaterial science, biological science, stem cell biology, tissue engineering, regenerative medicine with the advanced techniques in molecular and cell biology. His lab has been extensively funded by federal organizations, as well as private foundations, such as National Institutes of Health (R01 awards, and R21 awards), National Science Foundation (CAREER award, and NSF research grant), Department of Defense (Office of Naval Research, CDMRP), National Textile Center, Center for Innovative Technology, Michael J. FOX Foundation for Parkinson's disease, Wallace H. Coulter Foundation, March of Dimes Foundation, American Heart Association, AO Foundation, Orthopedic Trauma Foundation, South Carolina Spinal Cord Injury Research Fund, etc. He has filed over 70 disclosures (with 8 issued patents and 12 full patent applications). His research has been featured in numerous press releases.

(2) intracellular delivery of functional molecules, such as DNA, RNAi, peptides, proteins, and drugs that control the stem cell behaviors and fates;
(3) carriers for stem cell transplantation, and
(4) delivering drugs with stem cells for cancer treatment.[7,8]

In this review, we report recent research results on the applications of a variety of particles in tracking transplanted stem cells and labeling of endogenous stem cells *in vivo*. Discussions are focused on the recent advances in intracellular delivery of biological molecules for *in vivo* stem cell applications. Novel particle systems are introduced as carriers for stem cell transplantation and drug delivery in cancer treatment. Critical challenges and future directions are elucidated on the particle development for stem cell applications. All particles reviewed in this report are summarized in Table I.

STEM CELL LABELING AND TRACKING

Transplanted stem cells can survive, migrate, proliferate, differentiate, integrate with the host tissues, and then regain some functions of the lost cells, tissues, and organs. The safety of stem cells is one of the foremost concerns that need to be addressed before extensive clinical applications of this strategy. An important aspect is how to trace and identify transplanted stem cells *in vivo*. Noninvasive cellular imaging has been broadly used to track the transplanted stem cells. Compared to traditional histopathological methods acquiring cell information from invasive biopsy or postmortem analysis, noninvasive imaging is far more efficient since it can obtain unique information about cell behaviors over time. Noninvasive imaging techniques can also guide treatment for maximized therapeutic effect through timely evaluation of the migration and function of stem cells.[9,10] Current noninvasive imaging modalities available for *in vivo* tracking of the biological fates of stem cells include, but are not limited to, magnetic resonance imaging (MRI), ultrasound imaging, fluorescence imaging (FLI), bioluminescence imaging (BLI), computed tomography (CT), positron emission tomography (PET), and single photon emission computer tomography (SPECT). Compared to CT, PET, and SPECT, MRI has several advantages including high spatial resolution, widespread availability in clinics, and non-exposure of the patient to ionizing radiation.

In order to visualize stem cells in the body with MRI, cells are required to be labeled with a contrast agent. Contrast agents improve visibility, detectability and sensitivity of the image. There are many types of contrast agents or imaging probes, such as gadolinium chelates, iron oxide-based agents, iron platinum-based agents, and manganese containing agents. These elements (iron, manganese, and gadolinium) have been investigated as contrast agents for MRI due to their unpaired electrons with strong paramagnetic effects on the local magnetic field. All these agents have short life spans in body. Having these contrast agents in the particle form at micro- or nano-size scale greatly enhances their life span and offers the long-term tracking capability. The small size of particles also

Table I. The use of particles for stem cell specific applications.

Applications	Mechanism	Particles	Comments
Labeling and tracking stem cells	MRI	Iron oxide	+High spatial resolution −Easy to aggregate −Non-specific for *in situ* labeling −Cyto and tissue toxicity
	Optical imaging	Gold	+Inert character +Tunable optical property +High spatial and temporal sensitivity −Toxicity
	Fluorescent imaging	Quantum dots	+Tunable emission +Photo-stability −Light scattering −Cytotoxicity
Delivering genes and proteins into stem cells	Electrostatic interaction between positive particles and negatively charged DNA molecules to form polyplexes	Poly(L-lysine)	−Low transfection efficiency −High cytotoxicity
		Poly(ethylene imine)	−High cytotoxicity with high MW
		Poly(β-amino ester)	−Highly hydrolytically degradable +Low toxicity +High transfection efficiency
		Poly(amidoamine)	+Highly biocompatible +High transfection efficiency
		Silica	−Non-degradable
		Calcium phosphate	+Biocompatible +Bioresorbable +Cost effective to produce +High binding affinity for DNA
		Self-assembly peptide	+High bilcompatible
	DNA or protein embedded in particle matrix	Poly(lactic-co-glycolic acid)	+Biodegradability and biocompatibility +Control and sustained release of biomolecules +Possible targeting −Cytotoxicity of degradation parts
		Hyaluronic acid	+Non-immunogenic −Highly hydrophilic
		Chitosan	+Highly biocompatible +Easy modification −Inflammatory response
		Dextran	+Easy modification
		Self-assembly peptide	+Highly biocompatible
Carriers for stem cell transplantation	Particles providing physical support for stem cells	Poly(lactic-co-glycolic acid)	+Local and efficient control release biomolecules (VEGF for neovasculature in the stroke lesion cavity) −Cytotoxicity of degradation parts
		Silica	+Support long-time cell survival *in vivo* −Non-degradable
Drug delivery through stem cells as vehicles	Particles conjugated to the surface of or internalized into stem cells	Poly(lactic-co-glycolic acid)	+Local and efficient release of drugs −Cytotoxicity of degradation parts
		Silica	+Long retention lifetime and wide distribution of drugs −Non-degradable
	Tropism of mesenchymalstem cells for tumor	Poly(lactic acid)	+MSCs migrating toward glioma cells +Different drugs can be loaded into particles

allows for tissue penetration, tunable circulation half-life for different imaging needs, and large surface conjugation of functional groups for both imaging and therapeutic agents.[11]

Stem cell monitoring and tracking require high resolution and sensitivity for quantification and interpretation. Particles for this type of application should be non-toxic to stem cells and possess suitable properties for the contrasting mechanism. Ideally, particles should have reactive moieties that can conjugate with biologically active molecules for added functions in addition to tracking.[12] Particles can be loaded into stem cells before transplantation or directly used to target stem cells in the body for their versatility in functionalization.

Iron Oxide Particles

In the early 1990s, magnetic iron oxide particles were first reported for intracellular labeling of cells and detection by MRI. Their use in stem cell labeling and detection started after 2000.[13] Currently, a number of iron oxide particles have been used for stem cell imaging. Based on their sizes, iron oxide particles can be categorized as superparamagnetic iron oxide nanoparticles (SPIO; 50–200 nm in diameter), ultra small superparamagnetic iron oxide nanoparticles (USPIO; 10–40 nm in diameter), and micron-sized paramagnetic iron oxide particles (MPIO; approximately 1 μm in diameter). Since iron oxide nanoparticles are easily aggregated, which will be toxic to stem cells, different substrates, such as dextran, carboxydextran,[14] polyethylene glycol (PEG),[9,15] polystyrene, and silica, have been coated on the surface of these particles, in order to prevent their aggregation, enhance solubility, and improve biocompatibility.

Iron oxide particles enter stem cells in both passive or active fashion.[16] Passive uptake is non-specific, generally due to driving forces that arise from chemical, physiological, and biological gradient in the body. The particles adhere to the cell surface and penetrate the cell membrane without any machinery of the cells. The size of iron oxide particles affects the uptake in the cells. Smaller particles can enter the cells easier with better retention compared to larger ones. This is an important feature to consider since it influences the life span of the enhanced imaging. Active uptake can take place by conjugating specific targeting molecules to iron oxide particles or some polymeric systems such as micelles and liposomes. However, non-specific binding can still occur due to the surface chemistry of both particles and cells. Iron oxide particles have been explored in tracking transplanted stem cells by MRI in a variety of disease models, including cardiovascular diseases, skeletal tissue injury and diseases, traumatic brain injury, stroke, spinal cord injury, and multiple sclerosis. Some of the major applications in these areas are summarized in Table II.[17–26]

To obviate the immune rejection and ethical issues associated with stem cell transplantation, amplification of the body's self-repair response has been proposed for injured or diseased tissue regeneration. Endogenous stem cells have been found to be present within the adult mammalian tissue, such as brain, spinal cord, heart, skeletal muscle and so on. For example, neural stem cells (NSCs) are mostly restricted to the subventricular zone (SVZ) along the wall of the lateral ventricle. These stem cells migrate through the rostral migratory stream (RMS) to the olfactory bulb, and then differentiate into neurons. Recent studies have demonstrated that after traumatic injury or ischemia, the non-neurogenic regions of brain, such as striatum and cerebral cortex, can also induce neurogenesis.[27,28]

To utilize endogenous stem cells for tissue regeneration, an important aspect is to identify these cells *in vivo*. Iron oxide particles have been widely applied to labeling of stem cells *in situ* and investigation of cell migration *in vivo*. MPIOs are one of particles that have been shown to be well tolerated for stem cell labeling.[29] In the case of direct *in situ* labeling, it is found to be quite nonspecific and only a small fraction of targeted stem cells can be labeled with MPIOs. For example, intraventricular injection of a large amount of MIPOs (1.4×10^8 in 50 μL) only labeled about 30% of the migrating NSCs.[30] In addition to the targeted NSCs, MPIOs also nonspecifically labeled ependymal cells, microglia, and oligodendrocyte progenitor cells.[31,32] High concentrations of particles can result in significant signal distortion, leading to hindered visualization of the RMS near the injection site. It is thus difficult to identify the cells migrating along RMS. Some transfection agents, such as poly(L-lysine), when combined with MPIOs, can increase labeling efficiency. The entire RMS can be visualized by MRI using the aforementioned approach.[33–36] As shown in Figure 1, using quantitative intensity metrics, the labeled stem cells travel at the speed up to 100 μm/h en route to the olfactory bulb, but within which they are slowed down.[37] Moreover, *in situ* labeling of endogenous NSCs with MPIOs also revealed cell migration toward a hypoxic-ischemic insult.[35,38–43]

Although iron oxide particles have been shown to be useful in tracking cell location and migration *in vivo*, they have several inherent limitations:

(1) Their MRI contrast cannot provide any information on the viability of the labeled cells or cell-type due to non-specific binding. Macrophage can engulf the particles from the dead stem cells and lead to non-specific labeling;

(2) The contrast is reduced by the division of stem cells *in vivo*;[30]

(3) The eventual fate of the particles inside stem cells is still not well understood. Depending on the size and the coating materials on them, some are eventually biodegraded within the cells and others are removed through exocytosis. Particles inside stem cells cannot maintain a high level within a sufficient time, which inevitably affects the tracking of the labeled stem cells,[44] and

Table II. Application of iron oxide particles in different disease models.

Tissue	Particle type	Modification of particles	Type of stem cells	Results	References
Heart (myocardial infarction)	SPIO	Poly-L-lysine	Mouse ESCs	Similar cardiogenic capacity and calcium-handling property of ESCs labeled with SPIO compared to their unlabeled counterparts	[20]
Heart (myocardial infarction)	SPIO (Endorem)	Poly-L-lysine	Rat bone marrow MSCs	Both labeled and unlabeled cells attenuated left ventricular dilatation and dysfunction after myocardial infarction; At 4 weeks the transplanted MSCs labeled with SPIO are not present in the scar and the enhanced MRI signals arise from cardiac macrophages that engulfed the SPIO	[17]
Cartilage	SPIO (Endorem)	Protamine sulfate	Human bone marrow MSCs	SPIO labeling was effective and did not impair hBMSC secretion profile; SPIO released from dead, labeled cells could be taken up by synovial cells	[130]
Bone	USPIO	PEG and Dextran; Protamine sulfate	Human adipose stem cells (ASCs)	USPIO labeling did not affect cell viability and osteogenic differentiation; *In vivo* MRI can detect that transplanted stem cells until 28 days after implantation	[131]
Traumatic brain injury	SPIO	Protamine sulfate	Human marrow stromal cells	Human marrow stromal cells diminished hemodynamic abnormalities in the brain regions adjacent to and remote from the impact site, and reduced generalized cerebral atrophy, contributing to the observed improvement of functional outcome	[118]
Spinal cord injury	SPIO Endorem	None	Rat bone marrow MSCs	The lesions populated by grafted MSCs	[132]
Stroke	Ferumoxides	Protamine sulfate	Human MSCs	Human MSCs migrated to the infarcted area extensively in both ipsilateral and contralateral injections, exhibiting a pathotropism	[21]
Multiple Sclerosis	Ferumoxides	None	Mouse glial-committed neural precursor cells (NPCs)	Ferumoxides labeling did not affect NPC survival and pluripotency *in vitro;* Ferumoxides-labeled NPCs responded to inflammatory cues in a similar fashion as unlabeled cells; Ferumoxides-labeled NPCs migrated over comparable distances in white matter tracts and differentiated equally into the glial lineages	[133]

(4) Although limited toxicity has been demonstrated following *in vitro* labeling, recent studies have found decreased cell proliferation and migration, as well as signs of inflammation *in vivo*.[45]

Gold Nanoparticles

Gold nanoparticles can be used as contrast agents for their tunable optical property and distinctive advantages including biocompatibility, low cost, easy accessibility, as

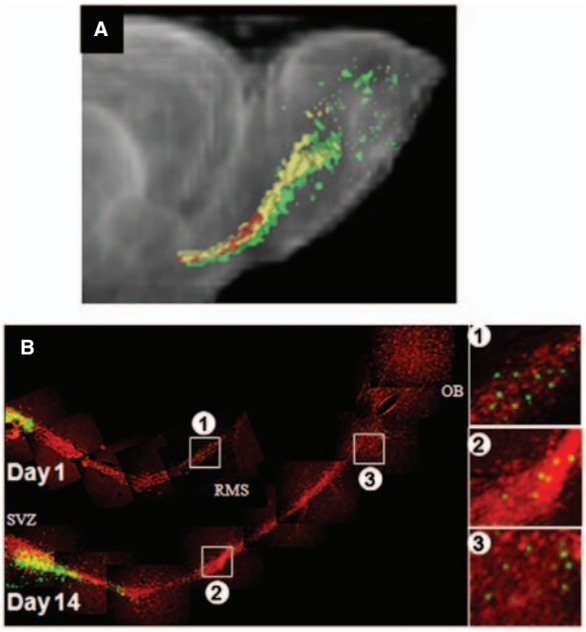

Figure 1. Tracking stem cells migration with magnetic nanoparticles using cellular MRI. (A) The cell migration into the olfactory bulb is visualized using the overlay of serial progression at 1 day (shown in red); 3 days (yellow), and 8 days (green) after injection of magnetic nanoparticles, guided with MRI. (B) BrdU labeling in rat neurogenic system is visualized using fluorescence microscope through RMS (left) to olfactory bulb (right) at 1 and 14 days post injection of MPIOs. Close-up images are the areas indicated with numbers shown in B. Reprinted with permission from [43], D. Granot, et al., Serial monitoring of endogenous neuroblast migration by cellular MRI. *Neuro. Image* 57, 817 (2011). © 2011, Elsevier.

well as high spatial and temporal sensitivity in comparison to other modalities like MRI, PET, and CT.[46] As a result of these advantages, they hold high promise in clinically labeling and tracking of stem cells *in vivo*. Gold nanoparticles can enter cells via a receptor-mediated clathrin-dependent endocytosis pathway.[47] It is hypothesized that the intracellular biocompatibility of gold nanoparticles are influenced by the presence of free radicals which can lead to oxidative stress and cell damage. These reactive species are exceedingly toxic *in vivo* as they can oxidize lipids, proteins, and DNA. The toxicity of the gold nanoparticles intensifies with the decreasing particle size and reaches a maximum at 1.4 nm.[48,49]

Photoacoustic imaging has been used to image cells labeled with gold nanoparticles at reasonable depths, as well as for longitudinal studies.[50] For example, Nam et al. have demonstrated the efficacy of ultrasound-guided photoacoustic imaging with high detection sensitivity (1×10^4 cells/mL) of the gold nanoparticles-labeled mesenchymal stem cells (MSCs). When the labeled MSCs were encapsulated in the PEGylated fibrin gel and injected intramuscularly into the lower limb of the Lewis rat, they were still detectable after one week.[51]

In addition, through surface-enhanced Raman spectroscopy, nuclear-targeted gold nanoparticles, as intracellular probes, have been employed to distinguish progenitor and differentiated cell types. In addition, cell differentiation is also detected by identifying the change in DNA/RNA ratio and the proteins transcribed.[52]

Quantum Dots

Quantum dots (QDs) have been widely used as fluorescent imaging agents.[53] QDs, with a size range of 2–10 nm, are structurally composed of three layers: an inorganic core, a shell of metal, and an outer organic layer. With appropriate excitations, they emit fluorescent lights from 525 to 800 nm. The tunable emission of QDs, especially at the near infrared region (~800 nm), avoids the background signal of auto fluorescence from the animal tissues (emissions are mainly at the visible region, ~300–550 nm). Good photo-stability allows QDs for the long-term tracking of stem cells.

QDs can enter stem cells through passive loading, receptor-mediated endocytosis, lipid-based transduction, microinjection, electroporation, and peptide-mediated delivery.[54–57] Rosen et al. have demonstrated that passive incubation was more effective than electroporation and receptor-mediated uptake in the labeling of human bone marrow stromal cells (hMSCs) with QDs. The labeled hMSCs were identified in histological sections of canine ventricle, and the fluorescence signals were visible for at least 8 weeks following the injection.[58] Hsieh et al. have shown that, through liposome-mediated transfection, hBMSCs were efficiently labeled with CdSe/ZnS QDs. Uptake of QDs into hBMSCs did not affect their proliferation, but prevented the cell response from induced osteogenic differentiation.[59,60] Figure 2 shows the *in vivo* fluorescence imaging of QD-labeled adipose stem cells (ASCs) indicating the organ-specific accumulation of these cells in mice with acute liver failure. Fluorescence imaging has confirmed accumulation of the transplanted ASCs in lungs within 10 min. When heparin was used in combination with these cells, in addition to the lungs, the transplanted cells were also detected in liver.[56] Although QDs represent a fairly novel progress in tracking stem cells, light scattering of QDs makes it difficult to locate the labeled cells in three-dimension (3D) and to estimate the cell survival in quantity. Another concern involving QDs for stem cell labeling is their potential cytotoxicity generated from the leakage of toxic heavy metal ions.[61]

INTRACELLULAR DELIVERY OF GENES AND PROTEINS

Viral carriers including retroviruses, lentiviruses, and adenoviruses have been widely investigated in delivery of specific genes for modification of stem cells. Although these carriers have been proved to be extremely efficient in transfecting stem cells and directing their fates, from

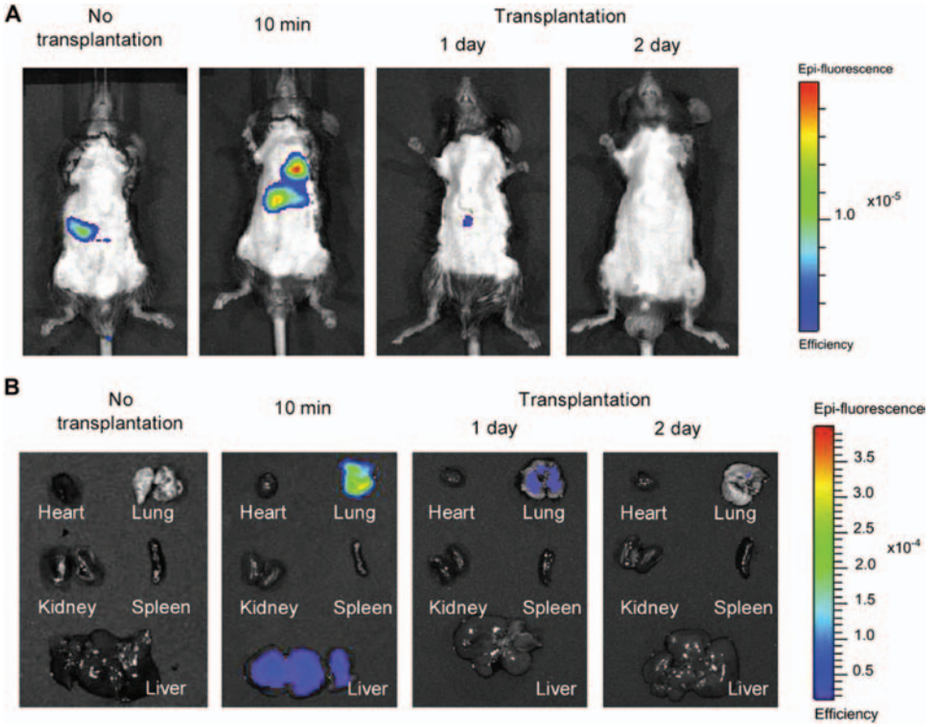

Figure 2. **Tracking ASCs with quantum dots. (A) Live imaging of animals transplanted with quantum dots 800 labeled ASCs. (B) *Ex vivo* fluorescence images of the key organs after transplantation of ASCs labeled with QDs800 in combination with heparin. Reprinted with permission from [56], H. Yukawa, et al., Monitoring transplanted adipose tissue-derived stem cells combined with heparin in the liver by fluorescence imaging using quantum dots. *Biomaterials* 33, 2177 (2012). © 2012, Elsevier.**

a clinical translation perspective, non-viral carriers are preferred on the consideration of viral vectors regaining reproductive potential or tumor formation by insertional mutagenesis.[62, 63]

Polymer Particles

Polyplexes

Non-viral polymer carriers are typically cationic in nature. They electrostatically interact with negatively charged DNA molecules to form polyplexes. The polyplexes with condense DNA molecules in a relatively small size facilitate passage of DNA through cell membranes and protect DNA against degradation in extracellular matrix (ECM). However, due to the high affinity between cationic polymer and DNA, it is difficult to separate DNA from the polymer inside the cytoplasm or nucleus, thus successful transfection is limited.[64] The most potent polyplex formulations still cannot reach efficiencies of viral vectors. Different types of cationic polymers, such as poly(L-lysine) (PLL), poly(ethylene imine) (PEI), poly(β-amino esters), poly(amidoamine), etc., have been studied for delivery of specific genes in order to manipulate stem cell behaviors and fates *in vitro* and *in vivo*.[51, 65–69] Some recent progress is introduced on polymeric particles as gene delivery vehicles for stem cells.

Poly(L-lysine) Based Particles. As a gene delivery agent, complexes of PLL and DNA have fairly low transfection efficiencies but high toxicity. Especially large molecular weight PLL (MW 25 kDa) exhibits relatively high toxicity. Different approaches have been developed to lower cytotoxicity of PLL and improve its transfection efficiency. One approach is by attaching hydrophobic and biodegradable poly(lactic-co-glycolic acid) (PLGA) grafts to the polymer backbone.[70–72] Another approach involves developing the multiblock copolymers composed of low molecular weight alternating PLL and poly(ethylene glycol) (PEG) blocks. The block polymer of PLL-PEG can enhance transfection efficiency over 6-fold compared to PLL only. Its cytotoxicity is much lower compare to PLL.[73, 74]

Recently, Clements et al. used palmitic acid (PA)-modified PLL to deliver plasmid DNA to rat bone marrow stem cells. Conjugating PA, a naturally occurring lipid, to PLL is hypothesized to enhance the transport of the complexes across the plasma membrane. The complexes are reported to deliver plasmid enhanced green fluorescent protein (EGFP) to approximately 80% of the cells, achieving a maximum transfection efficiency of about 22%, significantly higher than that of Lipofectamine 2000 (11%).[75–78] Three times dosing of complexes to BMSC enhanced the transfection efficiency by 2–3 folds,

without compromising cell viability. Long-term evaluation of EGFP expression showed 17% transfection on day 1 and disappearance on day 12.[75–78]

Poly(ethylene imine) Based Particles. PEI was first applied to gene delivery by Behr et al. (1995).[79] High molecular weight PEI is considerable toxic, whereas less toxicity is reported on its low molecular counterpart, but with almost no transfection.[80] Similar to PLL, branched polymers consisting of low molecular weight PEI and PEG have been thoroughly studied to improve transfection efficiency and cytotoxicity.[81,82] Santos et al. have developed a nanoparticle system based on PEI and dextran sulfate to deliver retinoic acid (RA) for *in vitro* and *in vivo* NSC manipulation. The intracellular delivery of RA-loaded PEI nanoparticles was effective in driving the neuronal differentiation of NSCs *in vitro.* These particles, when injected in the SVZ of mouse brain, successfully induced endogenous NSCs toward neuronal progenitors with the expression of Mash1 and Neurogenin1.[65,66]

Recently Park et al. have applied PEI modified PLGA nanoparticles to a complex with plasmid carrying Nurr1 cDNA to support neurogenesis of hMSCs. A device of electrical stimulation through gold nanoparticles was designed in combination with the plasmid to further improve the nerve regeneration. When cells were transfected with Nurr1 genes and motivated by electrical stimulation (250 mV for 1000 s), they exhibited the highest level of neurite outgrowth (150 μm) compared to those treated with only one stimulus (approximately 10∼20 μm).[51]

RNA interference (RNAi) is regarded as a promising technique for gene silencing because the small interfering RNA (siRNA) can recognize its complementary mRNA with high specificity and degrade it in a short period of time.[83] Recently, Liang et al. used PEG-PEI to deliver siRNA-targeting NgR to NSCs. The highest transfection efficiency of the PEG-PEI/siRNA nanoparticles was obtained at an N/P ratio of 15, which was better than that achieved in the transfection using Lipofectamine-2000. The gene knockdown effect of PEG-PEI/siRNA nanoparticles was verified at the levels of NgR mRNA and protein.[84] With the similar approach, they also synthesized the PEG-PEI/ROCK-II-siRNA complexes and transfected NSCs *in vitro*. ROCK-II-siRNA was found to express effective gene silencing.[85]

Poly(β-amino ester) Based Particles. Another potent polymer for gene delivery is poly(β-amino esters) which is highly hydrolytically degradable. A library of poly(β-amino esters) end-modified derivatives was developed and optimized with high transfection efficiency and low cytotoxicity for three types of stem cells: hMSCs, human adipose-derived stem cells (hADSCs), and human embryonic stem cell-derived cells (hESCds). Leading end-modified C32 polymeric vectors exhibited high cell viability (87–97%) in all cell types but different transfection efficiencies: hMSCs ($27 \pm 2\%$), hADSCs ($24 \pm 3\%$) and hESCs ($56 \pm 11\%$), respectively.[68] Furthermore, with the same C32 poly(β-amino esters), hMSCs and hESCds were transfected with angiogenic factor (vascular endothelial growth factor, VEGF) gene. VEGF-expressing stem cells improved 2 to 4 fold higher vessel densities in the subcutaneous implantation model compared to non-transfected counterparts. These cells also significantly enhanced limb salvage and reduced muscle degeneration and tissue fibrosis (Fig. 3).[86,87] Using the same poly(β-amino esters) as transfection agents, Montserrat et al. have delivered a single CAG-driven polycistronic plasmid expressing Oct4, Sox2, Klf4 and c-Myc to human fibroblast and successfully produced iPSCs from these fibroblasts.[67–69]

Poly(amidoamine) Based Particles. Poly(amidoamine)s with pendant primary amine, are new types of highly efficient gene delivery vectors.[32] They show improved cytocompatibility and tissue compatibility compared to PEI.[88] Recently, a poly(amidoamine) dendrimer was synthesized.[89,90] Peptides, such as RGD, were conjugated to this poly(amidoamine) dendrimer. The RGD functionalized dendrimer exhibited receptor-mediated gene delivery into MSCs and higher transfection efficiency than those presented by native and partially degraded dendrimers.[91,92] Furthermore, dexamethasone, loaded into the carboxymethyl chitosan/poly(amidoamine) dendrimer nanoparticles, have been shown to enhance osteogenic differentiation of MSCs, both *in vitro and in vivo*.[93–95]

Other Polymeric Particles

The previous section describes the DNA loaded particles which arise from self-assembling of negative DNA and cationic polymers. Other types of particles are developed with DNA embedded in the polymer matrix. In addition to DNA, several biomolecules, such as proteins, with the capacity to differentiate stem cells toward desired lineage-specific types, may also be encapsulated into the polymer matrix. These bioactive factors are difficult to administrate because of their short half-life and potentially undesirable side effects in the body if delivered systemically.

Hyaluronic Acid Based Particles. Hyaluronic acid (HA), a high molecular weight glycosaminoglycan, is naturally found in extracellulr matrix (ECM) of the mesenchymal tissues and central nervous system (CNS). HA has been broadly used as a hydrogel due to its neutrality and high affinity to water. Recently, Jha et al. prepared HA-based hydrogel particles with inherent nanopores through an inverse emulsion polymerization technique. Heparan sulfate-bearing perlecan domain I (PlnDI) was covalently conjugated to the HA hydrogel particles (HGP-P1). Bone morphogenetic protein 2 (BMP-2) was loaded into this HGP-P1 to induce the chondrogenic differentiation of MSCs. BMP-2 loaded HGP-P1 stimulated more robust cartilage specific ECM production when compared to BMP-2 loaded HGP because HGP-P1 can control release of BMP-2 with a near zero-order release pattern.[96,97]

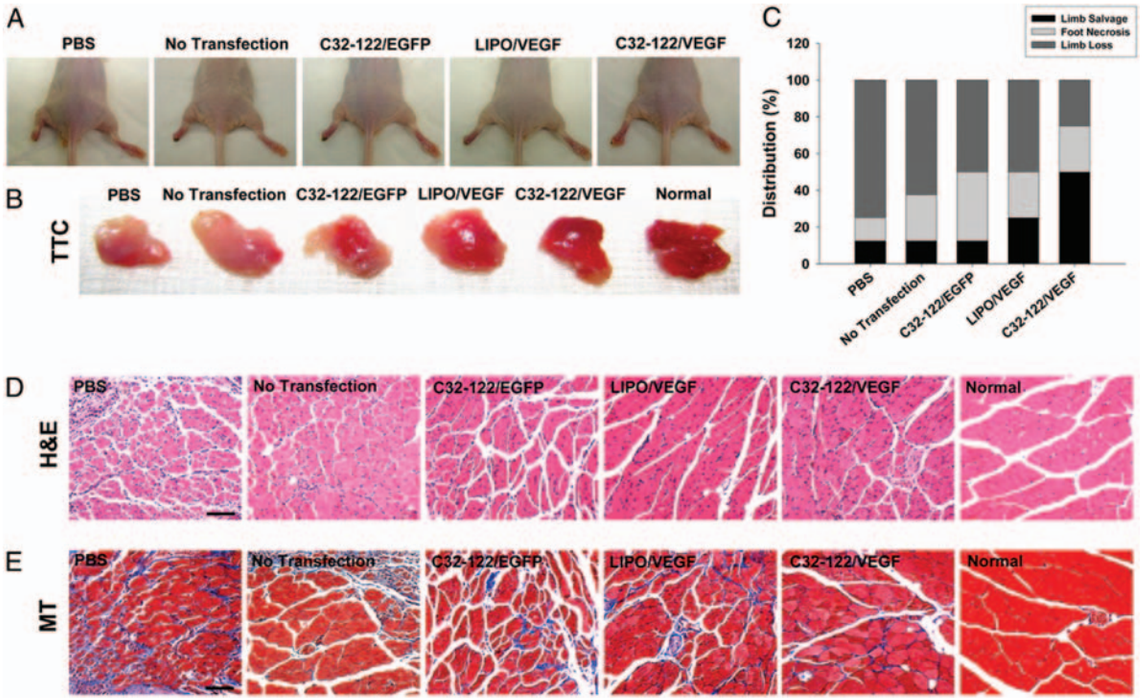

Figure 3. **Poly(β-amino ester) based particles for stem cell transfection. (A) Gross images of animals treated with VEGF transfected stem cells. (B) Triphenyltetrazolium chloride staining of muscle tissues harvested from VEGF transfected stem cell limbs and no-VEGF transfection control limbs at 4 weeks. (C) Physiological status of lesioned limbs treated under different protocols is evaluated 4 weeks. (D) H and E staining shows muscle tissues under different treatments and compared to normal muscle tissue. (E) Masson's trichrome staining shows fibrotic tissues in blue color under different treatment. VEGF-loaded, nanoparticle-transfected stem cells promote regeneration. Reprinted with permission from [87], F. Yang, et al., Genetic engineering of human stem cells for enhanced angiogenesis using biodegradable polymeric nanoparticles. *Proc. Natl. Acad. Sci. USA* 107, 3317 (2010). © 2010, National Academy of Sciences.**

Chitosan Based Particles. Chitosan is a natural polymer produced by alkaline deacetylation of chitin. The degree of deacetylation, and the resulting positive charge, significantly affects the properties of chitosan. Santo et al. prepared nanoparticles based on chitosan and chondroitin sulphate for controlled release of platelet lysates. Platelet lysates, which can be easily achieved from the blood of the patient, is a cost-effective source of bioactive molecule. Platelet lysates was prepared through three repeated thermal cycles from liquid nitrogen temperature to 37 °C. The release of platelet lysates from nanoparticles proved to enhance *in vitro* osteogenic differentiation of hASCs.[98, 99]

Dextran Based Particles. Dextran is a complex, branched glucan with chains of varying lengths. Spermine was introduced to dextran for the formation of the cationized polysaccharide. This cationized polysaccharide was internalized in cells through a sugar-recognizable receptor. Spermine-dextran has been successfully used to deliver plasmid DNA of adrenomedullin (AM), an anti-apoptotic and angiogenic peptide, to MSCs. Compared to MSC alone, AM-engineered MSCs were found to significantly improve the cardiac function after myocardial infarction.[100–102] Spermine-dextran was also applied for the delivery of siRNA to cells. The adipogenesis of MSCs was promoted when MSCs were cultured with the complex of spermine-dextran and a specific siRNA. The siRNA is an anti-transcription coactivator containing PDZ-binding motif for osteogenic differentiation.[103]

Degradable Polyester Based Particles. Degradable polyester, such as PLGA, can be made into the micro- and nano-particles for many stem cell related applications. PLGA nanoparticles have been approved by FDA and European Medicine Agency in drug delivery systems for parenteral administration. The PLGA nanoparticles have attractive properties of biodegradability and biocompatibility. Well described preparation methods have been used to load hydrophilic or hydrophobic small molecules or macromolecules. These systems are able to control the sustained release of loaded and protection molecules from degradation. Targeting to specific organs or cells is also possible via these methods.[104, 105] Sarkar et al. have shown that primary hMSCs can efficiently internalize the PLGA particles of a few microns that are loaded with differentiation factors, such as dexamethasone. The particles remained in the cells for at least 7 days. The releasing

of dexamethasone not only promoted differentiation of the particle-carrying cells, but also the neighboring and distant cells.[106] Leukemia inhibitory factor (LIF) has been loaded into the PLGA nanoparticles for sustained release. In combination with the hepatocyte growth factor (HGF), which controls the release from PEG hydrogels, LIF was found to significantly mobilize hNSCs and thus enhance their migration *in vitro*.[107]

Ceramic Particles

Silica Particles

Silica nanoparticles have been functionalized with amino groups in order to bind with plasmid DNA. They can prevent DNA from enzymatic digestion and affect cell transfection.[108] Recently, Shah et al. have successfully used modified silica nanoparticles to deliver chimeric protein for the control of NSC proliferation. The chimeric protein GFP-FRATtide was designed, with FRATtide bonded to the active site of glycogen synthase kinase-3β, a protein kinase involved in Wnt signaling. The chimeric protein uptake led to increased transcription of Wnt target genes, such as c-Myc, which instructed the cells to actively proliferate and maintain an undifferentiated state.[109, 110] Amine modified silica nanoparticles were also used to manipulate the NSC fates *in vivo*. A plasmid expressing of the nucleus-targeting fibroblast growth factor receptor type 1 was tansfected into endogenous NSCs, which significantly inhibited *in vivo* proliferation of the cells in SVZ and adjacent RMS.[110]

Calcium Phosphate Particles

Calcium phosphate has been applied for DNA transfection in a range of stem cell lines.[111, 112] Hydroxyapatite, a calcium phosphate mineral, is biocompatible, bioresorbable, non-toxic, cost effective to produce, and with a high affinity to DNA.[113] Recently, Curtin et al. developed a collagen nano-hydroxyapatite scaffold to act as a gene-activated matrix for BMP-2 delivery. This scaffold successfully transfected MSCs with BMP-2 leading to high levels of calcium production.[114]

Self-Assembly Peptide Based Particles

As discussed above, the use of cationic polymers as transfecting agents, such as PEI and PLL, have a detrimental effect on cell viability. Recent strategies for gene delivery systems intend to reduce cytotoxicity by applying a peptide-based delivery system. For example, Dash et al. fabricated tunable mono-dispersed elastin-like polypeptide (ELP) hollow spheres. The hollow spheres displayed a higher loading efficiency of plasmid DNA than that of the self-assembled ELP solid particles. The plasmid DNA released from the hollow spheres was triggered by protease and elastase. Polyplexes are developed based on poly(methacrylate) and pCMV-GLuc plasmid. When loaded in hollow spheres, they exhibited higher cell viability and luciferase expression compared to using polyplexes alone, since the spheres protected the polyplexes against endosomal degradation.[115, 116]

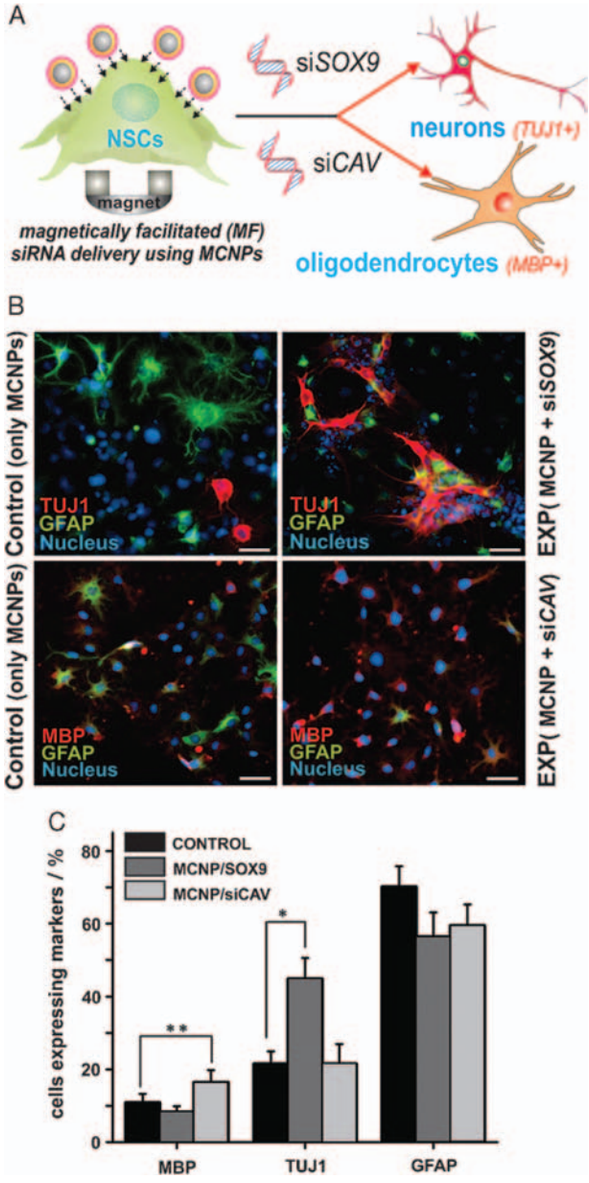

Figure 4. Magnetic facilitated delivery of siRNA. (A) Magnetic nanoparticles loaded with siRNA against *SOX9* (si*SOX9*) or *CAVEOLIN-1* (si*CAV*) for neuronal specific or oligodendrocyte specific differentiation of neural stem cells, respectively. (B) Neuronal (top row) and oligodendrocyte differentiation (bottom row) of the NSCs following delivery of si*SOX9* or si*CAV* siRNA, respectively, using magnetic nanoparticles. (C) Quantitative data of different neural cell markers when treated magnetic particles loaded with siCAV or siSOX9 versus control. Reprinted with permission from [109], D. A. Shah, et al., Regulation of stem cell signaling by nanoparticle-mediated intracellular protein delivery. *Biomaterials* 32, 3210 (2011). © 2011, Elsevier.

Metal Particles

Recently, metal particles have been post-synthetically modified with a biocompatible material (for example, SiO_2, gold, polymer) resulting in a core–shell structure for delivery of nucleic acids in order to control the stem cell fates. The shell acts as a platform for the surface functionalization of the particles.[117–119] Shah et al. have synthesized magnetic core–shell nanoparticles consisting of a highly magnetic $ZnFe_2O_4$ core surrounded by a gold outer shell. The nanoparticles were then coated with a cationic polyamine dendrimer to complex with negatively charged siRNA. Two different types of siRNA, namely, CAVEOLIN-1 and SOX9, were successfully delivered into NSCs via these nanoparticles, and selectively directed NSC differentiation into oligodendrocytes and neurons, respectively (Fig. 4).[109] As is well known, the gold outer-shell can enhance biocompatibility of the nanoparticles. The presence of gold nanoparticles within stem cells was also confirmed through dark-field imaging.

PARTICLES AS CARRIERS FOR STEM CELL TRANSPLANTATION

Two major difficulties of stem cell therapies are the low survival rate and the limited functionality of the transplanted stem cells. Recently, a novel strategy has been developed for stem cell transplantation using particle carriers. The particles not only provide physical support to stem cells, but also control the biomolecules released for modulation of stem cells *in vivo*. Bible et al. showed that the plasma polymerised, allylamine-treated PLGA microparticles (50–200 μm) can provide a structural support for NSCs that are injected directly into the stroke lesion cavity through MRI-derived coordinates. Upon implantation, the NSC-particle structure was found to integrate efficiently within the host tissue forming a primitive neural tissue.[120, 121] VEGF was further loaded into the PLGA microparticles. VEGF released from the particles attracted host endothelial cells into this primitive tissue and established a neovasculature in the lesion cavity. This particle system is useful for brain tissue regeneration, but the particle degradation may exert deleterious effects on the behaviors of cells in the brain.[122]

Silica beads have been explored as a transplantation vehicle for stem cells. These beads (45 μm) can provide large growth surfaces for stem cells to adhere, grow, and mature. The use of these beads allows transplanting stem cells without disrupting and damaging their delicate biological processes. For example, Jgamadze et al. have demonstrated that neurons, transplanted with colloidal beads, had a high survival rate of 76% one week post-surgery in rat hippocampus. Most transplanted neurons had migrated out of their beads, elaborated long processes, and further formed functional transplant-host synaptic connections. Interestingly, even after 6 months, these transplanted cells were still functionally integrated with host neurons with unchanged cell survival and distribution.[123]

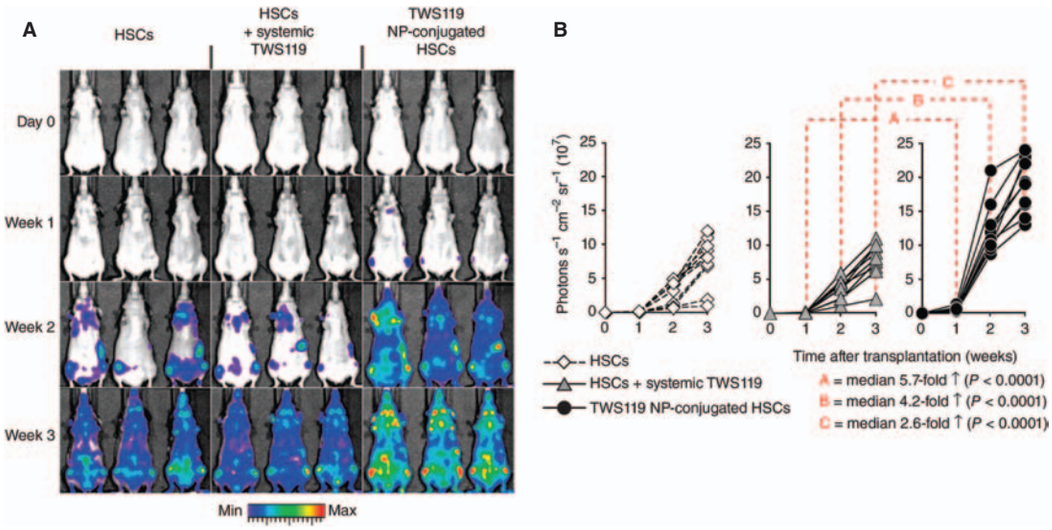

Figure 5. **Engraftment kinetics HSC labeled with luciferase in lethally irradiated recipients. One group of mice is treated with HSC transplantation alone. One group of mice is treated with HSC transplantation and a single systemic injection of TWS119, a GSK-3β inhibitor, on the day of transplantation. The third group is treated with HSC conjugated with nanoparticles loaded with an equivalent TWS119. Animals are imaged every 7 d for 3 weeks using for whole-body bioluminescence. Data from *in vivo* imaging pictures (A) and whole-mouse photon counts (B) indicate the significant higher survival for the transplanted HSCs with nanoparticles loaded with TWS119 (*$P < 0.001$). Reprinted with permission from [124], M. T. Stephan, et al., Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nat. Med.* 16, 1035 (2010). © 2010, Nature Publishing Group.**

STEM CELLS AS VEHICLES FOR PARTICLE DELIVERY

Recently, researchers have proposed to conjugate drug-loaded particles onto the surfaces of transplanted therapeutic cells, enabling continuous pseudoautocrine motivation of these cells *in vivo*, for enhanced cell therapy. PLGA nanoparticles, with drug-loaded core and phospholipid coated surface layer including maleimide headgroups, have been immobilized on the surface of hematopoietic stem cells via the reaction between maleimide and free thiols. With this method, as shown in Figure 5, *in vivo* repopulation rate of hematopoietic stem cells is increased with an adjuvant drug (glycogen synthase kinase-3β inhibitor) in extremely low doses, which is ineffective when given systemically.[124, 125]

In tumor therapy, nanoparticles have been applied to protection of the therapeutic drugs and their sustained release. Targeting to specific tumor and extensive intra tumor tissue distribution has been a major issue that needs to be addressed. MSCs have been demonstrated with the capacity of tropism for tumors. Recently MSCs have been used as the targeting vehicles for delivery of nanoparticles to tumors. Two types of nanoparticles, poly(lactic acid) and lipid, loaded with coumarin-6, have been efficiently internalized in MSCs. These nanoparticle-loaded cells were able to migrate toward glioma cells in an experimental model.[126–128] Another example involves MSCs as the targeting vehicle and silica nanorattle as the drug carrier. The doxorubicin loaded nanorattle was efficiently conjugated to the surface of MSCs by specific antibody–antigen recognitions. When compared to free doxorubicin and silica nanorattle-encapsulated doxorubicin, *in vivo* study confirmed higher efficiency of the burdened MSCs in tracking of the U251 glioma tumor cells. Longer retention lifetime and wider distribution of delivered doxorubicin within tumor tissues resulted in significantly enhanced tumor-cell apoptosis.[46, 129]

SUMMARY

Currently, over 2,000 clinical trials involving stem cells are underway. For clinical efficacy, it is extremely important to determine the final locations of stem cells and their eventual fates *in vivo*. MRI imaging with magnetic particle-labeled stem cells may provide an appropriate means to identify the location. However, several concerns need to be addressed in future studies. These include the loss of the MRI signals due to stem cell division *in vivo*, low efficiency and nonspecific *in situ* labeling, and toxicity of the magnetic particles. To modulate stem cell fates *in vivo*, particles, including polyplexes, have been extensively explored to deliver special genes into cells and to control the release of particular proteins. Although these particles exhibit great potentials for clinical use, their toxicity and low transfection efficiency need to be thoroughly investigated. Biomaterials in form of particles can provide a temporary physical support for transplanted stem cells, which ultimately benefit the *in vivo* engraftment of these transplanted stem cells. Biomolecules can also be loaded into these particles for release at specific times and locations in order to control the migration, differentiation, and fate of stem cells. The design, selection, synthesis, and optimization of these particles for stem cell applications present great challenges in future studies.

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