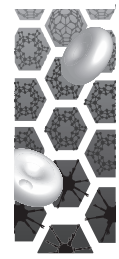

Nanotechnology for regenerative medicine: nanomaterials for stem cell imaging

Aniruddh Solanki[1], John D Kim[1] & Ki-Bum Lee[1,2,3†]

†Author for correspondence
[1]Rutgers, The State University of New Jersey, Department of Chemistry & Chemical Biology, Piscataway, NJ 08854, USA
[2]Institute for Advanced Materials, Devices & Nanotechnology, 607 Taylor Road, Piscataway, NJ 08854, USA
[3]The Rutgers Stem Cell Research Center, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA
Tel.: +1 732 445 0281;
Fax: +1 732 445 5312;
E-mail: kblee@rci.rutgers.edu

Although stem cells hold great potential for the treatment of many injuries and degenerative diseases, several obstacles must be overcome before their therapeutic application can be realized. These include the development of advanced techniques to understand and control functions of microenvironmental signals and novel methods to track and guide transplanted stem cells. The application of nanotechnology to stem cell biology would be able to address those challenges. This review details the current challenges in regenerative medicine, the current applications of nanoparticles in stem cell biology and further potential of nanotechnology approaches towards regenerative medicine, focusing mainly on magnetic nanoparticle- and quantum dot-based applications in stem cell research.

Keywords: magnetic nanoparticle, quantum dots, regenerative medicine, stem cell imaging

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Why nanotechnology for regenerative medicine?

The recent emergence of nanotechnology has set high expectations in biological science and medicine; many scientists now predict that nanotechnology can solve many key questions concerning biological systems that transpire at the nanoscale. Nanomedicine, defined broadly as the approach of science and engineering at the nanometer scale towards biomedical applications, has been drawing considerable attention in the area of nanotechnology [1]. Given that the sizes of functional elements in biology are in the nanometer scale range, it is not surprising that nanomaterials interact with biological systems at the molecular level [2]. In addition, nanomaterials have novel electronic, optical, magnetic and structural properties that cannot be obtained from either individual molecules or bulk materials. These unique features can be tuned precisely to explore biological phenomena through numerous innovative techniques. One of the major goals of biology is to address the spatial–temporal interactions of biomolecules at the cellular and integrated systems level [3]. However, to apply nanotechnology to biology and medicine, several conditions must be considered:

- Nanomaterials must be designed to interact with proteins and cells without interfering with their biological activities
- Nanomaterials must maintain their physical properties after surface modification
- Nanomaterials must be nontoxic

Cells are single living units of organisms that receive the input signals from disease and injury and then return the output signals to their microenvironments. Conventional experimental studies for specific cellular responses are typically conducted on large cell populations, which inevitably produce data measured from an inhomogeneous distribution of cellular responses. Unless cellular responses and processes are isolated from inhomogeneous signals at the single cell level, it would be extremely difficult to elucidate the intricate cellular systems and to analyze the complex dynamic signaling transductions. Furthermore, conventional biomedical approaches reveal very little concerning genotypic aspects that transcend into cell phenotypes. Thus, to better understand and control the responses of cells towards external stimuli at the single cell or single molecule level, it is imperative to characterize the full range of cell behaviors (e.g., self-renewal, differentiation, migration and apoptosis).

Recently, stem cells have gained much attention for the treatment of devastating injuries and damage caused by degenerative diseases, diabetes and aging [4]. Stem cells self-renew for long periods of time and then further differentiate into specialized cells and tissues on stimulation by appropriate microenvironmental cues. They are typically categorized as embryonic stem cells (ESCs) or tissue-specific adult stem cells, depending on their origin and differentiation capability. ESCs, which originate from the inner-cell mass of the blastocyst-stage embryo, are able to differentiate into all cell lineages found in the three primary germ layers of the embryo (e.g., endoderm, mesoderm and ectoderm) [5]. Although it has been shown that human ESCs (hESCs) can differentiate into many interesting cell types, such as cells of

heart, brain or bone [5], the therapeutic potential of hESCs has not been fully realized owing to numerous restrictions, including biological issues concerning immunogenicity and rejection and social issues concerning ethics and morality [6,7]. Adult stem/progenitor cells (e.g., mesenchymal [MSCs], hematopoietic and neural stem cells [NSCs]) reside in mature tissue compartments and are known to function as the replication resources for cell renewal during normal homeostasis of tissue regeneration. In contrast to ESCs, adult stem cells can only proliferate for a few passages and their differentiation ability is limited to certain cell types, depending on where they are located (e.g., bone marrow, brain or epithelial tissues) [8].

Intrinsic regulators (e.g., growth factors and signaling molecules) and cellular microenvironments, such as extracellular matrices (ECMs), are two prime factors that have critical roles in the regulation of stem cell behaviors. To harness the unique potential of stem cells, it is important to understand the functions of intrinsic regulators and extracellular microenvironments during stem cell fate [9]. Furthermore, to fully achieve the therapeutic promise of stem cells, several critical issues (**Box 1**) need to be addressed.

Nanostructures and nanomaterials can interact intrinsically with biological systems at the single molecular level with high specificity. The unique properties of nanomaterials and nanostructures can be particularly useful in controlling intrinsic stem cell signals and in dissecting the mechanisms underlying embryonic and adult stem cell behavior (**Figure 1**).

Herein, we have summarized nanotechnology approaches for stem cell research and have further addressed some of the challenges concerning these research efforts. Owing to the extensive scope of the topic and space limitations, we have focused primarily on cellular imaging from the numerous applications of nanotechnology in stem cell biology.

Box 1. Critical issues for the therapeutic applications of stem cells.

- The long-term behavior of transplanted stem cells in the target tissues
- The pluripotency/multipotency of stem cells to differentiate towards homogeneous populations of specific cell types
- The control of transplanted stem cells to migrate to the correct microenvironmental places
- The tracking of transplanted stem cells by labeling techniques
- The optimal time period for stem cell-replacement therapy for degenerative diseases

Nanomaterials for molecular & cellular imaging

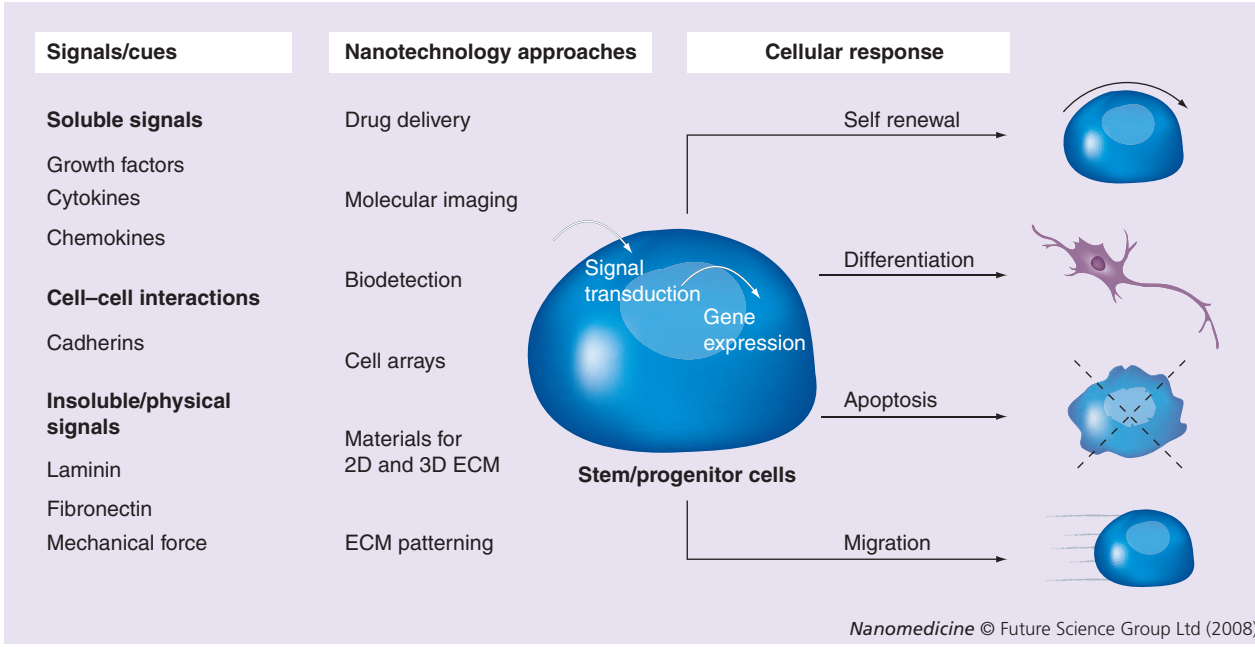
Although nanoparticles can be synthesized from various materials using several methods, the coupling and functionalization of nanoparticles with biomolecules should be carried out in controlled conditions, such as a specific salt concentration or pH. For this purpose, interdisciplinary knowledge from molecular biology, bioorganic chemistry, bioinorganic chemistry and surface chemistry must be used to functionalize nanoparticles with biomolecules. With significant advancements in synthetic and modification methodologies, nanomaterials can be modified to desired sizes, shapes, compositions and properties [10,11]; they can then be functionalized readily with biomolecules through combined methodologies from bioorganic, bioinorganic and surface chemistry.

Magnetic nanomaterials: iron oxide nanoparticles

Inorganic nanoparticles, especially iron oxide nanoparticles and quantum dots (QDs), are one of the most promising materials for stem cell research because they can be synthesized easily in large quantities from various materials using relatively simple methods. The dimensions of the nanoparticles can be tuned from one to a few hundred nanometers with a monodispersed size distribution. Moreover, they can comprise different metals, metal oxides and semiconducting materials, whose compositions and sizes are variable.

Iron oxide nanoparticles can either bind to the external cell membrane or can be internalized into the cytoplasm. Particles that are bound externally do not affect cell viability, although, they may interfere with cell-surface interactions or may simply detach from the cell membrane [12]. However, iron oxide nanoparticles that can be internalized within cells have their surfaces modified to ensure high uptake efficiency with minimum deleterious effects on the cells [13]. For example, coating the surface of superparamagnetic iron oxide nanoparticles (SPIONs) with dextran or other polymers enhances stability and solubility [14] and also prevents aggregation [15]. The coated SPIONs are useful for tracking and studying stem/progenitor cells with MRI. In this regard, magnetic iron oxide nanoparticles and their composites are emerging as novel contrast agents for MRI and are much more sensitive than conventional gadolinium-based contrast

Figure 1. Regulation of stem cell fate by microenvironmental signals and the corresponding applications of nanotechnology.



ECM: Extracellular matrix.

agents [16]. The use of SPIONs as *in vivo* cellular-imaging agents is increasing rapidly. Since their unique properties enable precise control of size and composition, magnetic nanoparticles offer great potential for highly specific MRI to track stem/progenitor cells. The major transfer mechanism of nanoparticles through the cell membrane, to label stem cells, is endocytosis or, more specifically, pinocytosis [17–19].

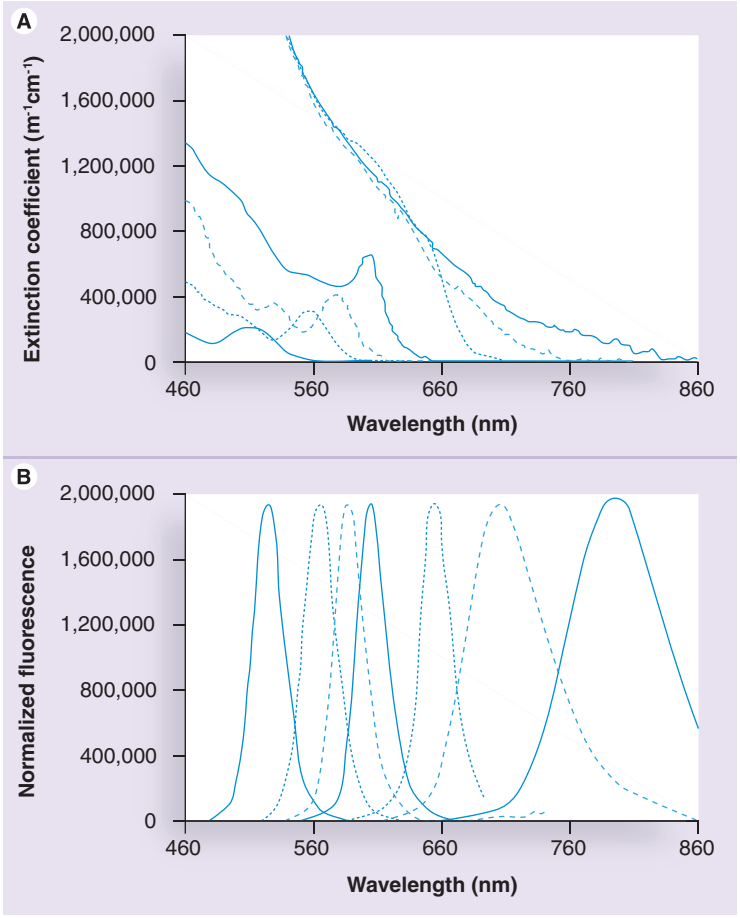
Dextran-coated SPIONs, which are commonly used to label stem cells, may be unfavorable to endocytosis, thus reducing their labeling efficiency. Therefore, the stem cells would require higher concentrations of nanoparticles and additional transfection agents. In addition, several studies have found that iron oxide nanoparticles, which are dissolved within the cells, may increase the formation of free hydroxyl radicals and reactive oxygen species. These may have toxic effects, such as an increase in the rate of apoptosis or cell death and alterations in cellular metabolism [20]. Moreover, dissolved Fe^{2+} ions from the dissolved iron oxide nanoparticles may have potential toxic effects on the cells. To protect stem cells from the toxic effects of SPIONs and to track the behavior of stem cells successfully *in vivo*, the SPIONs can be coated with gold. Coating the SPIONs with gold provides an inert shell around the nanoparticles and protects them from rapid dissolution within the cytoplasmic endosomes [21]. In addition, the gold-coated shell enhances MRI contrast significantly. More importantly, gold has well-defined surface chemistry with thiol or amine moieties. This offers an attractive and convenient route for further functionalization of the SPIONs with biomolecules through thiol- or amine-coupling chemistry [22]. One of the advantages of using SPIONs to label stem cells is that the migration of stem cells after implantation can be detected noninvasively using MRI. The stem cells can be further retrieved from excised tissues, such as spleen and bone marrow, by using magnetic-sorting techniques [23].

Semiconductor nanomaterials: QDs

In addition to magnetic nanoparticles, QDs are being used extensively for applications in cell biology, such as cell labeling, cell tracking and *in vivo* imaging, owing to their potential in imaging and detection applications. QDs are robust fluorescent semiconducting nanocrystals with broad absorption spectra and narrow emission spectra (**Figure 2**) [24].

QDs overcome the limitations of conventional imaging methods, such as fluorescence microscopy and differential interference contrast microscopy. Conventional methods are limited by a lack of quantitative data, high

Figure 2. Excitation and emission spectra of QDs.



(A) Broad excitation spectra of QDs. **(B)** Narrow emission spectra of QDs.
QD: Quantum dot.
Reprinted with permission from BMC [24].

background noise from labeled biomolecules and a requirement for long observation times owing to photobleaching, which gradually leads to a loss of signals [25]. However, QDs exhibit extreme brightness and resistance to photobleaching, which permits the use of lasers with low intensities over long periods of time, thus making them extremely useful for live-cell imaging. In addition, QDs offer many advantages, such as high fluorescent intensity from high quantum yields and high molar extinction coefficients, resistance to chemical degradation and long fluorescence lifetimes (>10 ns). Multiple QDs with different emission wavelengths can be used in parallel for multiplex imaging [26–29]. The interesting optical properties of QDs originate from the interactions between electrons, holes and their local environments, which can be controlled precisely to generate desired emission and absorption spectra. Because absorption and emission spectra exhibit sensitive changes depending on particle size, a wide range of emission spectra from ultraviolet to infrared can be obtained. Therefore, unique emission spectra, due to synthesizing particles with different diameters, have been acquired [30–32]. Generally, QDs have a core composed of heavy metals, such as CdSe or CdTe, with a surrounding ZnS shell. The thickness of the shell can be tuned depending on the reaction time. Typically, the core/shell QDs with sizes ranging from 2 to 8 nm in diameter are synthesized by changing reaction conditions, such as temperature, duration and ligands. The unique photophysical properties of QDs stem from their nanometer-scale size; by changing sizes and compositions, their optical properties can be controlled precisely for many applications. For example, QDs can be used effectively in multiplexing experiments in which multiple biological units can be labeled simultaneously. Moreover, owing to their resistance to photobleaching, QDs have enabled scientists to study live cells and complex mechanisms of biological processes in a real-time manner [33,34].

Nanoparticle-based applications for regenerative medicine

Magnetic nanoparticles for in vivo *stem cell tracking*

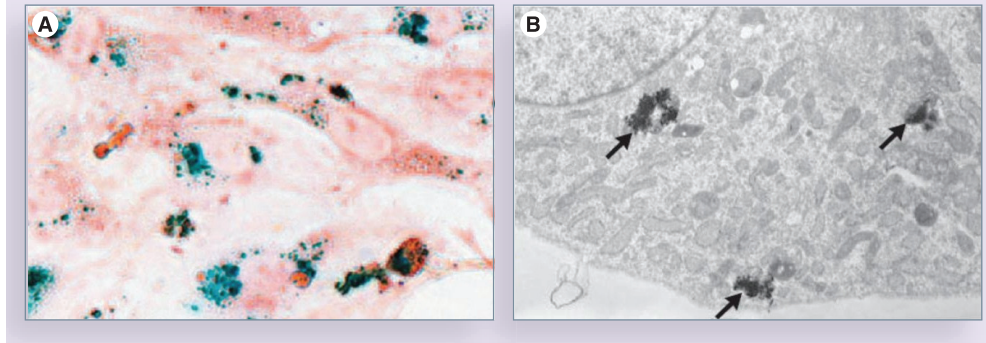
Transplanting various progenitor cells and stem cells for tissue regeneration is an extremely promising therapeutic strategy. One of the key factors in this approach is the availability of techniques that would enable long-term, noninvasive detection of transplanted stem/progenitor cells and, at the same time, enable monitoring of their differentiation, survival and proliferation within the desired organs. Several techniques, such as MRI, bioluminescence, positron emission tomography and multiple photon microscopy, are now available for *in vivo* cellular imaging; of these, MRI offers several advantages, such as high resolution, speed, easy accessibility and 3D capabilities [35,36]. In addition to providing information regarding the transplanted stem cells, a significant advantage of MRI is that it provides information regarding the surrounding tissues (e.g., edema, lesion or inflammation), which may have an effect on the fate of grafted stem cells or may hinder the recovery of damaged tissues [37]. Magnetic iron oxide nanoparticles, whose sizes can be tuned precisely, offer great potential for MRI applications. The

magnetic nanocrystals tend to behave as a single magnetic domain in which all nuclear spins couple to create a single large magnetic domain. At certain temperatures and crystal sizes, these moments wander randomly (superparamagnetic) or become locked in one direction, making the material ferromagnetic [38]. Magnetic nanocrystals of differing compositions and sizes can be synthesized to generate ultrasensitive molecular images, as shown in **Figure 3**.

Dextran and other polymer-coated SPIONs are currently used in a number of biomedical applications; for example, Endorem® (Geurbet, France) is a commercially available contrast agent based on SPIONs surface coated with dextran [39]. It is a suitable contrast agent for labeling human MSCs (hMSCs) and human ESCs (hESCs) as it does not need a transfection agent (which may damage the stem cells) to facilitate its cellular uptake. Feridex® and Sinerem® are other commercially available dextran-coated SPIONs that are combined with commercially available transfection agents, such as Fungene™, Superfect™ or Lipofectamine [39,40]. The use of transfection agents at higher concentrations may increase toxicity and, at lower concentrations, may not lead to sufficient cellular uptake [40]. Thus, the amount of transfection agent needed to enhance internalization is optimized carefully before combining it with SPIONs. The amount also depends on the stem cell type to be labeled.

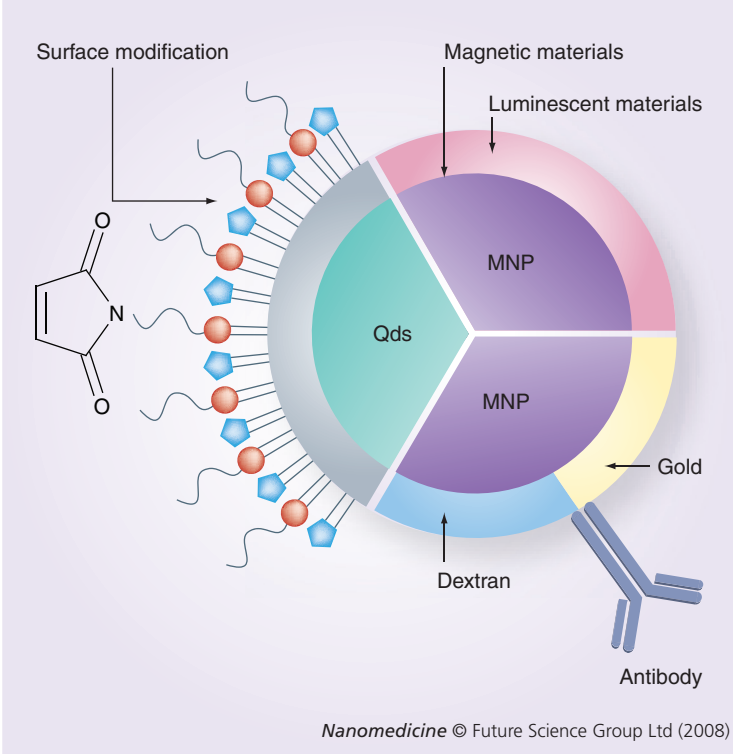
Stem/progenitor cells can be labeled with SPIONs by modifying their surfaces with internalizing ligands, such as the HIV-Tat peptide, dendrimers and polycationic transfection agents. In addition to internalizing ligands, the SPIONs can be multifunctionalized using fluorescent and isotope labels. These multifunctional nanoparticles (**Figure 4**) can be used to combine methods, such as optical and nuclear imaging, with MRI to validate the cellular behavior *in vivo*. This was demonstrated aptly by Weissleder and coworkers [23]. The magnetic nanoparticles used by this group consisted of small (5 nm) monocrystalline superparamagnetic iron oxide cores that were stabilized by coating with cross-linked aminated dextran. The overall size of the nanoparticles further increased to 45 nm. To modify the nanoparticles with a fluorescent label, the internalizing ligands, fluorescein isothiocyanate-derivatized HIV-TAT peptides, were attached to the coat of aminated dextran. In addition, the SPIONs were further modified for concomitant nuclear imaging by reacting the dextran coating with a chelator, diethylenetriamine penta-acetic acid, so as to label the nanoparticles with ^{111}In isotope. The modified SPIONs, with a triple label (magnetic, fluorescent and isotope), internalized into hematopoietic stem and neural progenitor cells efficiently. The group further demonstrated that the labeled neural progenitor cells retained their capability for differentiation and the iron incorporation did not have any effect on viability and proliferation of hematopoietic ($CD34^+$) cells. In another study, dextran-coated magnetic iron oxide nanoparticles with a core diameter of 4.6 ± 1.2 nm and an overall size, after coating with dextran, of 8–20 nm were attached covalently to OX-26, an

Figure 3. Neural stem cells labeled with iron oxide nanoparticles.



(A) Photomicrograph showing iron oxide nanoparticles in stem cells stained with Prussian blue and counterstained with neutral red. **(B)** Transmission-electron photomicrograph of stem cells showing the presence of iron oxide nanoparticles in nuclear and cell membranes.

Figure 4. Multifunctional inorganic nanoparticles.



antitransferrin receptor monoclonal antibody [41]. The antibody-functionalized nanoparticles were used to label oligodendrocyte progenitor cells by targeting the transferrin receptors on the cells. The progenitor cells were made highly magnetic by incubating them with iron oxide nanoparticles. Because the oligodendrocyte progenitor cells have been shown previously to myelinate large areas in the CNS significantly [42], they were transplanted into the spinal cord of myelin-deficient rats. After neurotransplantation, these cells could be tracked easily using MRI and the extent of myelination could be determined. The progenitor cells retain their capacity for myelination and migration fully *in vivo* [41].

Another important example of transfecting agents are dendrimers. Dendrimer composites of iron oxide nanoparticles, also known as magnetodendrimers, represent a versatile new class of contrast agent for MRI. They were developed by Frank and coworkers and label mammalian cells efficiently, including NSCs and MSCs [36]. They have an oligocrystalline structure of 7–8 nm. Labeling NSCs and MSCs with magnetodendrimers did not affect their growth rate and they exhibited a growth rate that was similar to that of unlabeled stem cells. The cellular uptake of these composites is through a nonspecific adsorption process, thus offering a great opportunity to label a variety of stem cells without regard to their origin or animal species. In a recent study, Kehr and coworkers labeled NSCs with gold-coated monocrystalline SPIONs [21]. The NSCs were infused into the spinal cord of rats and tracked by means of MRI for over a month. The MRI signals persisted 1 month postsurgery and the gold surface protected the nanoparticles from being digested by the glial macrophages. It was concluded that gold-coated SPIONs may represent a class of superior MRI labels for long-term *in vivo* tracking of stem cells. In another recent study, Zhu and coworkers labeled human NSCs with SPIONs using a nonliposomal lipid-based transfecting agent [43]. The labeled cells were then implanted in the region of brain damage in a patient suffering from brain trauma. The migration of NSCs from the site of injection to the border of the injured tissue of the brain was detected successfully [43]. One of the challenges of using SPIONs is the potential transfer of the contrast from the labeled stem cells to other cell types, such as macrophages, which metabolize iron after engulfing the stem cells. However, through detailed studies and experimentation, Zhu and coworkers excluded the possibility that magnetic signals could have been generated by macrophages engulfing the NSCs and thus concluded that the signals were indeed generated by the migrating stem cells and not by the engulfed stem cells.

Tracking of stem cell migration is not limited to NSCs/progenitor cells. It is also possible to study the migration of stem cells labeled with SPIONs in other systems, such as the cardiovascular system. Regenerative medicine for cardiac diseases will have enormous therapeutic potential in the future for situations involving ischemic cardiac injury, which involves irreversible cardiac damage. Bulte and coworkersdemonstrated the potential of MRI in tracking magnetically labeled MSCs in a swine model of myocardial infarction [44] . The MSCs were labeled with dextran-coated SPIONs (Feridex®) to noninvasively track the quantity and location of the MSCs after myocardial infarction. The MRI tracking of the MSCs labeled with Feridex® was feasible and represents a preferred method for studying engraftment of MSCs in myocardial infarction.

QD imaging for stem cells

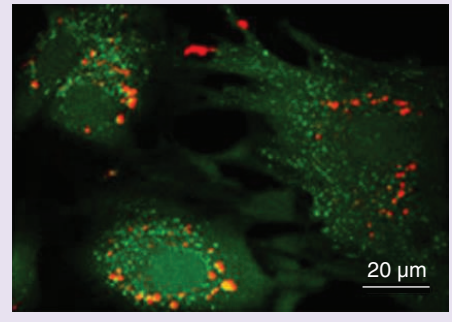
Quantum dots or semiconductor nanocrystals have opened doors to an array of diverse applications in biological sciences, such as live monitoring of physiological events taking place in cells by labeling specific cellular structures or proteins with QDs having different colors, monitoring cell migration, tracing cell lineage and *in vivo* cell tracking [45–48]. Their unique photophysical properties coupled with their diverse biological applications make QDs attractive nanoprobes for investigating stem cell behavior (**Figure 5**). Furthermore, QDs are advantageous for studying dynamic changes occurring in the membranes of stem/progenitor cells. Functionalized QDs bind selectively to individual molecules on the cell surface and help in tracking the motion of those individual molecules. In a study by Cho and coworkers [49], functionalized QDs were used to demonstrate changes in integrin dynamics during osteogenic differentiation of human bone marrow-derived progenitor cells. In this study, QDs conjugated with integrin antibodies enabled precise optical identification of integrin molecules, which led to a detailed examination of the molecular dynamics of integrin molecules involved in osteogenic differentiation of the progenitor cells [49]. In stem cell-based therapy, it is extremely important to monitor the survival and location of stem cells after they are transplanted to the desired location. Transplanted stem cells, which may be either embryonic or adult stem cells, are expected to remodel and differentiate in response to surrounding microenvironments, resulting in tissue regeneration and repair [43]. MSCs labeled with bright, photostable QDs couple functionally with cardiomyocytes in coculture, thus demonstrating the usefulness of QDs as labeling agents in culture (**Figure 6**) [50].

hMSCs were labeled with QDs bioconjugated with arginine–glycine–aspartic acid peptide during self-replication and multilineage differentiations into chondrogenic, androgenic and adipogenic cells in a long-term labeling study. Human MSCs labeled with QDs remained as viable as the unlabeled hMSCs from the same subpopulation, thus suggesting that QDs are useful probes from long-term labeling of stem cells [51].

QDs also elucidate the mechanisms involved in mechanical integration of stem cells to the surrounding tissues and their differentiation into specific cell lineages *in vivo* [45]. In addition, multiplex imaging (i.e., tracking different cell populations labeled with QDs that exhibit different emission wavelengths at the same time) is one of the biggest advantages of using QDs for tracking stem cells *in vivo*. Wu and coworkers successfully demonstrated *in vivo* multiplex imaging of mouse ESCs labeled with QDs [24]. They injected ESCs labeled with six different QDs subcutaneously, having diverse emission wavelengths of 525, 565, 605, 655, 705 and 800 nm, into various locations on the back of athymic nude mice and detected the labeled cells *in vivo* using a single excitation wavelength (425 nm), as shown in **Figure 7**. They also concluded that, within the sensitivities of the screening assays, the QDs did not affect viability, proliferation or differentiation capacity of the ESCs [24].

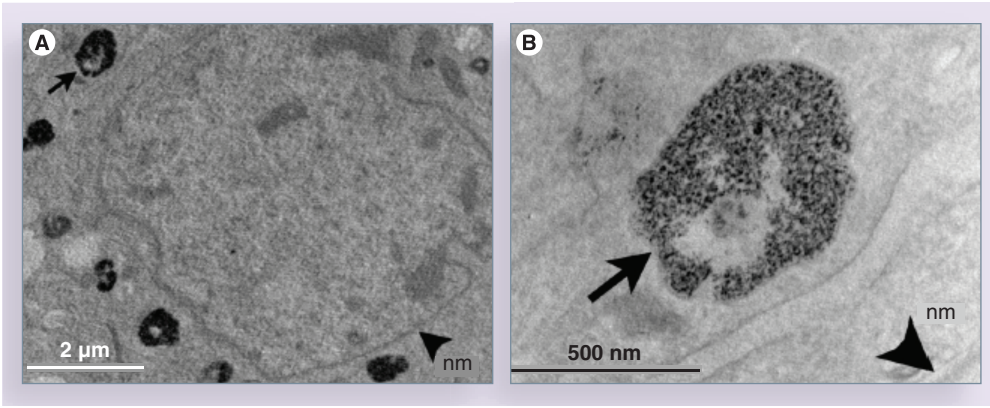
QDs can be used efficiently to label neural stem and progenitor cells (NSPCs) *in vivo* and can be used to study the migration and differentiation of NSPCs during mammalian development. However, direct QD labeling of NSPCs is a considerable challenge and not many techniques to label QDs directly and efficiently exist. Haydar and coworkers developed novel *in utero* electroporation and ultrasound-guided delivery techniques to label the NSPCs directly *in vivo* [46]. NSPCs labeled with QDs using the techniques described previously, were found to differentiate into three principle cell types: oligodendrocyte progenitors, astrocytes and neurons. QDs were found in all three types of cells after differentiation. The cells were also found to migrate away from the site of injection,

Figure 5. Confocal fluorescent image of MSCs labeled with QDs and colabeled with calcein.



The QDs are distributed in the perinuclear region within the stem cells. As the MSCs proliferate, the QDs remain bright and are easy to detect. MSC: Mesenchymal stem cell; QD: Quantum dot. Reprinted with permission from BMC [51].

Figure 6. TEM images of QD-labeled MSCs.



(A) Low magnification of MSC showing QD aggregates (arrow) in endosomal vesicles surrounding the nuclear membrane. **(B)** High magnification of single-enlarged vesicle showing presence of individual QDs (arrow).
MSC: Mesenchymal stem cell; QD: Quantum dot; TEM: Transmission-electron microscopy.
Reprinted with permission from BMC [51].

suggesting that neither the QDs nor the *in vivo* labeling techniques had any effect on migration and differentiation of the NSPCs. Furthermore, their method demonstrated a lack of toxicity and good tolerance of NSPCs for QDs, particularly during early embryonic mammalian development [46].

Despite having unique optical properties and a host of advantages over the conventional tracking agents, toxicity is a primary concern for the application of QDs in biology. Stem/progenitor cells tend to be extremely sensitive and thus toxicity is a primary determinant in deciding whether QDs would be feasible for stem cell tracking, especially *in vivo*. Some literature studies do suggest that QDs are nontoxic; nevertheless, recent data show that cytotoxicity is dependent on the physico–chemical properties, dose and exposure concentrations [52]. Although the mechanism of cytotoxicity is not yet clearly known and is under thorough investigation, concerns regarding the toxicity of QDs have been raised because they are used for cell-tracking studies in live animals. QDs contain heavy metals, such as cadmium and selenium, and the cytotoxicity is observed owing to the presence of Cd^{2+} and Se^{2-} ions [53,54]. Toxicity can be considerably reduced by coating the core made of CdSe with a shell of a material, such as ZnS, which reduces toxicity significantly by blocking the oxidation of CdSe by air [55]. Although the toxicity may not be critical at the low concentrations optimized for labeling, it could be detrimental for embryo development at higher concentrations. Nevertheless, the problem could be solved by coating the QDs and making them biologically inert [55]. Larger molecules, such as proteins (e.g., streptavidin and bovine serum albumin), further slow the photo-oxidation of the core [56]. Bioconjugation of QDs with biomolecules, such as arginine–glycine–aspartic acid, did not show any toxic effect on hMSCs as compared with unlabeled hMSCs [51]. In a dose-dependent study involving the labeling of MSCs with QDs, it was observed that if the exposure of QDs to MSCs was optimized and limited to low concentrations, then the QDs were not significantly toxic [50].

Other challenges & opportunities

Integration of nanotechnology and stem cell research should provide new opportunities for scientists to address fundamental questions of stem cell biology at the single-molecule level. Although many nanoparticle-based applications currently garner much discussion, other important applications of nanotechnology, regarding the regulation of stem cell fate, are also being developed. These applications include microenvironmental engineering and gene manipulation.

Surface engineering stem cell microenvironment

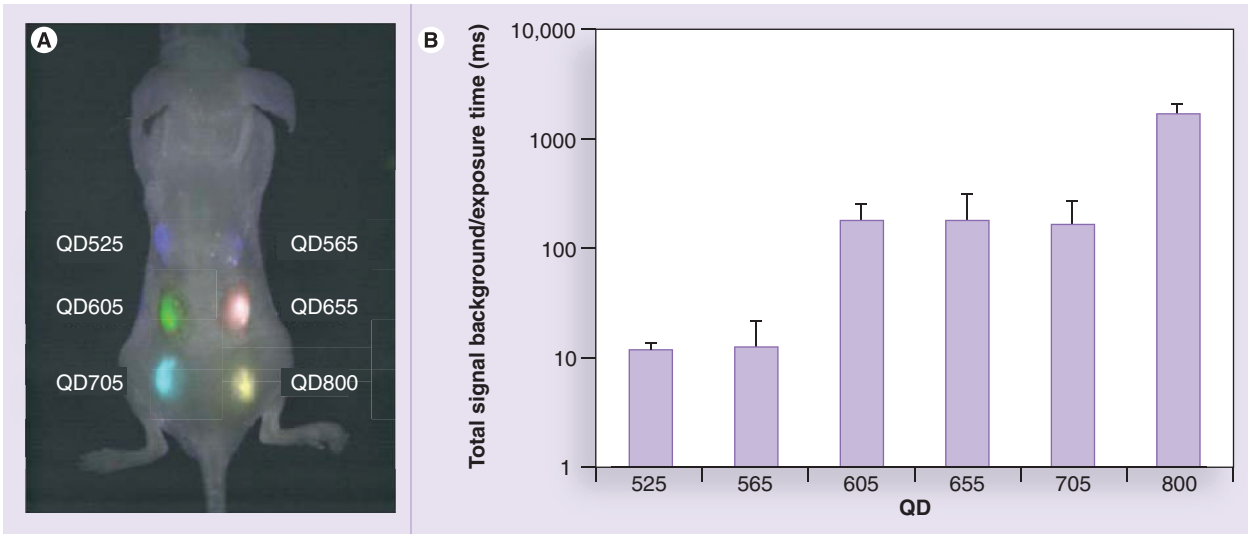
Stem cells normally reside within specific extracellular microenvironments that are typically referred to as stem cell niches [57,58], which are comprised of a complex mixture of soluble and insoluble ECM and signal molecules. It is well known that morphogenetic signaling molecules and ECM components can control stem cell

behaviors. For example, a variety of factors that regulate stem cell development have been explored in the context of stem cell fate regulation [58,59]. These factors include cadherins, laminins and morphometric protein families. Even though several combinatorial high-throughput screening methods probing the effect of soluble signal molecules on stem cell differentiation have been reported, similar approaches for screening the effect of insoluble cues are limited owing to technical difficulties. Only a few types of ECM arrays fabricated by conventional spot-array techniques have been used to probe stem cell differentiation and migration behaviors [60]. There is plenty of room for improvement in this approach in terms of pattern density, recognition sensitivity and small sample-volume requirement. Regarding this challenge, several soft micro/nanolithographic tools, such as microcontact printing and dip-pen nanolithography, are potentially useful. Both methods have been used successfully to generate ECM patterns on different surfaces at the micro- or nanoscale. With microcontact printing [61] and dip-pen nanolithography [62,63], single stem cells and their behavior on combinatorial ECM nanoarrays can be studied. Key scientific issues, such as the effects of ECM composition and temporal–spatial effects of ECM materials on stem cell differentiation, can be further investigated. It is critical to address these issues to enhance the feasibility of using stem cells for therapeutic purposes.

Gene manipulation of stem cells using nanomaterials

Gene delivery has an important role in recognizing the potential of regenerative medicine. To manipulate the expression level of key genes in stem cells, several biomolecules, such as gene vectors, siRNA, proteins and small molecules, have been developed. Because several transcription factors that regulate stem cell differentiation into specific cell types have been demonstrated, gene delivery could be an immensely powerful tool for specific differentiation of stem cells. The development of safe and efficient gene delivery systems, which can lead to high levels of gene expression within stem cells, is an urgent requirement for the effective implementation of regenerative medicine. Recently, Akaike and coworkers developed a biofunctionalized inorganic, apatite nanoparticle-based gene delivery system, which showed high affinity for mouse embryonic stem cell surface and led to accelerated trans-gene delivery [64]. Apatite nanoparticles by themselves are inefficient in transfecting the ESCs; however, when functionalized with biomolecules, such as fibronectin and E-cadherin chimera, the hybrid nanoparticle system shows enhanced trans-gene delivery, which is notably higher than that of a commercially available lipofection system [64]. Another nanomaterial-based novel approach to gene delivery was demonstrated by Miyake and coworkers [65].

Figure 7. Multiplex imaging by QDs.



(A) ESCs labeled with QD 525, 565, 605, 655, 705 and 800 injected subcutaneously in the back of athymic nude mice immediately after labeling. The image was taken with a single excitation wavelength straight after injection. **(B)** Quantified fluorescent signal intensities of QDs.
ESC: Embryonic stem cell; QD: Quantum dot.
Reprinted with permission from BMC [24].

In this approach, gold nanoparticles, 20 nm in diameter and conjugated with a DNA–polyethylenimine complex, were patterned on a solid surface (glass) and used as nanoscaffolds for the delivery of DNA into hMSCs through reverse transfection. The authors claimed that this method of delivering genes efficiently from a solid surface to stem cells might be useful in the development of tools for gene therapy in regenerative medicine [65].

Conclusions

In this review, we have summarized nanoparticle-based approaches for stem cell imaging. Considering that nanomaterials intrinsically enable cellular and molecular imaging with high sensitivity and high spatial resolution, it is not surprising that a growing number of imaging techniques based on nanoprobes are beginning to have an impact on stem cell-based therapies and research. Good examples of these include stem cell tracking using magnetic nanoparticles and QD-based imaging of stem cell interactions with other microenvironmental cues. In addition, other nanotechnology applications, such as surface engineering and drug delivery, have huge potential to further address challenges in the area of regenerative medicine. Collectively, advancements in nanotechnology enable the modulation of stem cell signaling pathways and improvement in their therapeutic applications.

Future perspectives

The application of nanotechnology in regenerative medicine has already begun to revolutionize several areas of stem cell research and will continue having great impacts on regenerative medicine. However, recognizing the optimum potential of nanotechnology in regenerative medicine requires several considerations. First, appropriate imaging methods should be designed carefully by considering the specific biological questions regarding stem cells that need to be addressed because each imaging technique has its unique set of advantages and disadvantages. Second, nanomaterials might have undesirable side effects on stem cells owing to their composition, size and physical properties. For instance, the cytotoxicity of QDs is a potential limitation for the application of molecular probes for both cellular and clinical use *in vitro* and *in vivo*. Finally, nanotechnology approaches for regenerative medicine essentially necessitate synergetic effort and interdisciplinary expertise from biology, chemistry and engineering. In particular, this approach would be beneficial to elucidate the complex cellular spatial–temporal dynamics and signaling pathways in more effective ways. Addressing the challenges ahead would accelerate the development of nanotechnology approaches toward regenerative medicine and facilitate the therapeutic application of stem cells.

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Executive summary

- To apply nanotechnology to stem cell biology, several conditions must be considered: nanomaterials must be designed to interact with proteins and cells without perturbing their biological activities; nanomaterials must maintain their physical properties after the surface conjugation chemistry; and nanomaterials must be biocompatible and nontoxic.
- Magnetic iron oxide nanoparticles, the size of which can be tuned precisely, are used to label stem cells and offer great potential for tracking them *in vivo* using MRI to generate ultrasensitive images.
- Quantum dots, with their unique photophysical properties and resistance to photobleaching, can be used for multiplex imaging of stem cells *in vitro* and *in vivo*.
- Combinational exracellular matrix micro/nanoarrays, generated by soft-lithography, have great potential in studying and controlling the behavior of single stem cells.
- Nanomaterial-based gene delivery for manipulating stem cells has a vital role in recognizing the potential of regenerative medicine.

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