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Nanoparticles as Tools to Study and Control Stem Cells

L. Ferreira[1,2*]

[1]Center of Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal
[2]Biocant-Center of Biotechnology Innovation Center, Cantanhede, Portugal

ABSTRACT

The use of nanoparticles in stem cell research is relatively recent, although very significant in the last 5 years with the publication of about 400 papers. The recent advances in the preparation of some nanomaterials, growing awareness of material science and tissue engineering researchers regarding the potential of stem cells for regenerative medicine, and advances in stem cell biology have contributed towards the boost of this research field in the last few years. Most of the research has been focused in the development of new nanoparticles for stem cell imaging; however, these nanoparticles have several potential applications such as intracellular drug carriers to control stem cell differentiation and biosensors to monitor in real time the intracellular levels of relevant biomolecules/enzymes. This review examines recent advances in the use of nanoparticles for stem cell tracking, differentiation and biosensing. We further discuss their utility and the potential concerns regarding their cytotoxicity. J. Cell. Biochem. 108: 746–752, 2009.

KEY WORDS: STEM CELLS; NANOTECHNOLOGIES; CARBON NANOTUBES; QUANTUM DOTS; NANOPARTICLES; REGENERATIVE MEDICINE

Nanoparticles are organic or inorganic materials on the scale of approximately 1–1,000 nm. Representative examples of nanoparticles for stem cell research includes, organic and inorganic nanoparticles [Ferreira et al., 2008b; Kutsuzawa et al., 2008], liposomes [Ahrens et al., 2005], polyplexes [Green et al., 2008], quantum dots [Chakraborty et al., 2007; Rosen et al., 2007], and carbon nanotubes [Zhu et al., 2007; Mooney et al., 2008]. Some potential application of these nanoparticles in stem cell research includes: (a) non-invasive tracking of stem cells and progenitor cells transplanted in vivo; (b) intracellular delivery of DNA, RNAi, proteins, peptides and small drugs for stem cell differentiation or survival, (c) biosensing of the physiological state of stem cells.

In general, all these applications require the cellular uptake of nanoparticles (Fig. 1). Nanoparticles with a diameter ranging from a few nanometers (2–10 nm, e.g., quantum dots) up to 1 μm can be taken up by stem cells or their progenies [Ferreira et al., 2008a]. The surface chemistry of the nanoparticles is an important factor to control cellular uptake [Green et al., 2008]. In most cases the nanoparticle charge provides the driving force for the uptake (through electrostatic interaction with the negatively charged cell membrane) and defines the internalization process. The nanoparticle charge also contributes for the adsorption of specific proteins in the cell medium that might enhance or reduce their cellular uptake. Despite the information gathered in the last years, the internalization process and the final intracellular location of the nanoparticles within the stem cells is poorly understood. Scarce information has been collected about the effect of nanoparticle size, shape, charge, and surface chemistry in the internalization process by stem cells and this is an aspect to explore in the near future.

The internalization of these nanomaterials can be followed by one of the following pathways: (i) receptor-mediated endocytosis [Lewin et al., 2000], (ii) non-specific endocytosis [Lu et al., 2007; Ferreira et al., 2008b], and (iii) internalization under endocytosis-inhibiting conditions [Kostarelos et al., 2007] (Fig. 1). Nanoparticles internalized in endosomes are then trafficked to acidic and oxidative environments of lysosomes and peroxisomes. At this stage they have three possible fates: (i) degradation by enzymes or acidic pH, (ii) exocytosis, (iii) escape from the endo-lysosome compartment and travel to other intracellular locations including cell nuclei.

It remains to be shown the mechanisms under the internalization of nanoparticles on stem cells and the differences relatively to the ones found on differentiated cells [Gratton et al., 2008]. It is known that integrin expression and pattern as well as lateral mobility on the cell surface is correlated with cell differentiation [Chen et al., 2007]. Cell cytoskeleton organization and elasticity, cell shape, and adhesion strength change with the differentiation program of stem cells. Another important issue to be evaluated is the effect of the internalized nanoparticles in the biology of stem cells. In several

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*Correspondence to: L. Ferreira, Biocant-Center of Innovation and Biotechnology, Nucleo 4, Lote 3, 3060-197 Cantanhede, Portugal. E-mail: lino@biocant.pt

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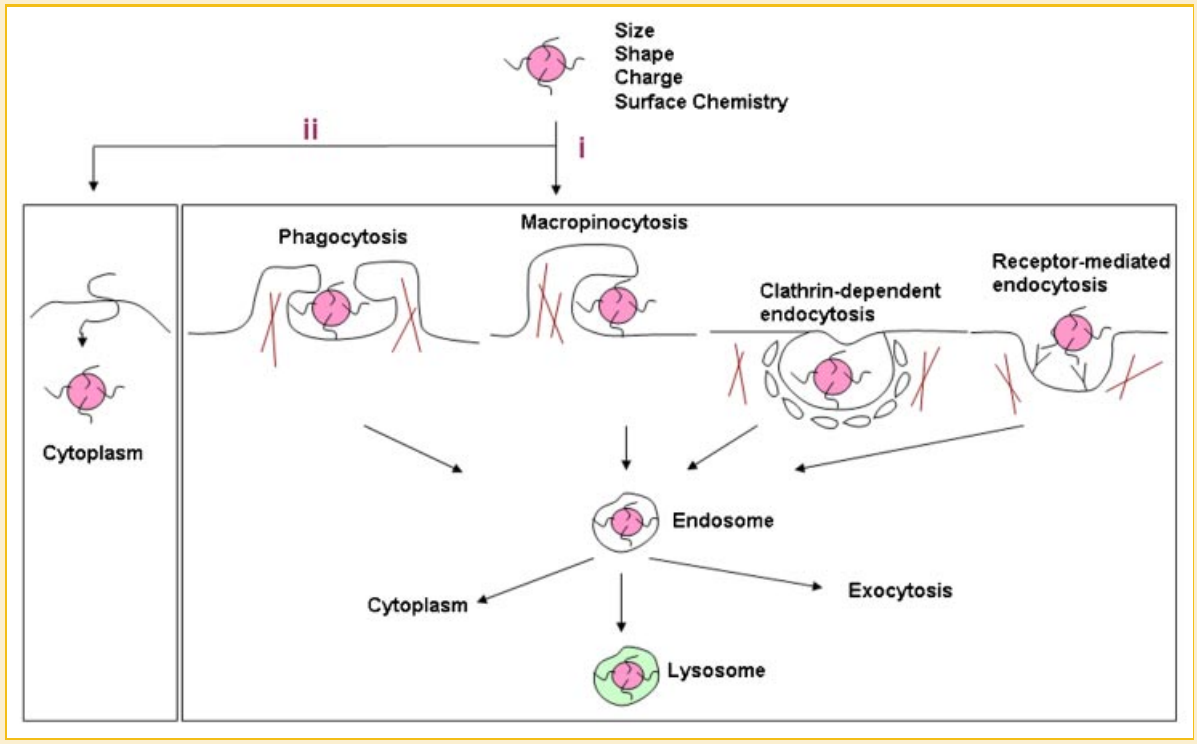

Fig. 1. Cellular internalization of nanoparticles. The nanomaterials can be internalized by endocytosis (i) or endocytosis-inhibiting conditions (ii). The endocytic pathway can be initiated by phagocytosis, macropinocytosis, clathrin-dependent endocytosis, and receptor-mediated endocytosis. The internalization of the nanoparticles might be affected by the nanoparticle properties (size, shape, charge, and surface chemistry), and type of stem (embryonic, fetal, or adult) or progenitor cells.

cases, nanoparticles are taken up by stem cells and accumulate in endosomes. Recent work in a variety of cellular models supports the thesis that endocytic organelles can play a direct role in signal propagation and amplification [Miaczynska et al., 2004]. Thus, it will be important to analyze the effect of nanoparticles in the endocytic signaling pathways of stem cells.

NANOMATERIALS FOR STEM CELL LABELLING AND TRACKING IN VIVO

Currently more than 2,000 clinical trials are being performed worldwide involving stem cells for the potential treatment of blood disorders, myocardial infarction, stroke, graft versus host disease, bone, and cartilage regeneration. Stem cells are generally tracked invasively by immunohistochemistry after the removal of tissues or organs from small animals. However, for pre-clinical and clinical trials, it will be important to track stem cells noninvasively in order to evaluate their therapeutic effect and grafting location to rule out potentially dangerous side effects. Magnetic resonance imaging (MRI) provides a noninvasive method for studying the fate of transplanted cells labeled with superparamagnetic iron oxide (SPIO) nanoparticles. Some nanoparticle formulations (e.g., Feridex/Endorem and Ferucarbotran) have been approved for human use by the U.S. Food and Drug Administration (FDA) as MRI contrast agents. Recently, a clinical study using stem cells labeled with nanoparticles in patients with neurological disease has been reported [Zhu et al., 2006]. Typically, the nanoparticles are taken up through endocytosis during in vitro cell cultivation and accumulate in the endosomes. In most cases, the internalization of nanoparticles requires the use of excipients, which include peptides and cationic agents [Ferreira et al., 2008a] (Fig. 2).

Nanoparticle-labeled stem cells/progenitor cells might contribute to our understanding of the cell migration processes in numerous diseases, such as neurologic diseases, myocardial infarction and cancer. In addition, this platform might give us important information and cues about the differentiation program of stem cells injected in small animals. For example, it has been demonstrated that stem/progenitor cells labeled with magnetic nanoparticles when injected in the blood stream of small animals can later be isolated by magnetic separation after in vivo migration to study the differentiation of the cells exposed to a biological environment [Lewin et al., 2000].

In some cases, it is difficult to distinguish SPIO-labeled cells from other hypointense regions on MRI images. Such signals can arise from regions containing blood hemoglobin, or blood clots/trombi [Gilad et al., 2008]. The development of new nanoparticle formulations based on probes other than iron oxide will be of great interest for stem cell applications. Some examples have been recently reported based on nanoparticles containing fluorine or manganese [Gilad et al., 2008; Ruiz-Cabello et al., 2008].

From a stem cell biology point of view it will be important to monitor the long-term effects of these nanomaterials, at the stem cell differentiation program level. It remains to be elucidated

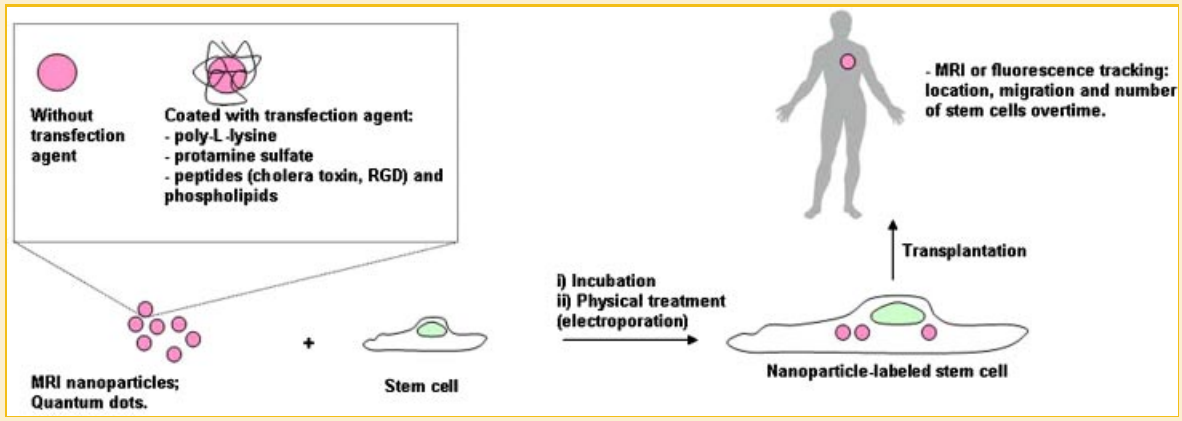

Fig. 2. Stem cell labeling with nanoparticles for MRI and fluorescence tracking. Nanoparticles must be biocompatible with a minimal release by the stem cells, they should not interfere with regulatory and differentiation programs of the stem cells and the number of nanoparticles must be enough for detection by MRI or fluorescence imaging. Depending on the type of stem cells and the characteristics of the nanoparticles, the cellular internalization of nanoparticles might require the use of transfection agents such as poly-L-lysine, protamine sulfate, peptides or phospholipids. Low voltages are also used to induce cellular internalization of the nanoparticles. The nanoparticles are internalized by mechanisms presented in Figure 1. Contrast agent-nanoparticle or qdot-labeled cells can be tracked in vivo by MRI and fluorescent imaging techniques, respectively.

whether the nanoparticles or its degradation products can activate endocytic signaling cascades and change the differentiation program of the stem cells. Although nanoparticles offer an excellent opportunity to track stem cells, their dilution by cell division and release by exocytosis might be a limitation of this approach. Therefore, the MRI results should be validated by a complementary technique such as fluorescence imaging. In addition, it will be important to identify strategies to retain the nanoparticles with the cells. The anchorage of the nanoparticles on the outer stem cell surface either by covalent or non-covalent linkages might be an interesting strategy to solve such issue as it was demonstrated for somatic cells [Gianolio et al., 2008].

Quantum dots (qdots), typically in the size range of 2–10 nm, are another class of nanomaterials for the long-term labeling of stem cells. These nanomaterials are commercially available and compare favorably to organic dyes or fluorescent proteins in terms of brightness, photostability (up to a few hours when using confocal microscopy), and large Stokes shift. The narrow emission and broad excitation spectrum of qdots allows simultaneous analysis of multiple cell targets by using a single wavelength activation [Michalet et al., 2005].

Qdots are powerful tools for imaging cellular events at the single-molecule level [Howarth et al., 2008]. Bioconjugate qdots can be used to track key biomolecules including growth factor receptors, integrins, phospholipids, and enzymes among others, when stem cells are exposed to different environments or soluble factors [Chen et al., 2007]. A method to generate monovalent qdots has been recently reported [Howarth et al., 2008]. Qdots with a hydrodynamic diameter of 11 nm were labeled with only one molecule of streptavidin, as demonstrated by gel electrophoresis and functional results. These nanomaterials might be a useful tool for imaging protein dynamics at the single-molecule level in the stem cells or their progenies.

Qdots are also attractive nanomaterials to monitor stem cell survival, location and differentiation either in vitro or in vivo due to their inherent long-term fluorescence intensity [Chakraborty et al., 2007; Rosen et al., 2007] (Fig. 2). In vivo tracking of qdots typically requires access to whole animal imaging like Caliper's IVIS. Stem cells are labeled with qdots in several ways, including receptor-mediated uptake, lipofection, electroporation, or passive loading. Passive loading of qdots resulted in uniform diffused cytoplasmic labeling of a population of human mesenchymal stem cells and this behavior is maintained for at least 6 weeks in vitro and 8 weeks in vivo [Rosen et al., 2007]. Stem cells labeled in this way had similar proliferative and differentiation capacities compared to unloaded human mesenchymal stem cells, indicating that the nanomaterials did not significantly affect the properties of the cells [Rosen et al., 2007]. Qdots-labeled mesenchymal stem cells injected intravenously in NOD/SCID mice (1×10^6 cells) accumulate after 24 h in the lungs, liver and spleen, but not in the heart, brain or kidneys [Lei et al., 2008].

Under appropriate conditions, stem cells labeled with qdots appear to maintain intact their self-renewal and differentiation potentials. For example, hMSCs labeled with a range of doses of qdots from 16 nM to 250 pM maintained their osteogenic differentiation potential [Chakraborty et al., 2007]. However, the cytocompability might be affected by the origin and surface modification of the qdots, mode of internalization and stem cells used [Hsieh et al., 2006; Chakraborty et al., 2007]. So far, most of the studies have reported on multipotent mesenchymal stem cells, and thus it will be important to extend these studies to pluripotent embryonic stem cells. Long-term effects of these nanoparticles and their degradation products on stem cells should be also assessed at gene and protein level. The oxidative degradation of qdots releases Cd^{2+} that might affect cell function [Derfus et al., 2004]. Cadmium can bind to the sulfhydryl groups of critical mitochondria proteins leading to mitochondrial dysfunction and ultimately cell poisoning [Rikans and Yamano, 2000]. The release of reactive oxygen species during the degradation of qdots also contributes for its cytotoxicity [Lovric et al., 2005]. However, it might be possible to coat the qdots in a way that circumvents its in vivo degradation.

NANOMATERIALS AS INTRACELLULAR BIOSENSORS OR EFFECTORS

The sensing of intracellular biomolecules, enzyme activity and pH in real time can contribute for a better understanding of key biological processes on stem cells and might potentially lead to the development of more effective strategies to control, at molecular level, their fate. Currently, most of the strategies to examine changes in the intracellular environment require processing (fixation, labeling, etc...) of the cells, and are less appropriate for high-throughput approaches. Nanoparticles can be used as nanosensors on stem cells. A sensor generally consists of two components: a recognition element for target binding and a transduction element for signaling the binding event [De et al., 2008]. The sensor is generally formed by a biological substrate molecule immobilized onto the nanoparticle surface. The conjugation of the substrate can be done either by covalent or non-covalent linkages. For enzymatic sensors, the substrate is modified by an intracellular enzyme with the subsequent change in the nanoparticles local environment, leading to the generation of an optical or electronic signals [Ghadiali and Stevens, 2008]. In the pH sensors, a nanosensor senses intracellular pH by a pH-responsive fluorescent probe [Coupland et al., 2008].

Nanosensors that have been developed for somatic cells [De et al., 2008] might be used on stem cells. For example, nanoparticles able to quantify kinase and caspase activities are of great interest to determine the activation of signal-transduction and apoptotic pathways, respectively, when the stem cells are exposed to different external factors [Kim et al., 2007; Boeneman et al., 2009]. A recent study reported the preparation of polymeric nanoparticles bearing a kinase peptide substrate and near-infrared fluorophore chemically coupled to the nanoparticle. In the nonphosphorylated state, these nanoparticles have low levels of fluorescence because of the short distance between each fluorescence probe in the nanoparticle. Upon nanoparticle phosphorylation, the nanoparticles dissolve because negatively charged phosphate groups are incorporated into the peptide substrate resulting in polymer solubilization [Kim et al., 2007].

The nanoparticles might also act as biological inductors. For example, carboxyfullerenes (molecules composed of large three-dimensional arrays of evenly spaced carbon atoms functionalized with carboxyl groups) can be uptaken by cells and act as antioxidant and free radical scavenger agents [Dugan et al., 1997].

NANOMATERIALS FOR THE INTRACELLULAR DELIVERY OF GENETIC OR PROTEIC MATERIAL

Gene delivery [DNA or RNA interference (RNAi)] can be a powerful strategy to study the basic biology of stem cells or to direct their differentiation into specific cell types [Hough et al., 2006; Meinel et al., 2006] (Fig. 3). The genetic material can be transferred to cells through viral and nonviral carriers, although nonviral carriers are a safer approach for most of the therapeutic applications. Typical examples of nonviral carriers include cationic polymers that interact electrostatically with negatively charged DNA/RNAi molecules forming polyplexes, cationic nanoparticles, and carbon nanotubes. Polymers that have been used for stem cell transfection include poly(L-lysine)-palmitic acid [Clements et al., 2007], chitosan [Corsi et al., 2003], polyethylenimine [Incani et al., 2007], poly(β-amino esters) [Green et al., 2008], and poly(L-lysine) [Incani et al., 2007]. Generally, the cytotoxicity profile is correlated with the nanomaterial chemistry and concentration [Corsi et al., 2003; Kutsuzawa et al., 2006; Incani et al., 2007; Mooney et al., 2008].

Current approaches for gene transfer to human embryonic stem cells (hESCs) are limited because there are safety concerns with viral approaches and nonviral methods have low efficacy. Gene delivery by commercial nonviral transfection agents (FuGENE, lipofectAMINE Plus, ExGen 500) has an efficiency below 10%. Recently a study has reported that polyplexes formed by poly(β-amino esters) and plasmid DNA had a gene delivery efficacy up to four times higher than that of the leading commercially available transfection agent, Lipofectamine 2000 [Green et al., 2008]. In contrast with viral gene delivery approach that raises some safety concerns associated with insertional mutagenesis after viral integration, the nonviral gene-delivery approach is relatively safe and is expressed transiently.

Nanoparticles are also effective vectors for gene transfection (Fig. 3). For example, apatite nanoparticles coated electrostatically with fibronectin and E-cadherin have been reported to be efficient gene delivery systems for embryonic stem cells [Kutsuzawa et al., 2006]. Specific binding to the cell surface integrin and E-cadherin molecules through double ligand created nanoparticles, resulted in synergistic acceleration of gene delivery and consequential expression into embryonic stem cells (59% of the cells expressed the gene) [Kutsuzawa et al., 2006, 2008]. Gene expression was almost three times higher for this system than what was observed for the commercially available lipofectamine TM 2000.

In the previous example, DNA is released from the nanoparticles in the cell cytoplasm; however, nanoparticles with covalently immobilized DNA or siRNA can be another very effective strategy to regulate gene expression [Rosi et al., 2006; Giljohann et al., 2009]. Polyvalent DNA-gold nanoparticle systems can be used for antisense gene regulation on stem cells, where the unique ensemble properties of the conjugate confer several important advantages in the context of intracellular target recognition and binding [Rosi et al., 2006]. For somatic cells, it has been reported that these systems have high resistance to nuclease degradation and high cellular uptake as a result of their oligonucleotide functionalization. These nanoparticle systems offer exciting opportunities for the regulation of stem cell genes and the control of their self-renewal/differentiation programs.

Carbon nanotubes (CNTs) discovered by Iijima in 1991 are another promising delivery strategy to transfect cells [Iijima, 1991]. CNTs are helical structures approximately 1–30 nm in diameter with lengths >100 nm, and able to encapsulate drugs and genetic material. Carbon nanotubes with different functional groups are transported towards the perinuclear region after a few hours of contact with cells, even under endocytosis-inhibiting conditions [Kostarelos et al., 2007]. After 24 h, a significant number of CNTs have been observed at the cell nucleus [Mooney et al., 2008]. Interestingly, a recent study has described a novel platform for the intracellular delivery of genetic material based on vertically aligned carbon nanosyringe arrays of controllable height [Park et al., 2009].

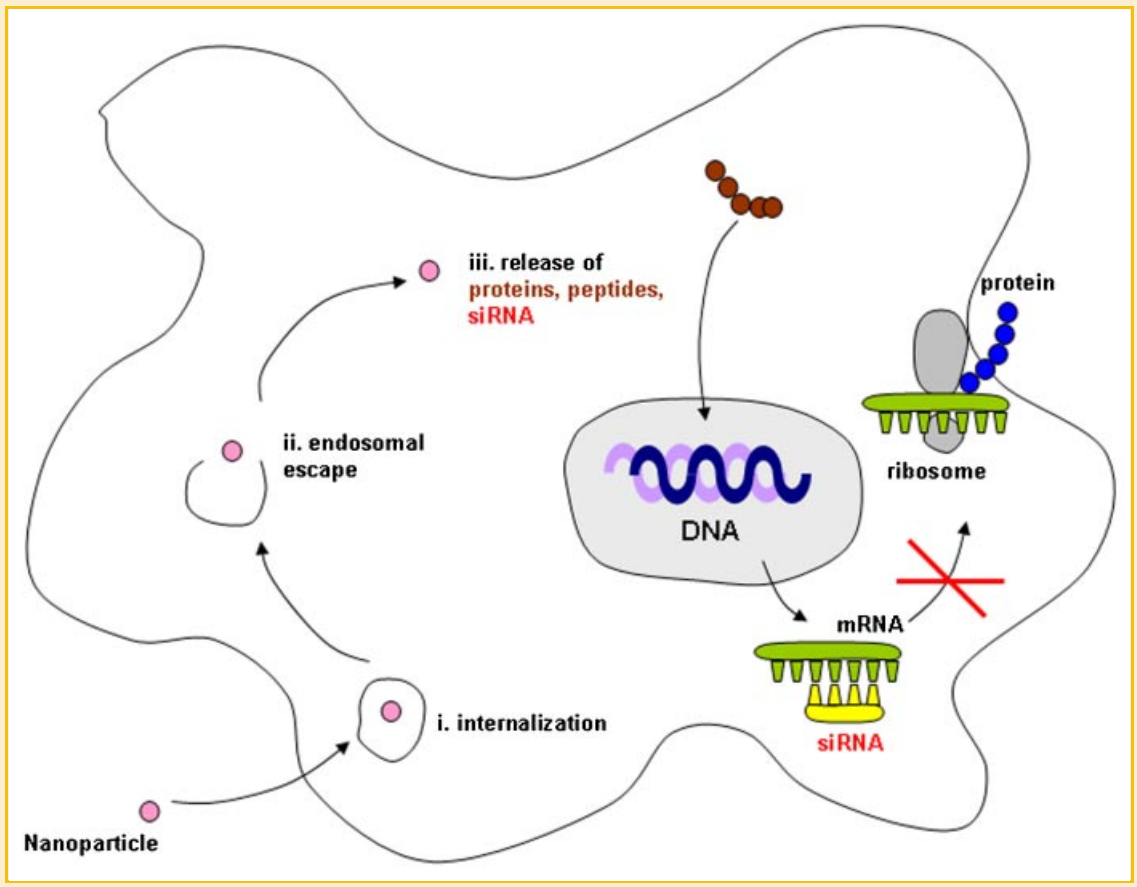

Fig. 3. Uptake of nanoparticle delivery systems. For efficient delivery, nanoparticles should escape the endo–lysosome compartment after being internalized by the cells. In the cytosol, the nanoparticles release their content, including siRNA, proteins and peptides (for simplification, DNA was not incorporated in this figure). The proteins and peptides might act on the signaling cascades of the stem cells, or migrate to the cell nucleus and control gene expression. siRNA released in the cytoplasm can interfere with protein expression.

This technology offers an opportunity to efficiently delivery the plasmid DNA into the cytoplasm of human mesenchymal stem cells (hMSCs) [Park et al., 2009].

Although very promising, CNTs can exert cytotoxic effects depending in their concentration, size, shape, and surface functionalization [Magrez et al., 2006]. For example, COOH-functionalized single-walled nanotubes (SWNTs) are not cytotoxic up to concentrations of 0.032 mg/ml when exposed to hMSCs while OH-functionalized SWNTs appeared to be more cytotoxic at smaller concentrations (above 0.0064 mg/ml) [Mooney et al., 2008]. In addition, multiwalled carbon nanotubes (MWNTs) accumulate in mouse embryonic stem cells (mESCs) activating the tumor suppressor protein p53 within 2 h of exposure and consequently leading to cellular apoptosis [Zhu et al., 2007]. The differentiation potential of hMSCs might also be affected by CNTs. CNT treated hMSCs differentiate into adipocytes, osteoblasts, and chondrocytes under specific media conditions; however, the chondrogenesis was slightly decreased as compared to the control [Mooney et al., 2008]. Further research is needed to elucidate the biological impact of carbon nanotubes on stem cells.

The ability to deliver biomolecules via an intracellular route, including proteins, growth factors, and small chemicals presents an excellent tool to control the differentiation of stem cells. Some of these biomolecules/chemicals have (i) poor solubility, (ii) can be quickly cleaved by cellular enzymes, (iii) and have side effects when administered systemically. Biodegradable and biocompatible nanoparticles able to target stem cells and release the payload in their cytoplasm with consequent activation of signaling cascades will be of great interest. We recently reported a new approach for the delivery of vascular growth factors into hESCs, by incorporating growth factor-release particles in human embryoid bodies (EBs) [Ferreira et al., 2008b]. We demonstrated that the incorporation of these polymeric biodegradable particles had a minimal effect on cell viability and proliferation but a large impact on differentiation. In some cases, the effect on vascular differentiation of particles containing growth factors was superior to the one observed by exposing EBs to large extrinsic doses of the same growth factors. In addition, we studied the intracellular trafficking of particles of different sizes within hESCs. We demonstrated that nanoparticles (diameter ~240 nm) could be taken up by hESCs and could accumulate in the perinuclear region [Ferreira et al., 2008b]. These nanoparticles could serve as a platform to deliver growth factors and other biomolecules within stem cells.

The localized delivery of biomolecules into specific domains or compartments of living cells without disturbing other parts of the

cell might be a great opportunity to manipulate cells at specific sites. Gao et al. [2008] reported that fluorescent magnetic nanoparticles can act as a vehicle to deliver biomolecules at different locations of the cell under a magnetic field and a fluorescent microscope to detect their positions. This technology has been applied to somatic cells and might be interesting to extend it to stem cells.

CONCLUSIONS AND FUTURE DIRECTIONS

According to the experimental results obtained in the last few years, nanoparticles are useful tools to study and control stem cells. The potential benefits of nanotechnologies for tracking, delivering and sensing can be enormous and may eventually offer the researcher and clinician novel therapeutic platforms that simply do not exist today. For further advancement of this area it will be important to strength the synergies between material scientists, stem cell biologists, and clinicians. Some products based in nanoparticles have been used in the clinic [Zhu et al., 2006] and hopefully new ones will be available soon, particularly for controlled drug delivery.

Small size, high surface-to-volume ratio, and high surface functionality are properties that make nanoparticles so interesting for regenerative medicine applications. However, nanoparticles can also have undesirable effects and might be cytotoxic, induce changes in the self-renewal and differentiation programs of the stem cells. Future research should evaluate carefully both sides. For example, polymeric nanoparticles and super paramagnetic iron oxide nanoparticles (SPIO) for MRI contrasting agents have been shown to degrade, but qdots, carbon nanotubes and gold nanoparticles are examples of nanotechnologies without clear indication of their cellular degradation profiles. The breakdown of the nanoparticles can exert molecular responses that are not predictable and thus it is of utmost importance to study this issue.

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