Published in final edited form as:

Adv Exp Med Biol. 2014 ; 811: 255–275. doi:10.1007/978-94-017-8739-0_13.

Stem Cells and Nanomaterials

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Abstract

Because of their ability to self-renew and differentiate into many cell types, stem cells offer the potential to be used for tissue regeneration and engineering. Much progress has recently been made in our understanding of the biology of stem cells and our ability to manipulate their proliferation and differentiation to obtain functional tissues. Similarly, nanomaterials have been recently developed that will accelerate discovery of mechanisms driving stem cell fate and their utilization in medicine. Nanoparticles have been developed that allow the labeling and tracking of stem cells and their differentiated phenotype within an organism. Nanosurfaces are engineered that mimic the extracellular matrix to which stem cells adhere and migrate. Scaffolds made of functionalized nanofibers can now be used to grow stem cells and regenerate damaged tissues and organs. However, the small scale of nanomaterials induces changes in their chemical and physical properties that might modify their interactions with cells and tissues, and render them toxic to stem cells. Therefore a thorough understanding of stem cell-nanomaterial interactions is still necessary not only to accelerate the success of medical treatments but also to ensure the safety of the tools provided by these novel technologies.

Keywords

Stem cells; Nanomaterials; Differentiation; Regenerative medicine; Toxicity

13.1 Introduction

Nanotechnology involves the fabrication and use of materials and devices on an atomic and molecular scale, with at least one dimension measuring from 1 to 100 nm [1]. Materials and tools created using nanotechnologies have at least two advantages. First, their minuscule sizes make them of interest in bioengineering and medicine, for example to build scaffolds for tissue engineering and to carry drugs that target specific cells and tissues [2–5]. Second, the fact that certain physical and chemical properties change as the size of the system decreases renders nanomaterials particularly useful in mechanical, chemical and electrical engineering, and ultimately life sciences [6]. Indeed nanotubes, nanowires, fullerene derivatives (buckyballs), and quantum dots are now used for the manufacturing of novel analytical tools for biotechnology [7–12]. Because of their novel properties, nanoscale materials can also be exploited to modulate cell proliferation or differentiation by influencing their attachment or manipulating their environment [13–16]. This feature is particularly applicable for the modulation of stem cell fate in regeneration studies.

Stem cells are undifferentiated cells that have the dual ability to self-renew to maintain their own pool, or to differentiate into functional mature cells. During early mammalian embryogenesis, the inner cell mass (ICM) of the blastocyst is made of pluripotent cells, or embryonic stem cells (ES cells) that are able to proliferate and differentiate into all cell lineages that will eventually generate the fetal organs [17]. As these pluripotent stem cells continue to divide, they start to specialize and become multipotent stem cells. Multipotent stem cells are found in the fetus and the adult animal; they are less plastic than ES cells and are able to differentiate only into specific lineages. For example, mesenchymal stem cells (MSCs) isolated from adult bone marrow or cord blood can generate only bone, cartilage, adipocytes, cardiomyocytes, nerve cells and supporting cells such as stromal fibroblasts (Fig. 13.1) [19]. Adipose tissue-derived stem cells (ADSC) are similar to MSCs and are found in the stromal-vascular fraction of fat tissue [20]. Hematopoietic stem cells, found in the bone marrow, produce both the lymphoid and myeloid lineages and are responsible for maintaining blood cell production throughout life [21]. The intestinal crypts contain stem cells that self-renew to continuously regenerate the gut epithelium, but can also differentiate into enterocytes, enteroendocrine cells, goblet cells and Paneth cells with distinct functions [22, 23]. Similarly, skin stem cells self-renew and/or differentiate to produce keratinocytes, hair follicles, sebaceous glands and sweat glands [24]. While multipotent stem cells usually produce several, but restricted, cell types, some stem cells are unipotent and give rise to only one kind of mature cells. For example, spermatogonial stem cells (SSCs) of the testis ultimately produce only sperm cells [25]. However, SSCs have the unique property to revert to an ES cell-like state when cultured in the appropriate conditions, and might become some day a source of adult pluripotent stem cells for use in regenerative medicine [26–29].

Induced pluripotent stem cells or iPS cells, are pluripotent stem cells derived from adult somatic cells, typically fibroblasts, by forcing the expression of pluripotent genes. In mice, these genes originally were OCT4, SOX2, c-MYC and KLF4 [30–32]. However, about 16 % of chimeric mice obtained after blastocyst injection of the iPS cells died of tumors within 100 days after birth, presumably because of the oncogenic properties of c-MYC. Therefore, mouse iPS cells were later obtained by omitting c-MYC in the gene transfection cocktail [33]. In humans, efficient production of iPS cells was demonstrated by forced expression of OCT4, NANOG, SOX2 and LIN28 [34]. Expression of these genes reprograms the cells, which are then able to differentiate into tissue types of the three embryonic germ line layers. Although progresses still need to be made to improve efficiency and ensure their safety, iPS cells will certainly be used in the future for tissue engineering purposes. Because stem cells constitute the building blocks for organ development and tissue repair, the past 15 years have seen growing interest in their biology and in the mechanisms that drive these cells into specialized differentiation programs. These cells are also surprisingly easy to isolate, culture and differentiate in vitro and in vivo, therefore the drive to translate findings into clinical therapeutics has led to an increase of their applications to regenerative medicine. Stem cells hold great promises for the treatment of Parkinson disease, congenital abnormalities and spinal cord injury, as well as liver and skin regeneration. While delivering on these expectations still needs extensive work, nanotechnology will without doubt help in visualization, fate tracking, and manipulating stem cells and their environment for building and repairing tissues [35, 36]. However, there is great concern about the health consequences of using nanomaterials due to their extremely small size, their high surface area and

increased surface reactivity (i.e., redox ability) as compared to larger materials. This review will describe how the combination of nanotechnology and stem cell research will dramatically advance our ability to understand and control stem cell fate decisions. This technology will lead to novel stem cell-based therapeutics for the prevention, diagnosis, and treatment of human diseases, provided that the toxicity of nanomaterials is properly assessed and understood.

13.2 Nanomaterials for Labeling and In Vivo Tracking of Stem Cells

Therapies using stem cell transplantations to regenerate tissues are currently being investigated to treat a multitude of degenerative disorders such as heart failures [37, 38], brain and spinal cord injuries [39, 40], diseases of the retina [41], liver failure [42], kidney dysfunction [43] and lower limb ischemia [44]. After transplantation, stem cells are expected to engraft, differentiate into specific cells or tissues in response to the surrounding microenvironment, and restore this tissue's functional properties. Despite these advances, the mechanisms underlying stem cell proliferation, differentiation, migration and integration within the host tissue are still poorly understood. This lack of knowledge is mainly due to our previous inability to monitor the in vivo behavior of stem cells in long-term studies because metabolic degradation and reduced photostability of cell markers such as DiI (1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate) or DiO (3,3'-Diocetadecyloxycarbocyanine perchlorate) compromised the quality of the signal over time.

The past decade focused on discovering and improving novel approaches for labeling and tracking stem cells for tissue engineering. These methods are now increasingly replacing earlier methods and mainly utilize nanoparticles. The first attempt to label and track cells injected into mice made use of superparamagnetic iron oxide (SPIO) particles for labeling of cancer cells, as well as neural and mesenchymal stem cells (Fig. 13.2) [45–48]. While the transplanted cells could be tracked with magnetic resonance imaging (MRI), poor resolution impaired the analysis of the labeled cells at the cellular level. Subsequently, various SPIOs and SPIONs (super-paramagnetic iron oxide nanoparticles) have been designed that carry specific alterations of their physical, biological and chemical properties to improve their functionality and MRI tracking [49]. In particular, sensitive, non-toxic SPIONs are now designed for future human therapeutic purposes. They can be dextran-coated to minimize aggregation [45, 50], or incubated with cationic compounds such as poly-L-Lysine [51, 52], protamine sulfate [53] or Lipofectamine [54] to facilitate absorption by non-phagocytic cells. While the sensitivity of MRI is lower than that of single photon emission tomography (SPECT), absence of exposure to ionizing radiation and long imaging window offer an obvious advantage for human clinical studies. Indeed, the labeled stem cells should retain the marker and remain viable for months to allow their long-term follow-up, and assessment of tissue function in pre-clinical and clinical trials.

Tracking transplanted cells or stem cells in vivo to monitor their tissue distribution, viability and differentiation is of great importance for tissue engineering and cell therapy. Although labeling with SPIONs has become the most common procedure for longitudinal tracking of stem cells, other nanotracers have been recently developed. These include silica and titanium nanoparticles, fluorescent gold nanoclusters, nanodiamonds and quantum dots, which all

have different degrees of toxicity [55–59]. In order to improve biocompatibility, alternative strategies are being developed to provide tracers amenable to clinical translation. For example, nanomaterials with dual paramagnetic and fluorescent characteristics were recently synthesized. In 2005, Vuu and colleagues reported the construction of a dual-modality contrast agent, Gadolinium-Rhodamine nanoparticles (Gd-Rd-NPs), which they used to label and track a breast cancer cell line after injection into the hind limb of mice (Fig. 13.3) [60]. Gadolinium allowed detection of the labeled tumor cells by whole animal MRI while Rhodamine was used to visualize the cells in whole animal optical imaging or in tissue sections. More recently, fluorescent biocompatible nanoparticles were produced by polymerization of methyl methacrylate-Rhodamine complexes [61]. These nanoparticles were used to label and track human amniotic fluid cells (hAFCs), which are known to contain a significant number of stem cells with pluripotent properties [62]. Labeled hAFCs were injected into the lateral brain ventricles of mice and could be tracked for at least 14 days. Labeling was strong and reliable, indicating that these novel nanoparticles, which are highly biocompatible, are a promising tool for cell therapy.

Cancer stem cells (CSCs) comprise a small fraction of cancer cells and are believed by many to be at the origin of most tumors [63]. A number of studies suggest that CSCs are also at the origin of metastases. Identifying and tracking metastatic cancer cells is a significant task that can also be achieved using fluorescent-labeled nanoparticles. Indeed, efficacy of treatment can be radically improved through earlier detection of metastatic foci, which depends on sensitivity and specificity of the analytic tool. Soster and colleagues recently developed Rhodamine and/or Cy5 fluorescent silica-poly(ethylene glycol) nanoparticles (SPNs) coupled to two metastasis-specific peptides [64]. A poly(ethylene glycol) (PEG) shell embeds a dye-bound silica core, and presents carbonyl groups to enable covalent attachment of targeting ligands, such as antibodies, proteins, or short peptides. These nanoparticles were coupled to the peptides CGIYRLRS and CGVYSLRS that are specific for metastatic colon carcinoma. They were intravenously injected into SCID mice bearing a spleen primary tumor (site of injection of colon cancer cells) and hepatic metastases. Whole organ imaging and immunohistochemistry clearly indicated that the circulating nanoparticles left the tumor capillaries and homed nearby the tumor epithelial cells. This method might therefore provide a reliable and safe way to detect small metastases before surgery.

Despite this progress, multi-imaging methods are still needed to follow the fate of different cell types simultaneously in the host tissue. For example, in tissue engineering there is the need of imaging stem cells together with endothelial cells since vascularization of reconstructed tissues is crucial for stem cell survival, which depends on oxygen and nutrient supply. Simultaneous labeling of mesenchymal stem cells (MSCs) and endothelial cells in polysaccharides-based scaffolds was recently performed by Di Corato and colleagues (Fig. 13.4) [65]. Paramagnetic gadolinium nanoparticles were used to label MSCs while SPIONs marked endothelial cells. The cells were embedded in polysaccharide-based scaffolds that were implanted subcutaneously in the flank of mice. Visualization by high-resolution MRI demonstrated that the two cell types remained distinct, with cellular level of resolution within the mouse body. Importantly, the authors demonstrated that the labeling of the MSCs did not alter their ability to differentiate into the osteogenic, chondrogenic or adipocytic lineages. In summary, our ability to trace stem cells in vivo using nanoparticles has

remarkably improved during the past 10 years. It is evident that manufacturing of more specific and efficient nanoparticles for the visualization of these cells was concomitant to improvement of high resolution imaging techniques. In addition to their reliability and efficiency, nanoparticles with potential use in regenerative medicine have become for the most part biocompatible.

13.3 Nanomaterials for Manipulating Stem Cell Commitment

Directing the fate of stem cells in vivo is of paramount importance for the success of tissue regeneration and engineering. Extrinsic cues from the natural microenvironment of many stem cell types have been recently elucidated and include activation of the Wnt, Hedgehog and Notch pathways, binding of growth factors such as nerve growth factor (NGF) or bone morphogenetic protein (BMP) to specific membrane receptors, and composition of the extracellular matrix (ECM) [23, 66–72]. Recently, nanomaterials have been manufactured with the intention to successfully mimic or even bypass the effect of biological molecules. For example, nanofibers can increase human umbilical cord blood stem cell expansion without inducing differentiation [73]. Incorporation of growth factors such as basic fibroblast growth factor (bFGF) during scaffold preparation by electrospinning techniques stimulates skin stem cells to accelerate wound healing [74]. Fibrin nanoscaffolds with different geometry of the fibrin structure can be prepared, which are amenable to encapsulation of growth factors [75, 76]. These growth factors can then be delivered to a target site to promote stem cell growth or differentiation where tissues need to be regenerated. Indeed, Fibrin scaffolds loaded with growth factors, such as vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) stimulated blood vessel and nerve repair [77, 78]. Gene therapy using a bone morphogenetic (BMP) expression vector embedded into a Fibrin nanoscaffold allowed bone regeneration [79]. In addition to creating nanosystems able to deliver growth factors, many efforts have been directed at the construction of scaffolds mimicking the ECM for directing stem cell fate. Increasing evidence has revealed that the ECM itself through its geometry at the nanoscale level and interactions with cellular receptors such as integrins can modulate the shape and therefore gene expression and fate of stem cells [80–85]. Mechanical forces and matrix elasticity are also of great importance [86–88]. Indeed, the degree of substrate rigidity can regulate cell phenotype, which was demonstrated by the fact that an increase of matrix stiffness results in preosteoblast proliferation, while a decrease of stiffness promotes their differentiation [89]. Yet another feature of the extracellular matrix that can be mimicked using nanomaterials to direct stem cell fate is the variety of its topography, which is at the nanometer scale. Cell responses modulated by nanotopography include alignment, survival, motility, proliferation and differentiation [90–93]. For example, human induced pluripotent stem cells (iPSCs) seeded onto nanostructured silicon substrates responded by elongating and aligning along the grating axis and expressed neuronal markers, while the same cells seeded on flat substrates spread randomly and conserved their pluripotent properties [94]. The same behavior can be obtained from human MSCs [90]. Interestingly, the dimension of TiO₂ nanotubes, in particular their diameter, is instrumental in directing proliferation or differentiation of human MSCs into osteogenic lineages, although the size at which differentiation occurs seems controversial [95–97]. Arrangement of nanopits is also

instrumental in determining stem cell fate. In a study using primary human osteoblastic cells, square and hexagonal nanopit arrays perturbed the formation of mature focal adhesions and the cell-spreading process, while groove/ridge arrays promoted polarised morphology and focal adhesion complexes [98]. Similarly, a square array of nanopits can support self-renewal of human MSCs, while a slightly disorganized array will promote their osteogenic differentiation [99]. Further, the genetic profile of human primary MSCs cultured on arrays presenting nanopits and raised nanomounds indicated that these cells differentiate into bone more efficiently than in planar surfaces with dexamethasone, a glucocorticoid commonly used to direct the differentiation of MSC into osteoblasts [100]. In another study, neural progenitors embedded into a nanofiber matrix engineered to contain the laminin-specific cell-binding domain (IKVAV) differentiated preferentially into neurons [101]. Similarly, a nanofiber scaffold that contained RGD, a binding domain for cellular integrins in many ECM proteins, significantly improved MSCs differentiation into osteoblasts [102]. Altogether, these studies clearly demonstrate that mimicking the ECM by nanotopography and engineering adhesion peptides is able to influence human adult stem cell fate, in many cases without addition of growth or differentiation factors. Therefore nanomaterials can efficiently replace the ECM and facilitate tissue regeneration and engineering protocols.

13.4 Nanomaterials Supporting Stem Cells for Regenerative Medicine

Healing and regeneration of injured or lost tissues require complex interactions between stem cells, growth factors, and extracellular matrix. Scaffolds mimicking the extracellular matrix must be constructed to establish form and structure of the organ or tissue to be regenerated. Scaffolds must be physically stable in the implanted site, allow the homing, expansion and differentiation of stem cells, and be non-toxic and biodegradable.

13.4.1 Nanofibers

Since they reproduce the morphology and structure of the natural ECM, polymeric nanofibers are among the most suitable nanomaterials for tissue engineering applications. Scaffolds made of nanofibers are three-dimensional meshes formed through the non-covalent assembly of peptides with nanometer diameters, and are commonly used in attempts of regenerating bone, skin and nerve tissue (Fig. 13.5). The most common methods for the fabrication of polymeric nanofibers are self-assembly, phase separation and electrospinning [104]. Self-assembly and phase separation allows the creation of short nanofibers with very small diameters (about 10 nm). For example, the RADA16 peptide nanofiber material self-assembles into scaffolds that were used for the growth of hippocampal neurons that subsequently produced neurites and synapses [105, 106]. This material contains the amino acids alanine, lysine, and glutamate in repeated sequences of Arg-Ala-Asp-Ala (RADA). RADA16-I nanofibers scaffold has been used for brain tissue reconstruction in mice after trauma [107], and has also been found to promote regeneration in experimental spinal cord and brain injuries [108]. Stem cell embedding into the material is feasible [109–111]. For example, Guo and colleagues embedded Schwann cells and neuroprogenitor cells (NPCs) inside a RADA16-I scaffold that integrated well within the injured spinal cord. They also demonstrated that the transplanted cells were able to survive and differentiate into neurons, astrocytes and oligodendrocytes [111]. In one of their study,

Garreta and colleagues embedded embryoid bodies derived from ES cells in RADA16-I, and achieved differentiation using osteogenic medium [109]. Several functional motifs can be attached to RADA16-I, which are known to promote cell adhesion, differentiation or stem cell homing. In these conditions, neural stem cells adhered well to their matrix environment, survived and differentiated toward both the neuronal and glial phenotypes [110]. Because it is made essentially from amino acids, this type of scaffold is well tolerated and does not elicit detectable immune response or inflammation in animals. However, despite other advantages such as easiness of use and affordability, self-assembled nanofibrous scaffolds are limited in their ability to form pores wide enough to allow cell proliferation and migration since the pore size is usually between 5 and 200 nm [112]. In addition, nanofibers produced by self-assembly are very short and are often phagocytosed by the cells [113].

Electrospinning is a reliable method to fabricate long continuous strands of nanofibers with a diameter ranging from nanometers to microns (50–1,000 nm) [104]. The nanofibers form a mesh with pore sizes ranging from several to tens of micrometers, which favors cell proliferation and migration [114]. Materials used in electrospinning are natural or synthetic biopolymers or combination of both. They include poly(L-lactic acid), alginates, silicon or chitosan, sometimes in combination with collagen or gelatin [13, 115–118]. ECM proteins/peptides and growth factors can also easily be incorporated into this type of scaffold [119]. Scaffolds made of electrospun nanofibers of different types have been widely used for bone and cartilage tissue engineering using MSCs [116, 120–124]. Even adipose-derived stem cells (ADSCs) could proliferate and differentiate along the osteogenic pathway in absence of any induction medium when electrospun poly(L-lactic acid)/collagen nanofibers were engineered with a cell adhesion peptide and functionalized to retain calcium phosphate (hydroxyapatite) [123, 125]. This confirms the positive influence of cell-matrix interactions on stem cell survival and proliferation [126]. To increase the tensile strength of the scaffold, McCullen and colleagues encapsulated multiwalled carbon nanotubes (MWNTs) in poly(L-lactic acid) nanofibers [123]. Interestingly, their data indicated that addition of MWNTs significantly stimulated ADSCs proliferation. Electrospun poly(L-lactic acid)/gelatin fibers were also recently used as scaffolds to differentiate neural stem cells into motor neurons [127]. Also, poly-L-lactide (PLLA) and hybrid PLLA/collagen (PLLA/Coll) scaffolds fabricated by electrospinning were recently used to differentiate MSCs into vascular endothelial cells [128].

13.4.2 Nanotubes

Nanotubes are cylindrical structures made of a single or several layers of carbon or silicon atoms. Carbon nanotubes are known for their electrical conductive capacity and strong mechanical properties, and they can be modified to accommodate stem cell adhesion. Single-walled or multi-walled carbon nanotubes (SWNTs or MWNTs) are used in regenerative medicine alone or as complement to poly(L-lactic acid) fibers to provide tensile or mechanical strength. As mentioned above, addition of MWNTs to poly(L-lactic acid) nanofibers seem to increase adipose stem cell proliferation [123], but they can also improve chondrogenic differentiation of MSCs [129]. When nanoscaffolds made of poly(L-lactic acid) and MWNTs were modified with poly-L-lysine to improve cell adhesion, the MSC-derived chondrocytes produced more glycosaminoglycans than the same cells in control

scaffolds without nanotubes or poly-L-lysine [129]. Combination of MSCs with the latter nanomaterial is therefore very promising for cartilage regeneration, one of the most difficult tasks in regenerative medicine since this tissue is not vascularized. MWNTs could also be combined to chitosan or hydroxyapatite to successfully mimic bone tissue with acceptable pore size amenable to periosteal stem cell proliferation [130]. Single-walled carbon nanotubes (SWNTs) were also functionalized with poly lactic-co-glycolic acid to form a biocompatible substrate allowing proliferation and differentiation of pre-osteoblasts that successfully expressed mature osteoblast markers [131]. Single-walled and multi-walled carbon nanotubes are also increasingly used as scaffolds for neural stem cell growth and differentiation. Neural stem cells (NSCs) can be isolated from the mammalian brain, propagated in culture and transplanted into damaged sites if provided with the necessary substrate. Differentiation of NSCs on single-walled carbon nanotubes layers was recently demonstrated by Jan and Kotov [132]. The stem cells were seeded onto this substrate using differentiation medium without epidermal growth factor (EGF), which is necessary for their proliferation. In these conditions, the NSCs could differentiate into neurons, astrocytes, and oligodendrocytes, with expression of neural markers such as nestin, microtubule-associated protein 2 (MAP2), glial fibrillary acidic protein (GFAP), and the oligodendrocyte marker O4. The same authors demonstrated that thin films of single-walled carbon nanotubes mixed with laminin induced NSCs differentiation into a functional neural network with synaptic connections. In addition, the neurons were excitable [133]. Another study recently established that carbon nanotubes seeded with subventricular zone neural progenitor cells (NPCs) could effectively repair damaged neural tissue after induced stroke [134]. Further, enhanced myotube formation from myoblasts and differentiation of MSCs into cardiomyocytes were possible after seeding these cells on carbon nanotubes mixed with polyurethane or poly-(L-lactic acid), followed by electric stimulation [135, 136]. Therefore, functionalized carbon nanotubes seem to fulfill the essential requirement for the regeneration of damaged nerve and muscle tissues, which is biocompatibility, induction of stem cell differentiation and excitation of the differentiated cells.

13.4.3 Use of ES Cells on Nanoscaffolds

Because of their pluripotent properties, ES cells have also been used in nanoscaffolds to probe their biocompatibility and potential for driving stem cell fate. Since these cells reliably differentiate into cardiomyocytes, they have been seeded into nanoscaffolds predominantly to assess the influence of the material on cardiac tissue regeneration [137–139]. Nanofibers made of biodegradable polyurethane, polyethylene glycol and polycaprolactone (PCL) alone or in combination could induce the formation of cardiomyocytes from ES cells. Also, nanofiber density seemed to play an important role [138]. Other applications using ES cells include the use of electrospun PCL nanoscaffolds to enhance their differentiation into neural lineages when cultured with retinoic acid [140]. In a study by Carlberg and colleagues, human ES cells cultured on electrospun fibrous polyurethane scaffolds differentiated predominantly into dopaminergic neurons without the need of hormones or growth factors [141]. Finally, human ES cells seeded into PCL scaffolds could be differentiated into adipocytes after induction with a hormone cocktail containing retinoic acid, insulin and triiodothyronine (T3) [142].

13.5 Stem Cell Toxicity by Nanomaterials

Because the physical and chemical properties of many compounds will change when their size is reduced to nanodimensions, nanomaterials are increasingly used for novel industrial, medical and military applications. This is particularly true for carbon- and metal-based nanotubes and nanoparticles. The small size of nanomaterials modifies their behavior to such extent that quantum physics rather than classical physics is needed to understand their properties. Characteristics of a material such as solubility, absorption, color, transparency, emission wavelength, conductivity, melting point and catalytic behavior will be drastically altered as the size is reduced [143]. It is therefore likely that these novel properties will change how the materials interact with living tissues [144]. For these reasons, there are concerns that certain nanomaterials might be harmful upon inhalation, ingestion or skin contact. Indeed, due to their very small size, nanomaterials can easily translocate across cell membranes and reach critical organelles such as mitochondria, the endoplasmic reticulum and possibly the nucleus [145–148]. It has also been demonstrated that the clearance of particles is reduced as the particle size decreases, therefore the deleterious effect of nanosized compounds, if any, will last longer [149]. Because stem cells are particularly sensitive to toxicants, the use of nanomaterials in bioengineering and medicine is a concern if their toxicity is not assessed.

13.5.1 Stem Cell Toxicity of Carbon Nanotubes

While toxicity of carbon nanotubes on cells and tissues in vitro and in vivo has been reported as early as 2003 [150–152], in 2007 the group of Y. Hong published for the first time the effects of MWNTs at the molecular level in ES cells [148]. Because stem cells are particularly sensitive to DNA damage, they have developed specialized mechanisms to preserve genomic integrity. One of these mechanisms is the expression and phosphorylation of the protein p53, which will arrest the cell cycle to allow DNA repair by the enzyme 8-oxoguanine-DNA glycosylase 1 (OGG1). The authors demonstrated that MWNTs significantly induce the expression and phosphorylation of p53, followed by an increase in expression of OGG1 in the nucleus and mitochondria, suggesting damages in the nuclear and mitochondrial DNA. Further, the authors demonstrated that DNA double-strand breaks occurred and that the frequency of mutations increased after repair. DNA alterations often happen in response to the formation of reactive oxygen species [153, 154]. A recent study testing the influence of SWNTs on myoblasts shows that these compounds also significantly alter DNA integrity [155].

Carbon nanotube manufacturing is rapidly increasing. Because of their fibrous-like shape and durability, there are concerns that their toxic properties may be similar to those of asbestosis or other fibrous materials [156]. Indeed, MWNTs induce lung inflammation and fibrosis in mice exposed to them by inhalation [157]. Therefore strict industrial hygiene measures should be taken to limit exposure during their manipulation. As mentioned above, efforts to functionalize carbon nanotubes have ensured their biocompatibility, especially when used with human NSCs or MSCs for tissue repair. Nevertheless their long-term toxicity after scaffold implantation into an animal body still needs to be assessed.

13.5.2 Stem Cell Toxicity of Metal-Based Nanoparticles

Metal and metal oxide nanoparticles (NPs) are increasingly used in medicine, consumer products, and industry. They are used for cell tracking within an animal, drug targeting, coating of medical devices, sunscreens, detergents, clothing, printer inks and fuel additives [158]. As for other nanomaterials, nanoparticles are defined as particulate matter with at least one dimension less than 100 nm. Most NP types in current use are metal-based NPs, such as nanosilver, zinc oxide, titanium dioxide, and iron oxide. Manufactured NPs have different properties than natural NPs because of their large surface area-to-volume ratio and size that is often smaller than 20 nm. These novel physical and chemical properties imply that their interactions with cells and organelles need to be critically analyzed since many undesirable adverse effects can be triggered. Metal NPs easily penetrate tissues, epithelia and cell membranes, and pass through the blood-brain and blood-testis barriers [159–161]. Ingested nanoparticles also generate DNA alterations due to production of ROS in the bone marrow, liver and lung, which are not necessarily due to the release of free metal ions [162, 163]. Therefore these compounds are likely to interfere with the biology, viability and fate of germ line and somatic stem cells. Our group was first to assess the in vitro cytotoxicity of metal nanoparticle in germ line stem cells, or spermatogonial stem cells (SSCs) [164]. Because these cells maintain the production of sperm throughout life, preserving their viability and integrity is of paramount importance. Adverse effects on germ line stem cells maintenance, maturation and differentiation can inhibit fertility, cause cancer, and may have negative effects on the development and fertility of offspring. Our work demonstrated that nanoparticles induced production of reactive oxygen species (ROS), decrease of metabolism and apoptosis in these cells, and that the degree of toxicity depended on the metal used for nanoparticle fabrication. Silver nanoparticles were significantly more toxic than aluminum oxide nanoparticles while molybdenum nanoparticles had no deleterious effect on these stem cells (Fig. 13.6).

13.5.2.1 Toxicity of Zinc Oxide Nanoparticles—Deng and colleagues were among the first to test the effect of ZnO nanoparticles on NSCs in vitro [165]. They showed that at low concentrations (<12 ppm) ZnO nanoparticles had no effect on cell viability, but that apoptosis and necrosis increased at concentrations above 12 ppm. They also concluded that free Zn^{2+} ions were responsible for high doses effects. While scientists generally agree that ZnO nanoparticles are neither toxic to differentiated cells nor to stem cells, it was recently demonstrated that their in vitro effects depend on the number and type of cells seeded [166, 167]. For example, Taccola and colleagues provided evidence that ZnO nanoparticles selectively kill rapidly proliferating cells such as MSCs, but have no effect on the cells once they are differentiated along the osteogenic pathway [167]. Similarly ZnO nanoparticles seem to selectively kill cancer cells, possibly through ROS production leading to cell apoptosis, and is now used for certain therapies [168].

13.5.2.2 Toxicity of Aluminum Oxide Nanoparticles—Aluminum oxide nanoparticles (ANPs) are widely used, and several studies have shown that ANPs negatively affect cellular morphology and cellular components. ANPs induce oxidative stress, which leads to apoptosis, DNA damage and protein degradation in vitro, while their ingestion affects neurobehavioral patterns [169–171]. However, few studies have tested their toxicity

specifically in stem cells. Only the recent investigation of Alshatwi and colleagues with MSCs indicated that ANPs might induce changes in cell morphology, decrease in cell viability and upregulation of apoptosis pathways. These effects were concentration-dependent but the physiological relevance of the doses used was not discussed [172].

13.5.2.3 Toxicity of Silver Nanoparticles—The anti-bacterial effects of silver have been known and exploited for centuries, in particular for the treatment of wounds and burns [173]. Silver is used as an antibacterial surface coating on medical devices such as stents, catheters and heart valves, and is non-toxic to human and animals in its bulk chemical form [174]. However increasing amounts of silver nanomaterials, in particular silver nanoparticles, are now finding their way into consumer products as additives to sprays, as surfactants for textiles and food containers, and as drinking water disinfectants to name a few applications. This trend is concerning because many studies have now demonstrated that the toxicity of nanosilver is much greater than that of most carbon-based and metal-based nanomaterials [143]. The cellular toxic effects of silver nanoparticles are size-dependent, and increase as the particle size decreases [175, 176]. They include reduction of mitochondrial function, increase of membrane leakage and increase of production of reactive oxygen species (ROS) [164]. They also can deplete cellular glutathione, an antioxidant [177]. As mentioned before, ROS cause DNA damages such as base oxidations, base changes or single and double-strand breaks that can lead to cell death or to mutations causing cancers. Recent studies in ES cells demonstrated that silver nanoparticles up-regulated expression and phosphorylation of the protein p53, induced DNA double-strand breaks, decreased cellular metabolism and promoted apoptosis [178]. In this work, silver nanoparticles with different surface chemistries were used: uncoated nanoparticles and particles coated with a polysaccharide to ensure biocompatibility and dispersion. Interestingly, in ES cells the coated silver nanoparticles produced more damages than the uncoated nanoparticles, but the cause remains unclear. Our group also recently investigated some of the mechanisms causing the toxic effects of silver nanoparticles in germ line stem cells [176]. Silver nanoparticles were hydrocarbon or polysaccharide-coated to study the influence of surface chemistry. We demonstrated that at low doses ($<10 \mu\text{g/ml}$), silver nanoparticles interfered with the glial cell line-derived neurotrophic factor (GDNF) signaling pathway, which is crucial for germ line stem cell self-renewal. More precisely, silver nanoparticles inhibited the phosphorylation of the membrane-associated FYN kinase downstream of the RET (REarranged during Transfection) transmembrane receptor after activation by GDNF. Silver nanoparticles did not interfere with GDNF binding to its receptor nor with receptor phosphorylation. Further, we demonstrated that silver nanoparticles reduced germ line stem cell viability and proliferation in a size- and concentration-dependent manner and that particle coating had no influence on the magnitude of the toxic effects. The effect of silver nanoparticles has since been tested on human MSCs [179]. When exposed to the dose of $10 \mu\text{g/ml}$, these cells experienced DNA damage and their viability decreased, as shown for other stem cell types, but their ability to migrate was not impaired. However, Samberg and colleagues recently demonstrated that silver nanoparticles exert minimal toxicity to human adipose tissue-derived stem cells (ADSCs) and do not impair their differentiation [180]. Therefore, for the same sizes and

concentrations, stem cell effects of silver nanoparticles might be cell type-dependent, with germ line stem cells particularly sensitive to this potential toxicant.

Conclusion

Nanomaterials have found many applications in stem cell research including tracking, delivering, and controlling the fate of these cells for tissue engineering. These applications have already started to transform several areas of cell biology and medicine. However, strategies allowing large-scale stem cell expansion before their differentiation are still lacking. In addition, the creation of complex tissues is still in infancy and will need the ability to direct the growth and differentiation of different cell types in three dimensions within biocompatible scaffolds. Furthermore, toxicity of certain nanoscaffolds such as MWNTs is still a concern since coatings that ensure their biocompatibility might not be stable within the tissue to be repaired. Finally, as the demand for nanomaterials is increasing, potential inhalation during manufacturing or packing, and their accumulation in the environment should be carefully monitored to ensure human safety.

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Fig. 13.1. Differentiation potential of mesenchymal stem cells (MSCs). MSC can differentiate in many tissues including bone, cartilage, adipocytes, cardiac cells and neurons (Adapted from Meregalli et al. [18])

Fig. 13.2.

Long-term monitoring of SPION-labeled mesenchymal stem cells (MSCs). Rat brain striata were injected bilaterally with quinolinic acid to induce neuronal death. After 1 h, SPION-labeled MSCs were injected into one side at the same location as quinolinic acid, while the other side received only saline. Hypointense signals (*black spots*) indicated the presence of MSCs in all analyzed periods. Seven days after cell injection, the black spots were also observed in the contralateral injured and non-transplanted striatum, and persisted for at least 60 days after lesion (DAL) and MSC injection (From Moraes et al. [45])

Fig. 13.3.

In vivo optical imaging of mouse hind limb after inoculation with unlabeled tumor cells or gadolinium-rhodamine nanoparticle-labeled tumor cells. The picture shows a female mouse bearing unlabeled tumor (left limb) and Gd-Rd-NP-labeled tumor (right limb) imaged 7 days post subcutaneous inoculation (1×10^6 cells in each limb). The fluorescent signal is localized only to the labeled tumor site (From Vuu et al. [60])

Fig. 13.4.

Simultaneous imaging of endothelial cells and mesenchymal stem cells (MSCs). Two contrast agents, iron oxide nanoparticles and gadolinium oxide nanoparticles were used to label endothelial cells and MSCs respectively. No impact on cell function, including their capacity for differentiation, was detected. The labeled cells were seeded together in a polysaccharide-based scaffold and visualized simultaneously by MRI. Labeled endothelial cells appeared in black while MSCs appeared in white in vitro and in vivo (From Di Corato et al. [65])

Composition	<u>Natural</u> <ul style="list-style-type: none"> • Collagen • Chitosan • Silk • Alginate 	<u>Synthetic</u> <ul style="list-style-type: none"> • PCL • PLLA • PLGA • PHBV 	<u>Blended</u> <ul style="list-style-type: none"> • Natural+Synthetic • Natural+Synthetic+Coating
Morphology	Solid	Porous	Core-shell
Assembly	Random	Aligned	Layered
	Yarn	Hollow yarn	Fiber bundle

Fig. 13.5.
Topography of different electrospun nanofibrous structures (From Reddy et al. [103])

Fig. 13.6.

Toxic effects of metal-based nanoparticles. The effect of silver and molybdenum nanoparticles on the metabolic activity of the C18–4 spermatogonial stem cells was evaluated after 48 h incubation. At the end of the incubation period, mitochondrial function was determined by the MTS reduction assay. (a) MTS reduction in presence of different concentrations of cadmium chloride and cadmium oxide. Cadmium chloride is a known toxicant and is used here as a positive control. (b) MTS reduction in presence of different concentrations of silver carbonate and silver nanoparticles (Ag—15 nm). (c) MTS reduction in presence of different concentrations of sodium molybdate and molybdenum nanoparticles. Overall silver nanoparticles were as toxic as cadmium chloride. Molybdenum nanoparticles were toxic only a high concentrations (From Braydich-Stolle et al. [164])