Biomedicine & Pharmacotherapy 137 (2021) 111236

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Nanotechnology shaping stem cell therapy: Recent advances, application, challenges, and future outlook

Yongqiang Dong [a,1], Xudong Wu [c,1], Xuerong Chen [b], Ping Zhou [b], Fangming Xu [c], Wenqing Liang [c,*]

[a] Department of Orthopaedics, Xinchang People's Hospital, Shaoxing, 312500, Zhejiang Province, PR China
[b] Department of Orthopaedics, Shaoxing People's Hospital, Shaoxing Hospital, Zhejiang University School of Medicine, 568# Zhongxing North Road, Shaoxing, 312000, Zhejiang Province, PR China
[c] Department of Orthopaedics, Zhoushan Hospital of Traditional Chinese Medicine Affiliated to Zhejiang Chinese Medical University, Zhoushan, 316000, Zhejiang Province, PR China

ARTICLE INFO

Keywords:
Nanotechnology
Stem cell
Cancer
Immunotherapy

ABSTRACT

Currently, stem cell nanotechnology is one of the novel and exciting fields. Certain experimental studies conducted on the interaction of stem cells with nanostructures or nanomaterials have made significant progress. The significance of nanostructures, nanotechnology, and nanomaterials in the development of stem cell-based therapies for degenerative diseases and injuries has been well established. Specifically, the structure and properties of nanomaterials affecting the propagation and differentiation of stem cells have become a new interdisciplinary frontier in material science and regeneration medicines. In the current review, we highlight the recent major progress in this field, explore the application prospects, and discuss the issues, approaches, and challenges, to improve the applications of nanotechnology in the research and development of stem cells.

1. Introduction

Nanotechnology and stem cell sciences are two of the contemporary and most prominent areas of research with major contributions towards the improvement of human health. While stem cells (SC) contain significant prospects for rejuvenating medicinal moieties, however, their applications have been restricted by the lack of effective ways to monitor the differentiation and long duration of engrafted cells or tissues *in-vivo* [1]. It is claimed that by integrating the two exciting fields of study, nanotechnology, and SC, our knowledge about the differentiation of SCs regulation will significantly advance, which in turn will potentially lead to SC-based treatment strategies for better understanding of the human disease, and their prevention, and treatment [2].

Nanotechnology-based methods have been developed by utilizing non-toxic and biodegradable nano-holds/ nano-fibers such collagen nanofiber [3], carbon nanofiber [4,5], graphene-oxide nanoparticles (GO-NPs) [6], poly-ε-caprolactone [7], Tri-$CaPSO_4$ (tricalcium phosphate) [8], tri-Ca-silicate [9] and auto-assembled peptide, for the stem cells differentiation and regeneration therapy [10]. Superparamagnetic-iron-oxide (Fe_3O_4) NPs could be applied for the labeling of grafted cells and studied by magnetic-resonance-imaging (MRI) [11]. Polymer-based scaffolds like heparin-hydroxyl-apatite chitosan NPs (CNPs), PLGA–nano-hydroxyapatite, and chitosan-based imageable NPs have significantly helped in differentiation, tagging, and monitoring of different kinds of SC, mainly hMSCs [12]. Another new technique in stem cell nanotechnology is nano-patterning. In the absence of specific media or chemical checks, nanopatterned coats or forms may be employed to the better direct attachment, spreading, auto-renewal, and led differentiation of pluripotent stem cells [13].

Studies have demonstrated that therapeutic cells are predicting transmitters in the active directed drug delivery [14]. Drug-NPs coats of therapeutic cells provide novel avenues in SC therapies to enhance the clinical efficacy of the transferred cells. In any SC therapies, we should be capable to study the delivery of mobes (cells) and monitor their distribution to their biological targets. Hence, it is significant to formulate NPs with special surface applications to ameliorate uptake and long-run monitoring of stem cells without involving their increment [15].

* Corresponding author.
E-mail addresses: farmxu@126.com (F. Xu), liangwq@usx.edu.cn (W. Liang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2021.111236
Received 29 October 2020; Received in revised form 29 December 2020; Accepted 31 December 2020
Available online 21 January 2021
0753-3322/© 2021 The Author(s). Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2. Nanotechnology application in cells isolation, purification, and differentiation

Cell isolation is crucial in stem cell-based therapies. Magnetic cell isolation is a widely used method to feed stem cells from a blended cell population [16]. Magnetic nanoparticles (MNPs) can label stem cells and the targeted cell types could be distinguished from a multi-cell mixture (magnetic-activated cell sorting (MACS) [17]. This procedure is carried out by mixing MNPs with monoclonal antibodies (MAB) against peculiar cell surface antigens, leading to the retention of the magnetic field of the identified cells expressing these antigens. It has been shown that MNPs, combined with anti-CD34 antibodies, can effectively mark and separate periphery blood progenitor cells from the whole blood of humans [18]. When MNP-conjugated anti-CD34 proteins to label CD34-cells were applied, an uninterrupted quadrupole magnetic flow sorter comprising a flowing carrier and a quadrupole magnet with 1.42 T maximum field loudness and optimum field strength were able to separate these cells from mononuclear cells suspension of whole blood. The collected CD34-cells were of 60–96 % purity, 18–60 % retrieval, 12–169 improvement rate, and a throughput of (1.7–9.3) 104 cells/s [18]. The purified cells with further optimization might be used for regenerative therapy in cell transplantation.

The use of scaffolds-dependent nanomaterials a well as their polymers for SC proliferation and differentiation has been of considerable importance. To regulate the differentiation of SC, various scaffolds have been examined to focus on nanotubes, NPs, and nanofibers. Given their exceptional mechanical ability, titanium dioxide (TiO2) and carbon nanotubes (CNTs) exhibit reasonable options for scaffold construction (such as bone replacement therapy) [19].

The SC fate is influenced by the shape of the biological molecules, and complex interactions with scaffold compounds. While numerous scaffolds based on nanomaterials have been utilized in tissue engineering applications, due to their unusual electrical, mechanical, and refractive indices and broad surface topographical features, this segment is primarily committed to graphene and graphene oxide (GO) as nontoxic scaffolds [20,21]. Table 1 enlists the various nanomaterials used for incorporating stems and their cell differentiation ability.

Table 1
Various nanomaterials used for stem cells and their differentiation ability. hMSC; human mesenchymal stem cells, MSC; mesenchymal stem cells, hNSCs; human neural stem cells, NSCs; neural stem cells, ES; embryonic stem cells.

S/No	Nanomaterial	Stem cells	Differentiation ability	References
1	Graphene oxide/ gelatin-hydroxyapatite		Increased osteogenic differentiation	[22]
2	Graphene surfaced Si/Sio2		Controlled and Speeded up the osteogenic differentiation	[23]
3	Gelatin immobilized poly (L-lactide-co-caprolactone)	hMSC	Increased osteogenic differentiation	[24]
4	PCL/PLA-3D scaffold		Elevated osteogenic differentiation	[25]
5	Monolayer graphene		Governed the growth and neural differentiation	[26]
6	Graphene/ grapheme oxide	MSC	Increased Osteogenic differentiation	[27]
7	3D-graphene scaffold	NSCs	supported neurospheres shaping and facilitated cell migration	[28]
8	Laminin-coated-graphene film		Increased differentiation into neurons	[29]
9	Graphene-oxide-pattemed substrate	hNSC	Improvement in integrin clustering, focal adhesion, and neuronal differentiation	[30]
10	Gold NP-coated collagen-nanofiber	Placental derived MSCs	increased neuronal differentiation and proliferation	[31]
11	Nanopore patterned (NPo) polystyrene (PS) surfaces	Human ES cells	Distinction into endodermal cells	[32]

3. Nanotechnology application in cell imaging and tracking

Cell-based therapies are currently alternative therapies to treat a variety of diseases and infections, where the conventional therapies failed. However, understanding the cell cycle, cell differentiation, and potential of cell engraftment is crucial to design promising cell-based therapies [33]. For instance, we need imaging techniques to track the fate of transplanted cells. Different methods have been developed to achieve these targets. Magnetic resonance imaging (MRI), photoacoustic imaging, fluorescent imaging, and radioactive cell imaging are widely used imaging methods for cell imaging [34,35].

The commonly used NPs in live-cell imaging are Quantum dots [36], MNP [37], and gold nanorods [38]. These nanoparticles have been used to find and map transplanted cells, and to enhance the effectiveness of imaging techniques. Till, nanoparticles like QDs, gold nanorods and MNPs can be applied to get imaging and monitoring of SCs (Fig. 1) [39]. As a representative, the QDs have also been further investigated because of their special dimensions and potential application aspects and have been successfully utilized in cellular imaging, optical barcoding, and immunoassays, and DNA hybridization. QDs provide a new operational platform for bio-analytical research and biomedical technology.

For visualization and chasing of stem cells, NPs like QDs, MNPs, and nanorods (gold) could be utilized [40,41]. Owing to their special properties and possible applications, QDs have been further investigated. DNA hybridization [42], immunoassays [42], optical barcoding, and Cellular imaging [43] have used QDs [44] successfully. In bioanalytical-sciences and biomedical technology, QDs provide a new functional framework. Ohyabu et al. [40] reported that QDs were linked covalently with an anti-mortalin peptide antibody (AB), preceding the development of the composite I-materials which is susceptible to be internalized by mesenchymal stem cells (MSCs) and tagged as MSC cells. *In-vitro* and *in-vivo*, the I-labelled MSCs got natural osteocyte, adipocyte, and chondrocyte differentiation, strongly indicating the I-labelled MSCs can be used for *in-vivo* diagnostic visualization and stem cell monitoring in rodent's delivery. QDs can be engineered as multidimensional nanoprobes capable to be altered by various biomolecules, including PEGs, liposomes, antibodies, peptides, having their special properties. Thus, they and can be utilized for molecular tracing, drug or gene delivery, and molecular imaging [45]

In addition to QDs, MNPs have also been used for the molecular imaging and tracing of SCs [46]. It has been reported that superparamagnetic iron-oxide NPs (SPIO) are capable of SC tagging, MRI, and tracking of transplanted SC [47]. For instance, dextran-covered iron--oxide nanoparticles were covalently linked with fluorescent molecules to characterize the labeling of Hematopoietic Stem Cells (HSCs) and track the phase of engraftment [48]. Fluorophores conjugation with dextran coating for purification of fluorescence-activated cell sorting reduced background of non-sequestered nanoparticles. A short-term specified incubation scheme was acquired which permitted both to be effectively labeled and of both cycling and quiescent HSCs, with no adverse event [49]. The transplantation of primary human cord-blood lineage-depleted and CD34+cells into immunodeficient mice made it possible to diagnose branded human-HSCs in the host tissues. Flow cytometry has been used for the measurement of cell populations that isolated the NPs specifically and used to track their post-transplantation

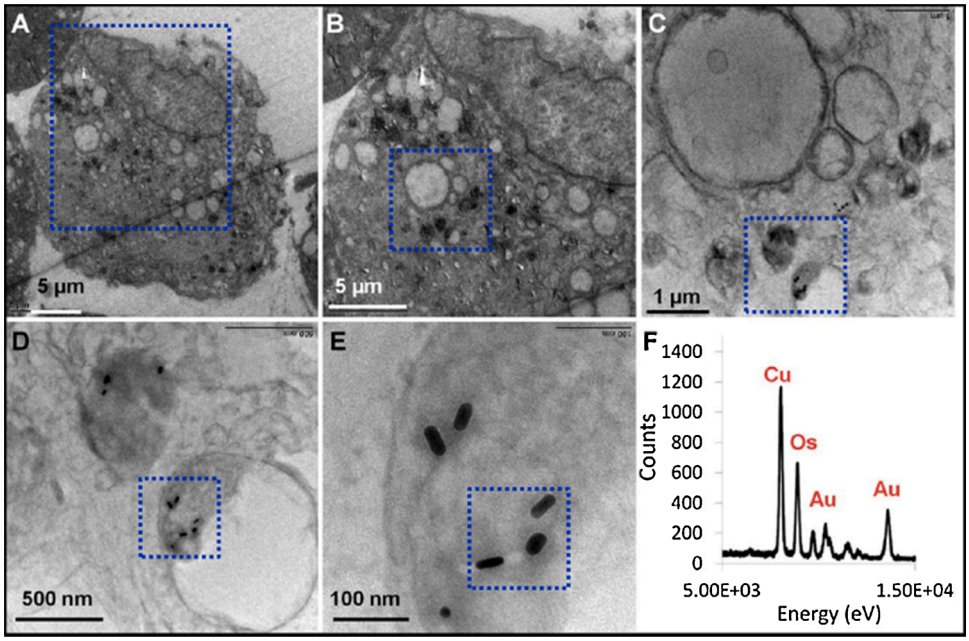


Fig. 1. Confirmation of Gold nanorods inside MSCs. (A–E) TEM images of MSCs loaded with Gold nanorods were collected at increasing magnifications. Panel F is a portion of the EDS spectra acquired on panel E (dashed inset) that verifies the presence of gold.
Adapted with permission from [39]. Copyright (2012) American Chemical Society.

destiny. The existence of MNPs-marked human SCs in marrow was confirmed by flow cytometry endpoint review [50]. The utilization of SCs-therapy has been thoroughly investigated in numerous central nervous system (CNS) diseases. In a stroke experimental model, rats were engrafted by GFP + MSCs labeled with Superparamagnetic iron oxide nanoparticles (Endorem) [50]. They intracerebrally grafted the cells into the contralateral (opposite side) hemisphere of the injury (lesion), or IV into the femoral vein, and continuously monitored the Rodents (grafted SCs) using a 4.7-T Bruker spectrometer for 3–7 weeks after transplantation. The lesion was noticeable as a hyper-intense signal on MR images. A hypointense signal was observed in the weak lesion after grafting, which increased during the 2nd and 3rd weeks, irrespective of the administration route. Prussian blue staining or GFP marking correlates to its severity. MSCs labeled with Superparamagnetic iron oxide nanoparticles (Endorem) were infused IV into the femoral vein later a week of transverse spinal-cord injury [46,51].

The lesion cavity was seen by MR photographs of longitudinal spinal cord parts from wounded non-grafted animals as a heterogeneous tissue with a clear hyper-intensive signal. The lesion of the grafted model was checked as shaded shadows of hypo intensity. Just a few other iron-containing cells in wounded control animals were confirmed by histological test, but high iron positivity in grafted animals was confirmed. In grafted specimens, lesions occupied by grafted MSCs were considerably smaller compared to control rats, indicating a beneficial impact of MSCs on lesion repair [52]. There are many effective MR tracking applications in other organs, including heart [53], liver [54], kidney [55], etc. Surface adjustment of MNPs with D-mannose (Dm), poly-L-lysine (PLL), or poly-dimethyl-acrylamide (PDMAAm) has been reported to have resulted in improved labeling performance as compared to SPIO coated with dextran. A previous study also found that fluorescent MNPs (FMNPs) could conjugate with anti-BARCA1 antibody, and formed brcaa1 antibody-labeled FMMNP probes. It was also observed that BRCAA1 protein exhibited over-expression in an embryonic stem cell line (Cellosaursus; CCE cells) [56].

4. Nanoparticle application in gene delivery systems for stem cells

Previously numerous studies have reported the therapeutic uses of embryonic SCs (ESCs) for the therapy of crippling inherited, painful and degenerative disorders, and the development of progenitor cells has been reported with *in-vivo* reconstitution properties [57]. Non-invasive efficient imaging of grafted cells to control biodistribution (*in-vivo* tracking) is a significant obstacle to the therapeutic applications of these pluripotent cells. Besides, reproducible strategies should be established that allow efficient intracellular distribution of biomolecules necessary to regulate ES cell differentiation, including RNA, DNA, peptides, and proteins.

Physical techniques like nucleofection and electroporation give the benefit of high performance in transmission, but also inflict significant harm to ES cells [58]. *In-vitro* results with viral vectors, considering retro-lenti- and adenoviruses showed the effective transfection and reproducible handling of ESC differentiation [44]. The chance of toxicity accelerated mutagenesis, and immunogenicity, however, greatly reduces the therapeutic feasibility for the clinical field of these viral vectors. Consequently, as being the most exciting nanotechnology medium, non-viral vectors including liposomes and polymeric NPs are presently explored to transform promising laboratory results with ESCs into real-time clinical applications [59,60]. No. 5 generations polyamidamine dendrimer-functionalized fluorescent multi-walled nanotubes of carbon (dMNTs-C) is highly effective in penetrating the CCE embryonic stem cell line in mice [61]. When the incubation time increases, it can reduce cell proliferation in a dose- and time-dependent manner and less than 5 μg/ mL dose will boost the distinction of embryonic stem cells. A dose of more than 20 μg/ ml will cause embryonic stem cells to get narrower. Dendrimers, a new and unique type of organic molecules, through a sequence of chemical changes, can take various functional groups, and their inner body cavities provide depot facilities for several genes and drugs [62].

Dendrimers could be a successful non-viral transmission vector as, compared to viral vectors which are more unsafe for therapeutic use, they have the benefits of ease of application and bulk production. Dendrimer-modified MNPs of polyamidoamine (PAMAM) have been

reported to dramatically increase the gene delivery efficacy [63,64]. The dMNTs could be an extremely expeditious form of gene transmission for ESCs and may have possible applications in ES science. NPs like MNPs [46] and QDs are capable to enter into the human MSC cells, and can sustain within ES cells for a longer time. Previously, it has been reported that SiO2 coated CdTe nanoparticles could join murine stem cells (MSCs) and sustain inside these induced- differentiated cells of the neurons, hematopoietic cells, and endothelial cells, showing no cytotoxicity within the concentration used. It can be easily demonstrated that teratomas consisting of tissues of all three primary germ strata were produced by such grafted stem cells with MNPs [47]. Recently, a biological delivery method to transfer genes into living cells was developed using nanoneedle and atomic force microscopy (AFM) [65]. A less-invasive method of gene delivery using etched AFM tip or nanoneedle for nucleus injection without inducing cellular injuries was identified by Han et al. The nano-needle had a 200 nm diameter with 6 μm length and was operated by utilizing an AFM device. The likelihood of nanoneedle can incorporate human MSCs and human embryonic kidney cells (HEK293) were greater than that of the capillaries used for microinjection. On a poly-L-lysine-altered nanoneedle base, inserted into the single human MSCs (primary cultured), a plasmid containing the green fluorescent protein (GFP) gene was observed. In human MSCs, over 70 % of effective gene delivery was achieved, which contrasted more favorably with other methods of non-viral gene delivery (lipofection ~50 % and microinjection ~10 %).

5. Nanopatterns to drive the fate of stem cells into a specific cell lineage and their application in tissue engineering

Different roles of SC including migration, adhesion, and propagation are strongly associated with niches surrounding them that are instructive and tissue-specific [66]. SCs are particularly susceptible to the composition of extracellular matrix (ECM) elements consisting of nanoscale feature-sized fibrillary collagens, elastin, and glycosaminoglycans [67]. ECM part structure and topography can force stem cells to differentiate into unique cell lineages [68].

In stem cell-based treatments, the key step is the pushing of SCs in a specific direction with more precision and production. Various artificial nanoplatforms imitating the topological features of the natural SCs niche have been established by nanotechnologists to trigger stem cell activity [69]. A core principle of the mechanism through which stem cells interpret and react to nanotopographical signals is the attachment of SCs surface proteins to topography [70]. Integrin-mediated cell attachment to ECM pieces known as focal-adhesion, playing a key role in stem cell control. Mechanical stimulation modulating focal-adhesions accompanied by a sequence of events will result in the changes in the level of genes and proteins and influence the stem cell differentiation program [71]. Cytoskeleton (CSK) stress, SC structure, and nuclear dynamics also affect the SCs state in addition to integrin-mediated adhesion signaling [72]. A key peptide episode in ECM proteins that mediates cell adhesion is arginine-glycine-aspartate (RGD). Recent research has focused on the impact on stem cell activity of RGD containing nanopatterns [73].

Cao et al. designed the synthesis of a series of charged or neutral oligopeptide motifs coupled with RGD and were used for surface modification using quartz substrates as a model. They demonstrated that the positively charged oligopeptide motif can inhibit osteogenic differentiation, whereas negatively charged and neutral oligopeptide patterns may facilitate osteogenic differentiation in the presence of RGD (Fig. 2) [74].

The impact of RGD nano-spacings from 37 to 124 nm on the conduct of MSC was explored by Wang et al. [75]. Nanopatterns of RGD were formulated on PEG hydrogels. Cells were exposed to these nanopatterns for 8 days at a maximum serum level. They differentiated SCs into adipogenic lineages and osteogenic with large and tiny nano-spacings [75]. Symmetry, scale, and regularity of surface nano-topographic attributes have proved to possess a significant effect, thus these variables influence stem cell activity [76]. Park et al. [77] demonstrated that MSC activity is highly reliant on the diameter (d) of self-assembled layers (SAL) of TiO2 nanotubes, includes differentiation, development, and spreading. They separated SCs into osteogenic cells through a tube having a 15 nm diameter and observed that osteogenic differentiation of MSC can be greatly decreased by raising the diameter of the tube to 50 nm or above [77]. The impacts on SCs self-renewal, differentiation, and proliferation potential of nanogrooves with different pitches have been investigated [78].

At present, the fusion of SC nanotechnology (SC-NTech) and tissue

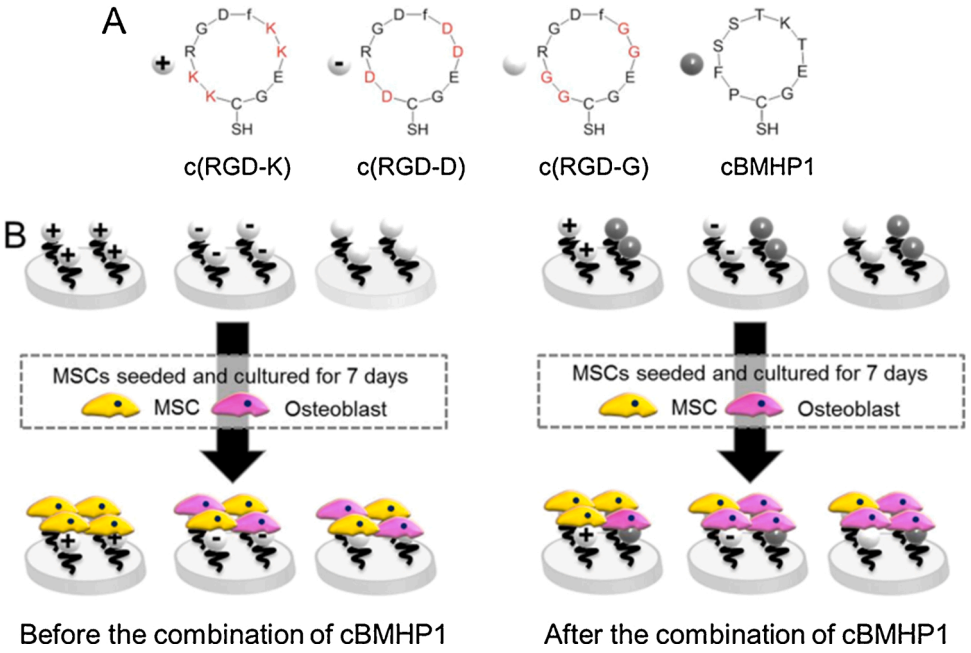


Fig. 2. (A) Chemical Structures of the Synthesized Cyclic Peptides Containing Both Different Charged Oligopeptide Motifs and RGD; and (B) Schematic Representation of the Different Peptides Tethered to the Substrates and Their Effects on Osteogenic Differentiation of MSCs.
Adopted with permission from [74]. Copyright (2015) American Chemical Society.

engineering concepts is an important area of study. Nanoengineered 3-D scaffolds are being commonly utilized to feasible the fate of SCs into the particular lineages of cells. These 3D scaffolds could be biodegradable, where cells could create their own ECM and decaying the artificial scaffold [79]. For instance, relative to the controls, the differentiation of SCs into osteogenic cells was significantly elevated utilizing nanofibrous-scaffolds in bone tissue engineering [80].

6. Application of nanotechnology in stem-cell-based therapy of neurodegenerative diseases

Neurodegenerative diseases (ND) are characterized by gradual degradation of neuron formation or activity arising from the central nervous system (CNS), degeneration of chosen neurons. Neurological disorders affect patients, their families, and society as a whole in terms of economic and social ways. There are no specific treatment strategies for neurodegenerative disorders, and only symptoms can be decreased or the progression of the condition can be slowed down by the commonly used drugs [81]. Effective treatment formulation for a patient group has to be closely examined by working with doctors, neuroscientists, and bioengineers to address all the conditions and the therapeutic requirements [82]. The understanding of neurogenesis has been established during recent cascades, and with the finding of the existence of adult neurons, the detection of the complex proliferation of progenitor cells, and new neuron production, the conventional definition of a stagnant brain has been dramatically altered [83]. Stem cells that can be generated from several sources can be self-renewed and differentiated from various types of cells and are ideal candidates for cell therapy [84].

In the CNS, the key aim of cell-based regenerative therapy (RT) is to enhance the protection of neurons, compensate for cell operation failure, and boost tissue regeneration capacity. Cell treatment for CNS requires the infusion of cells into the affected brain tissue to recover the loss of activity of the neurons [85]. The utilization of SCs in cell therapy (CT) for neurodegenerative disorders has drawn significant attention from scientists in recent years [86]. In several neurological disorders or brain trauma, SCs can cause neuroprotection [87]. The good impressions of SCs transplantation on the enhancement of sensory/motor and cognitive stroke functions, Alzheimer's disease (AD), Huntington's disease, Parkinson's disease (PD), spinal muscular atrophy, and amyotrophic lateral sclerosis (ALS) have been documented in many reports [81,88]. Thanks to the advancement of biomedical innovations and breakthrough new strategies like cell therapy for the treatment of degenerative disorders, the quality of life can be improved. There is presently no stem-cell-based treatment, considering positive findings in preclinical studies. Besides, because of their protection and ethical concerns, the use of stem cells on a therapeutic scale is limited. Tumorigenesis, SCs metastasis, undesired development, sequestration of essential organs, irreversibility of therapy, and long-term survival of transplanted cells are some of these issues [89]. Combining stem-cell therapy with other therapies could be a chairing option for overcoming some of these problems [89,90].

Due to the special properties of nanomaterials [91], nanotechnology can augment SC therapy and increase the performance of cell-based therapy. Also, the convergence of such technologies will create a new interdisciplinary area with an emphasis on intensive research [89]. Inside the stem cell niche, nanomaterials (NMs) and NPs can associate with proneurogenic factors and thus stimulate proliferation self-renewal, and differentiation of endogenous and exogenous neural stem cells (NSCs) [92]. Besides, usable peptide-labeled super--paramagnetic NPs can be injected intravenously into the wounded region and greatly traced by MRI techniques [93]. The internalized modified-NPs are capable to stimulate neurogenesis successfully and seem like a potential method in the treatment of neurodegenerative disorders for medicinal purposes and DDS (drug delivery system). Here, we will discuss the latest developments of SCT and the use of cell-based approaches to improve the efficacy of the treatments used for neurological disease. The relationship between stem cells and NPs remains obscure and, thus, requires further study, aimed at several attempts in the field of nanotechnology to increase the efficacy of SCT to prevent neuroregeneration. Fig. 3 highlights the various strategies and innovation for a better outcome while treating a neurodegenerative disorder

7. Nanomedicine in cancer stem cell therapy

Many traditional ways of cancer treatment, like chemotherapy and radiotherapy, are based on tumor cell removal, but not on cancer stem cells (CSC). Destroying CSC, nevertheless, will guarantee long-lasting remission of diseases, reduce metastases, and dramatically boost patients ' health status. CSC therapies are controlled by nanotechnology having the ability to exploit CSC and deliver them with therapeutic payloads (TPL) [95]. In this regard, numerous nanoparticles were engineered to attack the CD44 overexpressed CSC, such as all-transretinoic acid entrapped albumin nanoparticles surface coated with hyaluronic acid. Results showed that the hyaluronic acid content on the surface can lead to unique bonding and are attracted by B16F10 cells enriched with CD44, thus, these NPs are useful for the selective delivery of CSC suppression antitumor drugs [96].

Nucleus-targeted drug delivery (NTDD) for reversing CSC's drug resistance is another successful approach. A silica nanoparticles based system was engineered and was reported in a recent study to attack the nucleus of CSC directly. By surface modulation of anti-CD133 and thermal sensitive exposure of TAT peptides in the presence of opposing magnetic fields, they reached the nucleus. Results suggest that successful nucleus-targeted drug release eventually contributes to CSC apoptosis caused by combination chemotherapy and thermotherapy with hypoxia-activated [97].

8. Nanoparticles as macromolecular delivery systems for stem cells

In stem cell therapeutics, a big challenge is the discovery of an effective way to regulate their proliferation and differentiation. Various biomolecules have the capability of regulating SCs division, such as RNA, DNA, proteins, or peptides. To be functional, however, these macromolecules must be distributed effectively into the SCs. With several drawbacks, conventional methods of macromolecular delivery are usually not successful. Physical approaches can introduce biomolecules effectively through the cells, such as electroporation and nucleofection, but these procedures inflict harm to the cells. It is also possible to link different kinds of viral vectors to transfection agents. These vectors, however, possess the risk of toxicity, mutagenesis, and immunogenic responses. Agents used for chemical transfection often suffer from a lack of effectiveness and a low toxicity profile. On the other hand, nanoparticle delivery mechanisms have many benefits. These carriers have not only a suitable nature for biomolecules but also have the potential to undergo surface modification with targeting moieties. This technique will help to distribute the shipment to the desired cells and minimize susceptible toxicities or unexpected effects.

The NPs can be constructed from biocompatible, biodegradable, and safe polymeric materials. As carriers, NPs have been prepared with different shapes, designs, and construction blocks to deliver small and large biomolecules to SCs. Mouse embryonic fibroblasts were reprogrammed to pluripotency by Zhu et al. Using a common plasmid build carrying OSKM (pOSKM), an arginine-terminated polyamidoamine nanoparticle-based nonviral gene delivery system was created [98].

These experiments indicate that nanoparticles not only contribute to the effective generation of induced pluripotent cells but also greater efficiency of transfection than traditional transfection agents. Sohn et al. have also been experimenting with an acid-sensitive polyketal (PK3)-based nanoparticle device on the activation of pluripotency within the bone marrow mononuclear cells. This structure was transmitted to

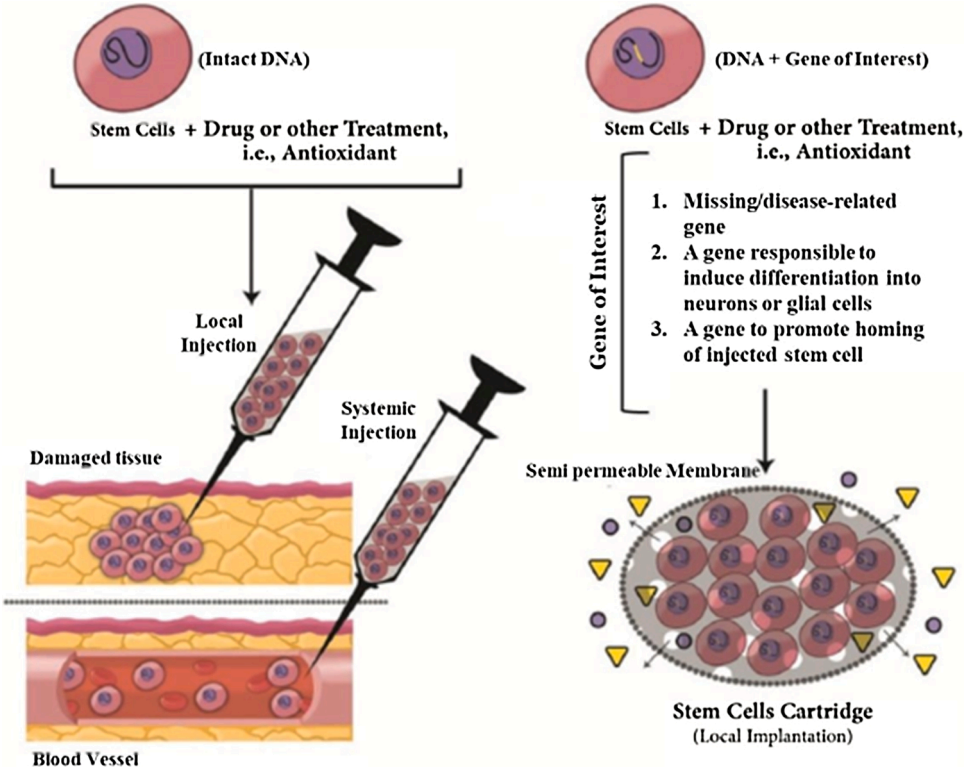


Fig. 3. Strategies and innovation for a better outcome while treating a neurodegenerative disorder.
Reproduced from [94].

miRNAs unique to mature embryonic stem cells [99]. The findings showed that without irreversible genetic modification, a polyketal-miRNA delivery mechanism would successfully produce multiple reprogrammed cells. Mesoporous silica nanoparticles are further utilized for nonviral cell marking and as differentiation agents for induced pluripotent stem cells because of their multifunctional properties. The efficiency and feasibility of FITC-conjugated mesoporous silica nanoparticles for labeling induced pluripotent stem cells were evaluated by Chen et al. [100], as shown in Fig. 4. The investigators also demonstrated that FITC-conjugated MSNPs with

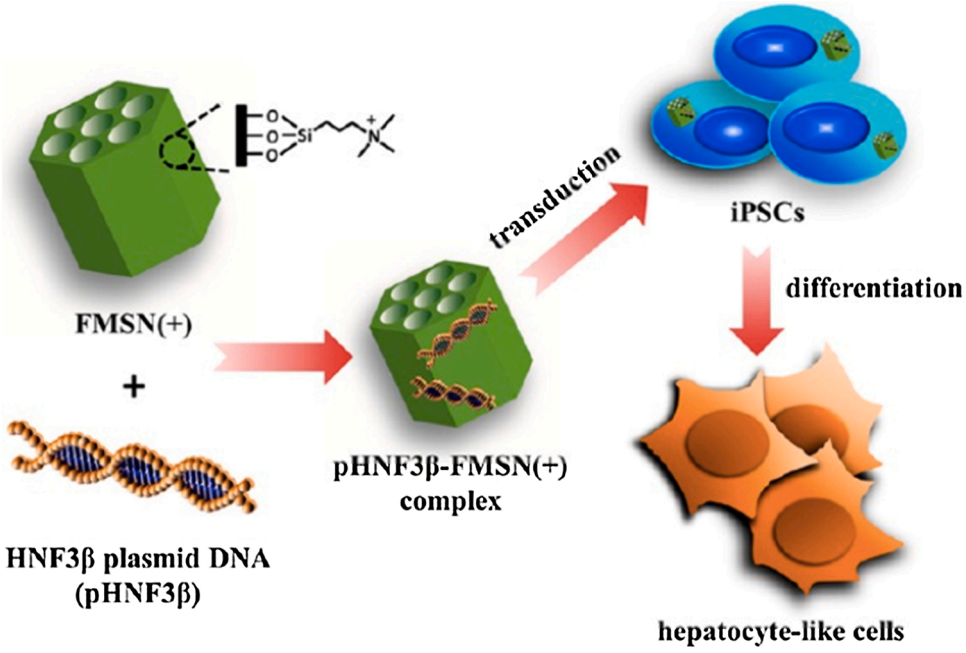


Fig. 4. HNF3β plasmid DNA (pHNF3β) was adsorbed on the positive-charged FMSN (FMSN(þ)) to form a pHNF3β- FMSN(þ) complex, which was internalized by iPSCs. The treated iPSCs exhibited significantly improved definitive endoderm formation and further quickly differentiated into hepatocyte-like cells with mature functions (low-densitylipoprotein uptake and glycogen storage) within 2 weeks in vitro.
Adopted with permission from [100]. Copyright (2013) American Chemical Society.

different surface charges were internalized effectively with limited cytotoxicity by the induced pluripotent stem cells. To deliver hepatocyte nuclear factor 3b into a plasmid, cationic nanoparticles were picked. They increased the level of mRNA expression in stem cells with liver-specific genes, quickly differentiating them into hepatocyte-like cells with matured functions.

9. Biosafety profiles of nanotechnology and stem cells therapy

The use of nanomaterials for the differentiation of stem cells is regulated primarily by three means (nanoparticle suspension, 2D culture, 3D culture). In addition to the intrinsic capacity to facilitate the differentiation of stem cells, nanomaterials with especially desired lineages or drug loadings can modulate the basic properties of the differentiation of stem cells, and their stiffness, orientation. A variety of other parameters have also proven to play an important role in the fate of stem cells. Owing to the complexity, the precise pathways connecting nanomaterials and the fate of stem cells are not well studied. Much of the literature on the mechanism of the differentiation of nanomaterial-promoted stem cells has not been extensively reviewed. The mechanism of interaction between nanomaterials and stem cells is still not well established. How nanomaterials and nanostructures influence the work of stem cells, how they are metabolized, are still major challenges. Generally, the NP technologies used to monitor *in-vivo* non-invasive stem cells must allow the long-term and sensitive position of cells to prevent cytotoxicity as much as possible. Conversely, it is important to remember that almost no NPs have been used to track patients for therapeutic stem cells. The explanation for this is that, before any acceptance for therapeutic use, there must be a crucial stage in the characterization of NPs for both chemical composition and biological effects on stem cells, including the rate of viability after loading, the impact on stem cell migration, differentiation, encoding and the determination of potential short-and long-term cytotoxicity.

Nanotoxicology has a subfield of toxicology that has been developed to address specifically the adverse effects produced by nanomaterials to lead to the production of sustainable and safe nanomaterials [101]. In particular, this specialty studies NPs mediated toxicity in both *in-vitro* and *in-vivo* laboratory models and aims to refine well-known toxicity tests or to develop new ones to be applied to nano-safety evaluations. Generally, the toxic effects of NPs depend on the basic characteristics of the NPs, for example, for bulk material, NPs have a higher surface-area-to-volume ratio and surface reactivity and are more prone to degradation or ion leaching [102,103]. In addition, regular NP agglomeration and/or sedimentation can affect subsequent absorption and can contribute to cytotoxicity.

The first mechanism of NP-induced cytotoxicity may derive from how NPs enter the cells. Even so, some NPs can be internalized via passive diffusion and possibly lead to toxicity by directly interacting with the cell cytosol, its structures, and/or DNA. Most types of NPs are instead endocytosed by cells and confined through clustering in cytoplasmic vesicles, especially lysosomes or late endosomes [104,105]. Nevertheless, some NPs can be prone to the oxidative environment of these organelles and, thus, undergo degradation or dissolution, resulting in the leaching of free ions or enhanced reactive surface groups. A second potential NPs toxicity mechanism is actin cytoskeleton disruption. Owing to endocytosis incidents, the cells undergo a reorganization of the cytoskeleton [106] which plays an important role in fundamental cellular physiology aspects of the cell such as shape, motility, division, adhesion, and connection with the surrounding environment [107].

Nanotechnology and stem cells have been widely studied for cellular therapies, gene therapy, and regenerative medicine [108]. NPs initiate toxic effects, which are secondary to altered ROS production, are (a) changes in gene expression mediated by either direct NPs-induced DNA damage [102,109,110] or interaction of NPs with the cellular transcription/translation machinery following perinuclear localization [110,111], (b) morphological modifications, including modulation of intracellular signaling cascades, protein denaturation or membrane injury [102], and (c) immunological effects via the upregulation of redox-sensitive transcription factors or proinflammatory kinases [112] or the initiation of an immune response directed to specific proteins localized in the outside NP corona [113].

Over the past few years, research teams investigating the results of NPs used for SC monitoring have concentrated on their potentially detrimental effects within the experimental model or even the host. Therefore, after the clinical use of NPs, it is particularly important to perform rigorous *in-vitro* studies to assess their toxicological properties and to determine their possible effects on the self-renewal and differentiation properties of SCs [114,115]. Probably the widest attempt to investigate the cytotoxicity of QDs in SCs has been has been carried out in human-derived MSCs (hMSCs). Many studies have demonstrated that QDs do not influence cell proliferation or cell-cycle spread, but do affect the chondrogenic and osteogenic differentiation capacity [116,117].

10. Challenges and future perspectives

Nanotechnology in a joint venture with stem cell therapy can face many challenges, similar to other novel interdisciplinary fields. The main reason is the cytotoxicity and safety profile of NPs and their unknown impact on stem cell differentiation [73,118]. Further analysis is needed into the process by which cells communicate with nanomaterials, how the NM is metabolized within a cell, and how cell function is affected by nanomaterials, making it challenging to monitor the effects of nanotechnology in cell-based therapies. There are also several problems such as methods for processing, characterizing, and tailoring 3-D nanostructures in tissue engineering. The substitution of gene nanotechnology-based stem cell therapy with traditional gene delivery systems is under active investigation and need future studies. Despite the numerous obstacles and hurdles, nanotechnology may have the ability to drive the advancement in cell-based and stem cell-based therapies in near future.

How to use existing skills and generate new multifunctional or homogeneous nanostructures, packaging, characterization, interface problems, the availability of best-quality nanomaterials, tailoring nanomaterials, and the processes regulating the behavior of these nano range complexes on the SC surface are also major challenges for designing effective SCN techniques. Though stem cells show great potential, they are evolving for regenerative medicine applications, stem cell nanotechnology is expected to be used in the treatment of degenerative diseases in near future.

11. Concluding remarks

Stem cell nanotechnology creates new possibilities for the production and study of SCs and accelerating the possible use of SCs in regenerative medication. Nanomaterials including fluorescent CNTs, QDs, fluorescent MNPs, and fluorescent CNTs, etc., have been utilized for imaging and labeling, drug or gene delivery, tissue engineering scaffolds, and monitoring of stem cell proliferation. Differentiation engineered nanostructures have been used, and are expected to accelerate the apprehension and monitoring of micro-environmental signals. Even though stem cell nanotechnology faces a lot of obstacles, nanotechnology-based stem cell therapies provide new opportunities and will significantly enhance the recognition and monitor SC-fate and develop innovative stem cell therapies, thus potentially contribute to the effective treatment of diseases based on stem cell-based therapy.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

This work was supported by Public Technology Applied Research Projects of Zhejiang province (LGF20H060010), Nature Science Foundation of Zhejiang Province (LY18H060013), Medical and Health Research Project of Zhejiang, Province (2020KY979, 2020KY995,2021KY1172), Science and technology project of Shaoxing (2018C30165).

References

[1] V. Khurana, et al., Chapter 5 - Emerging nanotechnology for stem cell therapy, in: A.K. Mitra, K. Cholkar, A. Mandal (Eds.), Emerging Nanotechnologies for Diagnostics, Drug Delivery and Medical Devices, Elsevier, Boston, 2017, pp. 85–103.
[2] Z. Wang, J. Ruan, D. Cui, Advances and prospect of nanotechnology in stem cells, Nanoscale Res. Lett. 4 (7) (2009) 593–605.
[3] Z. Bagher, et al., Differentiation of Wharton's jelly-derived mesenchymal stem cells into motor neuron-like cells on three-dimensional collagen-grafted nanofibers, Mol. Neurobiol. 53 (4) (2016) 2397–2408.
[4] D. Naskar, et al., Dual growth factor loaded nonmulberry silk fibroin/carbon nanofiber composite 3D scaffolds for in vitro and in vivo bone regeneration, Biomaterials 136 (2017) 67–85.
[5] G. Lalwani, et al., Three-dimensional carbon nanotube scaffolds for long-term maintenance and expansion of human mesenchymal stem cells, J. Biomed. Mater. Res. A. 105 (7) (2017) 1927–1939.
[6] X. Zhou, et al., 3D bioprinted graphene oxide-incorporated matrix for promoting chondrogenic differentiation of human bone marrow mesenchymal stem cells, Carbon 116 (2017) 615–624.
[7] J.H. Lee, et al., Tethering bi-functional protein onto mineralized polymer scaffolds to regulate mesenchymal stem cell behaviors for bone regeneration, J. Mater. Chem. B 1 (21) (2013) 2731–2741.
[8] A. Nuschke, et al., Epidermal growth factor tethered to β-tricalcium phosphate bone scaffolds via a high-affinity binding peptide enhances survival of human mesenchymal stem cells/multipotent stromal cells in an immune-competent parafascial implantation assay in mice, Stem Cells Transl. Med. 5 (11) (2016) 1580–1586.
[9] H. Wu, et al., Enhanced bacteriostatic activity, osteogenesis and osseointegration of silicon nitride/polyetherketoneketone composites with femtosecond laser induced micro/nano structural surface, Appl. Mater. Today 18 (2020) 100523.
[10] S. Prakash, A. Khan, A. Paul, Nanoscaffold based stem cell regeneration therapy: recent advancement and future potential, Expert Opin. Biol. Ther. 10 (12) (2010) 1649–1661.
[11] M.M. Yallapu, et al., Multi-functional magnetic nanoparticles for magnetic resonance imaging and cancer therapy, Biomaterials 32 (7) (2011) 1890–1905.
[12] D.K. Yi, et al., Recent progress in nanotechnology for stem cell differentiation, labeling, tracking and therapy, J. Mater. Chem. B 5 (48) (2017) 9429–9451.
[13] W. Chen, et al., Nanotopography influences adhesion, spreading, and self-renewal of human embryonic stem cells, ACS Nano 6 (5) (2012) 4094–4103.
[14] M.T. Stephan, et al., Therapeutic cell engineering with surface-conjugated synthetic nanoparticles, Nat. Med. 16 (9) (2010) 1035–1041.
[15] C. Liang, C. Wang, Z. Liu, Stem cell labeling and tracking with nanoparticles, Part. Part. Syst. Charact. 30 (12) (2013) 1006–1017.
[16] W. Davis Ronald, et al., Apparatus for Magnetic Separation of Cells, UNIV LELAND STANFORD JUNIOR, 2011.
[17] G.M. Rodrigues, et al., Purification of human induced pluripotent stem cell-derived neural precursors using magnetic activated cell sorting, Methods Mol. Biol. 1283 (2015) 137–145.
[18] Y. Jing, et al., Blood progenitor cell separation from clinical leukapheresis product by magnetic nanoparticle binding and magnetophoresis, Biotechnol. Bioeng. 96 (6) (2007) 1139–1154.
[19] C. Zhao, et al., Nanomaterial scaffolds for stem cell proliferation and differentiation in tissue engineering, Biotechnol. Adv. 31 (5) (2013) 654–668.
[20] H.Y. Mao, et al., Graphene: promises, facts, opportunities, and challenges in nanomedicine, Chem. Rev. 113 (5) (2013) 3407–3424.
[21] C. Chung, et al., Biomedical applications of graphene and graphene oxide, Acc. Chem. Res. 46 (10) (2013) 2211–2224.
[22] M. Nair, et al., Graphene oxide nanoflakes incorporated gelatin–hydroxyapatite scaffolds enhance osteogenic differentiation of human mesenchymal stem cells, Nanotechnology 26 (16) (2015) 161001.
[23] T.R. Nayak, et al., Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells, ACS Nano 5 (6) (2011) 4670–4678.
[24] Y.M. Shin, et al., Modulation of spreading, proliferation, and differentiation of human mesenchymal stem cells on gelatin-immobilized poly (l-lactide-co-ε-caprolactone) substrates, Biomacromolecules 9 (7) (2008) 1772–1781.
[25] Q. Yao, et al., Three dimensional electrospun PCL/PLA blend nanofibrous scaffolds with significantly improved stem cells osteogenic differentiation and cranial bone formation, Biomaterials 115 (2017) 115–127.
[26] J. Kim, et al., Monolayer graphene-directed growth and neuronal differentiation of mesenchymal stem cells, J. Biomed. Nanotechnol. 11 (11) (2015) 2024–2033.
[27] W.C. Lee, et al., Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide, ACS Nano 5 (9) (2011) 7334–7341.
[28] Z. Jiang, et al., Enhanced migration of neural stem cells by microglia grown on a three-dimensional graphene scaffold, ACS Appl. Mater. Interfaces 8 (38) (2016) 25069–25077.
[29] S.Y. Park, et al., Enhanced differentiation of human neural stem cells into neurons on graphene, Adv. Mater. 23 (36) (2011) H263–H267.
[30] K. Yang, et al., Graphene oxide hierarchical patterns for the derivation of electrophysiologically functional neuron-like cells from human neural stem cells, ACS Appl. Mater. Interfaces 8 (28) (2016) 17763–17774.
[31] A. Orza, et al., Electrically conductive gold-coated collagen nanofibers for placental-derived mesenchymal stem cells enhanced differentiation and proliferation, ACS Nano 5 (6) (2011) 4490–4503.
[32] J.H. Kim, et al., Nanotopography promotes pancreatic differentiation of human embryonic stem cells and induced pluripotent stem cells, ACS Nano 10 (3) (2016) 3342–3355.
[33] H.E. Daldrup-Link, et al., Detection of stem cell transplant rejection with ferumoxytol MR imaging: correlation of MR imaging findings with those at intravital microscopy, Radiology 284 (2) (2017) 495–507.
[34] V.K. Verma, et al., Fluorescent magnetic iron oxide nanoparticles for cardiac precursor cell selection from stromal vascular fraction and optimization for magnetic resonance imaging, Int. J. Nanomed. 10 (2015) 711–726.
[35] K. Pu, et al., Semiconducting polymer nanoparticles as photoacoustic molecular imaging probes in living mice, Nat. Nanotechnol. 9 (3) (2014) 233–239.
[36] A. Valizadeh, et al., Quantum dots: synthesis, bioapplications, and toxicity, Nanoscale Res. Lett. 7 (1) (2012) 480.
[37] E. Bull, et al., Stem cell tracking using iron oxide nanoparticles, Int. J. Nanomed. 9 (2014) 1641–1653.
[38] J.V. Jokerst, et al., Photoacoustic imaging of mesenchymal stem cells in living mice via silica-coated gold nanorods, ACS Nano 6 (7) (2012) 5920–5930.
[39] J.V. Jokerst, et al., Photoacoustic imaging of mesenchymal stem cells in living mice via silica-coated gold nanorods, ACS Nano 6 (7) (2012) 5920–5930.
[40] Y. Ohyabu, et al., Stable and nondisruptive in vitro/in vivo labeling of mesenchymal stem cells by internalizing quantum dots, Hum. Gene Ther. 20 (3) (2009) 217–224.
[41] C.L. Meisel, et al., Assessment of superparamagnetic Iron oxide nanoparticle poly (ethylene glycol) coatings on magnetic resonance relaxation for early disease detection, IEEE Open J. Eng. Med. Biol. 1 (2020) 116–122.
[42] S. Xu, et al., In situ generation and consumption of H2O2 by bienzyme-quantum dots bioconjugates for improved chemiluminescence resonance energy transfer, Anal. Chem. 88 (12) (2016) 6418–6424.
[43] R. Bakalova, et al., Designing quantum-dot probes, Nat. Photonics 1 (9) (2007) 487–489.
[44] A. Claxton, et al., The challenge of recognising sepsis: future nanotechnology solutions, J. Intensive Care Soc. 21 (3) (2020) 241–246.
[45] A. Hoshino, et al., Simultaneous multicolor detection system of the single-molecular microbial antigen with total internal reflection fluorescence microscopy, Microbiol. Immunol. 49 (5) (2005) 461–470.
[46] E. Syková, P. Jendelová, Magnetic resonance tracking of transplanted stem cells in rat brain and spinal cord, Neurodegener. Dis. 3 (1–2) (2006) 62–67.
[47] D.J. Maxwell, et al., Fluorophore-conjugated iron oxide nanoparticle labeling and analysis of engrafting human hematopoietic stem cells, Stem Cells 26 (2) (2008) 517–524.
[48] T.M. Coyne, et al., Marrow stromal cells transplanted to the adult brain are rejected by an inflammatory response and transfer donor labels to host neurons and glia, Stem Cells 24 (11) (2006) 2483–2492.
[49] H. Takizawa, et al., Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation, J. Exp. Med. 208 (2) (2011) 273–284.
[50] P. Jendelová, et al., Magnetic resonance tracking of transplanted bone marrow and embryonic stem cells labeled by iron oxide nanoparticles in rat brain and spinal cord, J. Neurosci. Res. 76 (2) (2004) 232–243.
[51] S. Ju, et al., In vitro labeling and MRI of mesenchymal stem cells from human umbilical cord blood, Magn. Reson. Imaging 24 (5) (2006) 611–617.
[52] J. Terrovitis, et al., Magnetic resonance imaging overestimates ferumoxide-labeled stem cell survival after transplantation in the heart, Circulation 117 (12) (2008) 1555–1562.
[53] Z. Berkova, et al., Labeling of pancreatic islets with iron oxide nanoparticles for in vivo detection with magnetic resonance, Transplantation 85 (1) (2008) 155–159.
[54] H. Castano, et al., Polypyrrole thin films formed by admicellar polymerization support the osteogenic differentiation of mesenchymal stem cells, Macromol. Biosci. 4 (8) (2004) 785–794.
[55] J.A. San Román, F. Fernández-Avilés, The role of noninvasive imaging techniques in the assessment of stem cell therapy after acute myocardial infarction, Nat. Clin. Pract. Cardiovasc. Med. (3 Suppl 1) (2006) S38–41.
[56] X. You, et al., Hydrophilic high-luminescent magnetic nanocomposites, Nanotechnology 18 (3) (2007) 035701.
[57] I.H. Park, et al., Generation of human-induced pluripotent stem cells, Nat. Protoc. 3 (7) (2008) 1180–1186.
[58] N. Nakatsuji, F. Nakajima, K. Tokunaga, HLA-haplotype banking and iPS cells, Nat. Biotechnol. 26 (7) (2008) 739–740.
[59] D. Cui, et al., Effects of antisense-myc-conjugated single-walled carbon nanotubes on HL-60 cells, J. Nanosci. Nanotechnol. 7 (4–5) (2007) 1639–1646.
[60] N.W. Kam, Z. Liu, H. Dai, Carbon nanotubes as intracellular transporters for proteins and DNA: an investigation of the uptake mechanism and pathway, Angew. Chem. Int. Ed. Engl. 45 (4) (2006) 577–581.
[61] D. Cui, et al., Effects of dendrimer-functionalized multi-walled carbon nanotubes on murine embryonic stem cells, ECS Trans. 13 (14) (2019) 111–116.

[62] R. Jimenez-Contreras, Nanotechnology Research Developments, Nova Science Publishers, 2008. Incorporated.
[63] J.W. Lee, et al., Convergent synthesis of symmetrical and unsymmetrical PAMAM dendrimers, Macromolecules 39 (6) (2006) 2418–2422.
[64] B. Pan, et al., Dendrimer-modified magnetic nanoparticles enhance efficiency of gene delivery system, Cancer Res. 67 (17) (2007) 8156–8163.
[65] S.W. Han, et al., A molecular delivery system by using AFM and nanoneedle, Biosens. Bioelectron. 20 (10) (2005) 2120–2125.
[66] S.H. Syva, et al., Microenvironmental factors involved in human amnion mesenchymal stem cells fate decisions, J. Tissue Eng. Regen. Med. 11 (2) (2017) 311–320.
[67] G.D. Prestwich, K.E. Healy, Why regenerative medicine needs an extracellular matrix, Expert Opin. Biol. Ther. 15 (1) (2015) 3–7.
[68] P. Viswanathan, et al., 3D surface topology guides stem cell adhesion and differentiation, Biomaterials 52 (2015) 140–147.
[69] P. Agarwal, R. Bhatia, Influence of bone marrow microenvironment on leukemic stem cells: breaking up an intimate relationship, Adv. Cancer Res. 127 (2015) 227–252.
[70] M.J. Dalby, N. Gadegaard, R.O. Oreffo, Harnessing nanotopography and integrin-matrix interactions to influence stem cell fate, Nat. Mater. 13 (6) (2014) 558–569.
[71] J. Lee, et al., Geometric guidance of integrin mediated traction stress during stem cell differentiation, Biomaterials 69 (2015) 174–183.
[72] A. Tijore, et al., Role of cytoskeletal tension in the induction of cardiomyogenic differentiation in micropatterned human mesenchymal stem cell, Adv. Healthc. Mater. 4 (9) (2015) 1399–1407.
[73] F.Y. Cao, et al., Evaluating the effects of charged oligopeptide motifs coupled with RGD on osteogenic differentiation of mesenchymal stem cells, ACS Appl. Mater. Interfaces 7 (12) (2015) 6698–6705.
[74] F.-Y. Cao, et al., Evaluating the effects of charged oligopeptide motifs coupled with RGD on osteogenic differentiation of mesenchymal stem cells, ACS Appl. Mater. Interfaces 7 (12) (2015) 6698–6705.
[75] X. Wang, et al., Effect of RGD nanospacing on differentiation of stem cells, Biomaterials 34 (12) (2013) 2865–2874.
[76] G. Abagnale, et al., Surface topography enhances differentiation of mesenchymal stem cells towards osteogenic and adipogenic lineages, Biomaterials 61 (2015) 316–326.
[77] J. Park, et al., Nanosize and vitality: TiO2 nanotube diameter directs cell fate, Nano Lett. 7 (6) (2007) 1686–1691.
[78] H.J. Levis, J.T. Daniels, Recreating the human limbal epithelial stem cell niche with bioengineered limbal crypts, Curr. Eye Res. 41 (9) (2016) 1153–1160.
[79] T. Memanishvili, et al., Generation of cortical neurons from human induced-pluripotent stem cells by biodegradable polymeric microspheres loaded with priming factors, Biomed. Mater. 11 (2) (2016) 025011.
[80] C. Gandhimathi, et al., Biomimetic hybrid nanofibrous substrates for mesenchymal stem cells differentiation into osteogenic cells, Mater. Sci. Eng. C Mater. Biol. Appl. 49 (2015) 776–785.
[81] H. Nguyen, et al., Stem cell therapy for neurological disorders: a focus on aging, Neurobiol. Dis. 126 (2019) 85–104.
[82] C.L. Nemeth, A.S. Fine, A. Fatemi, Translational challenges in advancing regenerative therapy for treating neurological disorders using nanotechnology, Adv. Drug Deliv. Rev. 148 (2019) 60–67.
[83] D. Carradori, et al., The therapeutic contribution of nanomedicine to treat neurodegenerative diseases via neural stem cell differentiation, Biomaterials 123 (2017) 77–91.
[84] O. Lindvall, Developing dopaminergic cell therapy for Parkinson's disease–give up or move forward? Mov. Disord. 28 (3) (2013) 268–273.
[85] S.B. Dunnett, A.E. Rosser, Clinical translation of cell transplantation in the brain, Curr. Opin. Organ Transplant. 16 (6) (2011).
[86] E. Hoveizi, et al., In vitro differentiation of human iPS cells into neural like cells on a biomimetic polyurea, Mol. Neurobiol. 54 (1) (2017) 601–607.
[87] J.G. Hunsberger, et al., Accelerating stem cell trials for Alzheimer's disease, Lancet Neurol. 15 (2) (2016) 219–230.
[88] K.A. Chang, J.H. Lee, Y.H. Suh, Therapeutic potential of human adipose-derived stem cells in neurological disorders, J. Pharmacol. Sci. 126 (4) (2014) 293–301.
[89] S. Farzamfar, et al., Will nanotechnology bring new hope for stem cell therapy? Cells Tissues Organs 206 (4–5) (2018) 229–241.
[90] X. Jin, T. Lin, Y. Xu, Stem cell therapy and immunological rejection in animal models, Curr. Mol. Pharmacol. 9 (4) (2016) 284–288.
[91] B. Corradetti, M. Ferrari, Nanotechnology for mesenchymal stem cell therapies, J. Control. Release 240 (2016) 242–250.
[92] T. Santos, et al., Nanomedicine boosts neurogenesis: new strategies for brain repair, Integr. Biol. 4 (9) (2012) 973–981.
[93] Z. Guilong, et al., The application of nanomaterials in stem cell therapy for some neurological diseases, Curr. Drug Targets 19 (3) (2018) 279–298.
[94] M. Mirahmadi, et al., Stem cell therapy for neurodegenerative diseases: strategies for regeneration against degeneration, Cell Ther. Regen. Med. J. 1 (1) (2016) 3.
[95] N. Tabassum, et al., Nanomedicine in cancer stem cell therapy: from fringe to forefront, Cell Tissue Res. 374 (3) (2018) 427–438.
[96] Y. Li, et al., Specific cancer stem cell-therapy by albumin nanoparticles functionalized with CD44-mediated targeting, J. Nanobiotechnol. 16 (1) (2018) 99.
[97] H. Li, et al., Nucleus-targeted nano delivery system eradicates cancer stem cells by combined thermotherapy and hypoxia-activated chemotherapy, Biomaterials 200 (2019) 1–14.
[98] K. Zhu, et al., Reprogramming fibroblasts to pluripotency using arginine-terminated polyamidoamine nanoparticles based non-viral gene delivery system, Int. J. Nanomed. 9 (2014) 5837–5847.
[99] Y.-D. Sohn, et al., Induction of pluripotency in bone marrow mononuclear cells via polyketal nanoparticle-mediated delivery of mature microRNAs, Biomaterials 34 (17) (2013) 4235–4241.
[100] W. Chen, et al., Nonviral cell labeling and differentiation agent for induced pluripotent stem cells based on mesoporous silica nanoparticles, ACS Nano 7 (10) (2013) 8423–8440.
[101] D.W. Hobson, Nanotoxicology: the toxicology of nanomaterials and nanostructures, Int. J. Toxicol. 35 (1) (2016) 3–4.
[102] A. Nel, et al., Toxic potential of materials at the nanolevel, Science 311 (5761) (2006) 622–627.
[103] R. Duffin, et al., Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: highlighting the role of particle surface area and surface reactivity, Inhal. Toxicol. 19 (10) (2007) 849–856.
[104] T. Wang, et al., Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry, ACS Nano 6 (2) (2012) 1251–1259.
[105] C. Schweiger, et al., Quantification of the internalization patterns of superparamagnetic iron oxide nanoparticles with opposite charge, J. Nanobiotechnol. 10 (1) (2012) 1–11.
[106] A.K. Gupta, et al., Effect of cellular uptake of gelatin nanoparticles on adhesion, morphology and cytoskeleton organisation of human fibroblasts, J. Control. Release 95 (2) (2004) 197–207.
[107] Y. Yang, et al., Integrators of the cytoskeleton that stabilize microtubules, Cell 98 (2) (1999) 229–238.
[108] W. Nawaz, et al., Nanotechnology and immunoengineering: how nanotechnology can boost CAR-T therapy, Acta Biomater. 109 (2020) 21–36.
[109] Y.-J. Gu, et al., Nuclear penetration of surface functionalized gold nanoparticles, Toxicol. Appl. Pharmacol. 237 (2) (2009) 196–204.
[110] N. Singh, et al., NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials, Biomaterials 30 (23-24) (2009) 3891–3914.
[111] T.R. Pisanic Ii, et al., Nanotoxicity of iron oxide nanoparticle internalization in growing neurons, Biomaterials 28 (16) (2007) 2572–2581.
[112] S. Lanone, J. Boczkowski, Biomedical applications and potential health risks of nanomaterials: molecular mechanisms, Curr. Mol. Med. 6 (6) (2006) 651–663.
[113] A. El-Ansary, S. Al-Daihan, On the toxicity of therapeutically used nanoparticles: an overview, J. Toxicol. 2009 (2009).
[114] K.L. Aillon, et al., Effects of nanomaterial physicochemical properties on in vivo toxicity, Adv. Drug Deliv. Rev. 61 (6) (2009) 457–466.
[115] L. Ferreira, et al., New opportunities: the use of nanotechnologies to manipulate and track stem cells, Cell Stem Cell 3 (2) (2008) 136–146.
[116] S.C. Hsieh, et al., The internalized CdSe/ZnS quantum dots impair the chondrogenesis of bone marrow mesenchymal stem cells, J. Biomed. Mater. Res. Part B: Appl. Biomater. 79 (1) (2006) 95–101.
[117] S.-C. Hsieh, et al., The inhibition of osteogenesis with human bone marrow mesenchymal stem cells by CdSe/ZnS quantum dot labels, Biomaterials 27 (8) (2006) 1656–1664.
[118] S.J. Soenen, et al., (Intra)cellular stability of inorganic nanoparticles: effects on cytotoxicity, particle functionality, and biomedical applications, Chem. Rev. 115 (5) (2015) 2109–2135.