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NANO REVIEW

Advances and Prospect of Nanotechnology in Stem Cells

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Abstract In recent years, stem cell nanotechnology has emerged as a new exciting field. Theoretical and experimental studies of interaction between nanomaterials or nanostructures and stem cells have made great advances. The importance of nanomaterials, nanostructures, and nanotechnology to the fundamental developments in stem cells-based therapies for injuries and degenerative diseases has been recognized. In particular, the effects of structure and properties of nanomaterials on the proliferation and differentiation of stem cells have become a new interdisciplinary frontier in regeneration medicine and material science. Here we review some of the main advances in this field over the past few years, explore the application prospects, and discuss the issues, approaches and challenges, with the aim of improving application of nanotechnology in the stem cells research and development.

Keywords Nanomaterials · Nanostructure · Nanotechnology · Stem cells · Regeneration medicine

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Introduction

Stem cells and nanotechnology are two different fields with the characterization of rapid development, highly interdisciplinary, and controversial. So far the two fields intercross and gradually form a new emerging field, that is, nanotechnology in stem cells or stem cell nanotechnology, which refers to the application of nanotechnology in stem cells research and development [1, 2]. Stem cells are the parent cells of all tissues and organs of the body and exist mainly to maintain and replace the cells in the areas where they are found such as blood, bone marrow, skin, muscle and organs like the brain, liver, etc. [3–6]. Stem cells are classified into two kinds, that is, embryonic stem cells (ESCs) and adult stem cells. ESCs is composed of somatic stem cells that are restricted to a set of lineages and normally arise from the tissue from which they are derived. Adult stem cells are not pluripotent but multipotent, which differentiate only into a limited variety of cell types [7, 8]. Since Evans, et al. [9] firstly reported the isolated ESCs in 1981, stem cells research have become a novel hotspot and provide a new chance for regenerative medicine, as shown in Fig. 1, the pluripotential nature of ES cells to differentiate into cell types of all three primary germ lineages exhibit attracting prospect for stem cells-based therapy for human injuries and degenerative diseases [10, 11]. Especially induced pluripotent stem cells (ips) was successfully established in 2007, which provided great progress for controllable man-made stem cells [12–14]. However, several obstacles must be overcome before their therapeutic application can be realized. These include the development of advanced techniques to understand and control functions of microenvironmental signals and novel methods to track and guide transplanted stem cells [15–18].

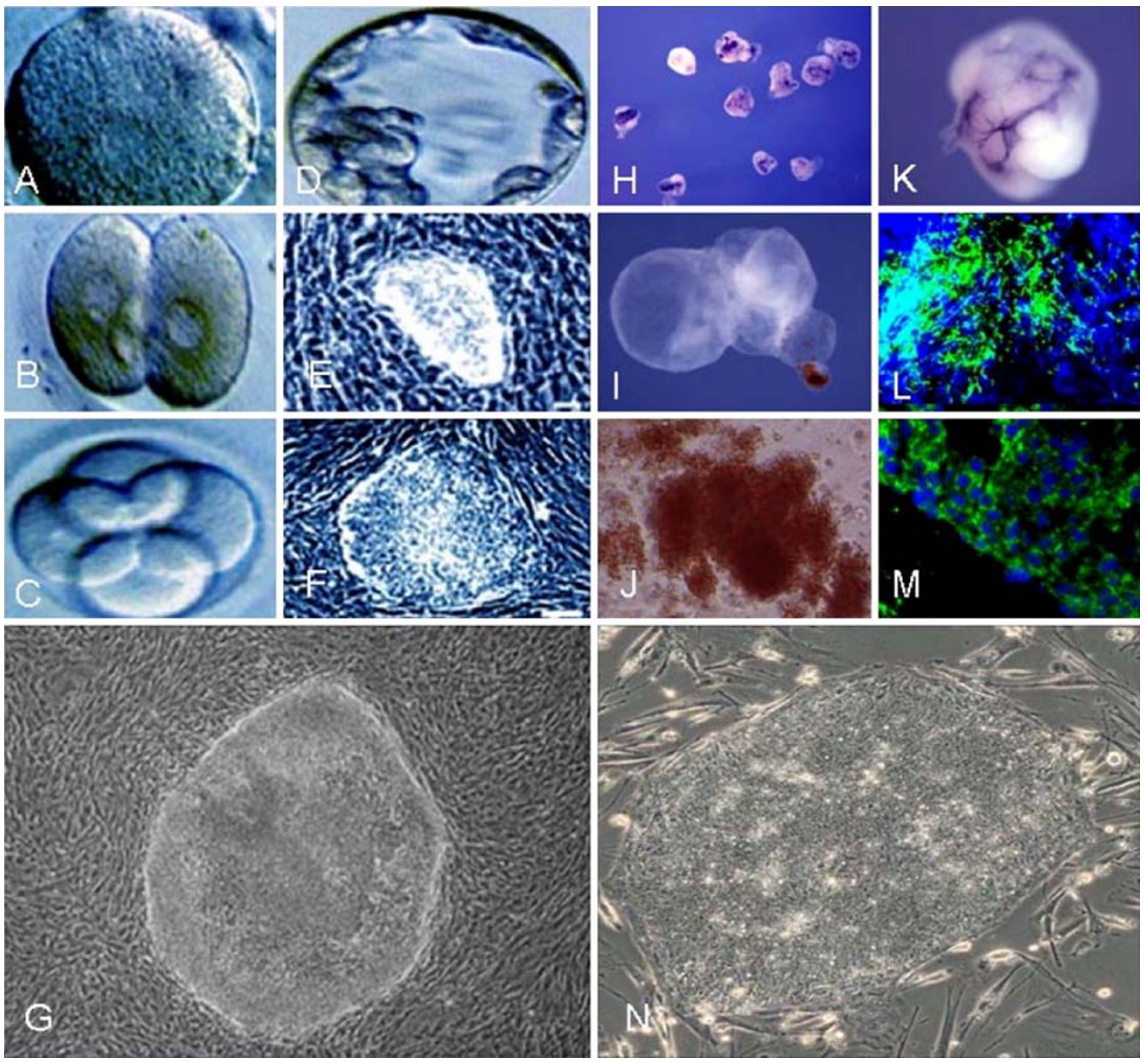

Fig. 1 Establishment of human ESCs (hESCs) showing (**a**–**f**) early stages of human embryo development, (**g**) established hESCs line. In vitro differentiation of hESCs toward hepatocyte-like cells showing (**h**) hESCs derived embryoid bodies, (**i**–**j**) hematopoietic differentiation, (**k**) endothelia differentiation, (**l**) neuron differentiation, (**m**) hepatocytes differentiation, (**n**) ips cells

Nanotechnology brings new chance to stem cells research and development. Nanotechnology is the term used to cover the design, construction, and utilization of functional structures with at least one characteristic dimension measured in nanometers [19, 20]. Such materials and systems can be designed to exhibit novel and significantly improved physical, chemical, and biological properties, phenomena and processes as a result of the limited size of their constituent particles or molecules. The reason for such interesting and very useful behavior is that when characteristic structural features are intermediate in extent between isolated atoms and bulk macroscopic materials, i.e., in the range of about 0.1–100 nm, the objects may display physical attributes substantially different from those displayed by either atoms or bulk materials. It is well-known that the nanomaterials own four basic unique effects such as small size effects, surface effects, quantum size effects, and tunnel effects, and ultimately these effects can lead to new technological opportunities as well as new challenges [21–23]. The application of nanomaterials and nanotechnology in stem cells research and development exhibits attracting technological prospects, which provide a new chance to solve current problems that stem cells research and development meet.

In particular, the effects of structure and properties of nanomaterials on the proliferation and differentiation of stem cells have become a new interdisciplinary frontier in regeneration medicine and material science [24, 25]. Here we review some of the main advances in this field over the past few years, explore the application prospects, and discuss the issues, approaches, and challenges, with the aim of

improving application of nanotechnology in the stem cell research and development.

Advance of Stem Cell Nanotechnology

In recent years, the application of nanotechnology in stem cell research and development have made great progress. For example, magnetic nanoparticles (MNPs) have been successfully used to isolate and sort stem cells [26], quantum dots have been used for molecular imaging and tracing of stem cells [27], nanomaterials such as carbon nanotubes (CNTs) [28], fluorescent CNTs [29] and fluorescent MNPs [30], etc. have been used to deliver gene or drugs into stem cells, unique nanostructures were designed for controllable regulation of proliferation and differentiation of stem cells, and all these advances speed up the development of stem cells toward the application in regenerative medicine.

Application of Magnetic Nanoparticles in Isolation of Stem Cells

Magnetic nanoparticles (MNPs), as shown in Fig. 2, a series of magnetism-engineered iron oxide nanoparticles were developed [31], because of their superparamagnetic

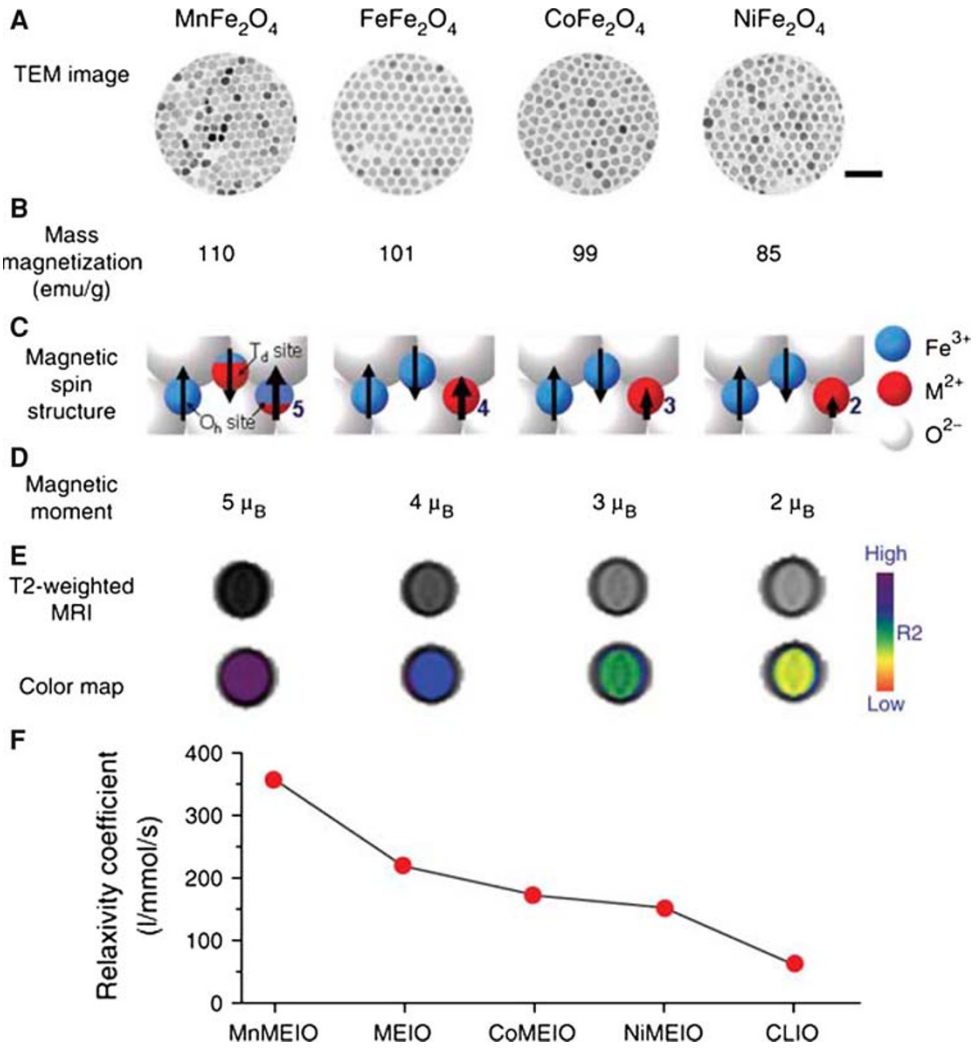

Fig. 2 Magnetism-engineered iron oxide (MEIO) nanoparticles and effects of their magnetic spin on MRI. **a** TEM images of $MnFe_2O_4$ (MnMEIO), Fe_3O_4 (MEIO), $CoFe_2O_4$ (CoMEIO), and $NiFe_2O_4$ (NiMEIO). All nanoparticles were synthesized to be ~12 nm with narrow size distributions ($0 < \sim 8\%$). Scale bar, 50 nm. **b** Mass magnetization values of MFe_2O_4 nanoparticles. In face-centered cubic lattices of oxygen, the magnetic spins at On sites aligned in parallel with the direction of the external magnetic filed whereas those at Td sites aligned antiparallel. $MnFe_2O_4$ had the highest mass magnetization value, with a magnetic spin magnitude of 5 μg (**e**, **f**) T2-weighted spin echo MR images, their color maps and relaxivity (R2) of a series of MEIO nanoparticles at 1.5 T. In **f**, the R2 of CLIO is also presented, for comparison. Consistent with the mass magnetion results, MnMEIO showed the strongest MR contrast effect (that is, darkest MR image, violet in color map) and had the highest R2 coefficient. Mass magnetization value, MR contrast and R2 coefficient gradually decreased as M2+ changed from Mn^{2+} to Fe^{2+}, to Co^{2+} and to Ni^{2+} [31]

property, have been widely explored potentials in applications such as hyperthermia [32], magnetic resonance imaging (MRI) [31], tissue repair [33], immunoassay [34], drug/gene delivery [35], cell separation [26], etc. Stem cells is a unlimited cell source in stem cell therapy, the key challenge is how to isolate stem cells from a multi-type cell mixture at a low cost, fast, and easily operated fashion. So far some reports show that MNPs can directly label stem cells, and then labeled stem cells were isolated by magnetic force or flow cytometry. For example, Jing, et al. [26] reported that MNPs combined with Cd34 antibody, successfully enriched peripheral blood progenitor cells (PBPCs) from human blood circulation. They tested CD34+ cell immunomagnetic labeling and isolation from fresh leukocyte fraction of peripheral blood using the continuous quadrupole magnetic flow sorter (QMS), consisting of a flow channel and a quadrupole magnet with a maximum field intensity of 1.42 T and a mean force field strength of 1.45×10^8 TA/m^2. Seven commercial progenitor cell labeling kits were quantitatively evaluated by measuring magnetophoretic mobility of a high CD34 expression cell line, KG-1a, using the cell tracking velocimeter (CTV). The commercial CD34 progenitor cell isolation kit from Miltenyi Biotec and Bergisch Gladbach in Germany was used to enrich the progenitor cells from 11 fresh and 11 cryopreserved clinical leukapheresis samples derived from different donors. Results showed that the KG-1a cells were strongly labeled, the CD34+ cells were isolated with a purity of 60–96%, a recovery of 18–60%, an enrichment rate of 12–16%, and a throughput of $(1.7–9.3) \times 10^4$ cells/s. Main parameters fall well within the clinical useful range. These isolated CD34 progenitor cells can be used for patient therapy.

Application of Nanoparticles in Imaging and Tracing of Stem Cells

Up-to-date, nanoparticles such as quantum dots, MNPs, and gold nanorods can be used for imaging and tracing of stem cells [27, 31, 36–38]. For example, quantum dots have been subject to intensive investigations due to their unique properties and potential application prospects. QDs have been used successfully in cellular imaging [39], immunoassays [40], DNA hybridization [41], and optical barcoding [42]. Quantum dots provide a new functional platform for bioanalytical sciences and biomedical engineering. Ohyabu et al. [27] reported that quantum dots conjugated with an antibody against mortalin protein, resulting in the formation of i-QD composites, which can be internalized by mesenchymal stem cells (MSCs), and finally labeled MSCs cells. The i-QD labeled MSCs underwent normal adipocyte, osteocyte, and chondrocyte differentiation in vitro and in vivo, which highly suggest that i-QDs can be applied to in vivo imaging diagnostics and tracing of stem cells in the distribution of mouse body. As shown in Fig. 3, QDs can be designed as multi-functional nanoprobes, which can be modified with different biomolecules such as liposome, PEG, peptides, or antibody, and own specific functions, can be used for molecular imaging, gene or drug delivery and molecule tracing [40].

Besides quantum dots, MNPs were also used for molecular imaging and tracing of stem cells [31, 43]. For example, superparamagnetic iron oxide nanoparticles (SPIO) have been used for stem cell labeling, MRI, and tracking of transplanted stem cells [44]. For example, fluorescent molecules were covalently linked to dextran-coated iron oxide nanoparticles to characterize HSCs labeling to monitor the engraftment process [45]. Conjugating fluorophores to the dextran coat for fluorescence-activated cell sorting purification eliminated spurious signals from nonsequestered nanoparticle contaminants. A short-term defined incubation strategy was developed that allowed efficient labeling of both quiescent and cycling HSCs, with no discernable toxicity in vitro or in vivo. Transplantation of purified primary human cord blood lineage-depleted and $CD34^+$ cells into immunodeficient mice allowed detection of labeled human HSCs in the recipient bones. Flow cytometry was used to precisely quantitate the cell populations that had sequestered the nanoparticles and to follow their fate post-transplantation. Flow cytometry endpoint analysis confirmed the presence of MNPs-labeled human stem cells in the marrow [46]. The use of stem cell therapy in different disorders of the central nervous system (CNS) has been extensively examined. Endorem-labeled GFP+ MSCs were grafted into rats in an experimental model of stroke [46]. The cells were grafted either intracerebrally into the hemisphere contralateral to the lesion, or intravenously into the femoral vein. Rats with grafted stem cells were examined weekly for a period of 3–7 weeks post-transplantation using a 4.7-T Bruker spectrometer. The lesion was visible on MR images as a hyperintense signal. One week after grafting, a hypointense signal was found in the lesion, which intensified during the second and third weeks, regardless of the route of administration. Its intensity corresponded to Prussian blue staining or GFP labeling. MSCs labeled with Endorem were also injected intravenously into the femoral vein 1 week after a transversal spinal cord lesion [43, 47]. MR images of longitudinal spinal cord sections from lesioned non-grafted animals showed the lesion cavity as inhomogeneous tissue with a strong hyperintensive signal. Lesions of grafted animals were seen as dark hypointense areas. Histological evaluation confirmed only a few iron-containing cells in lesioned, control animals, but strong positivity for iron in grafted animals. Compared to control rats, in grafted animals the lesion, which was populated by

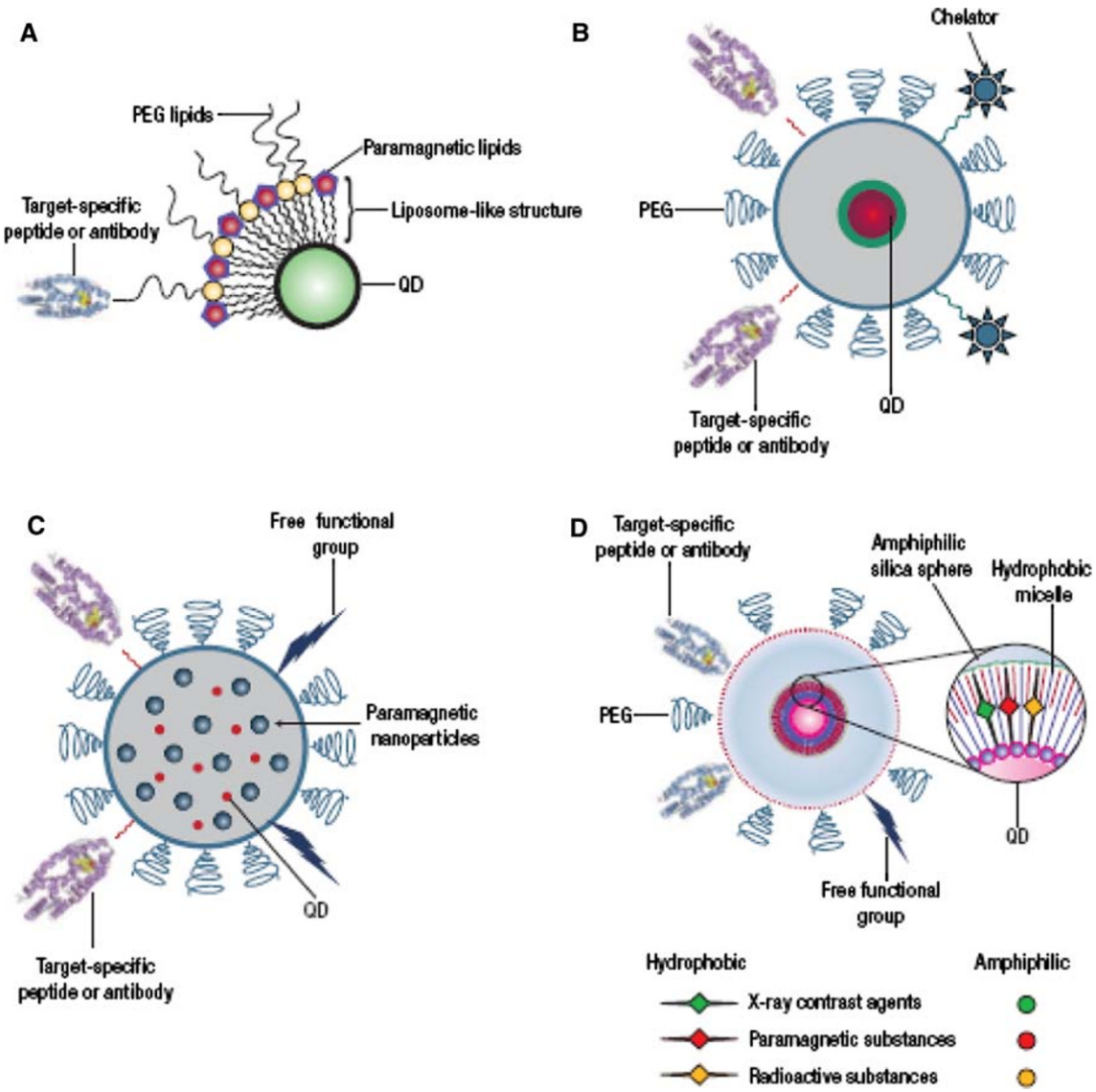

Fig. 3 Various designs of multimodal QD probes. **a**, **b** Quantum dots having different molecules for target-specific interaction, and, attached to the surface, paramagnetic lipids, and chelators for nuclear-spin labeling. **c** The silica sphere has QDs and paramagnetic nanoparticles inside and target-specific groups attached to the outside. **d** The structure of a multimodal QD probe, based on silica-shelled single-QD micelles [40]

grafted MSCs, was considerably smaller, suggesting a positive effect of the MSCs on lesion repair [48]. Several successful applications of MR tracking can be found in other organs, such as the heart [49], liver [50], kidney [51], and pancreatic islets [52]. It is reported that surface modification of MNPs with D-mannose, poly-L-Lysin (PLL) or polydimethylacrylamid (PDMAAm) resulted in better labeling efficiency than that seen with dextran-coated SPIO.

We also observed that fluorescent MNPs (FMNPs) [53] could conjugate with brcaa1 antibody, and formed brcaa1 antibody-labeled FMMNP probes. We also observed that BRCAA1 protein exhibited over-expression in ES CCE cells. As shown in Fig. 4e, while the prepared probes incubated with ES CCE cells for 30 min, the prepared probes can be internalized into CCE stem cells. Because of the internalized probes own superparamagnetic properties, these stem cells with fluorescent signals can be isolated directly under in vitro magnetic fields, and also can be traced (unpublished data). We also observed that the QDs covered CNTs can be internalized into stem cells, and realized labeling stem cells [28].

Application of Nanoparticles in Gene Delivery Systems for Stem Cells

Generating progenitor cells with in vivo reconstitution functions has been at the center of intense research to

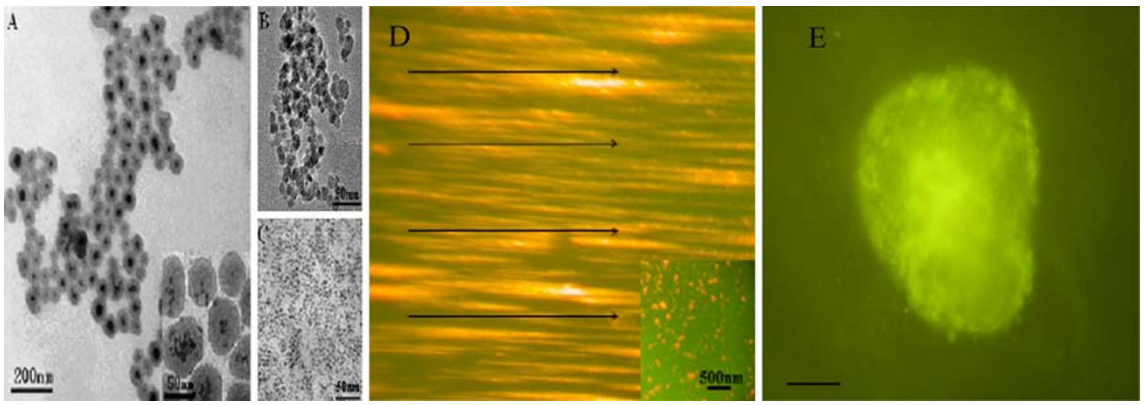

Fig. 4 TEM images of **a** FMCNPs **b** MNPs and **c** quantum dots. **d** FMCNPs are aligned in a magnetic field obtained with a fluorescent microscope3. The arrows are added to clearly show the orientation of the magnetic field. The inset image is obtained without a magnetic filed. **e** Fluorescent microscope image of FMCNPs inside murine ECC stem cells, scale bar: 10 μm [53]

accelerate biomedical applications of embryonic stem cells (ESCs) for the treatment of debilitating genetic, traumatic, and degenerative conditions [54]. A major challenge for clinical development of these pluripotent cells is effective, non-invasive imaging of transplanted cells to monitor biodistribution (i.e., in vivo tracking). Furthermore, reproducible approaches need to be developed enabling efficient intracellular delivery of biomolecules, including DNA, RNA, peptides, or proteins, required to control ES cell differentiation. Physical methods such as electroporation and nucleofection offer the advantage of high delivery efficiency but frequently cause severe damage to ES cells [55]. Viral vectors, including retro-, lenti-, and adenoviruses, result in successful transfection and reproducible manipulation of ES differentiation in vitro [56]. However, the risk of toxicity, immunogenicity, and increased mutagenesis significantly decrease clinical viability of these viral carriers for biomedical applications. Therefore, non-viral vectors such as polymeric nanoparticles and liposomes are currently pursued as the most promising nanotechnology platform to translate exciting laboratory findings with ES cells into clinically viable applications [57, 58].

We found that No. 5 generation of polyamidoamine dendrimer-functionalized fluorescent multi-walled carbon nanotubes (dMNTs) can enter into mice embryonic stem cell line CCE highly efficiently [28]. More than 20 μg/mL dose can cause ES cells become smaller and smaller as the incubation time increases, and inhibit cell growth in dose- and time-dependent means, less than 5 μg/mL dose can improve ES differentiation. Dendrimers is a novel special class of organic molecules: they can take different functional groups through a series of chemical modifications, and their interior cavities can serve as storage areas for a lot of genes or drugs [59]. Dendrimers may be a good nonviral delivery vector because it has the advantages of simplicity of use, and ease of mass production compared with viral vectors that are inherently risky for clinical use. Polyamidoamine (PAMAM) dendrimer-modified MNPs can markedly enhance gene delivery efficiency [60–62]. The prepared dMNTs may be a highly efficient gene delivery system for ES cells, have potential applications in ES research. As shown Fig. 5, nanoparticles such as MNPs [37, 49] and quantum dots can enter into human MSCs cells, and can keep longer time inside ES cells. As shown in Fig. 5d, i, j, and k, we observed that SiO_2 wrapped CdTe nanoparticles can enter into murine stem cells, and exist in those induced-differentiated neurons cells, hematopoietic cells and endothelia cells, and did not exhibit cytotoxicity within the used concentration.

As shown in Fig. 5a, b, and c, MNPs entered into ES cells, and also can be used to trace ES cells in the whole body of mouse. As shown in Fig. 5l, m, n, and o, we can clearly observe that those grafted stem cells with MNPs formed teratomas composed of tissues of all three germ layers [44].

More recently, a molecular delivery system by using atomic force microscopy (AFM) and nanoneedle has been developed to transfer gene into living cells [63]. Han et al. described a low-invasive gene delivery method that uses an etched AFM tip or nanoneedle that can be inserted into a cell nucleus without causing cellular damage. The nanoneedle is 200 nm in diameter and 6 μm in length and is operated using an AFM system. The probabilities of insertion of the nanoneedle into human MSCs and human embryonic kidney cells (HEK293) were higher than those of typical microinjection capillaries. A plasmid containing the green fluorescent protein (GFP) gene was adsorbed on a poly-L-lysine-modified nanoneedle surface, which was then inserted into primary cultured single human MSCs. A highly efficient gene delivery of over 70% was achieved in human MSCs, which compared very favorably with other major nonviral gene delivery methods (lipofection ~50% and microinjection ~10%).

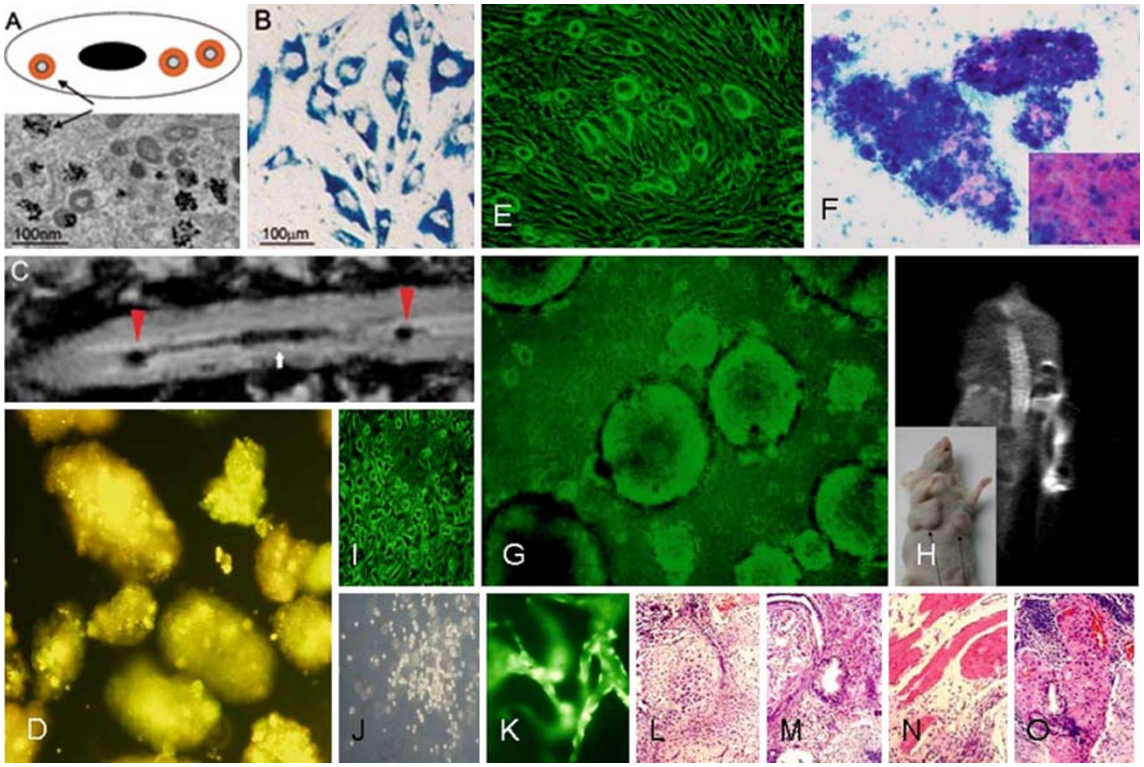

Fig. 5 Drawing and transmission electron microscopy (TEM) image (**a**) and Prussian blue-positive cells (**b**) showing nanoparticles inside the cell (arrow). **c**: T2-weighted image of a rat spinal cord injected with nanoparticle-labeled MSCs. Arrowheads mark the injection sites, arrow the lesion populated with cells; Implanted nanoparticles labeled mouse ESCs were labeled with SPIO (**e–g**). Cells were grafted intravenously. In vivo MRI was used to track their fate (**h**). Prussian blue staining confirmed the presence of iron oxide nanoparticles inside the cells (**f**). After 4 weeks post-implantation, grafted cells migrated to the lesion site and formed teratomas composed of tissue of all three germ layers (**l–m**); In vitro differentiation of quantum dots labeling of hESCs (**d**) into neurons (**i**), hematopoietic cells (**j**) and endothelia cells (**k**) [44]

Effects of CNTs on Proliferation and Differentiation of Stem Cells

Carbon nanotubes, because of unique mechanical, physical, and chemical properties, have great potential applications in various fields including molecular electronics, medical chemistry, and biomedical engineering [64–72]. Carbon nanotubes can be functionalized to achieve improved properties and functions such as biocompatibility and biomolecular recognition capabilities [73, 74]. Protein-conjugated CNTs can move across the cellular membrane and enter into cytoplasm and cell nucleus [75, 76]. Carbon nanotubes can be filled with DNA or peptide molecules, have high potential in gene or peptide storage, and delivery system in molecular therapy of diseases [57]. In our previous work, we investigated the effects of single walled carbon nanotubes (SWCNTs) on human embryonic kidney cell line HEK293 cells [77]. We observed that SWCNTs can inhibit HEK293 cell proliferation, and decrease cell adhesive ability in a dose- and time-dependent manner. HEK293 cells exhibit active responses to SWCNTs such as secretion of some 20–30 kd proteins to wrap SWCNTs, aggregation of cells attached by SWCNTs, and formation of nodular structures. As shown in Fig. 6, cell cycle analysis showed that 25 μg/mL SWCNTs in medium induced G1 arrest and cell apoptosis in HEK293 cells. Biochip analysis showed that SWCNTs can induce up-regulation expression of cell cycle-associated genes such as p16, bax, p57, hrk, cdc42, and cdc37, down-regulation expression of cell cycle genes such as cdk2, cdk4, cdk6, and cyclin D3, and down-regulation expression of signal transduction-associated genes such as mad2, jak1, ttk, pcdha9, and erk. Western blot analysis showed that SWCNTs can induce down-regulation expression of adhesion-associated proteins such as laminin, fibronectin, cadherin, FAK, and collagen IV. SWCNTs inhibit HEK293 cell growth by inducing cell apoptosis and decreasing cellular adhesion ability. It is also observed that SWCNTs stimulate human osteoblast cells and human fibroblast cells to appear many protuberance on the surface compared with the control, which is one kind of active protective reaction of stimulated cells. Regarding the mechanism of nanoparticles such as CNTs, etc., entering into cells, receptor-mediated endocytosis may be responsible for the phenomena, a

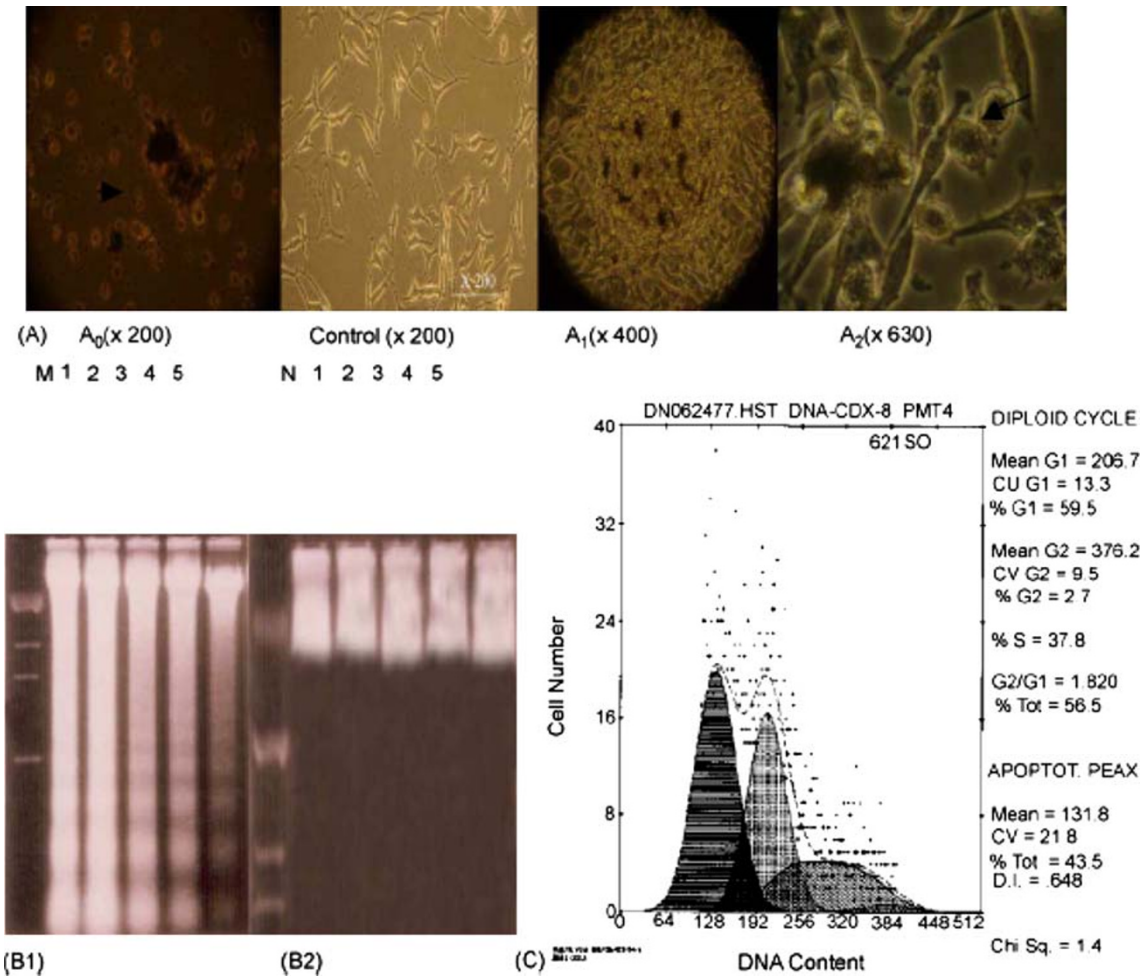

Fig. 6 Apoptosis of HEK293 cells induced by SWCNTs. **a** morphological changes of HEK293 cells cultured with 25 μg/mL SWCNTs for 3 days; **a0**: showing cells become round and floating with apoptotic characterizatics; control: showing normal morphological cells; **a1**: showing nodular structure composed of SWCNTs and apoptotic cells; **a2**: showing apoptotic cells attached by SWCNTs. **b1**: DNA electrophoresis of cells cultured with 25 μg/mL SWCNTs for 1–5 days, M: molecular Marker; no. 1–5 denote the results of cells cultured for day 1–5, respectively; **b2**: DNA electrophoresis results of control cells cultured for day 1–5; **c:** the cell cycle distribution of HEK293 cells cultured with 25 μg/mL SWCNTs for 4 days, the percentage of sub-G1 cells (apoptosis cells) was 43.5% [77]

theory model is also suggested, the optimal size of particles entering into cells is between 25 nm and 700 nm or so, too small nanoparticles are very difficult to enter into cells because of cellular surface tension force and adhesion. The further mechanism of effects of CNTs on human ES cells is being investigated from the following four scales such as molecular, cellular, animals, and environment levels.

Crouse et al. [78] investigated effects of a range of different types of CNTs, including single walled nanotubes (SWCNTs), multi-walled nanotubes (MWCNTs) and functionalized CNTs on hMSCs, and revealed that at low concentrations of COOH functionalized SWCNTs, the CNTs had no significant effect on cell viability or proliferation. In addition, by fluorescently labeling the COOH functionalized SWCNTs, the CNTs were seen to migrate to a nuclear location within the cell after 24 h, without adversely affecting the cellular ultrastructure. Moreover, the CNTs had no effect on adipogenesis, chondrogenesis, or osteogenesis. So far CNTs was considered to be one novel and emerging technology in gene or drug delivery, tissue engineering, and regenerative medicine. At low concentrations, CNTs have minimal affect on MSCs viability and multipotency [79–81]. Therefore, they have great potential to advance the field in a number of ways including: (1) Development of nanovehicles for delivering biomolecule-based cargos to MSCs; and (2) Creation of novel biomedical applications for electroactive CNTs in combination with MSCs. Since CNTs are electrically conductive, there is a huge potential for the manipulation of MSCs differentiation pathways to create electroactive cells such as those found in the heart. In particular, specific applications could result in novel MSCs based cell therapies for electroactive tissue repair; novel biomolecule delivery vehicle for manipulation of MSCs differentiation

pathways; and electroactive CNT scaffolds for damaged electroactive tissues.

Application of 3D Nanostructures in Stem Cell Tissue Engineering

The combination of stem cells with tissue engineering principles enables developing the stem cell-based therapeutic strategy for human diseases. Stem cell and progenitor cell directional differentiation is currently one hotspot, the differentiation of stem cells that conjugate 3D materials is considered as the most perspective tissue engineering. Up to date, various micro-/nanofabrication technologies have been used to guide stem cells to develop into 3D biodegradable scaffolds [82, 83]. Nanostructured scaffolds are designed to trigger stem cells to become specific cell types compromising the tissues and organs in the body. Inside these scaffolds, cells deposit their own matrix and as the scaffold degrades, they form a 3D tissue structure that mimics the body's natural tissues. For example, Gelain et al. [84] reported that they had developed a 3D cell culture system using a designer peptide nanofiber scaffold with mouse adult neural stem cells. They synthesized 18 different peptides which directly incorporate various functional motifs to promote cell adhesion, differentiation, and bone marrow homing activities. These functionalized peptides self-assemble into nanofiber scaffolds where cells can be fully embedded by the scaffold in 3D. Without addition of soluble growth factors and neurotrophic factors, two of these scaffolds functionalized with bone marrow homing motifs not only significantly enhanced survival of the neural stem cells, but also promoted differentiation toward cells expressing neuronal and glial markers.

As shown in Figs. 7 and 8, carbon nanotube patterns can be used to guide growth and alignment of MSCs [85]. The MSCs exhibited preferential growth on CNT patterns, and the cell culture results suggested that the CNT patterns did not have a harmful effect on the MSCs. The results clearly show that CNT patterns have enormous potential as a new platform for basic research and applications using stem cells.

Stem cell differentiation is closely associated with their microenvironment. The regulation of stem cells depend largely on their interaction with a highly specialized microenvironment or niches [86]. Secreted factors, stem cell–neighboring cell interactions, extracellular matrix (ECM), and mechanical properties collectively make up the stem cell microenvironment. The niche secretes appropriate chemicals to direct the differentiation and development of stem cells. For example, Adams et al. [87] has identified the elements of the microenvironment that control the behavior of mammalian stem cells. Mineral components are important to stem cell localization; matrix components are important to constraint of stem cells; and bone-forming osteoblasts are also very important to the support and proliferation of stem cells, the calcium-sensing receptor, located on the surface of HSCs and other cells, is critical to stem cells finding their niche.

A key challenge in stem cell microenvironment research is to develop an in vitro system that accurately imitate the in vivo microenvironment [88]. Nanotechnology can be utilized to create in vivo-like stem cell microenvironment to determine mechanisms underlying the conversion of an undifferentiated cells into different cell types [89]. A better solution is currently under investigation: growing the stem cells on a so-called "lab-on-a-chip" [90]. This is a silicon chip with nanoreservoirs. The chip surface contains about a

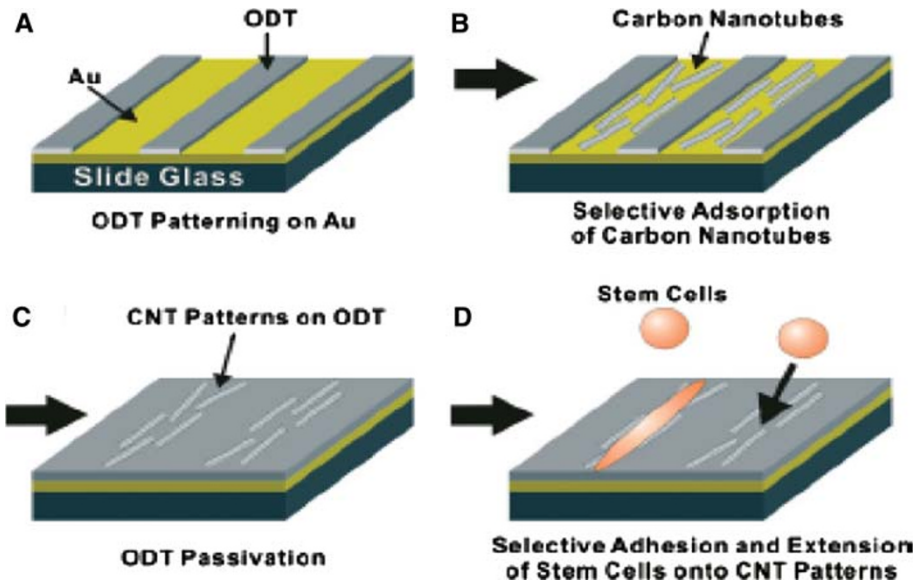

Fig. 7 Schematic diagram depicting the directed growth of MSCs on large-scale carbon nanotube patterns. **a** patterning of non-polar 1-octadecanethiol (ODT) SAM while leaving some bare Au area. **b** Selective adsorption and precision alignment of CNTs directly onto a bare Au surface. **c** Passivation of the exposed bare Au surface between the aligned CNTs with ODT. **d** Directed growth of MSCs onto the carbon nanotube patterns [85]

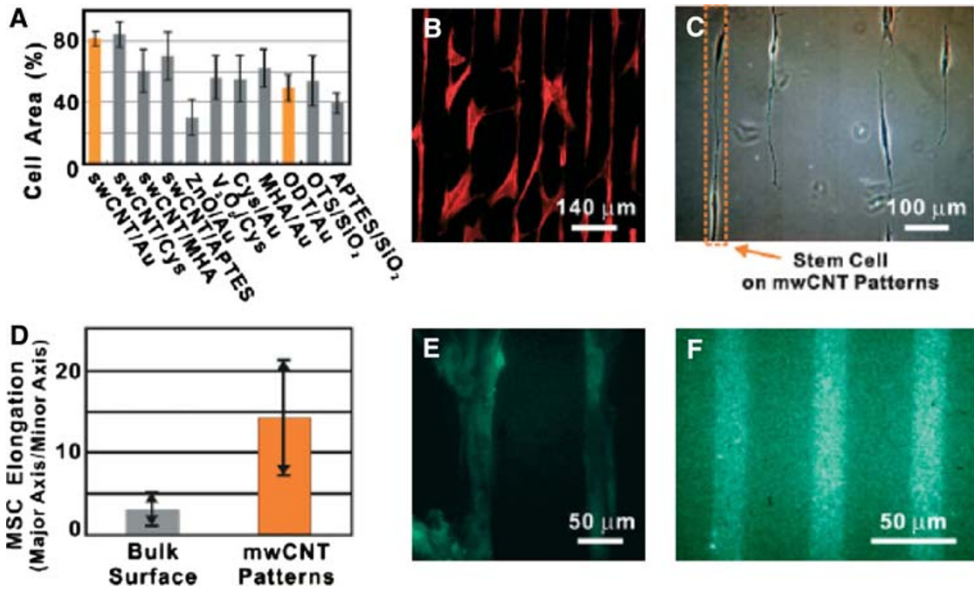

Fig. 8 **a** MSC adhesion on various nanostructures and self-assembled monlayer (SAM) on Au or SiO_2 surfaces. MSC spreading was characterized by measuring the cell area in actin filament fluorescence images. The surfaces studied are SWCNTs on Au (SWCNT/Au), SWCNTs on cystamine SAM on Au (SWCNT/Cys), SWCNTs on MHA SAM on Au (CNT/MHA), SWCNTs on APTES SAM on SiO_2 (CNT/APTES), ZnO nanowires on Au (ZNO/Au), V_2O_5 nanowires on cysteamine SAM on Au (V_2O_5/Cys), OTS SAM on SiO_2 (OTS/SiO_2), and APTES SAM on SiO_2 (APTES/SiO_2). **b** Fluorescence microscope image of actin filaments in MSCs adsorbed onto SWCNT patterns on Au surface. SWCNTs were adsorbed onto bare Au with ODT SAM as passivation layer. **c** Optical microscope image of MSCs adhered ontp mwCNTs/ODT SAM patterns (50 μm wide mwCNT regions and 100 μm wide ODT regions) with ODT passivation after 24 h of cell culture. The mwCNT regions appear as dark areas around the MSCs. **d** Elongation of MSCs on bulk swCNT substrates or swCNT line patterns as in (**b**). **e** Fluorescence microscope image of vinculins represaenting focal adhesions of MSC adsorbed onto swCNT patterns on Au. **f** Immunofluorescence image of the fibronectins adsorbed on the swCNT patterns on Au substrate [85]

thousand reservoir cavities, with each reservoir only about 500 nm across. A reservoir holds a small amount of liquid chemicals similar to what the stem cells would be exposed to in the niche. Each reservoir is sealed with a lipid bilayer equivalent to a cell membrane. These reservoir bilayers also contain the same voltage-gated channels found in cells. A small charge of electricity can then be applied to any individual reservoir to open the channels allow the chemicals to spill out, delivering them to any particular stem cell at any specified time of development. The nanoreservoir chip technology also allows the possibility of growing cells layer by layer, making compound tissues, which are otherwise difficult to produce.

Substrate topography influences a wide range of stem cell behaviors in a manner distinct from surface chemistry. One physical difference in the topography of divergent basement membranes is the size of pores and ridges. In vivo, cells never see flat surfaces: on the nanoscale, no basement membrane, or extracellular matrix is flat. The great majority of features in the extracellular environment are in the submicron to nanoscale range, ensuring that an individual cell is in contact with numerous topographic features [25, 91]. For example, Edgar et al. [92] focused on the thickness of polypyrrole films and their potential as a biocompatible material for rat MSCs. Others have investigated the potential of electrospun porous scaffolds of randomly oriented 500–900 nm diameter nanofibers for cartilage repair [57, 93]. Nanofibrous structures can favorably modulate osteoblast, osteoclast, and fibroblast activities toward implant and/or scaffold materials [94]. Nanofibrous matrices are introduced as scaffolds that may have a better structural resemblance to target tissues than their bulk counterparts, because major components in tissues are nanoscale structures and cells appear to attach and proliferate better on nanoscale structures than on bulk materials. So far there is a rapidly growing interest in synthesis of natural polymer based nanofibers because of their proven biocompatibility and resorbable biodegradation products. Advantageous attributes of natural polymers include hydrophilicity, nontoxicity, less immune reaction, as well as enhanced cell adhesion, and proliferation. However, fabrication of natural polymer nanofibers by electrospinning is challenging. Chitosan and alginate, two abundant natural polymers, have been widely used in tissue engineering, but none had been fabricated into nanostructured matrices until in recent 2 years. Li and Zhang [95] reported that they successfully used Chitosan- and alginate-based nanofibrous matrices to mimic the ECM of articular cartilage that primarily consists of type II collagen and proteoglycans (glycosaminoglycan, GAG). A kind of

nanopit template was etched with the special conglomeration surface and nanopits less than 100 nm in diameter. In the flat culture surface and nutrient medium of nanopit align ordered, the stem cell could not differentiate. But in the nutrient medium concurrent of ordered and unordered align nanopit, the stem cell could grow to the calcify ossature cell. The stem cell could obtain the signal from the template. The surface of the transplanted tissue is the nanoengineering surface, it can induce the stem cell grow into the ossature. Obviously, surface character play an important role on stem cell development and it is a relative simple way to control stem cell.

Application of Nanotechnology in Stem Cell Therapy

Nanotechnology plays more and more important role in stem cell therapy. Tysseling-Mattiace et al. [96] reported that paralyzed mouse which is lead by spinal cord injury recover walking function after injection the nanofiber which conjugating the laminin and nerve stem cell 6 weeks later. The neurite sprouting/guiding epitope combine the integrin which adjust cell differentiation could actuate signal and stimulate neuraxis extension. After 24 h, nerve stem cells begin to differentiate on damage position and generate new neuron which inhibit colloid cell form cicatrix and help recovery nerve. The nanofiber was degraded after 8 weeks. The experimental mice suffer sever spinal cord injury similar to human extremely sever damage caused by traffic accident. The regenerate method has great potential application in disease therapy such as Parkinson disease, apoplexy, cardiopathy, diabetes, and so on [97].

Nitric oxide (NO) has been shown to inhibit neointimal hyperplasia after arterial interventions in several animal models. NO-based therapies have great potential in clinical application. Combining nanofiber delivery vehicles with NO chemistry can create a novel, more potent NO-releasing therapy that can be used clinically. Primary experiment showed that the spontaneously self-assembling NO-releasing nanofiber gels can be used to prevent neointimal hyperplasia [98, 99].

Challenges and Prospects

In recent years, application of nanotechnology in stem cells has made great advances, which is becoming an emerging interdisciplinary field. Stem cell nanotechnology is developing toward imaging, active tracing, and controllable regulation of proliferation and differentiation of stem cells. However, like any emerging field, stem cell nanotechnology also face many challenges. The mechanism of interaction between nanomaterials and stem cells is still not clarified well, nanomaterials and nanostructures are how to affect the function of stem cells, nanomaterials inside stem cells are how to be metabolized, which are great challenges. How to use current knowledge and principles to fabricate novel multifunctional or homogenous nanostructures, the processing, characterization, interface problems, high quality nanomaterials availability, nanomaterials tailoring, and the mechanisms governing the behavior of these nanoscale composites on the surface of stem cells are also great challenge for present existing techniques. The ips cells were prepared by using HIV virus-based gene delivery system. Using nanomaterials-based gene delivery system to replace virus-based gene delivery system will also a great challenges. However, stem cell nanotechnology shows great attracting prospects, stem cells are developing toward application of generative medicine, we believe that stem cell nanotechnology will be broadly applied in treatment of injuries and degenerative diseases in the near future.

Concluding Remarks

Stem cell nanotechnology provides novel chance for stem cells research and development, speeds up the exploration of application of stem cells in generative medicine. Nanomaterials such as quantum dots, fluorescent CNTs and fluorescent MNPs, etc., have been used for imaging and tracing, gene or drug delivery, scaffolds for tissue engineering, designed nanostructures have been used to regulate the proliferation and differentiation of stem cells, which will speed up the understanding and controlling the microenvironmental signals, helping to solve the current bottleneck problems of stem cells-based therapy. Although stem cell nanotechnology faces many challenges, marriage of stem cells and nanotechnology have exhibited attracting technological prospects, and will dramatically advance our ability to understand and control stem cell-fate decisions and develop novel stem cell technologies, which will eventually lead to stem cell-based therapeutics for human diseases.

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