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Nanotechnology meets regenerative medicine: a new frontier?

Abstract: Regenerative medicine is the creation of a tissue or organ with normal structures and functions to replace the lost or impaired ones via biological modulation and tissue engineering. Indeed, many researchers have focused on exploring new techniques or approaches in this field. In recent years, numerous nanotechnologies have been incorporated into the field of regenerative medicine, aiming to replace tissues. The application of nanotechnology is important as many nanomaterials exhibit novel biological properties in the modulation of cellular events. The two main directions in this field are to provide biologically compatible scaffolds as an optimal environment for cell migration and proliferation; as well as to attempt to induce stem cell recruitment and differentiation. Thus, this review will focus on these two aspects, briefly describing the clinical applications.

Keywords: healing; nanoparticles; regeneration; scaffold; wound repair.

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1 Introduction

Nanotechnology is defined by the use or the manipulation of materials ranging from 1 to 100 nm [1]. The rapid development of nanotechnology in the past decade has resulted in expanding its applications into medical areas and has established itself into a new subbranch termed as nanomedicine [2, 3]. At the nanoscale, materials can interact and regulate the cellular/tissue events at the molecular level. Currently, nanomedicine is leading the way to a new perspective and advances in biomedical study and clinical application, including diagnostic imaging [4, 5], anti-inflammation/anti-infection [6, 7], cell therapy, and nano cryosurgery/laser surgery [8, 9], as well as the anticancer drug delivery systems or devices [10–13].

Furthermore, numerous nanotechnologies have also been incorporated into the field of regenerative medicine in recent years, aiming to replace cells or tissues lost, for example, after trauma, ischemic stroke, or myocardial infarction [14]. Indeed, wound repair and regeneration occur in any tissue and organs after injury to some extent. This is more apparent in the skin in daily life as it is the largest organ in the body and has the largest exposed area to the outside. The study of tissue repair and healing in the skin and bone after wounding plays an essential role in advancing knowledge in regenerative medicine. Nanotechnology can contribute to tissue regeneration in various ways, involving enhanced cell adhesion and proliferation/migration, improvement in growth factor production and delivery, provision of scaffold for cell repopulation, as well as induction of stem cell differentiation [15, 16]. This review will focus on the use of nanofiber scaffold in tissue engineering and nanotechnology-mediated effects on stem cells in wound regeneration, as well as their potential clinical applications.

2 Regenerative medicine

Tissue repair and regeneration are essential biological processes during wound healing. Conventional therapy for improving tissue repair after injury includes topical application of antibacterial drugs, to help maintain a relatively clean environment. This would allow the physiological process of healing to take place under normal conditions [17–19]. Nonetheless, for large wounds or those severe traumas with more tissue defects, the use of conventional therapy has not been universally successful, as regeneration of complex structures requires dramatic changes in cellular behavior. Many researchers have, thus, focused on exploring new techniques or approaches to enhance regeneration and function restoration in such severe wounds. Indeed, recent studies have shown that the promotion of cellular proliferation, migration, and differentiation could enable effective repair and regeneration, if used in the appropriate context [20, 21].

The definition of regenerative medicine is the creation of tissue or organ with normal structures and functions

to replace the lost or impaired ones via biological and tissue engineering [22]. In this regard, the two main directions in the development of this field are to provide biologically compatible scaffolds as an optimal environment for cell migration and proliferation, as well as to attempt to induce stem cell recruitment and differentiation [23]. Recent advances in nanotechnology have been effective in incorporating scaffold in tissue engineering and stem cell science [24], thus, expanding into the realms of regenerative medicine (Figure 1). The major applications of various nanomaterials currently being used in regenerative medicine are scaffolds, delivery devices, and applications for cellular modification.

3 Nanofiber scaffolds in tissue engineering

Tissue engineering involves the fabrication of scaffolds from synthetic and natural polymers through a variety of processing methods to act as biologically active substitutes for tissues and organs [25, 26]. Its initial theories and techniques of *in vitro* tissue constructs have already expanded with the development of the incorporation of nanotechnology. Indeed, the design and construction of the scaffolds are under intensive research in recent years, and its basic and important strategy is to construct biocompatible scaffolds, in combination with living cells and/or bioactive molecules, with the aim to replace, regenerate, or repair damaged cells or tissue [27, 28].

An ideal scaffold should have similar stromal properties comparable to target tissue. It should also have biocompatibility and permeability, controlled porosity, as well as support for cell attachment and proliferation. In this way, subsequent cell adhesion and growth will be greatly enhanced, and thus, acceleration of tissue repair and wound regeneration will be prompted. In this regard, nanotechnology-derived scaffolds have been proven to possess specific physiochemical properties [29, 30], satisfactory biocompatibility [31–33], improved permeability [34, 35], and adjustable porosity [36–38]. Moreover, recent study also indicated that nanotechnology could control the scaffold to have better nanotopographies, with which the surface roughness would further enhance cell attachment and spreading [39]. Zhu et al. regarded that an enlarged surface area of nanostructured materials in the scaffold would absorb and attach more adhesive proteins including fibronectin and vitronectin, which would increase cell-cell interactions, and tissue regeneration, as well as following the repair process in healing [40].

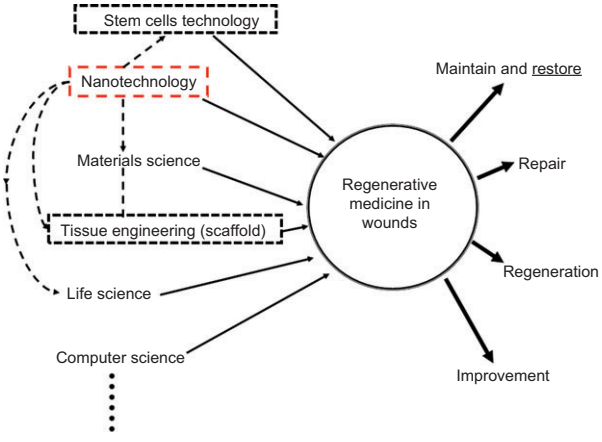

Figure 1 Schematic diagram demonstrating multidisciplinary sciences are involved in wound regenerative medicine. The advance of nanotechnology further innovates the development of various sciences.

3.1 Biochemical properties of nanofiber scaffolds

The chemical properties of various nanofiber scaffolds all mimic the fibrous architecture of the extracellular matrix (ECM) in normal tissues [41–43]. For instance, collagen types I, II, III, and IV, as the main ECM in the skin and bone tissues, are the most abundant natural polymers in the human body (Figure 2). They provide cells not only with the appropriate microenvironment for growth and tissue regeneration but also function to impart structural integrity and strength. As a result, collagen has been

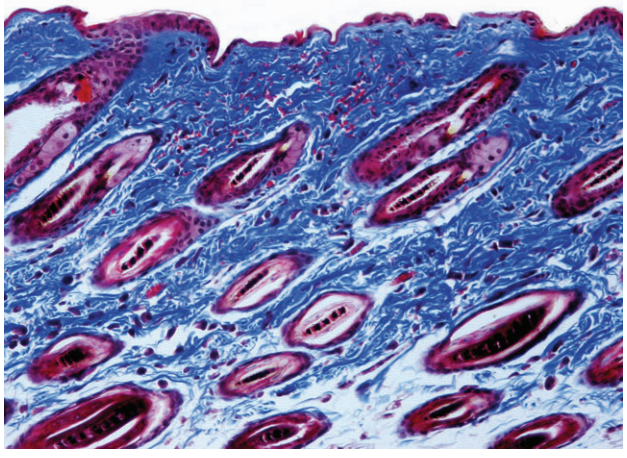

Figure 2 Masson trichrome of normal skin. Staining showed the distribution and density of collagen protein in the skin dermal layer. Under this staining, collagen protein was stained blue, the nuclei were stained black and background (muscle, cytoplasm and keratin) (200×).

utilized widely to fabricate nanofiber scaffolds. In addition, other natural polymers, including globular proteins and elastin, and synthetic polymers, such as poly (glycolic acid) (PGA), poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), polydioxanone, and polycaprolactone, have also been used [44].

Regarding the processing of nanofiber scaffolds, electrospinning and self-assembly techniques have been employed to fabricate for the regeneration of bone, skin, cartilage, and cardiac as well as nervous tissues [45].

3.2 Electrospun nanofiber scaffolds

Electrospinning is a widely used technique for the production of nanofiber scaffolds. It is a controllable technique through adjusting processing conditions and polymer properties. The nanofiber scaffold properties can be changed according to the practical requirement, especially for the size, porosity, surface topography, biodegradability, solubility, and mechanical function. These advantages make it possible to modify the spatial alignment and orientation, by which cell adhesion and proliferation as well as growth are directed and controlled [46].

Just like other nanomaterials, the electrospun nanofiber scaffolds have larger surface to volume ratio, which directly increases the exposure area to the surrounding cells. Therefore, they can be adapted to modulate the cellular event to promote proliferation, differentiation, and migration. Meanwhile, the larger area of these nanofiber scaffolds also increases the capability of cell adhesion. Furthermore, recent studies also indicated that these nanofibers could be also used to deliver various drug molecules, genes, and peptides, thus further enhancing cell regeneration.

During the exploration of the functionalization strategies to improve the delivery efficacy of the nanofiber scaffolds, the idea of coelectrospinning of the active surface agents and polymers, and the immobilization of bioactive ligands, have been sprung [47]. These could more effectively control the delivery efficacy. Meanwhile, the utilization of composites to control the shape of the nanofiber scaffolds has also been demonstrated to enhance the delivery of drug and biomedical properties. In this regard, Zhang et al. synthesized electrospinnable bioactive macromolecules (collagen and fibronectin) as the shell and a synthetic polymer with mechanical and structural properties as the core [48]. This functionalized composite scaffold, on one hand, enables cell growth and differentiation; on the other hand, the controlled release of encapsulated drugs further contributes toward cell regeneration and tissue repair.

Sahoo et al. targeted the natural ECM to fabricate into electrospun nanofiber scaffolds and deposited β-fibroblast (FB) growth factor, an important growth factor involved in mesenchymal stem cell proliferation and differentiation in tissue repair. Their study indicated that the electrospinning technique could effectively prolong the growth factor release from the scaffolds, and a sustained release positively influenced the stem cell behavior and fate [49]. Liu et al. further investigated the feasibility of incorporating neurotrophin-3 and chondroitinase ABC onto the electrospun collagen nanofibers for the treatment of spinal cord injuries. Their results showed that it was feasible to promote nerve regeneration through the provision of topographical signals and multiple biochemical cues arising from both nanofiber scaffolds and attached cytokines [50].

The traditional electrospinning technique is mainly for the fabrication of the 2-D nanofibrous solution or sheet. However, the size of the pores is, in general, too small for cell infiltration, resulting in the less protein absorption and cell adhesion. The seeded cells tend to grow in a monolayer on the surface of the mesh [14]. In recent years, research has been focused in producing 3-D nanofiber scaffolds for both *in vitro* and *in vivo* studies and applications, aiming to improve cell infiltration. Yang et al. explored a 3-D multilayered cell-nanofiber scaffold with alternating layers of human dermal FBs or keratinocytes seeded on PCL/collagen nanofibrous sheets. The results indicated that both kinds of cells seeded could effectively proliferate and contribute to the formation of new ECM, which would indicate the potential to be a substitute of the skin [51].

Horne et al. demonstrated that when compared to the classical 2-D cultureware, the modified 3-D electrospun poly-ε-caprolactone (PCL) nanofiber scaffolds were more superior in enhancing *in vitro* proliferation and differentiation of cortical cells. When neurotrophin, brain-derived neurotrophic factor (BDNF) were tethered onto the modified 3-D scaffolds, they further found neural stem cell proliferation and differentiation toward neuronal and oligodendrocyte lineages, indicating that modified PCL nanofiber 3-D scaffolds were capable of supporting neural stem cells and their derivatives [52]. Furthermore, Hurley et al. cocultured human microvascular endothelial cells (ECs) and FBs in peptide-made 3-D nanofiber scaffolds for up to 6 days and found that FBs in scaffolds enhanced capillary network formation by improving EC migration and increasing vascular endothelial growth factor and angiopoietin-1 expression in a temporal manner, while EC-FB interactions attenuated FB matrix metalloproteinase-2 expression and increased collagen I deposition, resulting in better construct stiffness and a more stable

microenvironment in cocultures [53]. This finding supported cell-cell interactions, and cell migration in 3-D nanoscaffolds contributed to an optimal environment for regeneration and tissue repair.

3.3 Self-assembling nanofiber scaffolds

In addition to electrospun nanofiber scaffolds, self-assembling nanofiber scaffolds provide another intensive research target in scaffold fabrication [54]. When topically applied onto the wound, these self-assembling nanofiber scaffolds would encourage wound edge tissue contraction. Furthermore, addition of functional ligands to the self-assembling peptides could enhance scaffold self-assembling effect.

Davis et al. designed a self-assembling peptide nanofiber scaffold for prolonged delivery of insulin-like growth factor 1 (IGF-1), a growth and differentiation factor for cardiomyocyte, to the myocardium using a biotin sandwich approach. After injection into the rat myocardium, the scaffolds provided sustained IGF-1 delivery for 28 days. When combined with transplanted cardiomyocytes, IGF-1 delivery by the scaffolds decreased caspase-3 cleavage and increased the myocyte cross-sectional area, through which systolic function was improved in an experimental myocardial infarction model [55].

Segers et al. also targeted stromal cell-derived factor-1 (SDF-1), which is a well-characterized chemokine for attracting stem cells and, thus, an ideal candidate for promoting regeneration, to investigate the efficacy of self-assembling nanofiber scaffolds containing SDF-1 on myocardial stem cell regeneration. They designed a chemokine named S-SDF-1 (S4V) that was resistant to matrix metalloproteinase-2 and exopeptidase cleavage but retains chemotactic bioactivity, thereby reducing the neurotoxic potential of native SDF-1. Self-assembling peptides were used to entrap and deliver S-SDF-1 (S4V). Their results demonstrated a nanofiber scaffold-induced delivery that promoted recruitment of stem cells and improved cardiac function in the myocardial infarction area [56]. This suggested that the chemotaxis of stem cells could be a promising strategy for tissue regeneration.

In addition to the above nanoscaffolds, some protein- and peptide-modified synthetic polymeric biomaterials were also used for the tissue regeneration and repair, including hydrogels based on protein-protein interactions and conformational changes of a protein. These conjugations at nanolevel of polymerics and protein/enzyme, not only were used as an ideal delivery system for macromolecules for cell adhesion but also provide a better "niche" to release important cytokines to local tissues [57]. Thus, these nanomaterials could further promote tissue repair due to their protein-protein interactions and conformational changes of a protein.

The 3-D printing technology is another area of intensive research in tissue engineering-mediated regeneration and repair. For example, this technology now enables the accurate 3-D organization of some components, which are important for bone formation, involving graft porosity and vascularization [58]. In this regard, bone printing is regarded as a promising therapeutics in orthopedics because it combines rapid prototyping technology to produce a scaffold of the desired shape and internal structure with the incorporation of multiple living cell types that can form the bone tissue once implanted [59].

4 Nanomedicine and effects on stem cells

The biomedical effect of nanoparticles on stem cells in wound is another important direction in regenerative medicine. Stem cells exist in most of the tissues and organs in the human body, including the skin and bone, cardiac tissue, etc. Even after severe injury, a few stem cells can still be found remaining in the local environment. These can proliferate and, together with the recruitment of the circulating stem cells, help in tissue repair. For example, in the skin, both the epidermal and dermal layers contain a subgroup of stem cells in each layer (Figure 3). These epidermal stem cells in the skin include two parts: the epithelial stem cells, found in the epithelial basal layer, and the bulge stem cells, located in the hair follicle [60]. The

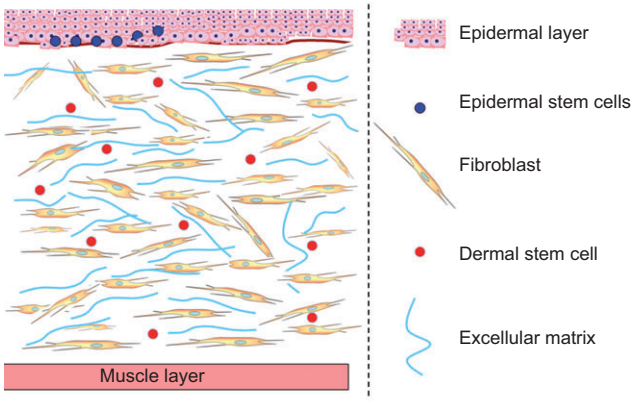

Figure 3 Schematic diagram showing the histological structures in the skin. The skin is composed of epidermal and dermal layers containing stem cell niches (blue: epidermal stem cells; red: dermal mesenchymal stem cells).

hair follicle consists of three anatomical structures involving the outer root sheath, inner root sheath, and hair shaft (Figure 4), the bulge stem cells are a group of specific cell population outer root sheath and have the potential to proliferate and differentiate into keratinocytes after wounding [61, 62]. When the epidermal tissue is damaged, the bulge stem cells can migrate to the epidermal layer to differentiate [63] (Figure 5). These populations of stem cells have self-renewing potential and amplify rapidly in response to skin injury and further undergo several cell divisions for the reconstruction of the epidermal barrier [64, 65]. For the dermal mesenchymal stem cells, they have been successfully isolated from the mouse and human skin [66, 67] and could be induced to form FBs, cardiomyocytes, adipocytes, and neurons [68, 69]. Furthermore, stem cell grafting is a specific technique for those wounds with severe damaged tissue or loss. The combination of nanotechnology with stem cell science may provide a new therapeutic approach for regenerative medicine.

4.1 Nanoparticles and stem cells differentiation

Stem cells in the undifferentiated state have the unique ability to differentiate into various kinds of cell types and the potential to develop into tissues eventually. They can be activated under the influence of drug molecules or cytokines. For the skin, the epidermal stem cell differentiation toward the keratinocyte lineage will contribute to the re-epithelization process during wound regeneration. The underlying signaling mechanisms of skin cell regeneration are beginning to be understood. Nguyen et al. reported that Notch/p63 cross-talk played an essential role in the differentiation of skin stem cells into keratinocytes [70]. Schafer and Werner found that c-myc knockout mice with the absence of the stem cells in the skin epidermal basal layer would display delayed re-epithelization [71]. This would support that c-myc was a regulator of the skin epidermal stem cell fate [72]. Apart from Notch and c-myc, Wnt/β-catenin [73, 74] and the Hedgehog signaling pathway [75, 76] have also been implicated in stem cell differentiation during skin regeneration.

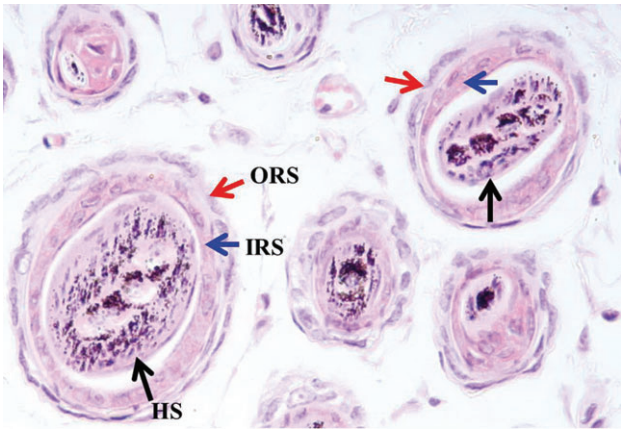

Figure 4 HE staining of normal skin hair follicles. Staining showed the anatomical structures of transverse section in hair follicles, including the outer root sheath (ORS), inner root sheath (IRS), and hair shaft (Hs) (400×).

Some nanoparticles have the potential to promote the differentiation of stem cells. For instance, Tian et al. reported that the topical application of silver nanoparticles (AgNPs) could accelerate healing in a burn wound model and that regenerated hair follicle tissue could be observed in the granulation tissue in AgNP-treated mice [77]. This finding would suggest that AgNPs might trigger various types of skin stem cell differentiations. Following this, we also observed a faster differentiation of keratinocytes deriving from skin stem cells during regeneration [78]. Moreover, others have also observed the concentration-dependent activation of human mesenchymal stem cells (hMSC) by AgNPs [79], which suggested that the ECM remodeling process in healing could be optimized through the effects of AgNPs, with consequent enhancement of the quality of healed skin.

For the bone injury, boron-containing compounds were shown to have the potential to contribute to osteoblast regeneration. Wu et al. explored boron-containing mesopore bioactive glass (B-MBG) scaffolds and evaluated the response of osteoblasts to these scaffolds. Meanwhile, the effect of dexamethasone (DEX) delivery in B-MBG scaffold system on the proliferation, differentiation, and bone-related gene expression of osteoblasts were also investigated. Their experiment suggested that boron-containing nanoparticles promoted osteoblast proliferation and contributed to the DEX release from the MBG scaffolds. Their study also suggested that the potential differentiation of the MSC induced by this nanoscaffold system into the osteoblasts was another factor contributing to bone tissue regeneration [80].

4.2 Nanotechnology-based microenvironment enhances stem cell differentiation

Stem cell niches are the microenvironment to the resident, maintenance, and development in cell circle. In recent years, intensive research targeted the creation of stem cell microenvironment to modulate cellular proliferation, differentiation, and maturation. In this regard, studies focused on the utilization of nanotechnology to mimic the

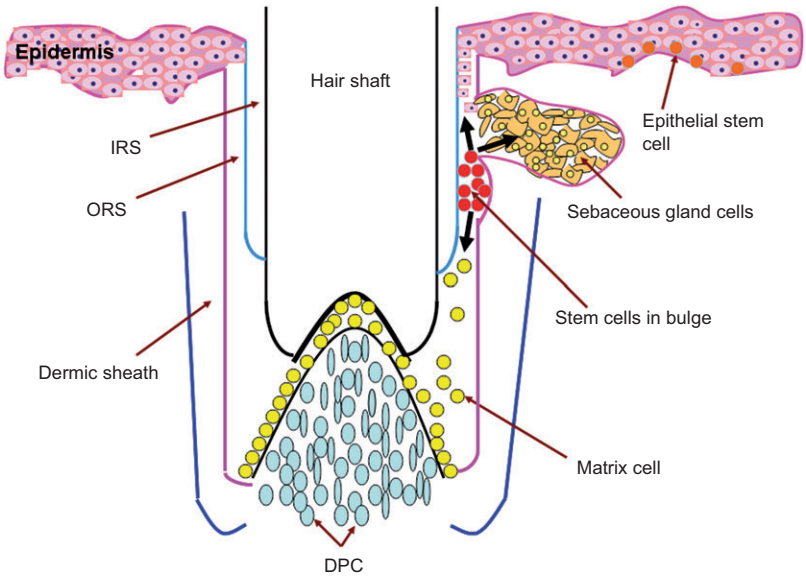

Figure 5 Schematic diagram showing the epidermal stem cell in the skin. Epithelial stem cells and bulge stem cells, respectively, distribute in the skin epithelium basal layer and in the outer root sheath in hair follicles (pink: epithelial stem cells; red: bulge stem cells).

in vivo stem cell microenvironment for the investigation of the mechanisms underlying the differentiation into different cell types [81].

Silva et al. utilized biologically compatible self-assembling peptide nanofiber scaffold (SAPNS) to create the ECM and used them for the neural cell regeneration. They found an effective migration of neural stem cells and growth of blood vessels in this scaffold [82], which suggested that SAPNS could establish a better 3-D microenvironment for the migration and differentiation of stem cells. Gelain et al. also reported the development of a 3-D cell culture system using the peptide nanofiber scaffold with mouse adult neural stem cells. They attached several functional motifs to self-assembling peptide RADA16. These functionalized peptides underwent self-assembly into nanofiber scaffolds similar to matrigel, and cells were fully embedded in the 3-D environment of scaffold. The results from gene expression profiling array experiments showed better neural stem cell adhesion and differentiation [83].

In recent years, Srouji et al. explored the effects of nanoscaffold on hMSC differentiation. It was found that this 3-D electrospun porous scaffold (composed of poly ε-caprolactone and collagen) could support hMSC cell attachment and proliferation with better distribution. When these nanoscaffolds were subcutaneously implanted into nude mice, good integration with surrounding tissues and neovascularization were seen [84]. Taken together, the combination of nanotechnology and stem cell science will bring promise for the wound regenerative medicine.

4.3 Biological effect of nanotechnology on various types of stem cells

Nanotechnology-mediated delivery of biomolecules, including proteins/enzyme, growth factors, and cytokines present an excellent tool to control the differentiation of stem cells. Some biocompatible nanoparticles can gain access into stem cells and activate signaling cascades.

For the embryonic stem cells (ESCs), Ferreira already reported that the incorporation of these polymeric nanoparticles had a large impact on the differentiation in the human ESCs [85]. Sridharan et al. explored the use of a composite collagen carbon nanotube material as an *in vitro* cell culture matrix to induce early differentiation of human ESC to neural progenitor cells. They used carboxyl-modified single-walled carbon nanotubes (SWCNTs) to obtain a composite material with type I collagen. They found that carboxyl-modified SWCNTs contributed to the early differentiation of ESCs into neural progenitor cells [86]. Chao et al. found that polyacrylic acid (PAA) grafted onto carbon nanotubes has the ability to differentiate human ESCs into neurons, and when they grafted a new 2-D thin film composed of PAA onto the carbon nanotubes, they found that the differentiated neurons were more mature than those on the pure PAA control surfaces due to the lower levels of neural differentiation. Furthermore, this new type of thin-film scaffold enhanced ESC proliferation and adhesion [87]. In the following study, they targeted the ability of PAA-grafted MWCNTs, pure MWCNTs, as well as MWCNTs functionalized by polymethacrylic

acid (PMAA) in various differentiation potential of human ESCs into neuronal cells [88]. The results further suggested that carbon nanotubes could enhance cell adhesion and growth factor adsorption, as well as stem cell differentiation. In addition to the ESCs, modern regenerative medicine research has been targeting the generation of patient-specific induced pluripotent stem cell (iPSC) lines [89]. Indeed, the protocol for the derivation of neural crest cells from human iPSCs has been developed [90, 91], which indicated that these could be induced to differentiate toward the neuron, while nanotechnology will continue to contribute to the induction and differentiation.

For the mesenchymal stem cells, they have the ability to differentiate into several specific cell lineages in the local microenvironment [92]. Voge and Stegemann found that nanotopography provided by carbon nanotubes could influence the mesenchymal stem cell differentiation [93]. Furthermore, the nanofiber scaffold fabricated by the electrospinning technique was indicated to be matrix conducive to the differentiation of hMSCs into hepatocyte-like cells, and the expression levels of liver-specific markers (albumin, α-fetoprotein) were higher, demonstrating that the nanofiber scaffolds enhanced hMSC differentiation into a hepatocyte lineage [94]. Their study suggested that the differentiation of mesenchymal stem cells induced by nanotechnology might be used in the treatment of liver failure. While for the neural stem cell, Kam et al. explored the influence of laminin-SWCNTs films on neural stem cells, their study indicated that compared with pure laminin substrates, laminin-SWCNT films could enhance the growth and proliferation of neural stem cells; moreover, layer-by-layer of films consisting of SWCNTs and laminin could promote their adhesion and differentiation, like that of the presence of large amounts of differentiated neurons and glial cells. These results suggested that thin composite SWCNT-laminin films can be employed as materials in the foundation of neural electrodes to long-term integration with neural tissue [95].

5 *In vivo* applications of nanotechnologies in regenerative medicine

5.1 Skin tissue regeneration and wound dressing

For the nanofiber scaffolds, enhanced cell adhesion and proliferation due to larger surface, better local adsorption of wound liquids due to controllable porosity, and comparability to ECM, make it possible to be used as wound dressing. Meanwhile, these nanofiber scaffolds also have the potential to deliver various drug molecules to the wound site, which can further contribute to the wound tissue regeneration and repair. For example, some nanofiber scaffolds could deliver antibiotics [96–99], some nanoparticles have antibacterial properties [100–102], and some contain cytokines [103], as well as growth factors [104]. Khil et al. initially explored the coating of polyurethane membrane on wound dressing using electrospun nanofibrous technique. Their study indicated that this wound dressing led to an increased rate of epithelialization and dermis organization in wound tissue [105]. In a subsequent study, Chen and Chiang modified the polyurethane membrane by adding AgNPs [106]. The polyurethane membrane's antimicrobial activity improved to approximately 100% and suggested that this modified product was a better collagen sponge wound dressing.

In theory, any polymer with better biocompatibility and antibacterial properties can be conjugated to nanofiber scaffolds for the fabrication of wound dressing. For instance, chitosan is a natural biodegradable polymer with ideal biocompatibility, antibacterial, hemostatic, as well as wound-healing properties [106, 107]. Meng et al. found an improved healing of deep second-degree burns in rats using wound dressing coated with RADA16-I peptide self-assembling nanofiber hydrogel scaffolds [108]. Zhou et al. used electrospun carboxyethyl chitosan/polyvinyl alcohol to construct wound dressing and found that the electrospun matrix could be used as a potential wound dressing for skin regeneration [109]. This finding was further supported by the Kang et al. study [110]. Other candidates for wound dressing applications include silk fibroin/hydroxybutyl chitosan nanofibrous scaffolds. They have also been shown to have a good biocompatibility with better cell viability and fast skin wound healing rate [111, 112].

5.2 Bone regeneration

Bone is a mineralized organic matrix composed of collagenous fibers and calcium phosphate and exists in the format of hydroxyapatite (HA). A large amount of osteoblasts, osteocytes, and osteoclasts are embedded in the bone tissue, which is endowed with both elasticity and hardness. Therefore, in terms of designing the bone scaffolds in reconstruction, several factors need to be considered. These include suitable biophysical properties (elastics and hardness), porosity to support cell growth,

and differentiation [14]. Furthermore, for those nanoscaffolds with delivery function, the releasing kinetics of drug molecules from scaffolds and sustainability of cellular differentiation also need to be considered. In this regard, osteoblast ossification induced by nanofiber scaffolds has been investigated in various HA composites, including with collagen [113–115], PLA [116], and PLGA [117, 118]. Furthermore, Gentile and Zou explored HA/gelatin composite nanoscaffolds in both *in vitro* and *in vivo* experiments and found that these nanoscaffolds could effectively promote osteoblast proliferation and adhesion, and the ossification process was accelerated [119, 120].

Li et al. targeted silk fibroin fiber scaffolds containing bone morphogenetic protein 2 (BMP-2) to explore the effect on hMSCs. They found that the scaffold could support hMSC growth and differentiation toward osteocytic lineage. Enhanced calcium deposition *in vitro* and upregulation of the transcript levels of bone-specific markers were demonstrated, indicating that these scaffolds provided an efficient delivery device for BMP-2 and induced improved bone formation [121].

5.3 Neural tissue regeneration

Ellis-Behnke et al. initially explored the effect of self-assembling peptide nanofiber scaffold on the repair of injured brain tissue. They found in a hamster model of severed optic tract that the nanofiber scaffold could enhance regenerated axon reconnection to target tissues with sufficient density to promote functional return of vision, indicating that the nanofiber scaffold could contribute to tissue repair and restoration, and would be helpful to the central nervous system trauma [122]. Wang et al. fabricated the chitosan nano/microfiber mesh tubes with a deacetylation rate (DAc) of 93% and used them to bridge injured rat sciatic nerve, observed sufficient mechanical properties to preserve tube space and provision of a better scaffold for cell migration and attachment, and enhanced nerve regeneration [34], which further provided the evidence that nanofiber scaffold had the potential for the therapy of severe regeneration.

In recent years, Sakai et al. found that conduits reconstructed using hyaluronic acid (HA) had better cell adhesion and differentiation, through the contribution from axonal regeneration in the peripheral nerves [123]. Ding et al. also targeted the rabbit sciatic nerves damaged model to explore the tissue-engineered scaffold-mediated regenerative effect, and they found superior functionality of the nanosilver-collagen scaffold in the adsorption to laminin and subsequent regeneration of damaged sciatic nerves [124].

5.4 Cardiac tissue regeneration

Cell-based therapies in cardiac tissue engineering represent a potential cure for patients with cardiac diseases, including myocardial infarction and heart failure. Currently, stem cell science has started to play an essential role in cardiac tissue regeneration and repair. Various stem cell graftings, including iPSC immobilization for myocardial infarction (MI) therapy, have achieved satisfactory efficacy.

Initially, it was found that injection of human ESC-derived vascular cells in a bioactive hydrogel could form capillaries in the infarcted zone in a rat model [125]. In this regard, Caspi et al. have engineered vascularized cardiac muscle using hESC-derived cardiomyocytes and hESC-derived ECs, and increased cardiomyocyte and EC proliferation, as well as the formation of vessel-like structures could be observed in engineered tissues [126]. Currently, iPS cells have been shown to be promising for use in cardiac tissue-engineering strategies, as they can give rise to functional cardiomyocytes [127]. The use of iPS cells would not only overcome the ethical concerns related to the use of hESCs, but it might also allow for the generation of an unlimited supply of functional, proliferative, and possibly autologous human cardiomyocytes and vascular cells, thus, overcoming any immunogenic concerns as well [128].

6 Conclusions

The advance of nanotechnology and its incorporation into other sciences, have greatly contributed to the progress of regenerative medicine, especially in controlling stem cell differentiation, modification of biocompatible materials, and the enhancement of drug delivery. Current interest of nanotechnology in the regenerative medicine field is growing at a great pace due to its capacity to mimic natural tissues. In the future, nanotechnology will be even more reliable, stable, and imaginative. Nonetheless, the nanomaterials used should be natural, non-immunogenic, and nontoxic. Before translating the findings of basic laboratory study to clinical trials, comprehensive assessment on the potential toxicity and side effects should be taken to ensure the absolute safety of our patients.

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