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Nanobiomaterials: State of the Art

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1.1 Introduction

Nanoscience and nanotechnology, an interdisciplinary research activity that deals with sub-nanometer to several hundred nanometer materials, has been developing explosively worldwide in the past decade. Biomaterial is the material used for diagnosis or treatment of disease, evaluation, repair, or replacement of any tissue, organ, or function of the body [1]. Nanobiomaterial – the combination of nanotechnology and biomaterials – has provided great opportunities to improve the preclusion, diagnosis, and treatment of various diseases. Nanobiomaterial, traditionally defined as a *special category of biomaterials* with constituent or surface sizes not more than 100 nm [2], is a new class of extraordinary materials with unique structures and properties such as mechanical, optical, and electrical compared to bulk traditional materials with microscopic or macroscopic structures. It has been broadly applied in a wide range of biological and biomedical applications such as tissue engineering, drug delivery, imaging and biosensor, and so on. These nanobiomaterials include nanoparticles, nanotubes, nanofibers, and so on.

Although nanobiomaterials have been applied to many aspects of biomedical fields, the accurate interface interaction between cells/tissues and materials is not completely clear. The safety and toxicity of nanobiomaterials have caused extensive concern at both occupational and research levels. Biocompatibility is an essential issue that requires evaluation for a nanobiomaterial under consideration for clinical application. Currently, researches on nanobiomaterials have entered a more comprehensive and systematic stage. The researchers are seeking further understanding of the mechanism behind the biological response to biomaterials and better design of such materials.

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The absolute efficiency of nanobiomaterials on the human body has not been confirmed completely and the full benefit of nanobiomaterials cannot be evaluated precisely at this stage. Therefore, it is meaningful to review the current state of the art regarding the application of nanobiomaterials. This chapter provides a discussion on prospective applications of nanobiomaterials in different biomedical fields covering tissue engineering, drug delivery, imaging, and so on. In addition, an overview of the unique properties of nanoscale materials, the assessment of biocompatibility and toxicity, and the future development is also presented.

1.1.1 Properties of Nanobiomaterials

Nanomaterials refer to those materials with constituent components or surface sizes within 1–100 nm in at least one dimension [3], and the definition has been extended to several hundred nanometers today. Nanomaterials possess numerous novel and significantly changed properties, such as mechanical, electrical, magnetic, optical, and others [4], compared to those traditional materials in the micron or larger scales. Firstly, nanomaterials have much larger specific surface area than their conventional forms, which is beneficial to greater biochemical reaction. Secondly, the mechanical properties such as yield strength and ductility are enhanced because of the many mechanisms hinging on their chemistry such as grain boundary sliding and short-range diffusion healing. Thirdly, the nanostructure can lead to novel optical, electrical, and magnetic properties for materials due to the quantum effects playing a prior role in determining the properties and characteristics in nanoscale. In addition, the homogeneousness and purity in ingredient and structure are improved due to reaction or mixture at the molecular and atomic levels. These novel and unique properties enable nanomaterials to be suitable candidates for applications in electronics, medicine, and other fields. Specifically, nanobiomaterials possess some important properties provided by nanoscale structures. First, the chemical properties and structure are similar to the native tissues with nanometer hierarchical components. For example, the collagen fibers and nanosized hydroxyapatite (HA) can mimic the components of bone tissue. Second, researchers can easily identify, handle, and mediate biocomponents because of the comparable size of nanoscale materials to biomolecules and bio-microstructures. At last, it is possible to modify the surface properties of nanostructured materials through advanced techniques [5].

1.1.2 Interaction between Nanobiomaterials and Biological System

The nanometer-scaled functional elements in the biological system determine that the interaction between nanobiomaterials and the biological system is at the molecular level [6], and the understanding of the interactions between them is of great importance. For example, embryonic and adult stem cell behavior can be controlled by modifying the material surface with intrinsic signals (e.g., growth factors and signaling molecules) if the interaction between a particular nanobiomaterial and stem cells could be understood [5, 7]. Up to now, details of the reaction at the interface between nanobiomaterials and biological systems (e.g., cells, blood, and tissues) have not been completely understood. Given the

current knowledge, the interaction between cells and biomaterials surface at the cellular and molecular level can be described as the interaction between the binding sites on the surface of the cell membrane and nanobiomaterials. In the physical environment, the interaction between cells and biomaterials is actually the molecular recognition between the receptors on the cell membrane and the ligand on the biomaterials surface, followed by a series of biological specific and nonspecific interactions. The previous researches showed that a sequence of events occur at the interface between biomaterials and cells [8, 9]. Firstly, the proteins in blood and tissue fluids are adsorbed onto the nanomaterial surface and protein desorption also usually occurs in the meantime. Then the tissue cells and/or immunocytes come close to the biomaterials. Next, the matrix proteins released from the biomaterial and specific proteins are adsorbed selectively. Eventually, the cells adhere to the surface of biomaterials and commencement of subsequent cell functions (the proliferation, migration, differentiation, and phagocytosis) occurs. These are a series of host responses toward the nanobiomaterials. Correspondingly, there is also a sequence of material responses to the host such as material decomposition that exists at the interface between cells and nanobiomaterials [9]. These events truly reflect the cytocompatibility and inflammatory/immune host responses that eventually determine the efficiency and safety of nanobiomaterials, which are vital for the successful design and application of nanobiomaterials. Thus, the deep understanding of the interaction between nanobiomaterial surface and cells is the key to clinical application of nanobiomaterials.

The response between cells/tissues and biomaterials can be altered or controlled by the surface properties of materials [3, 10, 11], such as topography, surface chemistry, charge, and energetics, which are closely related to cell or tissue responses [3, 10–16], due to the fact that cells/tissues can recognize the surface properties and synthesis nature of nanobiomaterials both *in vitro* and *in vivo*. Surface modification of biomaterials can make specific recognition sites for cellular and molecular responses, which has been widely applied in modulating cell and tissue responses by nanobiomaterials both *in vitro* and *in vivo*.

1.1.3 Biocompatibility and Toxicity of Nanobiomaterials

Nanobiomaterials have been applied to tissue engineering applications, and the researches demonstrated that nanobiomaterials can enter the body through different ways [17]. There is a well-developed system called the *immune system* in the human body which can protect it from invading organisms such as bacteria, viruses, and other parasites. The nanomaterial implanted into the body may be identified as foreign matter and consumed by immune cells. The pathway and route of biomaterial-like particles into the human body rest with the size, even at the nano-level. The agglomeration of nanobiomaterials is one of the vital factors that can affect their toxicity [18]. A research showed that the aggregation of nanoparticles can be problematic and even cancer may be induced because of the shape of nanomaterials [19]. The biocompatibility and toxicity of nanostructured biomaterials are important issues that require investigation for clinical development. For example, the nanoparticles used to deliver drugs

to targeted cells can normally traverse the cell membranes and be uptaken by the cells. Moreover, many implants undergo biodegradation *in vivo*. The effect of degradation on the cells and tissues in the physiological environment should be investigated [20]. The toxicity of nanobiomaterials is mostly dependent on the materials. In addition, the toxicity levels of a nanobiomaterial can also be affected by surface modification and functionalization. The evaluations of biocompatibility and toxicity of the nanobiomaterials are indispensable.

Presently, a series of *in vitro* and *in vivo* researches have been launched on the biocompatibility and toxicity of nanobiomaterials. As for the *in vitro* investigations, the influence of nanobiomaterials on cell morphology and cellular functions including proliferation, differentiation, and mineralization will be studied by microscopy and the gene/protein expressions with various biochemical analyses. The negative effects of nanobiomaterials *in vivo* usually include oxidative stress, inflammation, granulomas, and fibrosis. In order to see if nanobiomaterials trigger severe inflammation reaction and cause significant effects on the normal functions of the surrounding tissues or main organs, the materials are implanted into the animal body, and further histological, histopathological, and immunohistochemical studies are conducted [20]. Although there are existing methods to assess the biocompatibility and toxicity of the nanobiomaterials, they are nowhere near enough. Further researches such as deeper analytical approaches to animal experiments and much more convincing mechanisms on this issue are necessarily needed. For example, it has been shown that the toxic effects of carbon nanobiomaterials partially depend on the aspect ratio [21], but the actual toxicity levels of carbon nanotubes (CNTs) is still a debatable issue. It is necessary to improve the current measurement accuracy of biocompatibility and toxicity, and it is essential to establish more appropriate methods to evaluate the long-term safety of nanobiomaterials both *in vitro* and *in vivo*. Most importantly, it is urgent to find more effective methods to improve the biocompatibility and reduce the cytotoxicity of nanobiomaterials.

1.2 Nanobiomaterials for Tissue Engineering Applications

Tissue engineering, with the goal of developing or identifying appropriate biomaterials able to facilitate the desired cell behaviors and tissue functions, is promising to restore partial or even full functionality once a defect has occurred in tissues or organs [22]. The fine structure of nanobiomaterials, allowing direct mechanical interactions with cell surface receptors and cellular components and providing guidance for cells, usually serves as a microenvironment in which rich extracellular matrices (ECMs) and various cell types reside for tissue regeneration application [23]. Nanobiomaterials have been used in a wide range of tissue engineering applications in various basic structural units, such as nanoparticles, nanofibers, nanotubes, and nanofilms, to fulfill the specific requirements of different biological substitutes that repair or replace malfunctioning tissues and organs with separate physiological functions [24], which are reviewed in this section.

1.2.1 Vascular Tissue Engineering

There are three distinct layer structures in native blood vessels. The inner layer is composed of an endothelial cell layer with an anticoagulant function, the middle layer is composed of smooth muscle cells (SMCs) embedded in a three-dimensional ECM, and the outer adventitial layer is connective tissue composed of fibroblast cells. There are nanostructured collagen and elastin in ECM. Some studies found that cells of vascular tissues indeed can interact with nanomolecules *in vivo* [25–28]. There is an urgent requirement for an appropriate approach to replace vascular tissues that have been damaged or lost due to injury or disease. Currently, the therapy for damaged vessels involves replacement of the vessels with autografts or allografts or artificial vascular grafts with a structure similar to that of the native blood vessel [29]. However, due to the formation of thrombus and compliance incongruity, most synthetic materials used in vascular grafts have been indicated to be prone to clot and fail, and do not function well in the long term [30, 31]. Numerous methods have been used to fabricate artificial blood vessels with structures and functions similar to that of the native ones [32–35]. The vascular tissue engineering approach is used to overcome the defects of traditional vascular substitutes, particularly referring to small-diameter ($\leq$6 mm) vascular grafts. Nanomaterials have been used to mimic these actual nanostructures in vascular tissues. Various nanobiomaterials have been designed, fabricated, and modified to promote and control the function of vascular endothelial cells and SMCs and to overcome associated problems such as inflammation and thrombosis [24].

The desired vascular graft should have good mechanical property, which enables it to resist long-term blood pressure [36, 37]. Nanofibers, nanopatterns, and nanostructured materials have been fabricated to increase the mechanical strength of vascular grafts [38]. Besides, good biocompatibility is an important consideration for the design of vascular grafts, which requires that these constructs possess structures similar to that of native blood vessels and natural ECM [39]. A number of recent studies have indicated that nanomaterials are able to increase vascular cell (especially endothelial and SMCs) function such as the adhesion, proliferation, and synthesis of related collagen and elastin [40–44]. Choudhary *et al.* reported that the nanostructured surface on Ti greatly promoted the adhesion and proliferation of vascular endothelial cells compared to conventional Ti. It was also found that endothelial cells showed greater competitive functions than that of SMCs on the nanostructured Ti surface, which indicated that vascular endothelial cell functions were improved over that of vascular SMCs. Therefore, the endothelialization on nanostructured stents may be increased and vascular restenosis can be limited [41]. Miller *et al.* created poly(lactic-*co*-glycolic-acid) (PLGA) vascular grafts [43, 45, 46] with nanometer surface features that stimulated proliferation of both vascular endothelial cells and SMCs compared to the conventional PLGA scaffold [45]. It also proved that the PLGA scaffold with nanostructure improved adsorption of fibronectin and vitronectin from serum compared to the conventional PLGA scaffold [46]. Hence, the nanostructured PLGA can lead to greater vascular cell response. In addition, the influence of PLGA with different nanometer surface features

(500, 200, 100 nm) on vascular responses was studied. It showed that the vascular cell response was promoted by PLGA with 200-nm surface features and there was greater fibronectin interconnectivity than with smooth PLGA and PLGA with 500-nm surface structures [43].

Apart from nanoscaled surface L structures, 3D nanofibrous scaffolds have been fabricated for vascular tissue engineering application via electrospinning [47, 48]. Xu *et al.* fabricated poly(L-lactide-*co*-ε-caprolactone) P(LLA-CL) scaffolds with diameter of 400–800 nm by electrospinning [49], and they found that the adhesion and proliferation of vascular endothelial cells and human SMCs were both supported by these nanofibrous scaffolds that could mimic the nanoscaled dimensions of native ECM (Figure 1.1). Cells cultured on nanofibrous scaffolds could preserve their phenotype and then be integrated with nanofibers to form 3D ECM. Hashi *et al.* fabricated poly-L-lactic acid (PLLA) nanofibrous scaffolds [50] for culturing vascular SMCs and mesenchymal stem cells (MSCs) for 2 days, suggesting that cells had a cellular organization like that of native blood vessel. In addition, the nanofibrous grafts were also implanted in the carotid artery of rats for up to 60 days, and results showed that the nanofibrous scaffold combined with MSCs possessed antithrombotic and anti-immune functions. The nanofibrous structure enhanced recruitment of vascular cells *in vivo* and promoted the organization of a layered structured similar to that of the native blood vessel. In addition, P(LLA-CL) nanofibrous tubular grafts,

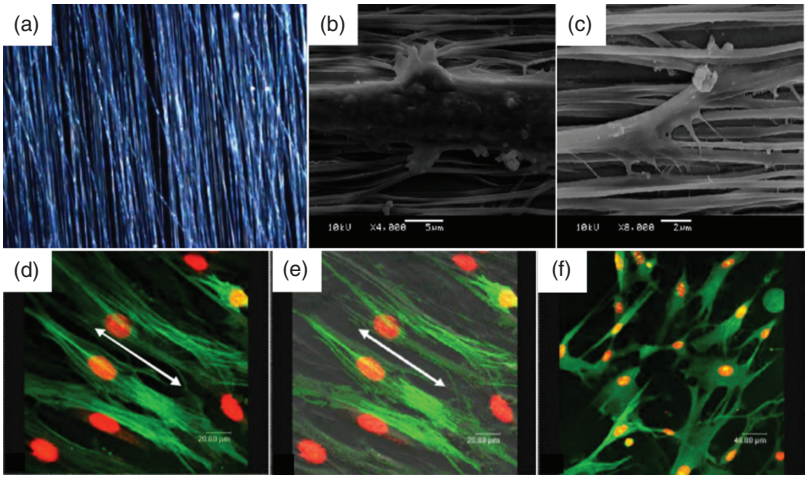

Figure 1.1 3D nanofibrous scaffolds fabricated by electrospinning for vascular tissue engineering application. (a) Optical microscope micrograph of aligned P(LLA-CL) nanofibrous scaffold; (b) and (c) SEM micrographs showing the cell–matrix adhesion between the SMCs and the aligned P(LLA-CL) nanofibrous scaffold; Laser scanning confocal microscopy (LSCM) micrographs of immunostained a-actin filaments in SMCs after 1 day of culture, (d) on aligned nanofibrous scaffold, (e) on aligned nanofibrous scaffold, overlay image on the aligned fiber, and (f) on TCPS. (With permission from Xu *et al*. 2004 [49], Elsevier.) Currently, nanobiomaterials have been fabricated into 2D and 3D scaffolds for vascular tissue engineering applications, indicating enormous promise to promote the efficiency of vascular stents or grafts for tissue regeneration.

with mechanical properties similar to that of the native coronary artery that were able to withstand high blood flow pressure and supported the attachment and proliferation of vascular SMCs as a temporary matrix, were used to repair damage to blood vessels [51]. Consequently, 3D nanofibrous scaffolds fabricated via electrospinning method can be used for vascular tissue engineering application with great potential. Another technique used to fabricate nanoscaffold is self-assembly. Self-assembled peptides with excellent cytocompatibility properties have been used along with the basement membrane of blood vessels for vascular tissue engineering [52]. Self-assembled nanostructured scaffolds of three functional peptide sequences were created from laminin and collagen IV proteins by Genové *et al.* [53], which could enhance the endothelialization. Meanwhile, laminin and collagen IV deposition and nitric oxide release by the vascular endothelial cell monolayer were also promoted. All these results indicate the great potential of nanostructured scaffolds to mimic the native artery for vascular tissue engineering application.

1.2.2 Neural Tissue Engineering

Patients with nerve injuries or traumas often suffer from neuropathic pains and eventually face losing sensory or motor function due to the regeneration ability of the nervous system being limited. Repair of damaged nerves and recovery of full function of the nervous system are great challenges. The nervous system, consisting of the central nervous system (CNS) and the peripheral nervous system (PNS), is a sophisticated network that can receive, elaborate, and respond to all information coming from the external and internal environments [54]. The brain and spinal cord are constituent parts of the CNS, whereas the nerves branching out from the CNS and going to the periphery form the PNS. The repair procedures of these two systems after injury are completely different, which is shown in Figure 1.2 [55–57]. For the PNS, the first step of regeneration is called *Wallerian degeneration*, where the Schwann cells (SCs) detach from the axons because of interruption of the myelin sheaths. Then the myelin sheath is phagocytized by resident and recruited macrophage. The next step is the formation of bands of Büngner, which are columns of cells aligning the endoneurial tubes due to proliferation of detached SCs [58, 59]. Later, these newly formed columns can guide the regeneration of axons. However, for the CNS, the recovery of full functions by re-extension and reinnervation of axons is very difficult because of the absence of SCs. Even more importantly, astrocytes, meningeal cells, and oligodendrocytes will lead to the formation of thick glial scar tissue around the materials, which will hinder the growth of proximal axon and limit the regeneration of the neuron [56]. For all these reasons, repair of CNS injuries is much more challenging than the repair of PNS injuries.

As always, autograft is the gold standard for nerve tissue repair. However, full recovery of functions is still not realizable even applying this method. The ability of the donor nerve to achieve full functional recovery is limited by its size and sensory nature [60]. As for the allograft, inflammatory reaction, infections, and even tumor formation may frequently occur; thus, systemic and prolonged immunosuppression is required to avoid rejection of the graft [61, 62]. A variety

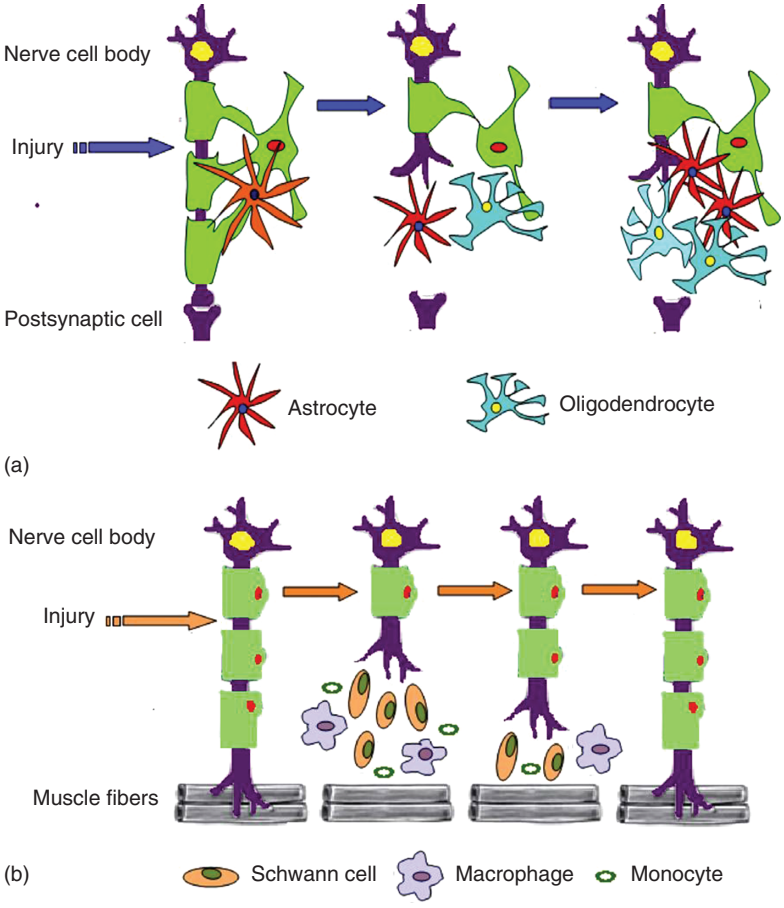

Figure 1.2 Schematic graphs of injured nerve regeneration in the central and peripheral nervous systems. (a) Central nervous system recovery process with glial scar tissue formation and (b) peripheral nervous system recovery process involving the activity of Schwann cells, macrophages, and monocytes. (With permission from Zhang and Webster 2009 [24], Elsevier.)

of biomaterials have been used to fabricate different nerve grafts to bridge nerve gaps and guide neuron outgrowth for nerve repair application [63–65], but these natural and synthetic biomaterials still have several limitations. For instance, polymers used as nerve conduits for nerve repair are limited because of the formation of glial scar tissue around the materials. In addition, the absence of optimal mechanical and electrical properties prevents nerve regrowth. Nanobiomaterials with exceptional mechanical and electrical properties and cytocompatibility may offer better chances of healing damaged nerves. With good mechanical property, the materials can last long enough to physically support neural tissue regeneration. With excellent electrical property, the materials may help enhance and regulate neuron behavior under electrical stimulation and guide neural tissue repair more effectively. With good cytocompatibility,

the materials can promote neuron growth and, in the meanwhile, will not cause inflammatory response and infection.

Nanobiomaterials with various morphologies have been fabricated by different techniques such as electrospinning, phase separation, and self-assembly and used to improve axonal regeneration and promote neural regeneration [66]. These nanobiomaterials, including nanoparticles, nanotubes, and nanofibers, can bio-mimic the natural structures of native neural tissues [67]. Chitosan–heparin nanoparticles containing nerve growth factors were able to improve neuron outgrowth in mice sciatic nerve, and these nanoparticles can be used as the functional drug delivery system for neural repair [68]. A nano-silver-embedded collagen scaffold coated with laminin and fibronectin proteins, with a structure similar to that of an autologous nerve graft, could increase the axonal outgrowth and the quantity of newly formed nerves [69]. In another study, better adhesion of embryonic stem-cell-derived neural precursors and faster differentiation were found on gold thin films with nanoscaled roughness than those on planar gold surface [70]. The axonal regeneration of embryonic stem cells could be guided by cooperation of micron-scale channels and nanometer surface structures. As for the neural regeneration, the provided surface area of nerve guidance conduit with bundles of nanotubes was higher than that of conduits alone [71]. CNTs, cylindrical structures with diameters of 1–100 nm [72], have been used as biomimetic scaffold at damaged neural tissues to direct axonal outgrowth and enhance neural functions due to their outstanding electrical properties, strong mechanical properties, and nanostructured features similar to that of native neurites [73]. Mattson *et al.* discovered that embryonic rat brain neurocytes can grow on multiwalled carbon nanotubes (MWCNTs) [74]. It was found that the total neurite length increased over 200% and the number of branches and neurites increased almost 300% on MWCNTs coated with 4-hydroxynonenal than on uncoated MWCNTs. Work by Lovat *et al.* demonstrated that purified MWCNTs potentially enhanced electrical signal transfer of neural network [75]. Various functional groups such as carboxyl and amidogen can be used to modify chemical and electrical properties of CNTs to enhance neuronal outgrowth [76]. MWCNTs with different surface charges could affect neural growth, and the number of growth cones and neurite branches increased significantly on positively charged MWCNTs compared to negatively charged ones. In addition, Gheith *et al*. prepared a separate positively charged SWCNT/polymer thin-film membrane using the layer-by-layer assembly technique and investigated its biocompatibility [77]. They found that the survival rate of neurons was up to 94–98% on the SWCNT/polymer films. The results revealed that the SWCNT/polymer films promoted neuron differentiation, guided neuron extension, and directed more elaborate branches than control. More importantly, nanostructured carbon materials have the ability to limit activated astrocyte function that causes the formation of glial scar tissue. For the first time, studies on carbon nanofiber (CNF) and polycarbonate urethane composites demonstrated that adhesion and proliferation of astrocytes decreased greatly with the presence of carbon nanofibers [78]. Similar to nanotubes, nanofibers can also mimic the tubular structure associated with ECM. Nanofibers made from PLLA or polycaprolactone (PCL)

polymers by electrospinning and phase separation techniques showed outstanding cytocompatibility for neural regeneration application [79–81]. A biomimetic laminin-incorporated PLLA nanofibrous scaffold was used to repair damaged peripheral nerves, on which the axon outgrowth increased [81]. Recently, SCs were found to be able to proliferate well on PCL/chitosan nanofibrous scaffolds produced by electrospinning [80]. In addition to electrospun nanofibers, self-assembled peptide nanofiber scaffolds could also support neuron functions, axon outgrowth, and functional neural synapse formation [82].

Nanobiomaterials are promising in the enhancement of neural repair and regeneration, and it is undeniable that the use of nanostructured biomaterials with excellent properties, especially electrical properties, will be a great breakthrough in neural tissue engineering.

1.2.3 Cartilage Tissue Engineering

Articular cartilage defects are a serious clinical problem. Minor cartilage injuries might result in further damage and joint degeneration. The self-repair capacity of damaged tissues is limited because of limited chondrocyte mobility, lack of progenitor cells, as well as the absence of an efficient vascular network structure to support cartilage growth [83]. A lot of attempts have been made to repair articular cartilage defects, including subchondral drilling, osteochondral allografting, and periosteal or perichondrial tissue grafting [84–86]. However, certain shortcomings and degeneration such as fibrosis and calcification can frequently be found by long-term follow-up [87, 88]. Tissue engineering has demonstrated most promising results for articular cartilage repair and regeneration [89–91]. Numerous nanobiomaterials have been used to fabricate biomimetic scaffolds with the ability to repair cartilage defects by supporting proliferation of chondrocytes and differentiation of progenitor cells.

Nanoparticles have been used to deliver drugs, growth factors, or genes to the defect sites for cartilage repair. For example, Park *et al.* fabricated transforming growth factor β1 (TGF-β1)-loaded nanoparticles along with chondrocytes [92], which on being implanted into nude mice showed that a large amount of ECM including polysaccharides was accumulated by chondrocytes. In another study, poly(ethyleneimmine)-coated poly(lactide-*co*-glycolide) microspheres were physically attached with heparinized nanoparticles, which was proved to be able to support both adhesion and growth of MSCs [92]. Nanoparticles can be used as good delivery systems for molecules like peptides, proteins, and DNA in cartilage tissue engineering application. Nanoparticles have also been combined with polymer materials to fabricate composite scaffolds. Nanoparticles can enhance the mechanical properties of scaffolds and increase their lifetime and performance. For instance, HA nanoparticles were used to improve the mechanical property of poly(vinyl alcohol) (PVA) gel using the *in situ* precipitation method. As a result, the mechanical property of the nano-HA/PVA gel composite scaffold was comparable to that of the native articular cartilage tissues [93].

Nanobiomaterials have also been fabricated into 3D nanofibrous scaffolds to support chondrocytes and the differentiation of progenitor cells. For instance,

electrospun PCL nanofibrous scaffolds [94] were able to maintain the chondrogenic phenotype of fetal bovine chondrocytes (FBCs) in terms of the expression of cartilage-specific ECM genes such as aggrecan, collagen II, collagen IX, and cartilage oligomeric matrix proteins. A more cartilaginous matrix rich in sulfated proteoglycan was produced by FBCs cultured on nanofibrous PCL scaffolds than that on tissue culture polystyrene (TCPS), which indicated that the nanofibrous structure could support the proliferation and maintenance of the chondrocytic phenotype of FBCs. It was observed that the chondrocyte functions such as adhesion, proliferation, and ECM formation were all increased on NaOH-treated PLGA scaffolds with nanostructure and anodized titanium with nanoscaled roughness compared to conventional untreated materials [95], indicating that the nanostructured scaffold may promote the growth of cartilage. Nanostructural hydrogel scaffolds of self-assembling peptides KLD-12 (sequence AcN-KLDLKLDLKLDL-CNH_2) and chondrocytes were encapsulated inside the scaffolds. Four-week *in vitro* results showed that the chondrocyte differentiation was promoted and formation of ECM consisting of proteoglycans and type II collagen was also improved [96]. The ability of nanofibrous 3D scaffolds to support chondrogenesis of MSCs was also studied *in vitro* [97]. Human MSCs were cultured on electrospun nanofibrous PCL scaffolds to engineer the superficial zone in articular cartilage [98]. As a result, cell orientation was guided by the nanofibrous structure even after 5 weeks and its chondrogenic phenotype was also maintained. Adipose mesenchymal stem cell (AMSC)-embedded 3D PLGA/nano-HA composite scaffold was used to repair osteochondral defects in rat knees [99], and it was found that the defects were filled with smooth and hyaline-like cartilage rich in collagen and glycosaminoglycan deposition. According to the aforementioned results, nanofibrous scaffolds with biomimetic structure similar to native cartilage may be functionally used for cartilage tissue engineering applications.

But so far the research on nanobiomaterials in cartilage regeneration is still in a preliminary phase, and a variety of challenges in constructing the complicated human cartilage using nanobiomaterials need to be addressed in the future.

1.2.4 Bone Tissue Engineering

Nanobiomaterials have also been used in hard tissue regeneration, such as bone tissue engineering, which is reviewed in this section. Bone tissue, with the ability to support the body and protect the internal organs from shock and injury [100], is a type of dense connective tissue that comprises the rigid organs that form part of the skeletal system of human beings. Nowadays, bone fractures and bone diseases are becoming common and major clinical problems. For example, traumatic bone damage happens frequently each year. Functionality of traditional implant materials can last only for 10–15 years on average, and inflammation and infection frequently occur [24], which is the motivation to develop new-generation materials applied for bone regeneration. Understanding of the components of natural bone tissue (Figure 1.3) has confirmed the feasibility of application of biomaterials in bone regeneration. Bone tissue is a nanocomposite composed of a protein-based soft hydrogel template (including

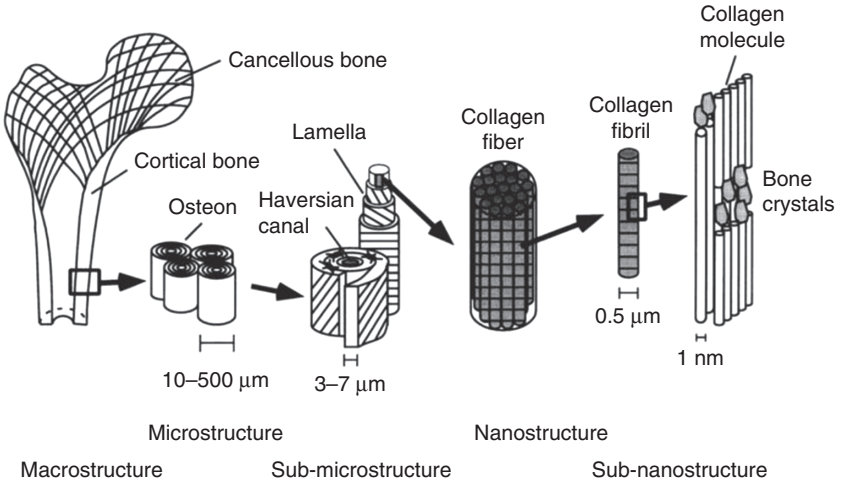

Figure 1.3 Hierarchical structural organization of bone. (With permission from Rho *et al*. 1998 [101], Elsevier.)

collagen, laminin, fibronectin, and vitronectin) and a hard inorganic constituent such as HA [102, 103]. The bone matrix consists of 70% nanocrystalline HA that is usually 20- to 80-nm long and 2- to 5-nm thick [103, 104]. In addition, the protein components of bone tissues are also ranged in nanoscale. Nanostructured materials, with great potential to satisfy a series requirement of scaffolds, can be potential candidates for bone tissue engineering applications.

Various synthetic and natural polymer materials such as PLLA, PCL, and PLGA, and gelatin, collagen, and chitosan have been fabricated into 3D porous nanostructured scaffolds by electrospinning, phase separation, and 3D printing techniques. For example, 3D PLLA electrospun nanofibrous scaffolds were reported to increase the adsorption of fibronectin and vitronectin from blood, leading to enhanced osteoblast function [105]. In addition, injectable nanoscaffolds or hydrogels, fabricated by the self-assembling technique, were also used to repair bone defects. Self-assembled nanobiomaterial is a promising candidate for bone regeneration due to the ease of modification by small molecules, such as peptides and proteins. For example, an injectable 3D peptide-amphiphilic (PA) nanofibrous scaffold loading bone morphogenetic protein 2 (BMP-2) was used to enhance bone regeneration *in vivo* [106].

Bone tissue integration was well mimicked by application of degradable polymers in combination with nanometer bioceramic particles [107]. The mechanical properties of scaffolds were enhanced by nanosized particles, and osteoblast proliferation and differentiation were both promoted. A nanophase titania/PLGA composite scaffold was reported to improve osteoblast adhesion, alkaline phosphatase (ALP) activity, and mineralization compared to a separate PLGA scaffold [108]. Similar results have been found both *in vitro* and *in vivo* on various nanostructured scaffolds such as HA/chitosan nanocomposites [109], PCL/HA/gelatin nanofibrous scaffolds [110], and PLA/CNT composites [111]. CNTs/CNFs are ideal materials for bone regeneration application because of

their excellent cytocompatibility and mechanical and electrical properties [112]. A study by Price *et al.* found that CNFs with a diameter of 60 nm were able to promote osteoblast adhesion and alleviate the adhesions of competitive cells such as fibroblasts and SMCs [113]. Other studies verified that CNTs were also beneficial for osteoblast function [114]. The application of CNTs as an osteogenic biomaterial was first reported by Supronowicz *et al.* [111]. In this study, the cell proliferation was effectively increased by 46% on CNTs in the presence of electrical stimulation. Concurrently, the expression of osteogenic markers including extracellular calcium, collagen, osteocalcin, osteonectin, and osteoprotegerin were all increased. In a recent study, ultrashort SWCNT polymer nanocomposites were implanted into rabbit femoral condyles and subcutaneous pockets for up to 12 weeks [115]. As a result, the bone volume was 300% greater than that of all other control groups after 4 weeks and the bone growth at defect sites was 200% greater than control scaffold without CNTs at 12 weeks. All these results indicated that the composites containing CNTs/CNFs were suitable to serve as biomimetic scaffolds for effectively improving bone tissue growth.

From the successful application of different nanobiomaterials, it is clear that nanostructured materials provide an innovative and effective approach for osseointegration or bone tissue regeneration. Although there are still many concerns to be solved for clinical practices, the application of nanobiomaterials in tissue regeneration is promising, and, thus, should be further studied.

1.3 Nanobiomaterials for Drug Delivery Applications

For applications in drug delivery systems, enhanced pharmacological effects and minimized negative effects are two key factors, for which numerous research efforts have been made [116, 117]. A variety of drug delivery systems have been developed at a tremendous speed to achieve the objective of enhancing water solubility of hydrophobic drugs, controlling drug release, and increasing rate of drug in target organs and tissues [118]. In this section, carbon nanobiomaterials, silicon nanobiomaterials, and polymeric nanobiomaterials for drug delivery applications are reviewed.

1.3.1 Carbon-Based Nanobiomaterials

As a new replaceable and efficient vector, CNTs have been appealing in the delivery of various therapeutic drugs or molecules in the family of nanomaterials. CNTs can be modified with multifarious bioactive molecules, such as proteins, peptides, and nucleic acids. CNT-based meshes or bundles, as a porous absorbent that entraps active substrate, were used to deliver drugs to cells, organs, and tissue for the realization of special biological functions. The low toxicity and non-immunogenicity enable such systems to be promising candidates in the area of drug transfer systems [116].

Single-walled carbon nanotubes (SWCNTs) were functionalized by various phospholipids (PLs) with a polyethylene glycol (PEG) chain segment and folic acid (FA) terminal group (SWCNTs-(PL-PEG-FA)), which selectively damaged

cancer cells without endangering normal cells, demonstrating that the transporting abilities of CNTs linked to proper chemical functional groups and their inherent optical properties could give rise to new types of nanomaterials for the application of drug delivery and tumor therapy in the clinic [119]. Amphotericin B (AmB) covalently bonded CNT was taken in by mammalian cells without showing any specific toxic effect, and the antifungal activity of AmB was highly reserved. It can be concluded that covalently linking different drugs to CNTs is a practical method that may be used to prompt the therapeutic effect of the agentia with interesting properties [120]. A neutralizing B-cell epitope (originating from the foot-and-mouth disease virus) that covalently linked peptide-functionalized CNTs held strong antipeptide antibody responses in mice model without obvious cross-reaction to the CNTs [121]. Nevertheless, high levels of virus-neutralizing antibodies only can be induced by the mono-derivitized CNT conjugate. Shaitan *et al.* [122] modeled non-immunogenic CNTs which encapsulated bioactive molecules of pentadecapeptide and cholesterol as well as an explosive agent for selective delivery to the cell membrane. The conformation of the bioactive molecule was studied in terms of chemical stability of the substance under shock conditions. Basically, modification through functional groups or ligands enables nanotubes to possess a selective landing area on the cellular membrane. Leonhardt *et al.* [123] incorporated a ferromagnetic material, a therapeutic agent, and a temperature sensor into CNTs, which would make it possible to control them by utilizing an additional magnetic field and a trigger device, to disrupt cancer cells hyperthermically. Venkatesan *et al.* [124] investigated the feasibility of nanoparticulate adsorbents with the existence of an absorption enhancer, as a kind of drug delivery vehicle for the escort of erythropoietin (EPO) to the small intestine. Liquid-filled nanoparticles (LFNPs) or liquid-filled microparticles (LFMPs) were fabricated by adopting CNTs as a porous nanoparticulate absorbent to control the delivery of EPO in the small intestine. The bioavailability of CNTs containing EPO formulations was improved to 11.5% in serum compared to the formulations without CNTs.

The unique enclosed nanochannels of CNTs make them a good candidate for drug delivery applications, and a lot of effort has been made in this regard. MWCNTs hold excellent drug loading capacity due to the features of cylindrical shape, hollow structure, and large surface area [125]. Kostarelos *et al.* fabricated copolymer-coated multiwalled nanotubes (MWNTs), which can form noncovalent supramolecular complexes with doxorubicin (DOX) for cancer therapy. The results revealed that the supramolecular complexes of MWNT-DOX achieved enhanced cytotoxic ability of killing human breast cancer cells compared to DOX alone [126].

Apart from MWCNT, attention is actively focused on another allotrope of carbon graphene, which appears to be a promising agent for successful delivery of biomolecules. Graphene-based materials have been linked to various natural biomolecules as functionalizing agents for applications of drug delivery [127]. Liu *et al.* [128] achieved the earliest research results in the field. PEG-functionalized nanoscaled graphene oxide (NGO) was attached by an analog of camptothecin (CPT), SN38 (NGO-PEG-SN38), which was water soluble and maintained cancer killing potential and efficiency in organic solvents compared to that

of the free SN38 molecules. The complex also expressed high cytotoxicity to HCT-116 cells and was approximately 1000 times more powerful than CPT. In another study, the anticancer drug was attached to the graphene nanosheets (GNSs) at a high loading capacity for drug delivery and cellular imaging. The doxorubicin/gelatin–GNS compound exhibited a high toxicity in MCF-7 cells and showed a gelatin-mediated controlled release process *in vitro*. Cellular toxicity test suggested that the gelatin–GNS was nontoxic for MCF-7 cells and underwent a gelatin-mediated controlled release process *in vitro*, even if at a high concentration of 200 mg ml^{-1}, displaying the potential for prompting the therapeutic efficiency. The gelatin–GNS could act as an ideal drug escort to be applied in the field of biomedicine [129].

1.3.2 Silica Nanoparticles

Among all the available nanomaterials, mesoporous silica nanoparticles (MSNs) are particularly attractive because of their unique properties, such as large surface area and pore volume to load drugs with high efficiency [129, 130], uniform and tunable pore size to accommodate molecules with various steric hindrance, and excellent physiochemical stability to protect the encapsulated drugs from degradation by endogenous enzymes [131]. Horcajada *et al.* reported the application of MSNs in the drug delivery system, in which an anti-inflammatory drug, ibuprofen, was attached into the small pore of MCM-41-type MSNs, displaying high drug loading capability and continuous drug release. MSNs have been proved favorable in loading a wide scope of pharmacological agents and have received a lot of attention. Besides, they found that drug molecules appeared to be in different geometrical morphology in pores with different sizes, which indicated that the amount of absorbed ibuprofen and the drug-releasing kinetics were dependent on the pore size of MCM-41 [132]. Shi *et al.* [133] developed MSNs with surface-attached high-density carboxyl groups, which served as compounds with platinum atoms in cisplatin, resulting in increased drug loading efficiency of cisplatin, durable and pH-responsive cisplatin release, and vastly improved growth-inhibiting effect against MCF-7 and HeLa cancer cell lines. Han *et al.* [134] functionalized MSNs by lipid bilayer coating, and the achieved lipid-bilayer-coated mesoporous silica nanoparticles (LMSNs) displaying good biocompatibility were promising nanocarriers in improving the cellular uptake and therapeutic efficacy of anticancer drugs. The loading efficiency can reach as high as 16% by encapsulating a model drug, DOX into LMSNs. The obtained LMSNs-DOX exhibited a pH-responsive release behavior and the presence of the lipid bilayer did not significantly delay the release of DOX. Furthermore, LMSNs greatly enhanced the cellular accumulation and cytotoxicity of DOX toward the MCF-7 cells.

MSNs as drug delivery systems can prevent biomacromolecules such as peptide and protein from degradation [135]. As an example, Zhang *et al.* [136] reported that basic fibroblast growth factor (bFGF) were loaded in MSNs by a water-in-oil microemulsion method for a sustained release via *in situ* route. The loading capacity of bFGF in MSNs was about 72.5%, and the MSNs were shown to be nontoxic. In an interesting study, nanotherapy has been used to target

delivery cargo in senescent cells by capped MSN S1 with high specificity and undetectable toxicity. The results suggested that it might be possible to prevent, remove, or replace senescent cells by choosing a proper cargo (cytotoxic drug or telomerase reactivation drug). These researches might open up new roads for developing innovative therapeutic protocols to treat age-related diseases [137].

1.3.3 Polymer-Based Nanomaterials

Natural and synthetic polymeric biomaterials, able to encapsulate a wide range of drug molecules and achieve distinct therapeutic effects in the aspect of controlling release of drugs, have been used for drug delivery systems, protecting drugs from premature degradation and reducing drug toxicity [138]. In terms of natural polymers, Rossi *et al.* designed a drug delivery system based on hydrogel loaded with the anticonvulsant drug ethosuximide (ESM) and studied the effect of design parameters on the adsorption and diffusion in gels and in water. The platform may provide a better way for device design [139]. Similarly, Hofmann *et al.* prepared dextran/protein-encapsulated silk fibroin (SF) films and studied the impact of crystallinity on the sustained release of dextran and protein. The research indicated that SF can act as an appropriate polymer for drug delivery of polysaccharides and bioactive proteins with well-protected unstable compounds because of the controllable crystallinity [140]. Besides, as for synthetic polymers, Nie *et al.* successfully fabricated polyacrylonitrile (PAN) monofilament fiber-encapsulated tamoxifen citrate (TAM) using wet-spinning technique. The constant drug release from the system can be observed for a long time in an *in vitro* release test, demonstrating that PAN fibers could be a kind of potential material used in drug delivery systems with high loading capacity and effectiveness in release [141]. Inspiringly, drug delivery based on three-dimensional (3D) technology has been rapidly developed and achieved certain advancement in the area of research. Rattanakit *et al.* designed a novel drug delivery system of biodegradable polymer (PLGA) and water-soluble PVA-encapsulated dexamethasone-21-phosphate disodium salt (Dex21P) based on extrusion printing technology. The ability to control the dexamethasone release through the system was monitored for more than 4 months, suggesting that it was a facile and effective method to design a novel drug delivery system by extrusion printing technique [142].

The success of nanobiomaterials in drug delivery has been achieved to a certain extent. However, many challenges must be settled before nanomaterials are applied clinically, for the biological behavior and toxicity of materials and loaded drugs are still indefinite. Therefore, reasonable safety evaluation and further study of nanomaterials for drug delivery are highly desired.

1.4 Nanobiomaterials for Imaging and Biosensing Applications

Medical imaging offers the possibility of dramatically improving existing strategies of cancer diagnosis and treatment at the cellular and molecular

level. Nanoscale contrast agents have shown supernormal superiority, such as biocompatibility, reduced toxicity, and selective accumulation in cancer cells [143], over single-molecule-based ones. Thus, plenty of imaging agents and novel imaging systems have been developed using nanomaterials, which can be utilized in applications such as computed tomography (CT) imaging, photothermal therapy (PTT), or magnetic resonance imaging (MRI) in cancer imaging. Compared with primeval catalyst system-based biosensors, the direct electrical detection of label-free, highly multiplexed biological and chemical agents over a broad dynamic range can be carried out based on the nanotechnology sensor platform. This platform adopts functionalized nanomaterials to monitor molecular binding in a higher sensitive and selective format, possessing the capability of detecting a wide range of molecules, such as ions, small molecules, proteins, DNA, RNA, cells, and even the pH values. Based on these outstanding performances of nanomaterials, a large number of new biosensors have been exploited [144].

1.4.1 Polymer-Based Nanobiomaterials

In contrast to small molecules labeled with various fluorophores, polymer-based imaging probes are more advantageous in terms of less toxicity, stability, large surface areas, and improved targeting [145]. Weissleder *et al.* reported a method to investigate tumor-related lysosomal protease activity *in vivo* using autoquenched near-infrared fluorescence (NIRF) probes, tied to a long circulating graft copolymer composed of poly-L-lysine and methoxypolyethylene glycol succinate. *In vivo* imaging displayed a 12-times increase in the NIRF signal, allowing the submillimeter-sized diameter detection of tumors. This method can be adopted to detect the early-phase tumors *in vivo* and to explore specific enzyme activity [146]. In one study, a multifunctional PLGA nanoparticle encapsulating inorganic nanocrystals and chemotherapeutic drugs was designed using solvent evaporation approach. The results indicated that the polymeric nanoparticles may be used as an optical imaging agent and simultaneously act as a drug delivery system [147]. A kind of complex of dual-modal fluorescent-magnetic nanoparticles was fabricated by encapsulating conjugated polymer (PFVBT) and iron oxides (IOs) to the mixture of poly(lactic-*co*-glycolic-acid)–poly(ethylene glycol)–folate (PLGA–PEG–FOL) and PLGA. *In vitro* results demonstrated that the complexes can be used as a kind of fluorescent probe without apparent cytotoxicity to MCF-7 breast cancer cells and obtain targeted imaging. Meanwhile, *in vivo* results of fluorescence and MRI indicated that these NPs can be detected in a living body with preferential accumulation in tumor tissues [148].

1.4.2 Quantum-Dot-Based Nanobiomaterials

Quantum dots (QDs) as a kind of semiconductor nanomaterial with several superiorities such as the broad, continuous excitation spectrum, and resistance to chemical degradation compared with conventional organic fluorophores [149], have been applied in biological imaging and labeling probes with increasing interest. Appropriate modification and optimization by targeting agents (such as peptides, antibodies, aptamers, etc.) have aroused more sensitive

and specific targeted imaging and diagnostic forms. With the continuous advancement by the conjugation and synthesis methods, undoubtedly, there will be some challenges to be addressed in the near future for the fabrication of compounds with improved sensitivity, stability, and binding specificity of QD-based assemblies combined the chemical sensors and biosensors [150].

Medintz *et al.* [151] prepared QD–protein/receptor as fluorescence resonance energy transfer (FRET) donors, and the ability to combine each QD FRET donor with a relatively large number of specific receptors with approximately symmetrical arrangement displayed an amazing possibility for constructing and optimizing novel classes of optically addressed nanosensors. By utilizing these methods, QD-biomolecule assemblies may facilitate the booming development of new sensing compound materials. In another study, researchers attempted to use QD nanocrystal grafted to bombesin or angiotensin II (ANG II)-labeled cognate G-protein-coupled receptors (GPCRs) in various live cell lines, demonstrating that the QD-bombesin compound could label the bombesin-preferring GPCR in live mouse Swiss 3T3 cells and in Rat-1 cells. Similarly, they used the QD–ANG II complex to label GPCR in different types of cells, demonstrating that QD–ANG II was brighter and more photostable in comparison with the organic dye Cy3-labeled agonist [152]. Kim *et al.* proved that with sentinel lymph node mapping a major cancer surgery could be performed in large animals under complete image guidance using QDs. The chemical, optical, and *in vivo* data demonstrated the potential of near infrared (NIR) QDs for biomedical imaging [153]. Later, researchers utilized QDs to distinguish tumor cells from both the perivascular cells and the circumambient matrix in mice, and investigated the relationship between particle size and uptake [154]. These examples display the versatility of QDs for studying tumor pathology and opening avenues for treatment. The unique and forceful optical properties of QDs, combined with the novel characteristics of the conjugated biomolecules, could be promising for biomedicine applications.

QDs provide a powerful platform for exploiting FRET-based nanosensors to monitor biological responses. In the QD-based biosensors, the biological processes were designed to regulate the physical distance between a QD and an energy transfer partner as well as their spectral overlap. Various DNA sensing methods with good selectivity and reduced effects of interfering molecules have been developed. In one study, they utilized CdSe/ZnS quantum dot-single-stranded DNA (QDs-ssDNA)-fluorescent dye conjugates as bioprobes to detect micrococcal nuclease (MNase), and further adopted the bioprobe to monitor the activity of MNase in the culture medium through fluorescence microscopy, which extended the development of a QD-FRET probe for the quantitative determination of MNase and other specific nuclease [155]. From the point of biology, exploiting QD technology to prompt the determination of the interaction between DNA and anticancer drugs is of vital significance. Rapid, highly efficient, and sensitive detection of DNA is decisive in diagnosing genetic disease. Zhang *et al.* reported a FRET-based nanosensor with the ability to monitor low concentrations of DNA in a free-separation format. In the system, QDs were linked with DNA probes to catch DNA targets. The target strand bound to a dye-labeled reporter strand forms a FRET donor–acceptor assembly. The QD also

served as a concentrator that amplified the target signal by limiting several targets in a nanoscale domain. The result of the study demonstrated almost zero background fluorescence and sensitive detection of as low as 50 copies of DNAs [156].

Apart from these, the modulation of QD-based biosensors has been explored to probe enzymatic activity. An excellent example was the proposal of a simple method for preparation of the first architecture of QDs combined with enzyme. Employing glucose oxidase (GOD) as a model enzyme biosensor for oxidase substrate, it was incorporated in thioglycolic acid (TGA)-capped CdSe QD films. The inherent cathodic electrochemiluminescence (ECL) of the QDs can act as the indicator of enzymatic procedures of oxidases for detecting their substrates. The architecture displayed a sensitive ECL response to glucose in a broad linear range. The suggested ECL sensor displayed exciting reproducibility and receivable stability. This strategy may be adopted in more stimulating systems [157].

Graft of luminescent proteins onto QDs has also become the focus of research. In the absence of *Escherichia coli* maltose, QD-linked proteins (maltose-binding protein (MBP)) function as sugar receptors and can interact with a β-cyclodextrin-QSY-9 dye conjugate, thus resulting in the quenching of the fluorescence of the QDs by QSY-9. The addition of maltose led to the replacement of β-cyclodextrin-QSY-9 and the restoration of the QD fluorescence. The FRET change induced by the emulative binding was able to monitor maltose in solution. QD-biomolecule assemblies thus developed may accelerate the development of new complex materials [151]. In addition to the examples mentioned, Christine E. Schmidt's group attached QD to live neurons using both antibody and peptide recognition molecules. Peptide recognition molecules offer nanometer-scale control of the target and separation distance between the QD and the cell. The creative design devices with specific, known attachment points and controllable nanometer-length separation distances open the avenue to develop future biological and electronic devices [158].

1.4.3 Magnetic Nanoparticles

Magnetic nanoparticles (MNPs), a kind of significant substance for the contrast enhancement of MRI in the area of medicine, are promising in the present clinical diagnostic and therapeutic methods. MNPs with unique magnetic features are able to function at the cellular and molecular level, making them a fascinating platform as contrast agents for MRI [159]. Greatly enhanced biocompatibility, stability, functionality, and applicability of these MNPs can be achieved by integrating highly specific target agents and some functional molecules [160].

Lee *et al.* reported fabricated thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPION) and found that 68% signal drop was detected in lung carcinoma tumor allograft mice, demonstrating that a great amount of nanomagnets accumulated within the tumor location. *In vivo* fluorescence images confirmed the highest level of accumulation of the Cy5.5 TCL-SPION in the tumor. It is worth noting that without loading any targeting agents on its surface, TCL-SPION was highly efficient for tumor detection and diagnosis *in vivo* by dual imaging [161]. Sun *et al.* [162] reported a biocompatible nanoprobe consisting of a PEG coated with iron oxide nanoparticles, capable of selective recognition

of glioma tumors through the surface-linked target peptide, chlorotoxin (CTX). The *in vitro* and *in vivo* experiments confirmed preferred accumulation of the nanoprobe in gliomas. High target specificity and benignant biological response made this nanoprobe a promising platform to facilitate the diagnosis and treatment. Jaffer *et al.* [163] utilized dextran-coated magneto-fluorescent nanoparticles (MFNPs) to investigate its cellular targeting and imaging capabilities in atherosclerosis, and inflamed plaques were visualized by MRI and optical imaging modalities. The cellular distribution of MFNPs in the atherosclerosis-targeted part can be quantified through *in vitro* and *in vivo* fluorescence imaging. Thus, the study provided a fundamental guide for using MFNPs to image cellular pathological changes in experimental atherosclerosis and for the future advancement of novel targeted nanomaterials for atherosclerosis.

1.4.4 Gold Nanobiomaterials

Gold nanomaterials have been undergoing extensive advancement for promising applications in the imaging and therapy of cancer *in vitro* and *in vivo* [164], for its attractive performances including biocompatibility, stability, unique adjustable optical properties, easy incorporation of bioactive molecules to the surface for tumor target specificity and diagnostic applications, and easily detectable backscattering of NIR reflection light [165]. Gold nanospheres and nanorods offer outstanding contrasts in the dark field optical and photothermal imaging of cells and tissues, while nanospheres, nanoshells, nanorods, and nanocages are optional for optical coherence tomography and photoacoustic imaging of deeper tissues, circulatory systems, and lymph nodes [166].

Kim *et al.* [167] fabricated gold nanoshells (GSNs) encapsulated with magnetic Fe_3O_4 nanoparticles, to which the targeting agent against cancer – anti-HER2/neu – was added for targeting MRI and NIR PTT of tumor cells. The encapsulated Fe_3O_4 nanoparticles led to high contrast in the MRI images, and the GSNs had an optical absorption cross section highly sufficient for NIR PTT. Tumor cells targeted with the GSNs-AbHER2/neu were detectable *in vitro* by MRI system. In another study, Liu *et al*. [168] synthesized innovative multifunctional GSNs, which were composed of a thin GSN and a monodispersed mesoporous silica nanoparticle (MSN) core. MSNs endowed GSNs with many merits because of their unique structure with movable cores and mesoporous shells, which can incorporate remote controlled PTT with chemotherapy like a "magic bullet" to exploit the excision of hepatocellular tumor both *in vivo* and *in vitro*. They also decreased drug negative effects by continuous drug release and offered a new multimodality tumor treatment with higher efficiency and less toxicity. More lately, with the progress in the synthesis and biograft of QDs, gold entered into the golden age of fluorescence-based imaging, and gold quantum clusters (QCs) have become closely relevant in bioimaging due to their nontoxic nature, simple synthesis, and high photostability. Biju's group [169] demonstrated the preparation of biotinylated NIRF gold QC-conjugated streptavidin-functionalized Fe_3O_4 nanoparticles and evaluated their intracellular delivery by GPCRs using NIRF imaging and MRI. Apart from the NIRF and MRI

contrasts provided by the probe, the endoperoxide-triggered green fluorescence provided a third modality for live-cell imaging.

1.4.5 Organic–Inorganic-Based Materials

Organic–inorganic-based biosensors have been developed by nanotechnology and processing. Yang *et al.* [170] fabricated a glucose biosensor via a surface-treated nanoporous ZrO_2/chitosan composite matrix, which took full advantage of inorganic nanoparticles – ZrO_2 – and organic polymer – chitosan. The immobilization of GOD in the material kept its activity and avoided the use of glutaraldehyde. The result revealed the biosensor retained roughly 75.2% of its primal response to glucose even when stored in a phosphate buffer saline for a month. Similarly, Chen and Dong [171] reported a new type of composite material based on sol–gel-derived titanium oxide/copolymer compound with poly(vinyl alcohol) grafting 4-vinypyridine (PVA-*g*-PVP) acting as a glucose biosensor. The GOD entrapped in the composite matrix maintained its bioactivity. Results demonstrated that the response time was less than 20 s and the linear range up to 9 mM of the biosensor with the sensitivity up to 405 nA mM^{-1}. The stability of the biosensor can last more than 1 month. Kim and Lee [172] developed a biosensor based on sol–gel silicate/Nafion composite film with tyrosinase immobilized for the detection of phenolic compounds. The biosensor can achieve 95% of stable current in about 15 s. The sensitivity of the biosensor for catechol and phenol were up to 200 and 46 mA M^{-1} separately. The enzyme electrode reserved 74% of its original activity even after being stored for 2 weeks in 50 mM phosphate buffer at pH7. Wang *et al.* [173] developed a new type of sol/gel/organic hybrid compound material based on the cross-linking of natural polymer chitosan with (3-aoryloxypropyl)dimethoxymethylsilane as a biosensor for the determination of amperometric H_2O_2. The biosensor maintained approximately 75% of its initial activity after about 60 days of storage in a phosphate buffer at 4 °C.

1.4.6 CNT-Based Nanobiomaterials

In the past few years, CNTs have been intensively explored for biosensor and biodetection applications due to the combination of unique structural, electronic, and mechanical properties [174]. In one study was fabricated a new and promising glucose biosensor by immobilizing GOD at the surface of a basal plane pyrolytic graphite (bppg) electrode modified by MWCNTs. The modified biocomposite electrode displayed wonderful sensitivity, notable stability, and rapid response in comparison with other forms of biosensors for detection of glucose; and the procedure of preparing the CNT sol–gel compounds is convenient, fast, and repeatable and this approach might be used in designing a wider range of novel biosensors [175]. Wang *et al.* utilized the combination of CNT and the perfluorinated polymer Nafion as a solubilizing agent to design a kind of CNT-based biosensor device. The CNT-/Nafion-modified glassy carbon electrodes displayed a strong and steady electrocatalytic response to hydrogen peroxide. The dramatic improved response to the hydrogen peroxide redox is very inspiring for designing oxidase-based amperometric biosensors. These

results opened a new door for exploiting a wider range of chemical sensors and nanoscale electronic devices based on CNT [176]. In another study, GOD was encapsulated in the compound of CNTs/chitosan, leading to the direct electron transfer reaction between GOD and electrode. The electron transfer rate of this system was greatly improved, which was double that of flavin adenine dinucleotide adsorbed on the CNTs. Therefore, the preprocessed electrode can act as a kind of glucose biosensor with excellent sensitivity and better stability. The facile procedure of immobilizing GOD might accelerate the electrochemical research for protein, biosensors, and other bioelectrochemical devices [177]. Wang's group developed a novel method to prepare DNA biosensors based on self-assembly, in which the probe DNA oligonucleotides were immobilized on MWCNTs. The results suggested that the DNA biosensors based on self-assembled MWNTs exhibited higher hybridization efficiency in comparison with those based on random MWCNTs. In addition, the novel DNA biosensors had an excellent selectivity of DNA hybridization detection [178]. Considerable advancements have been achieved in preparing and modifying CNTs, and more research is going on regarding how to effectively integrate the CNTs with biological systems.

In this section, several successful examples of nanobiomaterials, such as polymer-based nanobiomaterials, QDs, MNPs, gold nanobiomaterials, and organic–inorganic-based and CNT-based nanobiomaterials, used in imaging and biosensing applications were introduced and reviewed. We believe that the advances in nanoscience and nanotechnology will provide the nanobiomaterials with more extraordinary and controllable properties, which could further facilitate the development of nanobiomaterials-based imaging and sensing technologies.

1.5 Conclusions and Perspectives

The development of tissue engineering and regenerative medicine has brought nanobiomaterials into a new stage, in which smart or intelligent nanobiomaterials with the ability to respond to environmental changes (such as electrical, light, temperature, and other signals) are showing the potential for multidisciplinary applications. In the near future, automatically responsive nanomaterials may become a reality. In addition, multifunctional nanobiomaterials, with the ability to enhance tissue regeneration, minimize immune response, and inhibit infection [10], will remain an attractive research direction. Concurrently, the mechanisms of interactions between nanoscaled materials and biological systems are not completely understood and need further investigations.

The research on nanobiomaterials for tissue engineering application is still in the primary stage, and the influence of nanomaterials on human beings is not well understood. Emphasis will be given to the increasing concern for evaluation of safety and toxicity of nanomaterials. Toxic response to nanoparticles generated from degraded products of nanomaterials, wear remains from artificial joints, and residue from nanomaterials have been reported [24]. The toxicity of CNTs was found to be greater than that of carbon black in lungs in an *in vivo* study;

thus, it may be a serious health hazard in chronic inhalation exposures [179]. How and why nanomaterials exert toxic influences on the human body have been reported, but further investigation on health effects of nanobiomaterials is still necessarily needed before application of these nanobiomaterials in human subjects. Understanding of the interaction mechanisms between nanobiomaterials and biological systems is indispensable for understanding the effect of nanobiomaterials on the human body, which will be further investigated and understood at the molecular level in the future, more importantly, providing fundamental support for nanomaterials design to achieve more satisfactory properties.

In future, novel nanobiomaterials will be designed and fabricated using nanotechnology in combination with other advanced techniques, such as mathematical and computational models [180–182]. Therefore, the progress of such computer-aided tools will be a promising direction in the study, design, and creation of novel nanobiomaterials.

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