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Nanomaterials promise better bone repair

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Nanomaterials mimicking the nano-features of bones and offering unique smart functions are promising for better bone fracture repair. This review provides an overview of the current state-of-the-art research in developing and using nanomaterials for better bone fracture repair. This review begins with a brief introduction of bone fracture repair processes, then discusses the importance of vascularization, the role of growth factors in bone fracture repair, and the failure of bone fracture repair. Next, the review discusses the applications of nanomaterials for bone fracture repair, with a focus on the recent breakthroughs such as nanomaterials leading to precise immobilization of growth factors at the molecular level, promoting vascularization without the use of growth factors, and re-loading therapeutic agents after implantation. The review concludes with perspectives on challenges and future directions for developing nanomaterials for improved bone fracture repair.

Introduction

Bone fracture repair is a complex process of bone regeneration, that is, reconstruction of bone defects and nonunion, including the structural and functional reconstruction of bones [1,2]. Besides conventional fixation, bone grafts combined with appropriate physical therapy, like electrical stimulation, ultrasound, and gradient low oxygen environment, have been studied for bone repair [3]. Materials used for bone fracture repair include autografts, allografts, xenografts, and synthetic bone materials. Among them, autografts are considered the 'gold standard' of bone repair materials [4]. However, autografts are limited by bone sources and may also introduce new trauma and/or complications. The implantation of allografts and xenografts may be prone to trigger immune rejection. Although bone fracture repair may not need supportive materials other than implants, bone defects or large tissue voids may require materials as supports. As a result, a variety of synthetic bone materials have been developed and have shown great promise for bone fracture repair.

Nanomaterials are defined as having at least one dimension between 1 and 100 nm. They present unique physical and/or chemical properties that are different from conventional materials [5], and have emerged in recent years as promising breakthroughs to bone fracture repair. For instance, fluorescent nanoparticles like quantum dots and upconversion nanoparticles have shown promise for long-term *in vitro* and *in vivo* tracking of mesenchymal stem cells (MSCs) [6,7], and gold nanoparticles have promoted the differentiation of MSCs toward osteoblast cells over adipocyte cells [8]. Applications of nanomaterials in regenerative medicine, including bone fracture repair, are summarized in Fig. 1, including (A) isolation of target cells with nanoparticles, (B) *in vitro* instruction of therapeutic cells with nanomaterials as delivery vehicles, functional modules, and artificial extracellular matrixes (ECMs) with nanotopological cues, (C) delivering nanoengineered cells to target tissues for regenerative therapy, (D) building *in vitro* disease models for understanding disease pathology and drug screening, and (E) *in situ* cell engineering using nanomaterials directly within native tissue *in vivo* [1,9]. In this paper, the research progress of nanomaterials in the repair of bone fractures in recent years is reviewed.

Bone fracture repair

Bone fracture repair processes

Bone fracture repair is a complex process and has two mechanisms, that is, primary bone healing and secondary bone healing. In general, the primary bone healing is less commonly seen and

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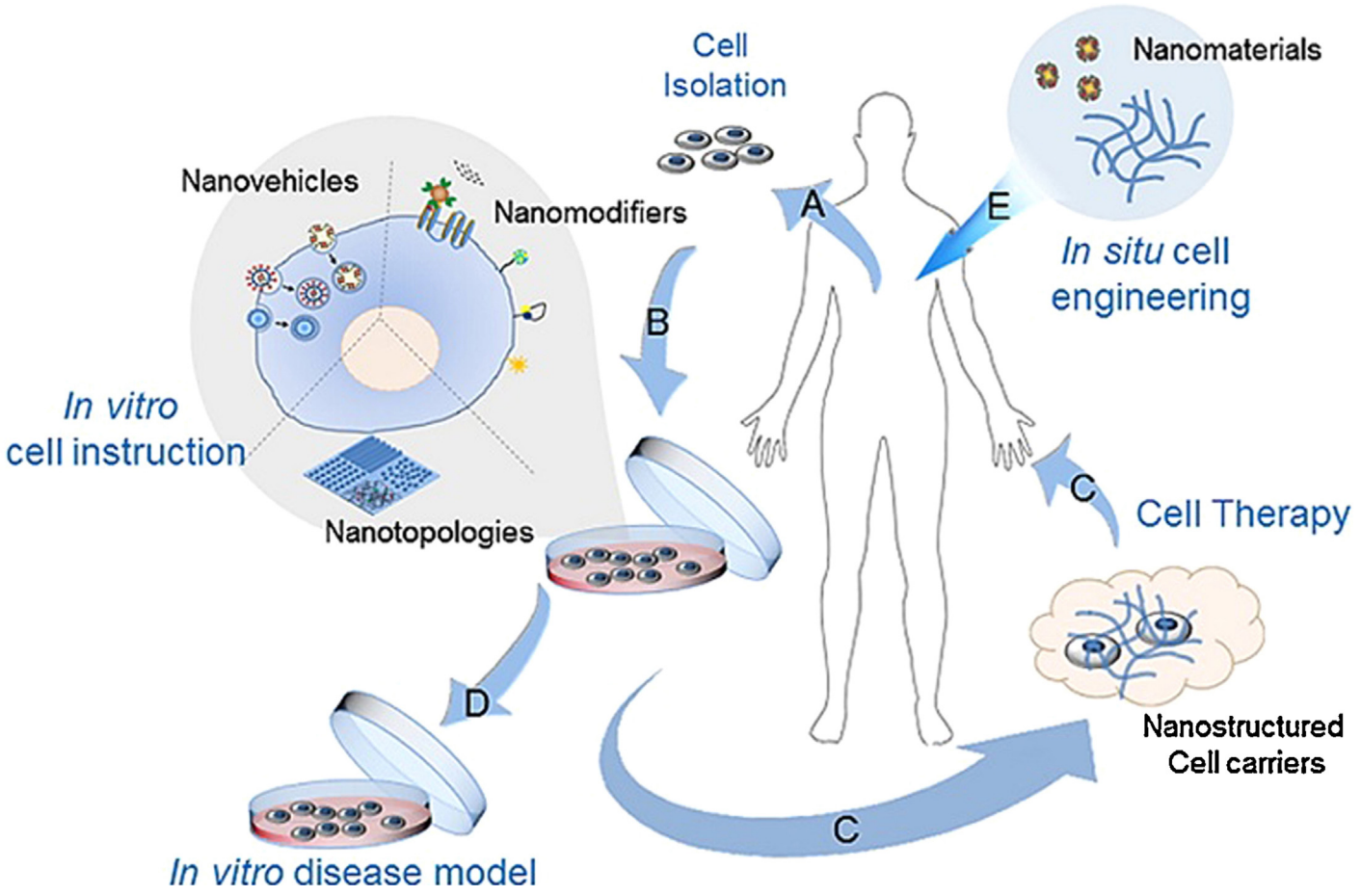

FIGURE 1

Multifaceted applications of nanomaterials in the cell engineering and therapy: from (A) isolation of target cells out of the heterogeneous cell populations, to (B) *in vitro* instruction of therapeutic cells with nanomaterials as delivery vehicles, functional modules, and artificial ECMs with nanotopological cues, and to (C) delivering nanoengineered cells to target tissues for regenerative therapy. (D) In addition to therapeutic applications, nanoengineered cells can also be used to build *in vitro* disease models for understanding disease pathology and drug screening. (E) *In situ* cell engineering, an emerging strategy to manipulate cell functions using nanomaterials directly within native tissue *in vivo*. (Reprinted with permission from [9]. © 2013 Elsevier B.V.)

the secondary bone healing process can be divided into four main steps (Fig. 2) [10]: (1) formation of hematoma at the fracture site. Inflammation is usually observed since tissues at the fracture site swell and bone cells die. (2) Formation of fibrocartilaginous callus, which serves as a splint for the fracture, over a three to four week period. During this time period, new capillaries start to grow into the hematoma followed by invasion and cleanup of debris by phagocytic cells at the injury site. Fibroblasts and osteoblasts also migrate into the injury site. (3) Formation of bony calluses about three to four weeks after injury. During this process, osteoblasts and osteocytes multiply and turn the fibrocartilaginous calluses into bony calluses. (4) Bone remodeling, during which excess material of the bony calluses is removed and compact bone is laid down to reconstruct the bone shaft [10,11].

Importance of vascularization in bone fracture repair

Adequate blood supply, which transports oxygen and nutrients and discharges metabolic products, is very important for the repair of bone fractures. Lack of blood supply is believed to result in delayed union of fractures or nonunion. Vascularization is essential to blood supply and includes the establishment of microvasculature and also macro blood circulation. Depending on the sources of endothelial cells forming blood vessels, vascular formation can be divided into two types: vasculogenesis and angiogenesis [12]. The former refers to the formation of new blood vessels via vascular endothelial cells derived from endothelial progenitor cells, and the latter refers to the growth of blood vessels from the existing microvascular endothelial cells which are formed by budding and microvascular growth of the already existing blood vessels. Besides the sources of endothelial cells, other factors such as integrins, scaffold materials, and growth factors may also influence vascularization and thereby bone fracture repair. Researchers, by modifying nanoparticles with peptides, have shown that nanoparticles can either speed up or slow down angiogenesis via selectively interacting with cell receptors that are responsible for activation or inhibition of angiogenesis [13]. These nanoparticles did not cause significant toxicity while altering the balance between naturally secreted pro- and anti-angiogenic factors [13].

Role of growth factors in bone fracture repair

Bone fracture repair or healing begins immediately after injury and involves a cascade of cellular events in which mesenchymal cells

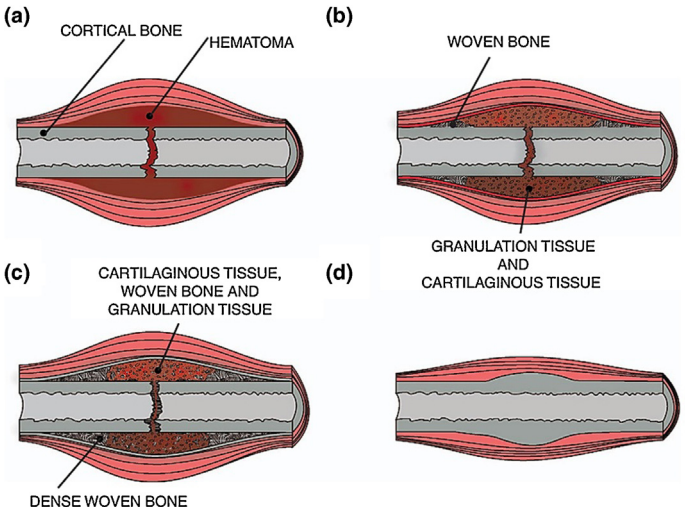

FIGURE 2

A sequential schematic of four classical stages of fracture healing. (a) After inflammation a hematoma is generated. (b) In the first stage of the reparative phase, the initial fibrin is gradually replaced by cartilaginous tissue and woven bone starts to form. (c) In a later stage of the reparative phase, the cartilaginous tissue mineralizes, more bone is formed and the volume of granulation tissue substantially decreases. (d) Eventually, once the bone is bridged, remodeling restores the original cortex. (Reprinted with permission from [10]. © 2012 Nature Publishing Group Ltd.)

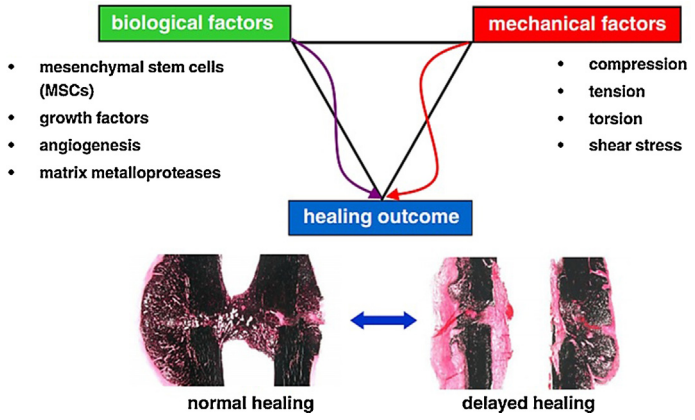

FIGURE 3

Both the biological and mechanical stimuli influence the endogenous regeneration pathway. The optimal mechanical and biological stimuli would result in fast and uncomplicated healing; an inappropriate stimulus leads to impaired/delayed healing. (Reprinted with permission from [27]. © 2012 Elsevier B.V.)

respond to a variety of regulators and proliferate, differentiate, and synthesize ECM [14]. Current concepts suggest that growth factors may regulate different steps in this cascade [15]. Recent studies confirmed the regulatory roles of platelet-derived growth factor (PDGF), acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), and transforming growth factor-β (TGF-β) in the initiation and development of fracture callus. Upon injury, growth factors including TGF-β1 and PDGF are released into the fracture hematoma by platelets and inflammatory cells. TGF-β1 and PDGF are generated by osteoblasts and chondrocytes throughout the healing process. TGF-β1 and PDGF may influence the initiation of fracture repair and the formation of cartilage and intramembranous bone in the initiation of callus formation. aFGF, synthesized by chondrocytes, chondrocyte precursors, and macrophages, appears to stimulate the proliferation of immature chondrocytes or precursors and indirectly regulates maturation of chondrocytes and expression of the cartilage matrix. Presumably, growth factors in the callus later in bone fracture repair regulate additional steps later in fracture repair [16,17]. These suggest that growth factors are central regulators of cellular proliferation and differentiation during bone fracture repair. The details about the functions of these important growth factors in bone fracture repair are listed in Table 1. It is noteworthy to mention that abnormal growth factor expression has been found to cause impaired or abnormal healing, indicating that an altered growth factor expression may be responsible for abnormal or delayed fracture repair.

Failure of bone fracture repair

Bone fracture repair may fail when the fractured bone lacks adequate stability or blood flow, or both. The biological and mechanical factors presented in Fig. 3 are believed to influence the

TABLE 1

Functions of growth factors in bone fracture repair.

Growth factor	Working period	Function	Refs
Bone morphogenetic protein (BMP)	Whole process	Induce undifferentiated mesenchymal cells to differentiate into osteogenesis and cartilage	[18,19]
Transforming growth factor-β (TGF-β)	Whole process	Enhance bone induction ability of BMPs	[20]
Fibroblast growth factor (FGF)	Hematoma formation and fibrocartilaginous callus formation	Stimulate the proliferation and differentiation of vascular endothelial cells and bone tissue cells and the formation of granulation tissue	[21]
Vascular endothelial growth factor (VEGF)	Hematoma formation	Induce angiogenesis	[22]
Platelet-derived growth factor (PDGF)	Hematoma formation and fibrocartilaginous callus formation	Promote bone cell differentiation, induce mature osteoblast synthesis of type I collagen, and accelerate bone formation	[23]
Insulin-like growth factor (IGF)	Bony callus formation	Promote division of cartilage cell proliferation and the synthesis of cartilage matrix	[24]
Interleukin-1 (IL-1)	Fibrocartilaginous callus formation	Inhibit the generation of type I collagen and activity of alkaline phosphatase	[25]
Tumor necrosis factor (TNF)	Fibrocartilaginous callus formation	Inhibit the synthesis of collagen osteocalcin and collagen synthesis of fibroblasts and cartilage proteoglycan synthesis	[26]

endogenous regeneration pathway and control the outcome of bone fracture repair [27]. Optimal mechanical and biological stimuli would result in quick, uncomplicated healing. An inappropriate stimulus, however, may lead to delayed healing or nonunion; the latter is a permanent failure of healing following a fracture where the normal process of bone healing is interrupted. In general, if a nonunion is still evident six months post-injury, it will remain unhealed without specific treatment. A nonunion which subsequently heals is called a delayed union and typically takes longer than usual to heal [28]. Bones like toe bones have inherent stability and an excellent blood supply; they can be expected to heal with minimal treatment. However, in other bones, like the upper thighbone (femoral head and neck) and small wrist bone (scaphoid), the blood supply can be destroyed when the bones are fractured, possibly leading to nonunion. Nonunion may also occur when the bone fractures are caused by a high-energy injury (such as motor vehicle accident) since severe injuries most likely impair blood supply to the broken bones.

A number of factors can increase the risk of nonunion and these factors include the use of tobacco or nicotine in any form (smoking, chewing tobacco, and use of nicotine gum or patches), old age, severe anemia, diabetes, low vitamin D level, hypothyroidism, poor nutrition, medications involving anti-inflammatory drugs (such as aspirin, ibuprofen, and prednisone), infection, and a complicated fracture that is open or compound [29,30].

Applications of nanomaterials in bone fracture repair

The ideal materials for bone fracture repair should possess the following six characteristics: (i) good biocompatibility. The material itself and its degradation products should be non-toxic. (ii) Appropriate biodegradability. The material should be able to degrade after fulfilling its targeted mission and its degradation rate should match the tissue growth rate. (iii) Optimal plasticity and mechanical properties. The material can be made into desired shapes and provide support for new tissue growth until the repair process is complete. (iv) Good osteoinductivity and osteoconductivity. The material is expected to induce osteogenesis and to stimulate bone growth. (v) A three-dimensional (3D) porous structure. The material is desirable if can be processed into a three-dimensional porous structure which mimics the structure of bones and is conducive for cell adhesion and extracellular matrix deposition and has passages for nutrients and oxygen. (vi) Easily sterilized. The material should be suitable for sterilization by currently available approaches (e.g. ethylene oxide sterilization) while maintaining its mechanical and biological properties.

Traditional bone fracture repair materials

Currently, the primary materials that have been used for bone fracture repair include bone, bone cement, metal, ceramic, and polymer. The advantages and disadvantages of these materials are compared and shown in Table 2 [31–35]. Scanning electron microscopy (SEM) images of several polymers are also provided in Fig. 4. Note that fracture healing may be achieved by implants, for instance, made from stainless steel; materials like bone cement are typically used as void fillers and drug delivery vehicles which may reduce possible implant-associated infection and speed bone healing.

Nanomaterials for better bone fracture repair

Nanomaterials have shown improved bone cell functions compared to their micron-sized counterparts [36,37] and have been emerging as a new viable class of materials for bone fracture repair. This is because nanomaterials may precisely mimic the hierarchical and nanoscale features of bones and nanomaterials and the introduction of magnetic nanoparticles may provide mechanical stimuli as needed or provide unique 'smart' functions.

TABLE 2

Traditional materials used for bone fracture repair.

Material		Advantage	Disadvantage
Bone	Autologous bone	High bone fusion rate, biocompatible	Limited sources
	Allogeneic bone	Relatively high bone fusion rate	Subject to immune rejection
	Heterologous bone	Wide variety of sources	Subject to severe immune rejection and poor bone formation
Bone cement	Non-bioactive bone cement	Easy fit; good hardening properties	Poor biocompatibility, non-osteoconductive and non-osteoinductive
	Bioactive bone cement	High strength, high stability, and good bone induction activity	Insufficient mechanical properties, relatively expensive
Metal	Stainless steel	Easy processing, inexpensive	High stiffness, relatively low biocompatibility
	Titanium alloy	Excellent biocompatibility, bone-like elastic modulus, and corrosion resistance	Poor wear resistance
	Cobalt chromium alloy	Good biocompatibility and high resistance to corrosion	Low ductility
Ceramic	Aluminum oxide	Inertness, high corrosion resistance, and low expansion	High elastic modulus, easy to cause local stress
	Apatite-wollas-tonite glass ceramic	Good biological activity	Brittleness, poor flexibility, and difficult to machine
Polymer	Poly(lactic-*co*-glycolic acid)	Good biocompatibility, degradable	Possibility of disruption
	Polymethyl methacrylate	Corrosion resistant, easy fit	Poor biocompatibility
	Chitosan	High biodegradability and biocompatibility, porous structure, and good mechanical properties	Non-osteoconductive, inadequate bone formation ability, and low solubility
	Alginate	Easy to manipulate, non-toxic, biodegradable, less expensive	Low mechanical stability

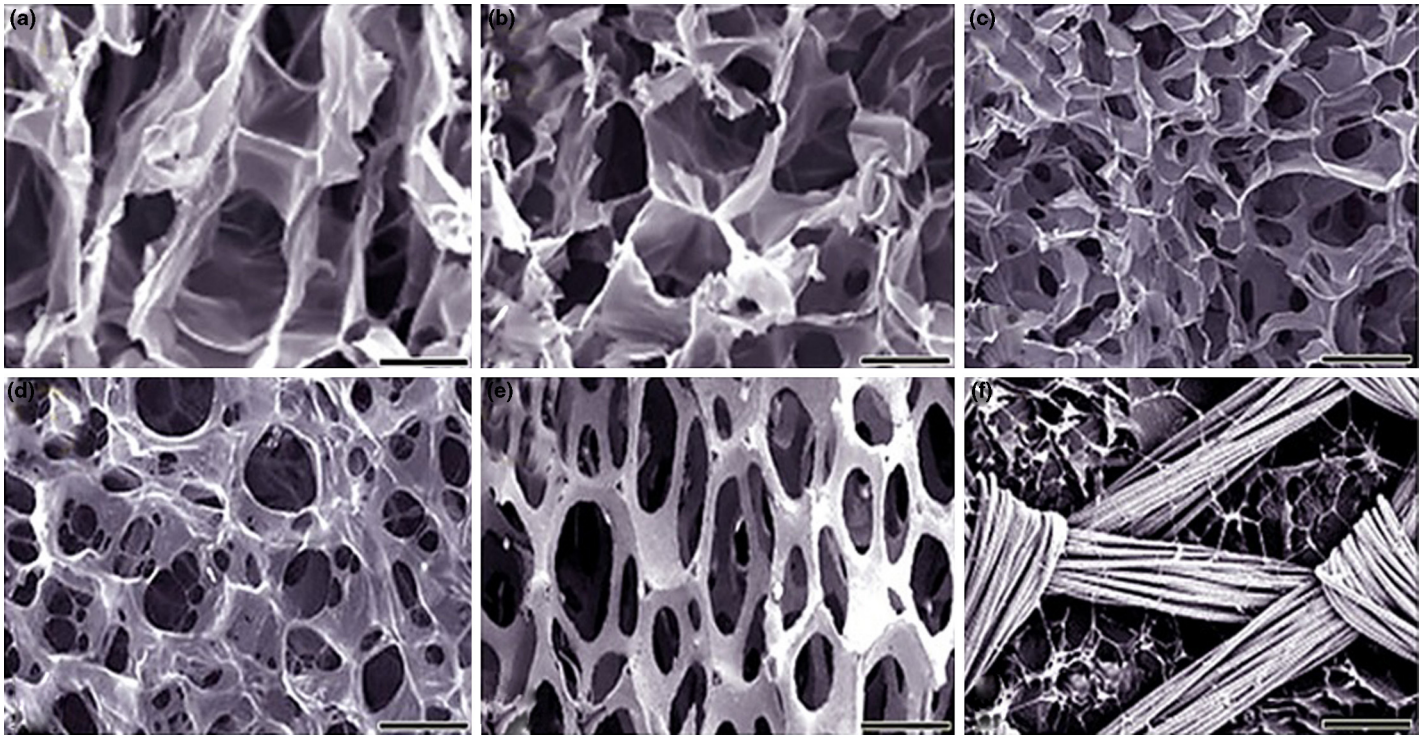

FIGURE 4

SEM images of (a) alginate, (b) alginate-chitosan, (c) chitosan, (d) chitosan-collagen, mesenchymal stem cells cultured on (e) poly(lactic-*co*-glycolic acid) scaffold and (f) porous hydroxyapatite (HA) scaffold. Scale bars (a–d, f) 100 μm, (e) 500 μm. (Reprinted with permission from [35]. © 2014 5 BioMed Central Ltd)

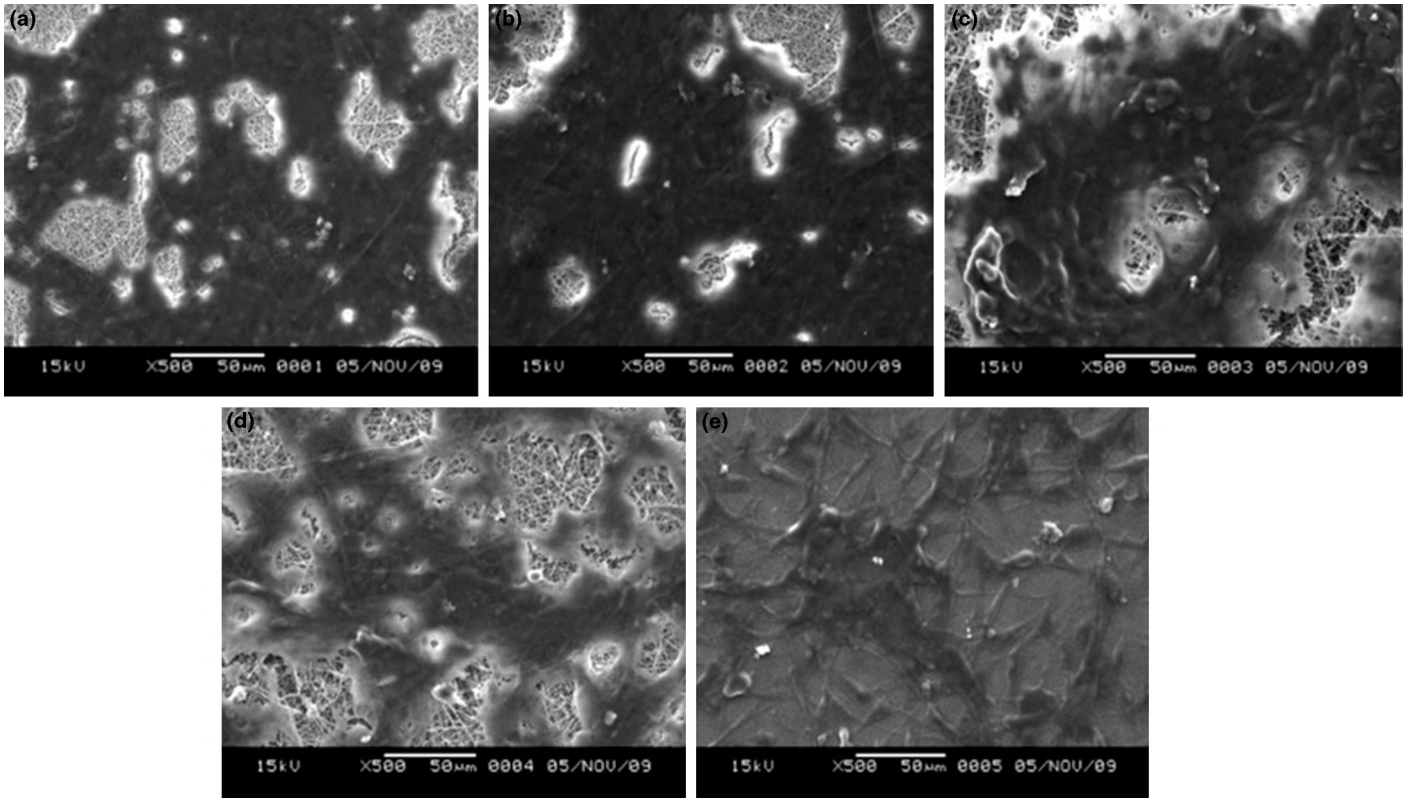

FIGURE 5

SEM micrographs of PIECs grown on nanofibrous scaffolds for 3 days: SF/HBC weight ratio = (a) 100:0, (b) 80:20, (c) 50:50, (d) 20:80, and (e) 0:100. (Reprinted with permission from [38]. © Koninklijke Brill NV, Leiden, 2011)

Biomimetic nanomaterials

Natural extracellular matrix presents a complex three-dimensional network structure and plays an important role in basic cell activities. Zhang et al. synthesized silk fibroin (SF)-hydroxybutyl chitosan (HBC) blend nanofibers, mimicking natural extracellular matrix, using the electrospinning technique [38]. Their SEM results showed that when the HBC content increased from 20% to 100%, the diameter of the nanofibers increased. Water contact angle measurements showed that the weight ratio of SF/HBC determined the hydrophilicity of the nanomaterials. Cell behavior on nanofibrous scaffolds showed that pig iliac endothelial cells (PIECs) proliferated well on the SF-HBC nanofibers and formed a typical confluent endothelial monolayer (Fig. 5).

Nanomaterials immobilizing growth factors at the molecular level

As aforementioned, growth factors play important roles in bone fracture repair. In biological systems, growth factors like BMPs exist in soluble as well as in matrix bound forms [39]. A variety of approaches have been developed to immobilize growth factors like BMPs onto implants, such as noncovalent (e.g. entrapment or ion complexation) and covalent immobilization strategies [40]. Excitingly, nanotechnologies like nanolithography allow the fabrication of substrates or implants with precisely spaced gold nanoparticle arrays and growth factors like BMP-2 could be selectively immobilized onto gold nanoparticles (Fig. 6) [41]. The spacing and the size of the gold nanoparticles could be precisely tuned thereby offering the unique advantage of controlling the amount of immobilized growth factors at the molecular level [41].

Nanomaterials promoting vascularization without the use of growth factors

As previously discussed, vascularization is important in bone fracture repair, and induction of angiogenesis is usually triggered by growth factors. Excitingly nanomaterials may offer new opportunities to promote angiogenesis and bone fracture repair even

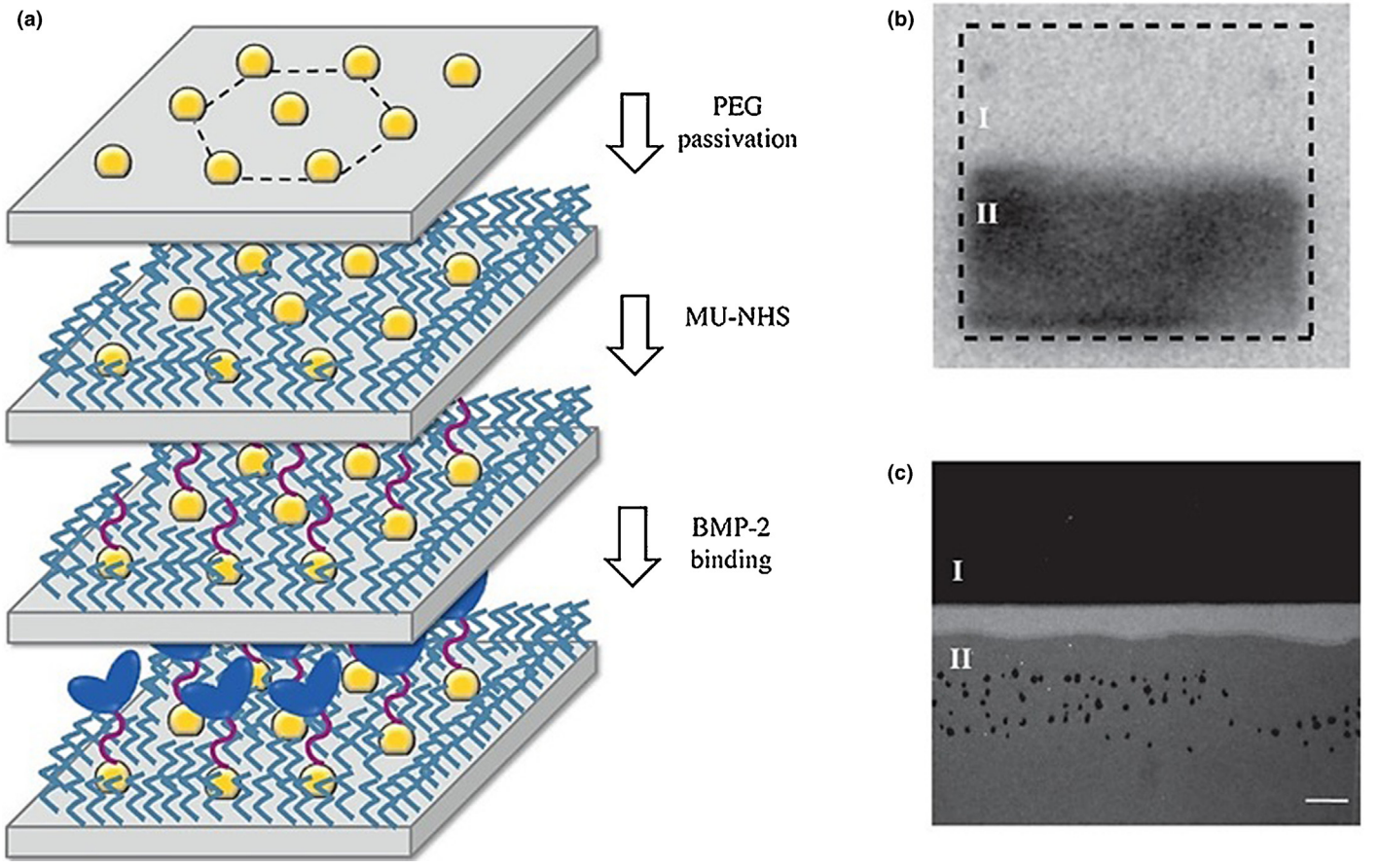

FIGURE 6

Preparation and detection of surface immobilized BMP-2. (a) Fabrication of nanostructured surfaces presenting BMP-2 covalently bound to gold nanoparticles. Gold nanoparticle arrays are produced by block copolymer micellar nanolithography. The space between the nanoparticles is covered with a layer of polyethylene glycol (PEG) to prevent unspecific adhesion of proteins and cells. Subsequently, the surfaces are incubated with a heterobifunctional linker (MU-NHS) that selectively binds to gold. Next, BMP-2 is immobilized on the functionalized gold nanoparticles via covalent binding of its primary amines to the linker. (b) Phosphorimaging of radiolabeled BMP-2. The protein is covalently immobilized on gold nanoparticles, which decorate only the lower part of a coverslip (darker area indicated with II), whereas the upper part is only coated with the PEG layer (brighter area indicated with I). (c) A representative fluorescence micrograph of part of a nanostructured coverslip presenting BMP-2 covalently bound to gold nanoparticles. Indirect immunofluorescence labeling of the protein, immobilized on the surface as in panel (b), results in a fluorescence signal from the part of the substrate where BMP-2 is covalently bound to the gold nanoparticles (bottom part of the image, II) and not from the protein repellent side of the substrate (upper part of the image, I). The scale bar in panel C is 20 μm, whereas the image in panel B shows the entire substrate (20 mm × 20 mm). (Reprinted with permission from [41]. Copyright © 2015 American Chemical Society)

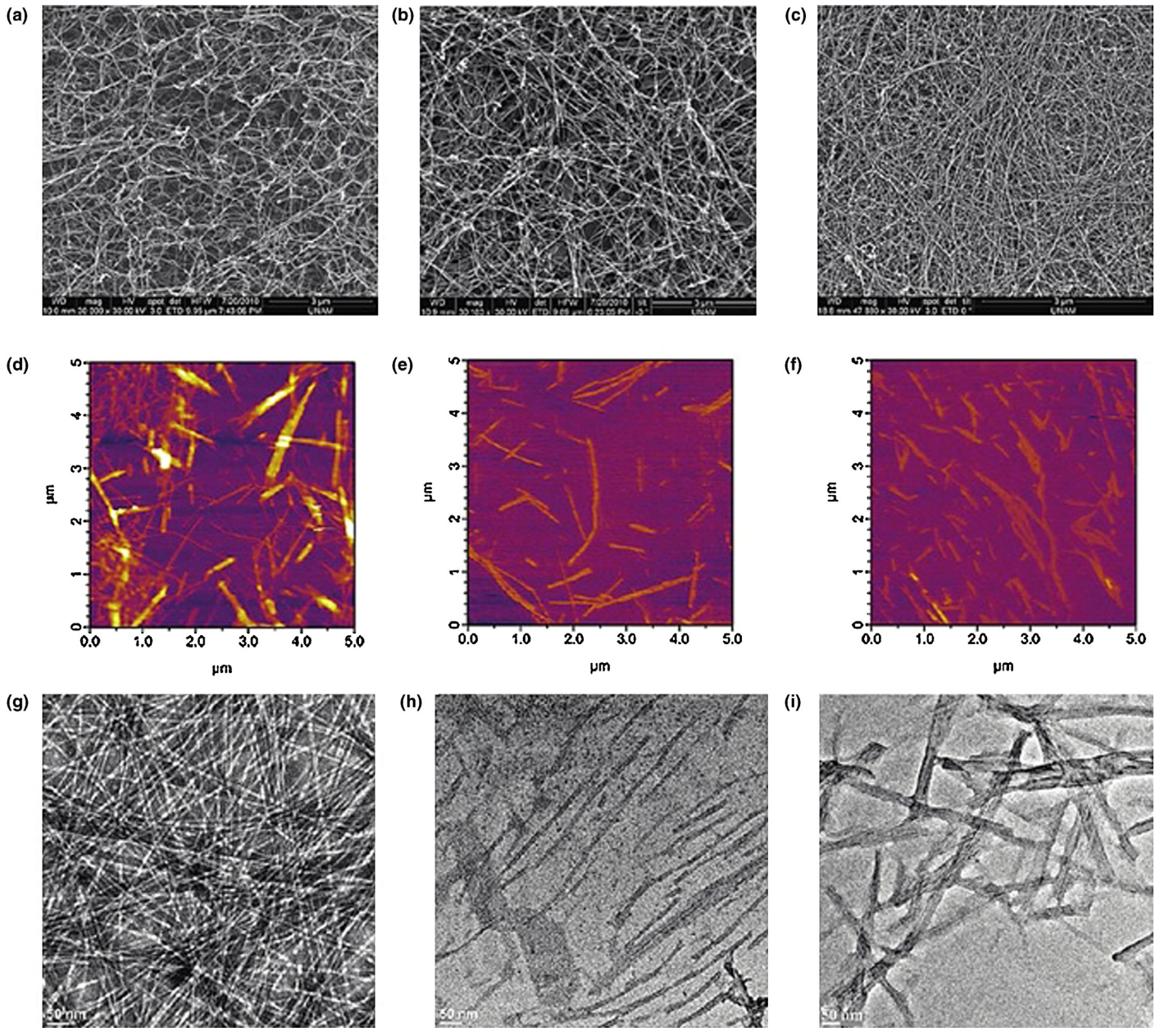

FIGURE 7

Characterization of peptide amphiphile nanofiber matrices using SEM, atomic force microscopy (AFM), and transmission electron microscopy (TEM). Imaging studies revealed that the gels formed by the PA molecules had similar structural properties in terms of individual fibers and formation of nanofibrous networks. SEM images of (a) heparin-mimetic PA, (b) Asp-PA, and (c) SO_3-PA. AFM images of (d) heparin-mimetic PA, (e) Asp-PA, and (f) SO_3-PA. TEM images of (g) heparin-mimetic PA, (h) Asp-PA, and (i) SO_3-PA. (Reprinted with permission from [44]. Copyright © 2015 American Chemical Society)

without the use of growth factors. For example, hydrogels are one of the important promising materials for bone fracture repair. During the bone fracture repair process, hydrogels may serve as excellent scaffolds for skeletal regeneration since the high water content presents a cell-friendly microenvironment to support cell functions. The ability to conveniently control the chemistry and functionality of polymers further allow tailoring the physical and mechanical properties of hydrogels for bone regeneration [42,43]. Very recently, Mammadov et al. [44] induced angiogenesis with a synthetic peptide nanofiber scaffold without the addition of any growth factors. They synthesized a self-assembling peptide amphiphile (PA) molecule that was functionalized with biologically active groups mimicking heparin. These peptide molecules formed nanofibers with a 3D network that mimicked structural proteins such as heparin in ECM (Fig. 7). As a result, angiogenesis was induced in the absence of exogenous growth factors *in vitro* and the bioactive interactions between the nanofibers and the growth factors led to robust vascularization *in vivo* [44].

Smart nanomaterials

Introducing 'smart' nanomaterials like magnetic nanoparticles, which are responsive to magnetic fields, into bone repair materials could be an innovative approach to improve the performance of bone fracture materials. Bone requires dynamic mechanical

stimulation to form and maintain functional tissue. However, mechanical stimuli are often lacking in many therapeutic approaches for bone fracture repair. Magnetic fields can cause changes in the cells' physiological and biochemical processes by affecting charge particle movement, the permeability of the membrane system, and the magnetic moment orientation of biological macromolecules [37]. Magnetic nanoparticles thereby can be used as a method to deliver mechanical stimuli by directly targeting

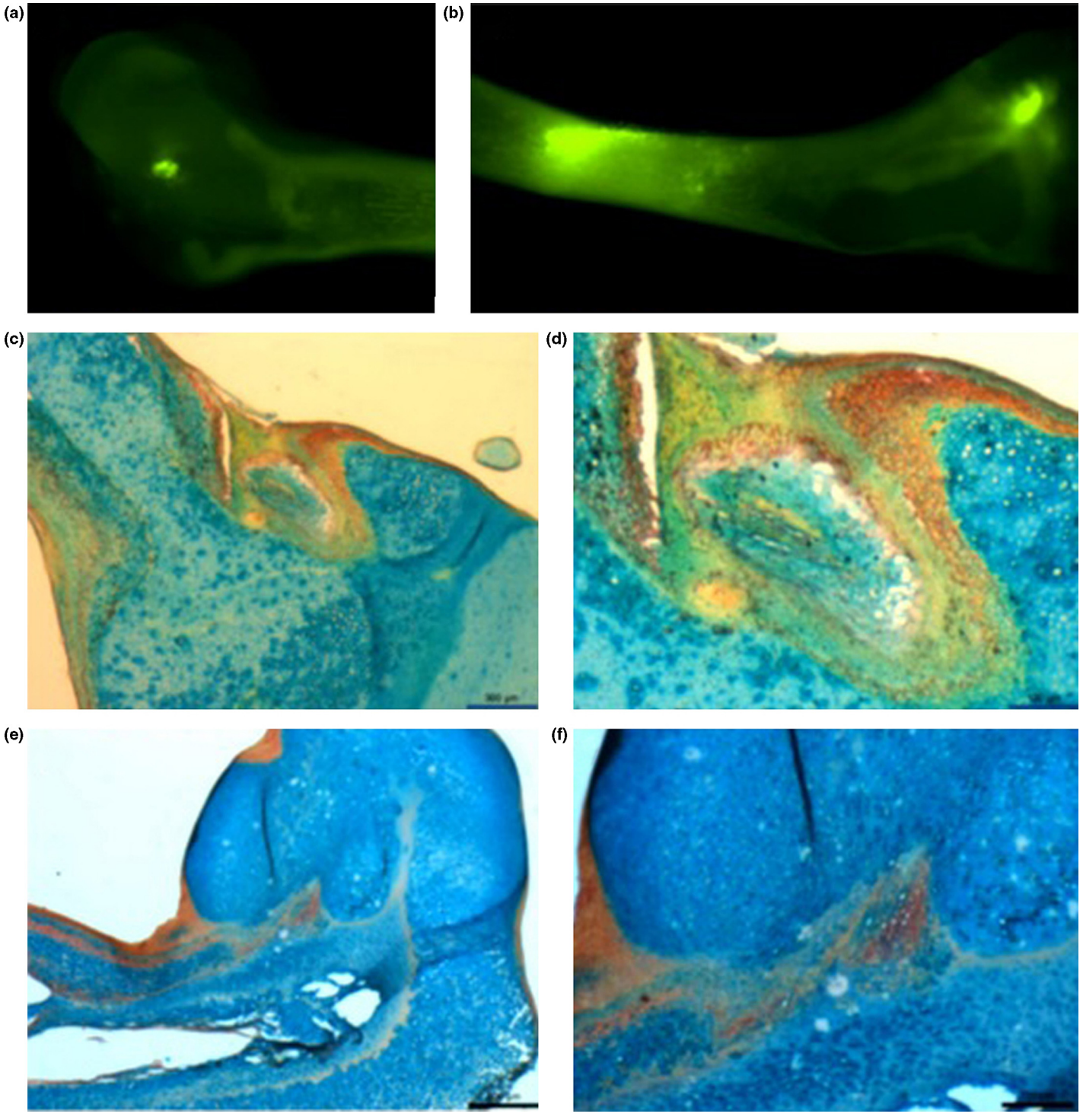

FIGURE 8

Microinjection into the chick fetal femur. (a, b) hMSCs labeled with the live cell tracker membrane dye DiL and injected into both cartilaginous epiphyses (a) and the mid-diaphysis (b), imaged immediately after microinjection. (c–f) After 2 weeks of *ex vivo* organotypic culture, (c, d) femur epiphyses from sham control groups and (e, f) femurs injected with TREK1 magnetic nanoparticle-labeled hMSCs were sectioned and histologically stained for glycosaminoglycans (blue) and calcium (red). Damage to the cell layers at the injection site appears to stimulate mineralization in the sham-injected control groups, whereas the nanoparticle-injected epiphyses show more widespread mineralization distal to the injection site. Scale bars = 300 mm (c, e) and 120 mm (d, f). (Reprinted with permission from [46]. Copyright © 2006 IOP Publishing, Ltd)

cell-surface mechanosensors and transducing forces from an external magnetic field, resulting in remotely controllable mechanotransduction. For example, Riegler et al. reported that superparamagnetic iron oxide nanoparticles could be used for *in vivo* labeling and imaging of human MSCs (hMSCs). Their nanoparticles did not present negative effects on cell viability, differentiation, or secretion patterns, but resulted in a sixfold increase in cell retention following balloon angioplasty in a rabbit model [45]. Henstock et al. also functionalized magnetic nanoparticles by attaching them to either the mechanically gated TREK1 K(+) channel or the (integrin) arginine-glycine-aspartic acid (RGD)-binding domains of human mesenchymal stem cells [46]. These cells were microinjected into an *ex vivo* chick fetal femur model. The cells receiving mechanical stimuli via the nanoparticles were found to mineralize the epiphyseal injection site more extensively than the unlabeled control cells (Fig. 8). The nanoparticle-tagged cells were also seeded into collagen hydrogels to evaluate osteogenesis in tissue-engineered constructs. It was found that inducing mechanotransduction by targeting TREK1 led to a 2.4-fold increase in mineralization and significant increases in matrix density. In both models, a significant additive effect on mineralization was observed by combining the mechanical stimulation with sustained release of BMP-2 from polymer microspheres. As a result, it was demonstrated that nanoparticle-mediated mechanotransduction can be used with pharmacological approaches to maximize bone formation [46].

Wu et al. [47] combined the bone repair ability of hydroxy calcium phosphate ceramics with magnetic fields and made new calcium phosphate ceramics (i.e. hydroxyapatite/calcium phosphate) by integrating super paramagnetic nanoparticles into the calcium phosphate ceramics. Their *in vitro* and *in vivo* studies found that, compared to the control calcium phosphate ceramics, the addition of magnetic nanoparticles led to significantly improved cell growth and differentiation as well as expression of BMP-2. Meng et al. [48] made paramagnetic nanofiber films with hydroxyapatite, poly(lactic acid), and γ-Fe_2O_3 using the electrospinning technique. They found that their paramagnetic nanofiber films significantly increased the differentiation of osteoblasts and the secretion of extracellular matrix in mice under a static

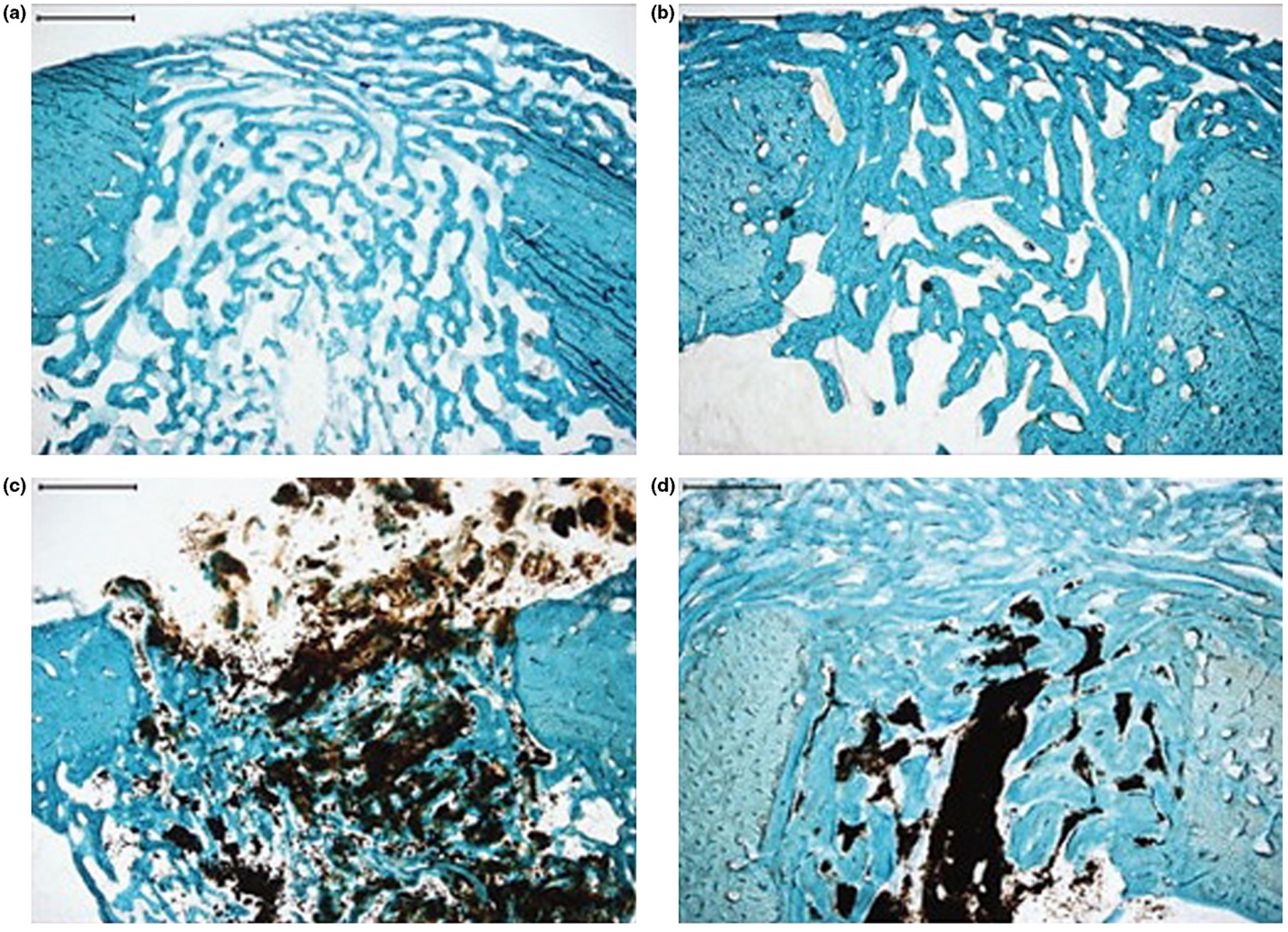

FIGURE 9

Diaphyseal implant analysis. Undecalcified histological patterns of scaffolds of (a, b) control material and (c, d) self-assembled collagen fibrils immobilized with magnetic nanoparticles in rabbit tibia diaphysis at light microscopy at 2 (a, c) and 4 (b, d) weeks after surgery (Toluidine Blue, Acid Fucsin, and Fast Green staining; bar: 200 μm). No inflammatory reaction against the scaffolds was observed. The newly formed bone tissue has a regular architectural pattern surrounding the implants without connective capsules or gaps. (Reprinted with permission from [49]. © 2013 Elsevier B.V.)

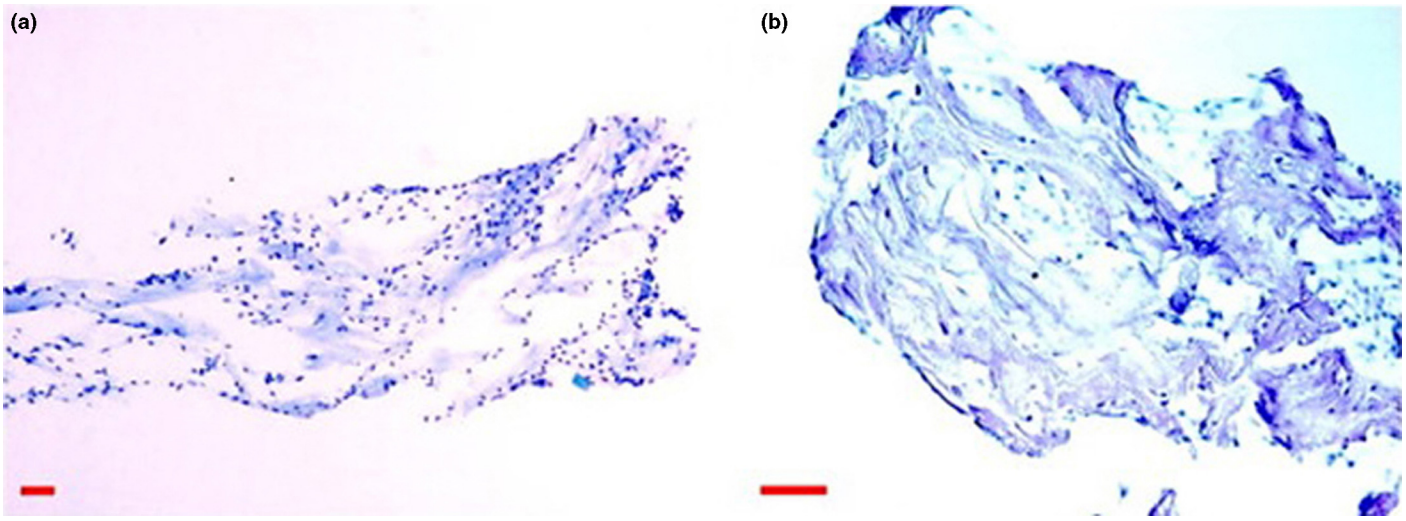

FIGURE 10

Morphological evaluation of hBMSCs cultured on a magnetic scaffold, by light microscopy and hematoxylin and eosin (HE) staining: (a) after 5 days of cultivation, the cells have covered the scaffold surface and have spread along the pore walls; (b) after 10 days of cultivation, the cells have colonized the surface and most of the inner pores. Scale bar 100 μm. (Reprinted with permission from [50]. © 2013 Elsevier B.V.)

magnetic field. Further, scaffolds with magnetic nanoparticles were found to significantly improve bone healing while not causing significant inflammation *in vivo* (Fig. 9) [49].

Magnetic nanoparticles can also be used to re-load therapeutic agents after implantation. Bock et al. coated iron oxide nanoparticles onto scaffolds made of hydroxyapatite and collagen which, like other conventional scaffolds, cannot re-load therapeutic agents after implantation. By contrast, the nanoparticle coated scaffold by Bock et al. was found to be able, via magnetic driving, to attract and take up *in vivo* growth factors,

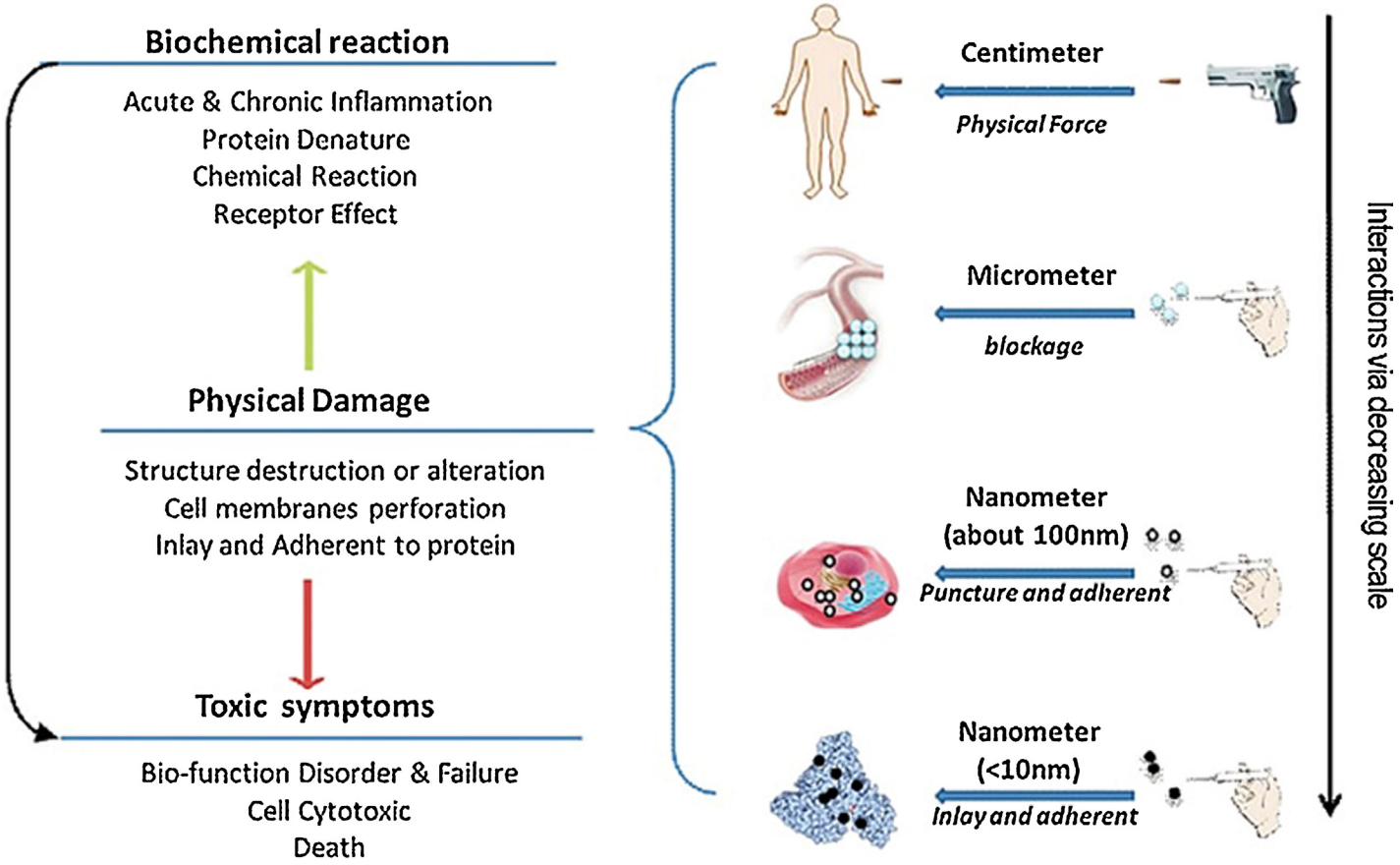

FIGURE 11

Physical damages exist in all sizes including meter, micrometer, and nanometer, and the origin of nanotoxicity is physical damage. (Reprinted with permission from [55]. © 2013 Wanfang Ltd.)

stem cells, or other agents that can bind to the magnetic nanoparticles. The developed magnetic scaffolds did not suffer from any structural damage during the preparation process and did not release the magnetic nanoparticles under a constant flow of simulated body fluids over a period of eight days. Studies also showed that the magnetic scaffolds supported adhesion and proliferation of human bone marrow stem cells/hBMSCs (Fig. 10) [50].

Conclusions and outlook

Bone fracture is a complex process of bone regeneration and a variety of conventional biomaterials including metals, ceramics, and polymers have been applied as bone fracture repair materials. In biological systems, regeneration of bone requires the coordinated effort of cells and growth factors in a time, concentration, and site specific fashion. The ideal bone fracture repair materials therefore should not only allow initial cellular infiltration and subsequent integration with native tissue, but also should stimulate vascularization and new bone formation. By mimicking the nanoscale features of bones and/or offering unique properties, nanomaterials have demonstrated improved bone cell functions compared to their microstructured counterparts and, more excitingly, have led to new breakthroughs. To name a few, nanomaterials have resulted in precise immobilization of growth factors at the molecular level, promotion of vascularization by simply mimicking ECM components (e.g. heparin), and re-loading of therapeutic agents into nanomaterials after implantation. In addition, as we previously reviewed, nanomaterials could lead to better treatment of intracellular diseases (e.g. intracellular bone infections) by offering unique properties such as targeting drug delivery to a specific intracellular compartment [51], and electrospun nanofiber scaffolds could offer improved tissue regeneration by mimicking unique features of the native ECM [52].

However, nanomaterials have also posed new challenges. One of the main challenges is the potential toxicity related to nanomaterials or nanotoxicity. Due to the nano-size, nanomaterials are expected to have different toxicity properties compared to their micron-sized counterparts. For instance, we have found that nanoparticles could cause significantly greater toxicity *in vitro* at lower concentrations and shorter exposure times compared to micron-sized particles where nanoparticles but not micron-sized particles were internalized by lung epithelial cells [53]. It has been known that endocytosis including clathrin-mediated endocytosis and post-endocytotic trafficking is the predominant route of nanoparticle uptake, and multiple internalization mechanisms may be involved. Furthermore, nanoparticle internalization has

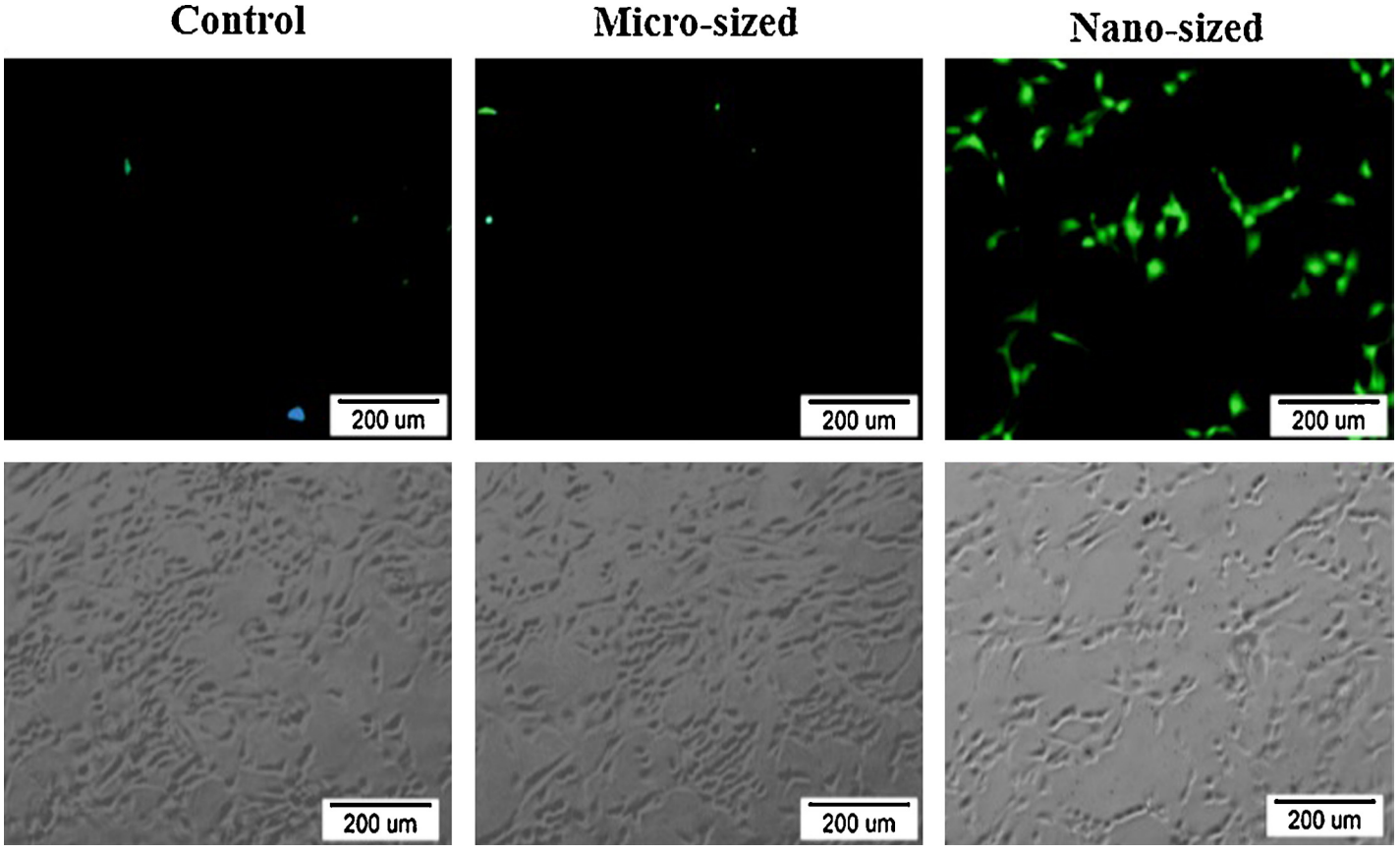

FIGURE 12

Nano-sized tungsten carbide-cobalt (WC-Co) increased ROS production. BEAS-2B cells were seeded onto coverslips in 6-well plate and incubated at 37°C for 24 hours. The cells were cultured in fresh serum-free medium with micron-sized or nano-sized WC–Co at 5 μg/cm^2 for 30 min. 2′,7′-Dichlorofluorescein diacetate (5 μM) was added and incubated for 15 min, and then, the cells were washed and fixed. The images were captured with a fluorescence microscope (upper panel). The corresponding bright field micrographs are shown in the bottom panel. Bar: 200 μm. (Reprinted with permission from [56]. © 2015 Springer International Publishing AG)

been confirmed to contribute to cellular toxicity. Among the mechanisms of nanotoxicity that have been investigated, production of reactive oxygen species (ROS), for example, as a result of chemical interactions, has been widely studied [54]. Such chemical interactions can induce *in vitro* apoptosis by the formation of free radicals, accumulation of peroxidative products, and the depletion of cell antioxidants. Despite the existence of chemical damage, physical damage from nanomaterials also play a key role in the creation of 'trauma' to the body (Fig. 11) [55]. Nanomaterials could cause cell damage due to the physical blockage of microcirculation, cell destruction due to membrane random insertion, and cell dysfunction due to physical contact with biological molecules. More recently, we found that, compared to micron-sized particles, nanoparticles could lead to increased ROS production (Fig. 12) which could further activate AKT and ERK1/2 signaling pathways in lung epithelial cells. Nanoparticles could also lead to increased transcriptional activation of AP-1, NF-κB, VEGF, and angiogenesis [56]. Unfortunately, *in vitro* nanotoxicity data may not necessarily predict what may happen *in vivo* [57], adding challenges to the dilemma of nanotoxicity. Moreover, appropriate *in vitro* cell culture models and *in vivo* animal models, which can be used for comparison among different nanomaterials, have not been established.

Therefore, future studies should focus on developing nanomaterials that not only mimic the nano-features of bone but also stimulate vascularization or new bone growth, establishing *in vitro* and *in vivo* models that could potentially be used for and compared among a variety of nanomaterials, investigating the toxicity of nanomaterials, and establishing standard protocols for nanotoxicity tests of biomaterials.

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