Society For Biomaterials

Review Article

Nanostructured scaffolds for bone tissue engineering

Xiaoming Li,[1] Lu Wang,[1] Yubo Fan,[1] Qingling Feng,[2] Fu-Zhai Cui,[2] Fumio Watari[3]

[1]Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China
[2]State Key Laboratory of New Ceramic and Fine Processing, Department of Materials Science and Engineering, Tsinghua University, Beijing 100084, China
[3]Department of Biomedical Materials and Engineering, Graduate School of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan

Received 31 October 2012; revised 25 November 2012; accepted 26 November 2012
Published online 1 February 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34539

Abstract: It has been demonstrated that nanostructured materials, compared with conventional materials, may promote greater amounts of specific protein interactions, thereby more efficiently stimulating new bone formation. It has also been indicated that, when features or ingredients of scaffolds are nanoscaled, a variety of interactions can be stimulated at the cellular level. Some of those interactions induce favorable cellular functions while others may leads to toxicity. This review presents the mechanism of interactions between nanoscaled materials and cells and focuses on the current research status of nanostructured scaffolds for bone tissue engineering. Firstly, the main requirements for bone tissue engineering scaffolds were discussed. Then, the mechanism by which nanoscaled materials promote new bone formation was explained, following which the current research status of main types of nanostructured scaffolds for bone tissue engineering was reviewed and discussed. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 101A: 2424–2435, 2013.

Key Words: scaffold, nanostructure, bone tissue engineering

How to cite this article: Li X, Wang L, Fan Y, Feng Q, Cui F-Z, Watari F. 2013. Nanostructured scaffolds for bone tissue engineering. J Biomed Mater Res Part A 2013:101A:2424–2435.

INTRODUCTION

Bone tissue engineering is a complex and dynamic process that initiates with migration and recruitment of osteoprogenitor cells followed by their proliferation, differentiation, matrix formation along with remodeling of the bone. Bone scaffolds are typically made of porous degradable materials that provide the mechanical support during repair and regeneration of damaged or diseased bone.

Researches on bone tissue engineering over the past decades have inspired innovation in new materials, processing techniques, performance evaluation, and applications. Significant progress has been made toward scaffold materials for structural support with desired osteogenesis and angiogenesis abilities. Bioresorbable scaffolds with controlled porosity and tailored properties are possible today due to innovation in scaffold fabrication using advanced technologies.

Natural bone derives its unique combination of mechanical properties from an architectural design that spans nanoscale to macroscopic dimensions, with precisely and carefully engineered interfaces. Many different groups have tried to manipulate the mechanical properties (e.g., stiffness, strength, and toughness) of scaffolds through the design of nanostructures (e.g., the inclusion of nanoparticles or nanofiber reinforcements in polymer matrices) to mimic bone's natural nanocomposite architecture.

To better mimic the nanostructure in natural ECM, over the past decade, scaffolds manufactured from nanofibers, nanotubes, nanoparticles and hydrogel, have recently emerged as promising candidates in producing scaffolds that resemble the ECM and efficiently replace defective tissues. Because natural tissues or organs are nanometer in dimension and cells directly interact with (and create) nanostructured extracellular matrices (ECM), the biomimetic features and excellent physiochemical properties of nanomaterials play a key role in stimulating cell growth as well as guide tissue regeneration. Even though it was a field in its

Correspondence to: X. Li; e-mail: x.m.li@hotmail.com or Y. Fan; e-mail: yubofan@buaa.edu.cn
Contract grant sponsor: National Basic Research Program of China (973 Program); contract grant number: 2011CB710901
Contract grant sponsor: National Natural Science Foundation of China; contract grant numbers: 31000431, 10925208
Contract grant sponsor: Beijing Nova Program; contract grant number: 2010B011
Contract grant sponsors: Program for New Century Excellent Talents (NCET) in University from Ministry of Education of China; State Key Laboratory of New Ceramic and Fine Processing (Tsinghua University); Beihang Graduate Innovative Practice Fund

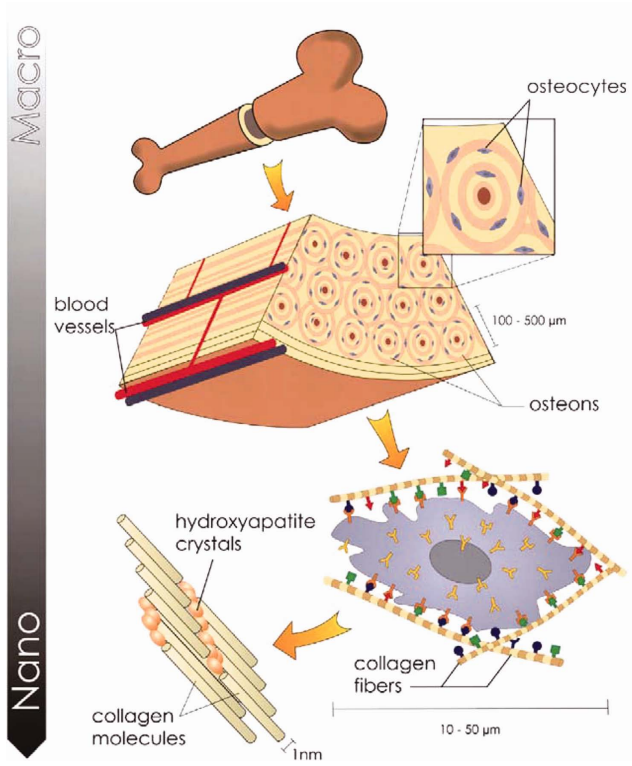

FIGURE 1. Hierarchical organization of bone over different length scales. Bone has a strong calcified outer compact layer (a), which comprises many cylindrical Haversian systems, or osteons (b). The resident cells are coated in a forest of cell membrane receptors that respond to specific binding sites (c) and the well-defined nanoarchitecture of the surrounding extracellular matrix (d). (Adapted with Permission from Ref. 2. Copyright 2008 Elsevier Ltd.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

infancy a decade ago, currently, numerous researchers fabricate cytocompatible[1] biomimetic nanomaterial scaffolds encapsulating cells (such as stem cells, chondrocytes and osteoblasts, etc.) for tissue engineering applications.

To give a general description of studies of nanostructured scaffolds for bone tissue engineering, we have composed this review. This review presents the mechanism of interactions between nanoscaled materials and cells and focuses on the current research status of nanostructured scaffolds for bone tissue engineering. And it should be emphasized that, the "nanostructure-scaffold" here refers that features or ingredients of which are nanoscale, that a variety of interactions can be stimulated at the cellular level. In some instances, some of those interactions also lead to toxicity, which can be of serious concerns.

BONE AND SCAFFOLD ENGINEERING

Structural properties of bone

Following the approach of scaffolding as a way of temporarily mimicking the extracellular matrix of bone, it is necessary to look at the chemical, mechanical and structural properties of bone.

Bone is a sophisticated composite on different hierarchical levels, as shown in Figure 1. From a structural perspective, bone tissue consists of two main parts, a compact shell called cortical bone ("compacta") and a porous core called spongiosa or trabecular bone ("trabecular" meaning "little beam" in Latin[3]). The combination of a dense shear stress-resisting shell and a cellular inner structure with a typical relative density of between 0.05 and 0.3[4] prevents buckling and results in a lightweight core analogous to a sandwich structure with excellent bending resistance. In contrast to most man-made sandwich cores, trabecular bone has an optimized structural anisotropy due to the trabecular orientation along the principal stress trajectories.[5] On a nanometer scale bone can be basically described as a composite between 70% calcium phosphate crystals and 20–30% collagen matrix with some water.[6] This geometrically complex combination of an elastic collagen matrix (elastic modulus $E = 1$–2 GPa, ultimate tensile strength UTS = 50–1000 MPa) with a hard and brittle calcium phosphate mineral ($E = 130$ GPa, UTS = 100 MPa)[6] leads to high mechanical bulk properties with ductile and thus failure-tolerant characteristics. According to a literature review the compressive strengths of cortical and cancellous bone are in the ranges 100–230 and 2–12 MPa, whereas the Young's moduli are in the ranges 7–30 and 0.5–0.005 GPa, respectively.[7] While macro mechanical parameters are well described in the scientific literature, the mechanical properties at the micro- (osteons, Haversian canals), submicron (lamellae) and nanostructural (collagen fibers) levels remain poorly understood[8] and are still a matter of extensive research.[9]

Bone regeneration requires four components: a morphogenetic signal, responsive host cells that will respond to the signal, a suitable carrier of this signal that can deliver it to specific sites then serve as a scaffold for the growth of the responsive host cells, and a viable, well vascularized host bed.[10] Bone tissue engineering, for the purpose of this review, is the use of a scaffolding material to either induce formation of bone from the surrounding tissue or to act as a carrier or template for implanted bone cells or other agents. Materials used as bone tissue-engineered scaffolds may be injectable or rigid, the latter requiring an operative implantation procedure.

Scaffold requirements

The goal of the scaffold is to provide a 3D environment for cells and tissue to grow on. In 2002, Hutmacher et al.[11] first reported the processing of bioresorbable scaffolds [Fig. 2(a–d)] for tissue engineering applications using FDM (Fused Deposition Modeling).

The key factors for an ideal scaffold for bone tissue engineering are:[12] (i) macro- (pore size $>$ 100 μm) and microporosity (pore size $<$ 20 μm); (ii) interconnected open porosity for *in vivo* tissue in-growth; (iii) sufficient mechanical strength and controlled degradation kinetics for proper load transfer to the adjacent host tissue; (iv) initial strength for safe handling during sterilizing, packaging, transportation to surgery, as well as survival through physical forces *in vivo*; and (v) sterile environment for cell seeding.

Unfortunately, it is at present obscure how this is applied in practice and in particular if a material fulfilling these criteria would really perform better than existing

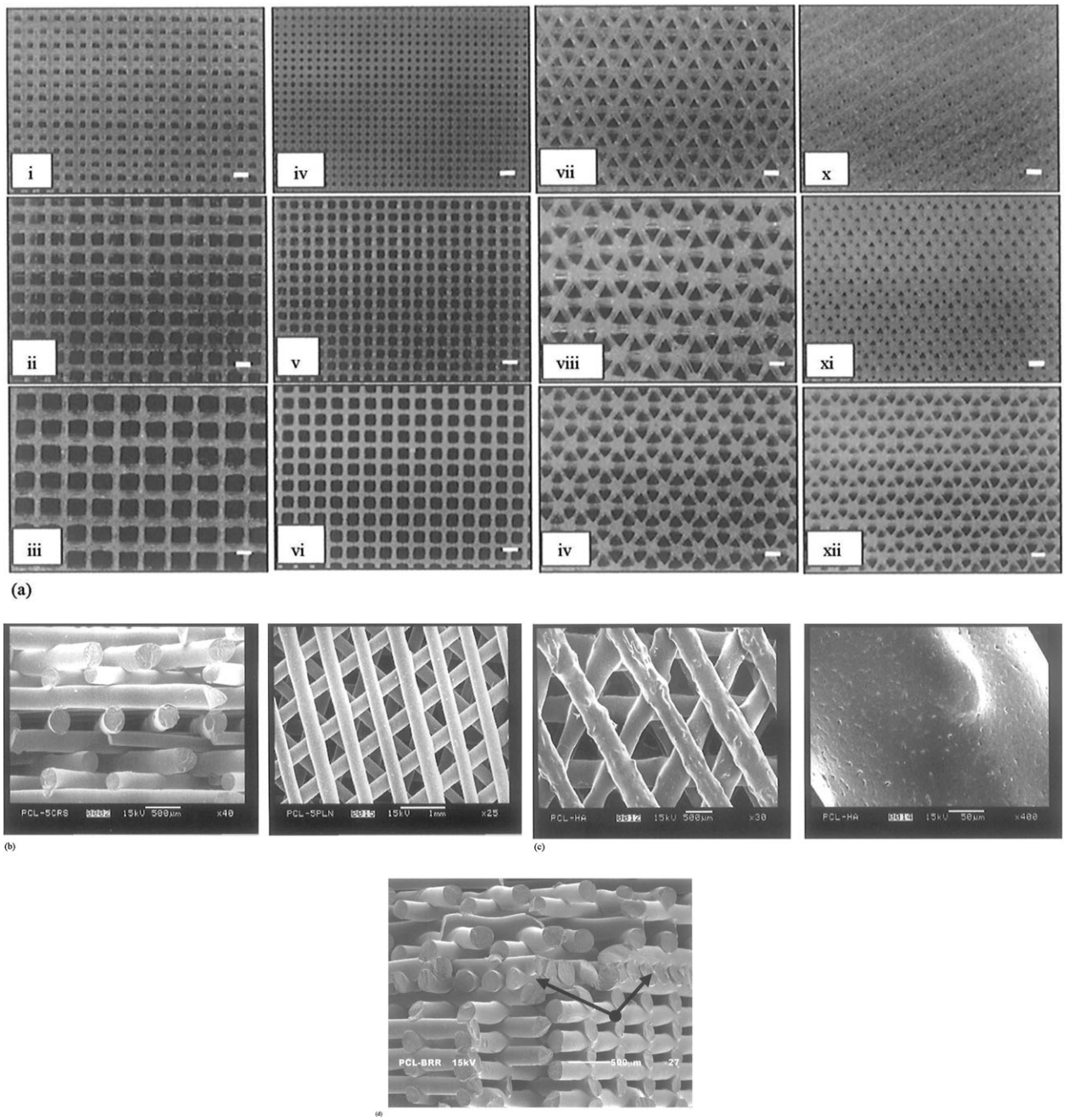

FIGURE 2. (a–d) 3D scaffold systems of various porosity and pore geometry fabricated by FDM (fused deposition modeling). (Adapted with Permission from Ref 11. Copyright 2000 Elsevier Ltd.).

materials. Furthermore, the distinction between the requirements for tissue engineering and bone graft substitution scaffolds is still far from clear. Nevertheless, there is a general consensus that 3D bone scaffolds should be highly open porous structures (>40–60%) to favor rapid diffusion or the flow of cell nutrients and to allow cell migration.[13] Pore sizes necessary to achieve suitable porosities are suggested to be in the range 50–1000 μm[14,15] for *in vivo* bone regeneration. In contrast, osteogenesis *in vitro* requires pore dimensions one order of magnitude lower than *in vivo*.[15] The size of the interconnections is still a matter of debate, with values of between 15 and 50 μm.[16,17] Taking into account the assumptions and simplifications in all methods [gravimetry, mercury intrusion, liquid displacement, scanning electron microscopy (SEM), and computed tomography (CT)][15] to determine pore parameters, an additional uncertainty arises. In this light it becomes obvious that instead of the "ideal" tissue engineering scaffold, different structures are needed for various applications.

NANOMATERIALS AND THE MECHANISM OF PROMOTING GROWTH OF BONE TISSUE

Biomaterials are widely used in repair, replacement, or augmentation of diseased or damaged bones. Bone and joint

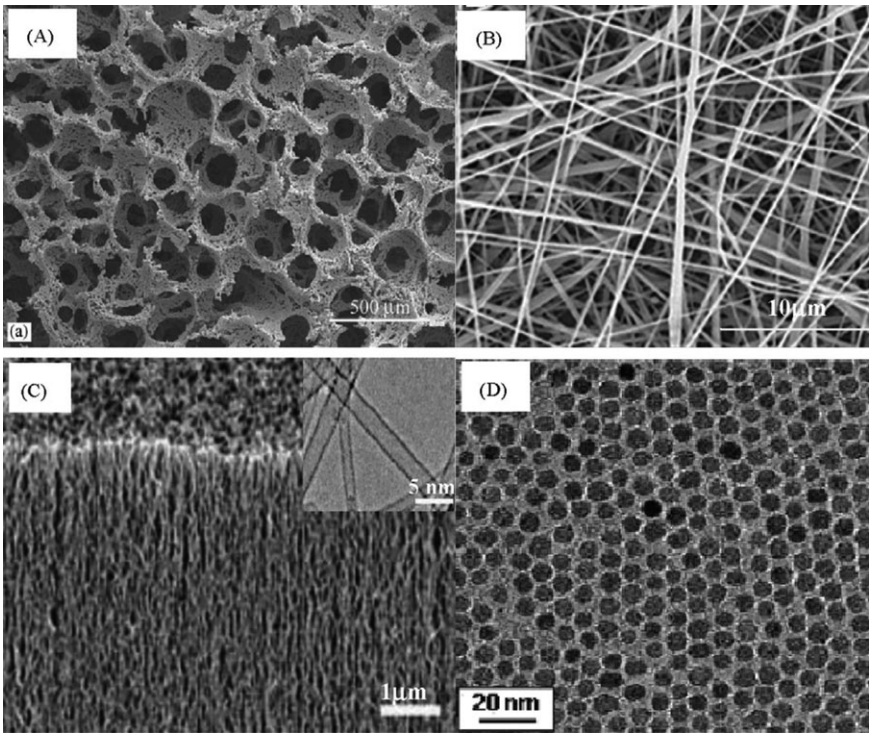

FIGURE 3. (A) Scanning electron microscopy (SEM) image of poly (L-lactic acid) (PLLA) nanofibrous scaffold with interconnected spherical macropores created by a phase-separation technique. (Adapted with Permission from Ref. 25. Copyright 2004 Elsevier Ltd.) (B) Electrospun polycaprolactone/hydroxyapatite/gelatin (PCL/HA/gelatin, 1:1:2) nanofibers which significantly improved osteoblast functions for bone tissue engineering applications. (Adapted with Permission from Ref. 26. Copyright 2008 John Wiley and Sons.) (C) Densely aligned single wall carbon nanotube (SWCNT) forest grown with novel water-assisted chemical vapor deposition in 10 min. (Adapted with Permission from Ref. 27. Copyright 2004 The American Association for the Advancement of Science.) (D) Transmission electron microscopy (TEM) image of mono dispersed magnetic Fe_3O_4 nanoparticles (6 nm) deposited from their hexane dispersion and dried at room temperature. (Adapted with Permission from Ref. 28. Copyright 2004 American Chemical Society.).

substitutes are commonly made of metals, ceramics, polymers, composites, and more recently, nanocomposite. The attention to these materials is due to their mechanical strength, porosity, biocompatibility, and bioactivity, which allows cell adhesion, migration, growth, and proliferation, resulting in excellent integration with surrounding tissues.[18,19]

Ideally, the materials should exhibit the same response to loading as real bone and should also be biocompatible with existing tissue in the form of highly porous scaffolds. These scaffolds will provide a suitable microenvironment to ensure cell adhesion, proliferation, and secretory activity of the cells' own extracellular matrices, thus replacing the biodegrading scaffold.[20–22] The compatibility issue includes surface and mechanical compatibility as well as osteocompatibility.[23]

Nanomaterials include nanoparticles, nanoclusters, nanocrystals, nanotubes, nanofibers, nanowires, nanorods, nanofilms, and so forth. To date, numerous top-down and bottom-up nanofabrication technologies (such as electrospinning, phase separation, self-assembly processes, thin film deposition, chemical vapor deposition, chemical etching, nano-imprinting, photolithography, and electron beam or nanosphere lithographies[24]) are available to synthesize nanomaterials with ordered or random nanotopographies (Fig. 3,[25–28]). Nanomaterials can also be grown or self-assembled into nanotubes/nanofibers which can even more accurately simulate the dimensions of natural entities, such as collagen fibers. After decreasing material size into the nanoscale, dramatically increased surface area, surface roughness and surface area to volume ratios can be created to lead to superior physiochemical properties (i.e., mechanical, electrical, optical, catalytic, magnetic properties, etc.).[29]

In addition to the dimensional similarity to bone/cartilage tissue, nanomaterials also exhibit unique surface properties (such as surface topography, surface chemistry, surface wettability, and surface energy) due to their significantly increased surface area and roughness compared to conventional or micron structured materials. As is known, material surface properties mediate specific protein (such as fibronectin, vitronectin, and laminin) adsorption and bioactivity before cells adhere on implants, further regulating cell behavior and dictating tissue regeneration.[30] Studies have demonstrated that nanostructured materials with cell favorable surface properties may promote greater amounts of specific protein interactions to more efficiently stimulate new bone growth compared to conventional materials[31–34] (Fig. 4). This may be one of the underlying mechanisms why nanomaterials are superior to conventional materials for tissue growth. Therefore, by controlling surface properties, various nanophase ceramic, polymer, metal, and composite scaffolds have been designed for bone/cartilage tissue engineering applications.

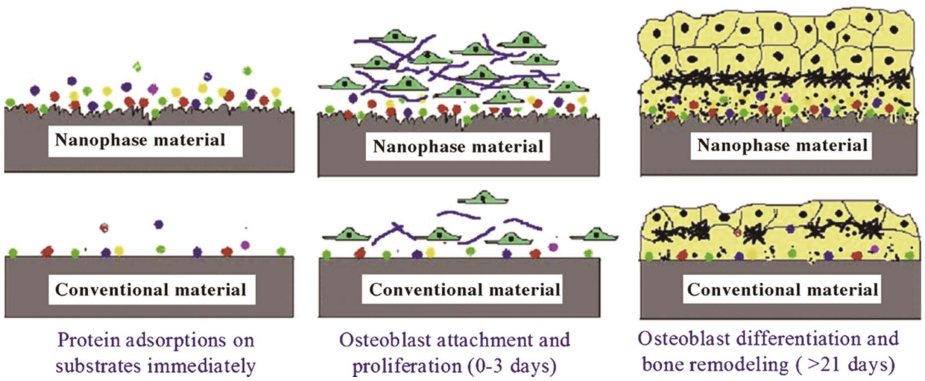

FIGURE 4. Schematic illustration of the mechanism by which nanomaterials may be superior to conventional materials for bone regeneration. The bioactive surfaces of nanomaterials mimic those of natural bones to promote greater amounts of protein adsorption and efficiently stimulate more new bone formation than conventional materials. (Adapted with Permission from Ref 35. Copyright 2009 Elsevier Ltd.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MAIN TYPES OF NANO-SCAFFOLDS MATERIALS FOR BONE TISSUE ENGINEERING

Nanocomposite

From the biological perspective the natural bone matrix is a combination of organic/inorganic composite materials and consists of a naturally occurring polymer (collagen) and a biological mineral (apatite).[36] Further blending with inorganic materials can modify the mechanical properties as well as the degradation rates of the materials. The designed composite scaffold should combine the advantages of both components. Moreover, the natural composite material should have an excellent balance between strength and toughness, both of which should be superior to those of the individual components.[37] For successful application of HA based ceramics as bone grafts, higher strength and toughness are desirable. Unfortunately, because of poor sinterability, HA ceramics show low strength and toughness, especially in wet environment under physiological condition, which makes them unsuitable even for low load bearing applications. Nanostructured material can improve the sinterability due to high surface energy and, therefore, improve mechanical properties.[38] Therefore, instead of using a single material type for synthesis it is a natural strategy to combine polymers and hydroxyapatite (HAp) to fabricate scaffolds that meet all the requirements desired for particular applications in TE. Thus polymer/inorganic composite scaffolds have attracted the attention of researchers. The emphasis today is in the areas of resorbable ceramics to make scaffolds-either bi-phasic (with HA and TCP) or just HA or TCP (tricalcium phosphate types of ceramics which can dissolve in the body after some time.

For instance, HAp, which has the general formula $Ca_{10}(OH)_2(PO4)_6$, is the main inorganic calcium phosphate mineral component of bones and teeth. The close chemical similarity of HAp to natural bone has led to extensive research efforts to use synthetic HAp as a bone substitute and replacement in biomedical applications.[39,40] Recent studies have suggested that nanocrystalline HAp (nHAp) powders exhibit improved sinterability and enhanced densification due to their greater surface area, which may improve fracture toughness as well as other mechanical properties.[41] However, nHAp is difficult to shape in the specific form required for bone repair and implantation because of its intrinsic hardness, fragility, and lack of flexibility, which limits its use as a load-bearing implant material. To capitalize on its advantages and simultaneously overcome the drawbacks nHAp is combined with various types of polymers to generate biocomposite materials that can be used for osteoconduction in the field of orthopedic surgery. In general, from the biomimetic point of view the designed nHAp composites potentially improve both the biocompatibility and mechanical properties of bone-grafting materials.[42]

So far, there have been many studies utilizing the incorporation of HAp into various synthetic polymers, including poly(D,L-lactic acid-*co*-glycolic acid) (PLGA),[43–45] poly(L-lactic acid) (PLLA),[46] poly(propylene fumarate) (PPF),[47] poly(caprolactone) (PCL),[48] a copolymer,[49] and a cyclic acetal hydrogel,[50,51] collagen, chitosan. The nanoscale features of HA particles induce advantageous cellular responses when compared with micro-sized HA particles. For example, HA nanoparticles coated on glasses demonstrated higher MG-63 cell attachment and proliferation than micro-sized HA particles due to the higher surface area for cell adhesion and lower crystallinity.[52] Similarly, HA nanoparticles embedded in 3D PCL scaffolds have shown enhanced levels of attachment, proliferation, alkaline phosphatase activity, and calcium deposition (i.e., mineralization) of mesenchymal stem cells (MSC).[48] Nanohydroxyapatite/collagen/poly-L-lactic acid (PLLA) composite reinforced by chitin fibers has shown better mechanical properties in previous study.[53] To enhance the strength of the scaffold further, PLLA was linked with chitin fibers by dicyclohexylcarbodimide (DCC). The crosslinked scaffolds exhibited a higher dimensional stability in aqueous medium due to chemical crosslinking as shown in Yang group's studies.[54] Figure 5 shows the structure and morphology of nanofibrous in biocomposite scaffolds of chitosan (CS), PVA, and hydroxyapatite (HA) in Yang's study. After the link treatment, pore and structure of the samples are more even than they do only

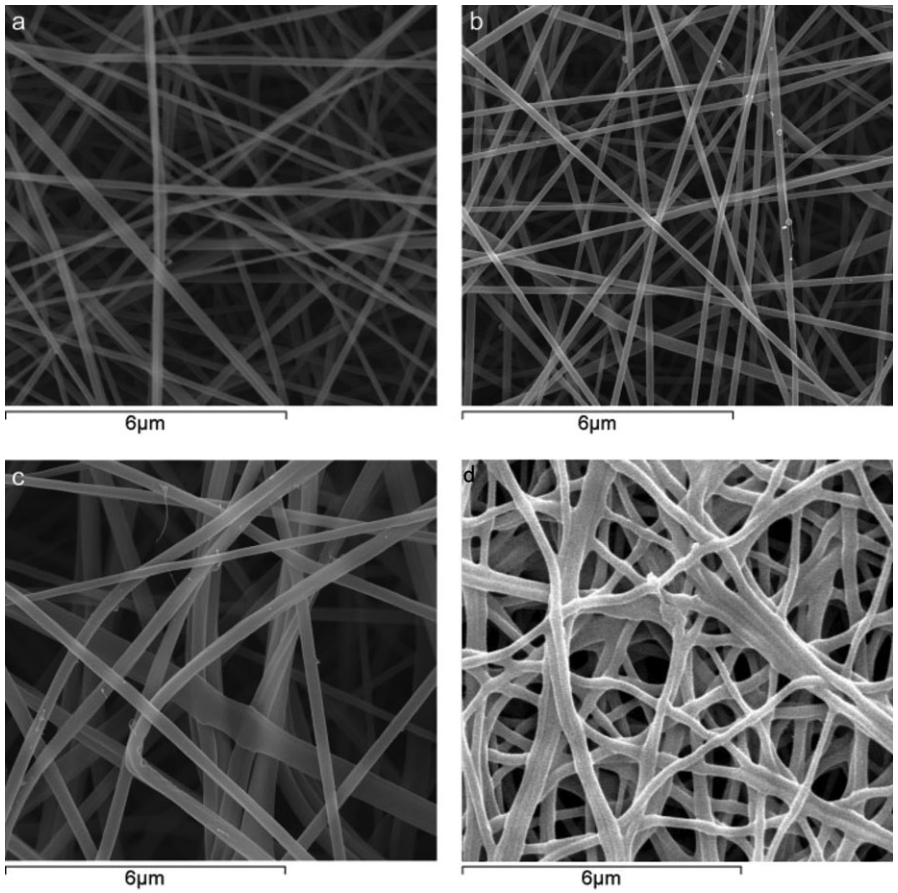

FIGURE 5. SEM photographs of electrospun scaffolds. (a) Chitosan/polyvinylalcohol (Chi/PVA) fibers, (b) Chi/PVA + 2% hydroxyapatite (HAp) (uncrosslinked), (c) Chi/PVA + 5% HAp (uncrosslinked), (d) Chi/PVA + 5% HAp (crosslinked). (Adapted with Permission from Ref. 54. Copyright 2008 John Wiley and Sons.).

after being mixed mechanically because the link treatment makes the chitin fibers and PLLA integrative. Uniformity of pore and structure of the scaffold will promote growth of tissues and degradation of the scaffold.

Numerous researchers are working on porous ceramic scaffolds primarily for bone-graft applications. To understand and delineate the influence of different porosity parameters on the cell growth behavior on bioresorbable TCP ceramics, Bose et al.[55] have identified pore size and pore volumes as two parameters. In their study, scanning electron microscopy was conducted with some of these alumina and TCP scaffolds. Figure 6(a,b) shows SEM images of alumina and TCP matrices, illustrating the cell-matrix interaction. It was observed that cells tend to attach more intimately with TCP matrix than alumina. Also it was noted that cells grew on both the top and the bottom surfaces of TCP scaffolds during cell growth experiments, but for the alumina scaffolds, it was primarily the top surfaces where most of the cell growth occurred.

To tailor the biodegradation behavior of the ceramic, Banerjee et al.[56] have synthesized a novel biphasic calcium phosphate ceramic composed of tricalcium phosphate (TCP) and calcium pyrophosphate (CP). An *in vitro* cell materials interaction study using human fetal osteoblast cells indicates that TCP/CP ceramic is cytocompatible. TCP/CP ceramic also show a good loading capacity for alendronate. Adsorption of alendronate (AD) on the TCP/CP surface is found to proceed via ligand exchange mechanism and the *in vitro* release profile of AD from TCP/CP surface is characterized by an initial fast release followed by a slow and sustained release. Strong electrostatic interactions between AD groups and surface Ca2+ ions enable the slow and sustained release of AD. Their results demonstrate that the newly developed biphasic ceramic, with its controlled strength degradation and drug release, shows promise for use in orthopedic and tissue engineering applications.

Nanofibrous scaffolds

The goal of the scaffold is to provide a 3D environment for cells and tissue to grow on. The composite structure of the mineralized collagen fibrils is what gives bone its lightweight strength.[57–59] The mineralized collagen fibrils then align and arrange in ways to form higher order structures and eventually a full bone.

Naturally then, scaffolds were developed to mimic the nanofibrous collagen ECM.[60,61] In addition to the nanofibrous architecture, these new scaffolds also had to have a key set of characteristics that all tissue engineering scaffolds need.

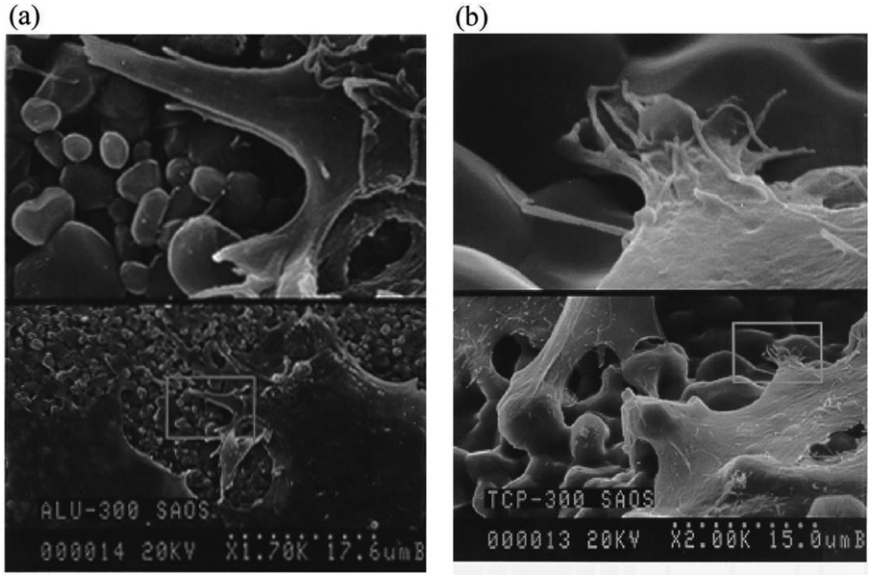

FIGURE 6. (a) and (b) shows the SEM images of alumina and TCP matrices showing the cell–matrix interaction. (Adapted with Permission from Ref. 55. Copyright 2003 Elsevier Ltd.).

They need to be highly porous to allow for cell ingrowth and efficient mass transport of nutrients, oxygen, growth factors, and waste products. For larger constructs, the pores must also facilitate vascularization to avoid necrosis at the core.[62]

Currently, there are two main fabrication methods used in bone tissue engineering to create nanofibrous scaffolds: electrospinning and phase separation.[62] In addition to being a function of processing, the requirements, mechanical integrity and biodegradability are also a function of material choice. There are myriad natural and synthetic polymers that can satisfy those characteristics, such as poly (L-lactic acid) (PLLA). Figure 7 shows the nanofibrous hollow microspheres fabricated by Liu et al.,[63] using a star-shaped PLLA (SS-PLLA) polymer. Poly (L-lactide) (PLLA) as a synthetic polyester has been widely used as scaffold materials for bone tissue engineering because of its superior mechanical properties, biodegradability and biocompatibility.[64–66] However, the acid degradation products and poor cells affinities limit the scope in tissue engineering applications.[67] In contrast, chitosan exhibits good cytocompatibility[68] and antimicrobial property but low mechanical strength as a biodegradable natural basic polysaccharide, which has been commonly used to complex with polylacitide for improving the cytocompatibility and neutralizing the acid degradation products of polylactides in biomedical application.[69,70] In previous work, chitosan nanofibers (CSNFs)[71] mimicking the ECM collagen structure with a diameter range of 50–500 nm have been first engineered via a new developed solid–liquid TIPS,[72] which provides the possibility to fabricate a new biomimetic composite nanofibrous scaffold taking advantage of the good mechanical property of PLLA and the excellent cytocompatibility of chitosan by introducing CSNFs network into the macropores of nanofibrous PLLA scaffold (NF-PLLA) via a dual TIPS technique. Among the scaffolds, NF-PLLA/CSNFs scaffold showed the largest protein adsorption and the highest mineralization ability, meanwhile CSNFs could also resist the fast degradation of NF-PLLA. The bone mesenchymal stem cells (BMSCs) *in vitro* culture suggested that NF-PLLA/CSNFs showed the highest cells viability and the best cytocompatibility maybe contributed to the biomimetic nanofibrous network and good cell affinity of CSNFs.[73] Figure 8 shows the SEM images of various PLLA-based scaffolds in this study.

Carbon nanotubes/nanofibers scaffolds

Because of their superior biocompatible,[74] mechanical and electrical properties, carbon nanotubes/nanofibers (CNTs/CNFs) are been seen as kind of promising scaffold candidates for bone tissue engineering applications.[75] In a recent study by Price et al., 60 nm diameter CNFs significantly increased osteoblast adhesion and concurrently decreased competitive cell (fibroblast, smooth muscle cell, etc.) adhesion to stimulate sufficient osseointegration.[76] Some research efforts have also demonstrated that CNTs are suitable to promote osteoblast functions and cellular functions.[77–79] And, the research conducted by Li et al.[80] also indicated that MWNTs might stimulate inducible cells in soft tissues to form inductive bone by concentrating more proteins, including bone-inducing proteins.

Recently, Sitharaman et al. reported an *in vivo* study of ultra-short SWCNT (single-walled carbon nanotube) polymer nanocomposites after implanting them into rabbit femoral condyles and subcutaneous pockets for up to 12 weeks.[81] The nanocomposites exhibited favorable hard and soft tissue responses after 4 and 12 weeks. They induced a 300% greater bone volume than all other experimental groups at 4 weeks and 200% greater bone growth at defect sites than control polymers without CNTs after 12 weeks. CNT/CNF reinforced polymer nanocomposites have also

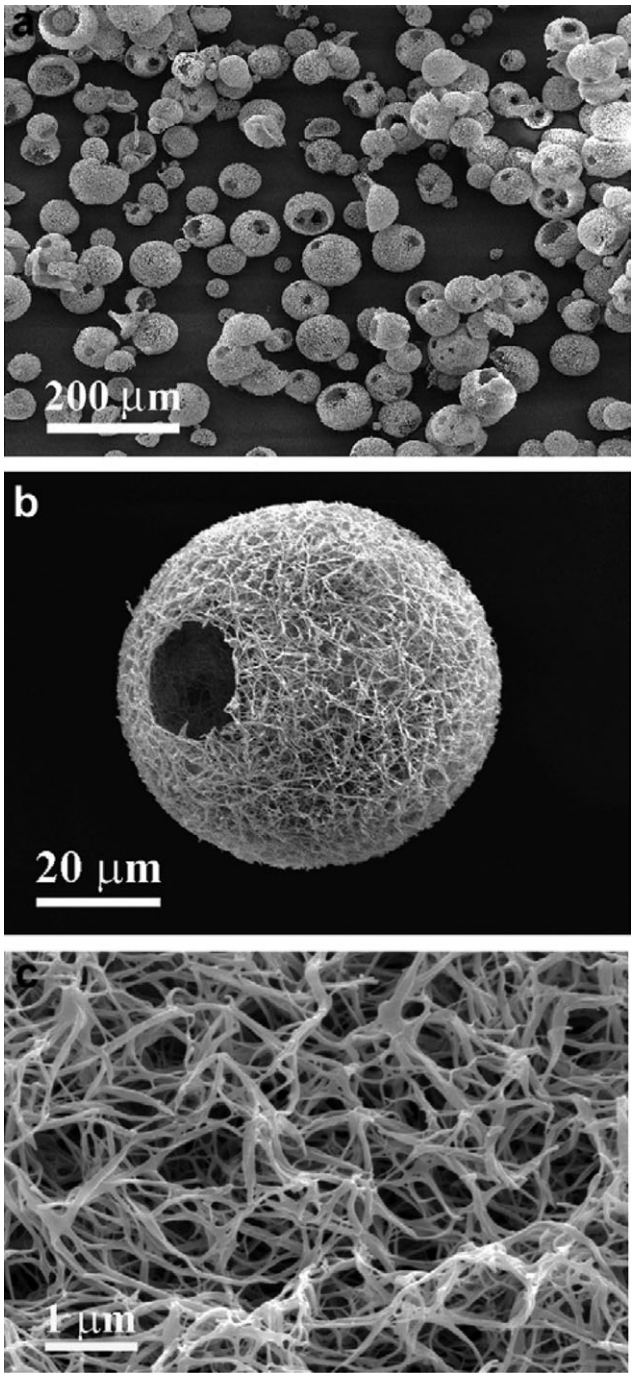

FIGURE 7. SEM images of nanofibrous hollow microspheres at (a) low, (b) medium, and (c) high magnification. (Adapted with Permission from Ref. 63. Copyright 2011 Nature Publishing Group.).

demonstrated excellent electrical conductivity for tissue regeneration. For instance, using biodegradable polylactic acid (PLA)/CNT composites as an example, an 80%/20% (w/w) PLA/CNT composite exhibited ideal electrical conductivity for bone growth while PLA was an insulator and not appropriate for electrically stimulating bone growth. Specifically, the PLA/CNT composite promoted a 46% increase in osteoblast proliferation and a 307% increase in calcium content after electrical stimulation for 2 and 21 days compared to PLA alone, respectively.[82] In chitosan/f-MWCNT (multiwalled carbon nanotube) scaffolds' study,[83] the water uptake ability and porosity of scaffolds increased with an increase the amount of f-MWCNT. The cell proliferation, protein content, alkaline phosphatase, and mineralization of the composite scaffolds were higher than chitosan scaffold due to the addition of f-MWCNT. These studies indicated that the CNTs/CNFs and their composites can serve as osteogenic scaffolds with good cytocompatibility properties, reinforced mechanical properties and improved electrical conductivity to effectively enhance bone tissue growth.

However, the toxicity of CNT is obscure till date and it does vary due to their size and impurities in the process of manufacturing.[35] To give full play to CNT's good biocompatibility, mechanical and electrical properties, necessary measures have been taken to avoid the potential toxicity. The functionalized CNT can be obtained by the surface modification of CNT with strong acidic condition such as Conc. Nitric acid and Conc. Sulfuric acid. In general, functionalization of CNT is carried out on surface area with COOH, OH, SH, and amide with polymers such as poly (lactic acid-glycolic acid), poly lactic acid, polyglycolic acid and various natural polymers. After functionalized CNT has good dispersibility in water, it can be considered for biomedical applications. Moreover, small addition of CNT in the polymeric matrix increases the materials beneficial properties.

Nanostructured hydrogel scaffolds

Recently nanostructured hydrogel based biodegradable scaffold materials have gained interest in the field of tissue engineering. Ehrlich et al.[84] reported that marine invertebrate organisms including sponges inspire investigators to develop biomimetic composites, scaffolds and templates for use in tissue engineering. Nanostructured hydrogel scaffolds are capable of swelling when immersed in liquid medium, which would help the cells to get entrapped inside the scaffolds and also promote the passage of oxygen and nutrients into the inner regions of the scaffolds. Thus, this kind of scaffolds can also provide necessary support for cells to proliferate and to maintain their differentiated function.

A variety of synthetic and naturally derived materials may be used to form nanostructured hydrogels for tissue engineering scaffolds. Synthetic materials include poly (ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), poly (acrylic acid) (PAA), poly(propylene furmarate-*co*-ethylene glycol) (P(PF-*co*-EG)), and polypeptides. Representative naturally derived polymers include agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid (HA).

Nanostructured hydrogel scaffolds have been suggested to biomimic the structure complexity that existed in the natural extracellular matrices (ECMs), and to impart important structural cues on the subsequent interactions between the material and cells.[85,86] For example, Zhang et al.[87] reported a nanoscale hydrogel network self-assembled from peptide nanofiber within 20 nm in diameter. These gels mimic natural ECM that is composed of an intricate interweaving of protein fibers with diameter ranging from 10 to several hundreds of nanometers. Chondrocytes seeded in these

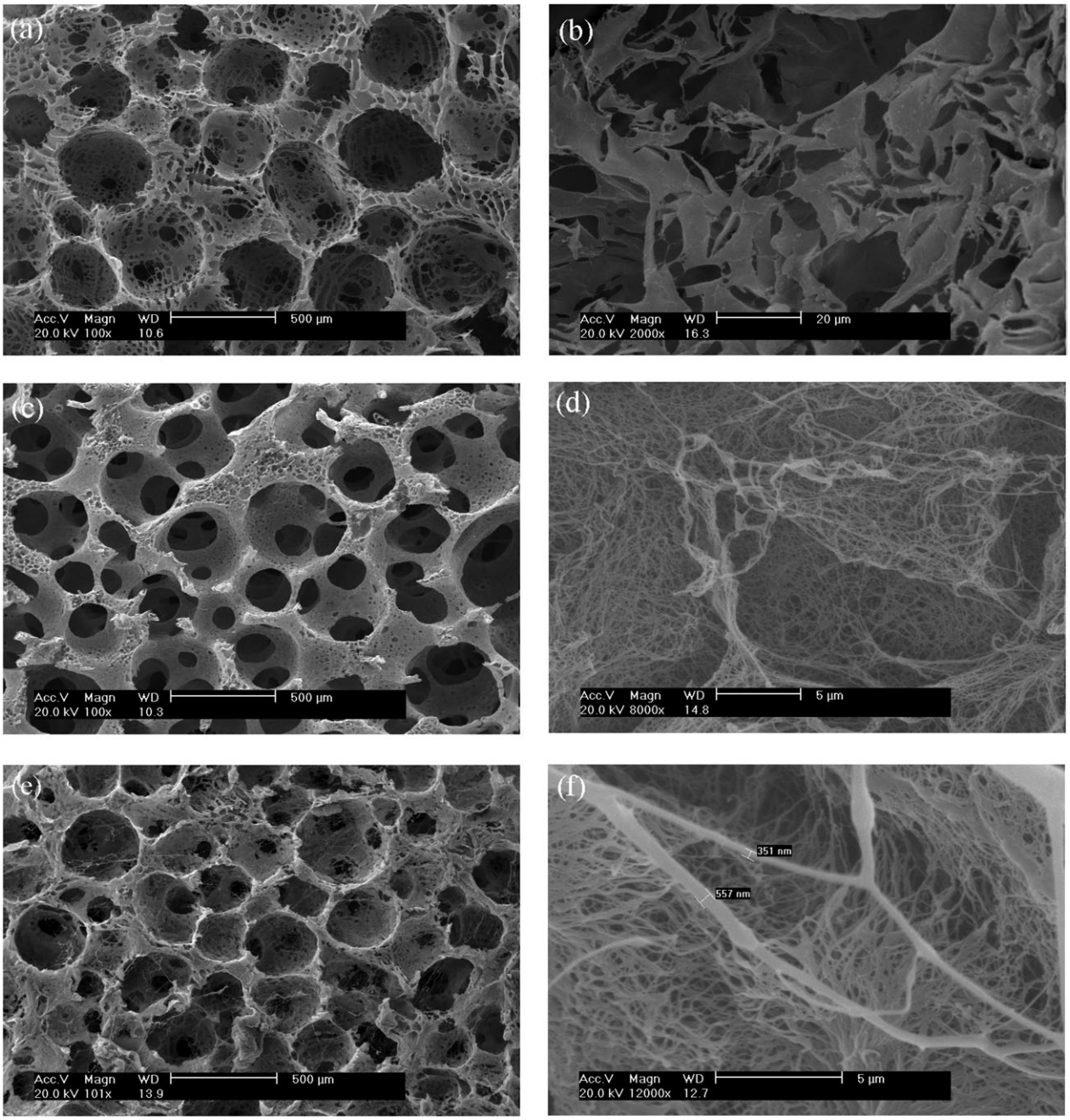

FIGURE 8. SEM images of various PLLA-based scaffolds. (a) and (b) PLLA scaffold, (c) and (d) NF-PLLA scaffold, (e) and (f) NF-PLLA/CSNFs composite scaffold. (Adapted with Permission from Ref. 73. Copyright 2012 Elsevier Ltd.).

peptide hydrogels maintained their phenotype throughout 4-week period of *in vitro* culture, and developed a cartilage-like ECM rich in type II collagen and proteoglycans. In addition, physical incorporation of polymeric micelles into PEG hydrogels was also used to tune the storage modulus, thereby influencing cell behavior in the hydrogel.[88] On the basis of these studies, Li'group[89] used a new strategy by covalently incorporating nanosized polymeric micelles self-assembled from an amphiphilic block copolymer of PEG and biodegradable polycarbonate into PEG hydrogel networks. This study proves that incorporating nanoparticles into the hydrogels is a useful strategy to control cellular behavior in a 3-D environment, and shows these biodegradable nanostructured hydrogels can be an excellent platform for the delivery of hMSCs. In recent studies chitin forms hydrogen bonds with other polymers and ceramics and with this capability β-chitin holds other polymers and ceramics intact to serve as a better composite. For example, Sudheesh Kumar et al.[90] have preparedβ-Chitin hydrogel/nano hydroxyapatite (nHAp) nanocomposite scaffolds by freeze-drying approach, which were found to have 70–80% porosity with well defined interconnected porous structure. The

scaffolds also showed a swelling ratio of 15–20, controlled biodegradation of about 30–40% with enhanced protein adsorption. In addition, the nanostructured hydrogel scaffolds showed well improved cell attachment and proliferation.

CONCLUSION AND PERSPECTIVES

The purpose of this article was to give a general description of studies of nanostructured scaffolds for bone tissue engineering.

Nanophase ceramics, especially nano-hydroxyapatite (HA), are popular bone substitutes, coatings and other filler materials due to their documented ability to promote mineralization. The nanometer grain sizes and high surface fraction of grain boundaries in nanoceramics increase osteoblast functions (such as adhesion, proliferation, and differentiation). Similar tendencies have been reported for other nanoceramics including alumina, zinc oxide and titania, thus, providing evidence that, to some extent, it may not matter what implant chemistry is fabricated to have nanometer surface features to promote bone growth. However, this need further studies. For applications, synthetic and natural polymers (e.g., polyglycolic acid (PGA), poly (lactic-*co*-glycolic acid) (PLGA), PLLA, PLA, gelatin, collagen, chitosan) are regarded as excellent candidates for bone tissue engineering applications due to their biodegradability and ease of fabrication. Nanoporous or nanofibrous polymer matrices can be fabricated via electrospinning, phase separation, particulate leaching, chemical etching and 3D printing techniques.

The clinical application of nHAp/polymer composites entails successful interplay between cells, biological signals, and the biomaterials. However, there are still many unanswered questions and unexplored frontiers which can greatly influence the role of nanostructured materials in clinical applications. It requires fundamental understandings in both the life sciences and materials sciences to develop successful regeneration technologies.

As mentioned in this article, features or ingredients of these scaffolds are nanoscale so that a variety of interactions can be stimulated at the cellular level. In some instances, some of those interactions also lead to toxicity, which can be of serious concerns. In particular, toxic responses to nanoparticles generated from the degradation of implanted nanomaterials, via wear debris from artificial joints with nanofeatures, and heavy metals (iron, nickel and cobalt catalysts) remaining in CNTs, have all been reported. Sometimes nanoparticle interactions with biomolecules *in vivo* or their aggregation states may change their toxicity to humans. But the often contradictory results of current studies are clearly not enough to provide the final answer concerning nanomaterial toxicity. In depth investigations of nanomaterials on human health and the environment are necessary to fully elucidate whether nanoparticles should be used in biomedical applications.

New frontiers of research should be directed towards better biomimicing the natural process of bone tissue regeneration such as coupling between angiogenesis and osteogenesis which may require progenitor cell recruitment and differentiation. Although it is difficult to mimic nature, recent scientific and technological findings show potential to achieve bone scaffolds that would encourage local and systemic biological functions. Proper selection of scaffold materials, their geometry, pore size and size distribution, and ability to release biomolecules at a desired rate will play critical roles in future development of bone scaffolds.

To better mimic the nanostructure in natural ECM, over the past decade, scaffolds manufactured from nanofibers, nanotubes, nanoparticles, and hydrogel, have recently emerged as promising candidates in producing scaffolds that resemble the ECM and efficiently replace defective tissues. Even so, the combination of these materials in the form of nanoscaffolds is an under explored arena. The design of stronger and tougher scaffold materials requires incorporation of a hierarchical design encompassing many length-scales from the nanolevel to generate strength (i.e., to mimic composite deformation of nanocrystals of HA and collagen) as well as micro-level structures to influence the crack path and generate toughness (e.g., to mimic osteons and cement lines). However, nanotechnology alone may not be the answer to improving the mechanical properties of scaffolds. The limitations in processing techniques, in part, have hampered the progress in the development of new scaffolds to form structures with a multidimensional architecture. The challenge is to use these technologies in combination with nanomaterials. It is possible that at the end an optimum scaffold should combine several materials and techniques (e.g., a complex polymer structure can be created by ice-templating or computer-assisted fabrication that can subsequently be mineralized to achieve the desired mechanical and biodegradation responses).

REFERENCES

1. Liu HN, Yazici H, Ergun C, Webster TJ, Bermek H. An in vitro evaluation of the Ca/P ratio for the cytocompatibility of nano-to-micron particulate calcium phosphates for bone regeneration. Acta Biomater 2008;4:1472–1479.
2. Stevens MM. Biomaterials for bone tissue engineering. Mater Today 2008;11:18–25.
3. Dornblüth O. Pschyrembel Klinisches Wörterbuch. Berlin: De Gruyter; 2004.
4. Gibson LJ. Biomechanics of cellular solids. J Biomech 2005;38: 377–399.
5. Wolff JDG. Transformation der Knochen. Berlin: Hirschwald; 1892.
6. Wegst UGK, Ashby MF. The mechanical efficiency of natural materials. Philos Mag 2004;84:2167–2181.
7. Hutmacher DW, Schantz JT, Lam CXF, Tan KC, Lim TC. State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. J Tissue Eng Regen Med 2007;1: 245–260.
8. Rho JY, Kuhn-Spearing L, Zioupos P. Mechanical properties and the hierarchical structure of bone. Med Eng Phys 1998;20:92–102.
9. Schneider P, Voide R, Stampanoni M, Muller R. Post-processing technique for improved assessment of hard tissues in the submicrometer domain using local synchrotron radiation-based computed tomography. Biomed Technol 2009;54:48–54.
10. Tiffany NV, Kasper FK, Mikos AG. Strategies for controlled delivery of growth factors and cells for bone regeneration. Adv Drug Deliv Rev 2012;64:1292–1309.
11. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials 2000;21:2529–2543.

12. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends in Biotechnology 2012;30:546–554.
13. Will J, Melcher R, Treul C, Travitzky N, Kneser U, Polykandriotis E, Horch R, Greil P. Porous ceramic bone scaffolds for vascularized bone tissue regeneration. J Mater Sci Mater Med 2008;19: 2781–2790.
14. Bohner M, van Lenthe GH, Grunenfelder S, Hirsiger W, Evison R, Muller R. Synthesis and characterization of porous beta-tricalcium phosphate blocks. Biomaterials 2005;26:6099–6105.
15. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26:5474–5491.
16. Lu JX, Flautre B, Anselme K, Hardouin P, Gallur A, Descamps M, Thierry B. Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo. J Mater Sci Mater Med 1999;10:111–120.
17. Bignon A, Chouteau J, Chevalier J, Fantozzi G, Carret JP, Chavassieux P, Boivin G, Melin M, Hartmann D. Effect of micro- and macroporosity of bone substitutes on their mechanical properties and cellular response. J Mater Sci Mater Med 2003;14:1089–1097.
18. Piskin E. Biomaterials in different forms for tissue engineering: An overview. Mater Sci Forum 1997;250:1–14.
19. Weber JN, White EW. Carbon-metal graded composites for permanent osseous attachment of non-porous metals, Mater Res Bull 1972;7:1005–1016.
20. Murphy WL, Kohn DH, Mooney DJ. Growth of continuous bonelike mineral within porous poly(lactide-*co*-glycolide) scaffolds in vitro. J Biomed Mater Res 2000;50:50–58.
21. Rezania A, Healy KE. Biomimetic peptide surfaces that regulate adhesion, spreading, cytoskeletal organization, and mineralization of the matrix deposited by osteoblast-like cells. Biotechnol Prog 1999;15:19–32.
22. Yang XB, Roach HI, Clarke NMP, Howdle SM, Quirk R, Shakesheff KM, Oreffo ROC. Human osteoprogenitor growth and differentiation on synthetic biodegradable structures after surface modification. Bone 2001;29:523–531.
23. Sethuraman S, Nair LS, El-Amin S, Nguyen MT, Singh A, Krogmanf N, Greishh YE, Allcockf HR, Browng PW, Laurencin CT. Mechanical properties and osteocompatibility of novel biodegradable alanine based polyphosphazenes: Side group effects. Acta Biomaterialia 2010;6:1931–1937.
24. Freeman JW, Wright LD, Laurencin CT, Bhattacharyya S. In: Gonsalves KE, Halberstadt CR, Laurencin CT, Nair LS, editors. Nanofabrication Techniques (chapter 1), Biomedical Nanostructures. New Jersey: Wiley; 2008. p 3–24.
25. Chen VJ, Ma PX. Nano-fibrous poly(L-lactic acid) scaffolds with interconnected spherical macropores. Biomaterials 2004;25: 2065–2073.
26. Venugopal JR, Low S, Choon AT, Kumar AB, Ramakrishna S. Nanobioengineered electrospun composite nanofibers and osteoblasts for bone regeneration. Artif Organs 2008;32:388–397.
27. Hata K, Futaba DN, Mizuno K, Namai T, Yumura M, Iijima S. Water-assisted highly efficient synthesis of impurity-free single-walled carbon nanotubes. Science 2004;306:1362–1364.
28. Sun S, Zeng H, Robinson DB, Raoux S, Rice PM, Wang SX, Li GX. Monodisperse MFe_2O_4 (M=Fe, Co, Mn) nanoparticles. J Am Chem Soc 2004;126:273.
29. Fahlman BD. Materials Chemistry. Dordrecht, Netherlands: Springer; 2007.
30. Webster TJ. Nanophase ceramics: The future orthopedic and dental implant material. In Ying JY, editor. Advances in Chemical Engineering, Vol. 27. New York: Academic Press; 2001. p 125–166.
31. Degasne I, Baslé MF, Demais V, Huré G, Lesourd M, Grolleau B, Mercier L, Chappard D. Effects of roughness, fibronectin and vitronectin on attachment, spreading, and proliferation of human osteoblast-like cells (Saos-2) on titanium surfaces. Calcified Tissue Int 1999;64:499–507.
32. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. J Biomed Mater Res 2000;51:475–483.
33. Webster TJ, Schadler LS, Siegel RW, Bizios R. Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. Tissue Eng 2001;7:291–301.
34. Li XM, Van Blitterswijk CA, Feng QL, Cui FZ, Watari F. The effect of calcium phosphate microstructure on bone-related cells in vitro. Biomaterials 2008;29:3306–3316.
35. Zhang LJ, Webster TJ, Nanotechnology and nanomaterials: Promises for improved tissue regeneration. Nano Today 2009;4:66–80.
36. Chu PK, Liu X. Biomaterials Fabrication and Processing Handbook. Boca Raton, FL: CRC Press; 2008.
37. Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 2008;60:184–198.
38. Kingery WD, Bowen HK, Uhlmann DR. Introduction to Ceramics, 2nd ed. Wiley, New York; 1976. p 469.
39. Foroughi MR, Karbasi S, Ebrahimi-Kahrizsangi R. Physical and mechanical properties of a poly-3-hydroxybutyrate-coated nanocrystalline hydroxyapatite scaffold for bone tissue engineering. J Por Mater 2012;19:667–675.
40. Habraken W, Wolke JGC, Jansen JA. Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007;59:234–248.
41. Legeros RZ. Biodegradation and bioresorption of calcium phosphate ceramics. Clin Mater 1993;14:65–88.
42. Sun FF, Zhou HJ, Lee J. Various preparation methods of highly porous hydroxyapatite/polymer nanoscale biocomposites for bone regeneration. Acta Biomater 2011;7:3813–3828.
43. Kim S, Kim SS, Lee SH, Eun Ahn S, Gwak SJ, Song JH, Kim BS, Chung HM. In vivo bone formation from human embryonic stem cell-derived osteogenic cells in poly(D,L-lactic-*co*-glycolic acid)/hydroxyapatite composite scaffolds. Biomaterials 2008;29: 1043–1053.
44. Lee JH, Rim NG, Jung HS, Shin H. Control of osteogenic differentiation and mineralization of human mesenchymal stem cells on composite nanofibers containing poly[lactic-*co*-(glycolic acid)] and hydroxyapatite. Macromol Biosci 2009;10:173–182.
45. Ngiam M, Liao S, Patil AJ, Cheng Z, Chan CK, Ramakrishna S. The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. Bone 2009;45:4–16.
46. Niu X, Feng Q, Wang M, Guo X, Zheng Q. Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2. J Control Release 2009;134:111–117.
47. Lee KW, Wang S, Yaszemski MJ, Lu L. Physical properties and cellular responses to crosslinkable poly(propylene fumarate)/hydroxyapatite nanocomposites. Biomaterials 2008;29:2839–2848.
48. Heo SJ, Kim SE, Wei J, Kim DH, Hyun YT, Yun HS, Kim HK, Yoon TR, Kim SH, Park SA, Shin JW, Shin JW. In vitro and animal study of novel nano-hydroxyapatite/poly (epsilon-caprolactone) composite scaffolds fabricated by layer manufacturing process. Tissue Eng 2009;15:977–989.
49. Gupta D, Venugopal J, Mitra S, Giri Dev VR, Ramakrishna S. Nanostructured biocomposite substrates by electrospinning and electrospraying for the mineralization of osteoblasts. Biomaterials 2009;30:2085–2094.
50. Patel M, Dunn TA, Tostanoski S, Fisher JP. Cyclic acetal hydroxyapatite composites and endogenous osteogenic gene expression of rat marrow stromal cells. J Tissue Eng Regener Med 2010;4: 422–436.
51. Patel M, Patel KJ, Caccamese JF, Coletti DP, Sauk JJ, Fisher JP. Characterization of cyclic acetal hydroxyapatite nanocomposites for craniofacial tissue engineering. J Biomed Mater Res 2010;94: 408–418.
52. Shi Z, Huang X, Cai Y, Tang R, Yang D. Size effect of hydroxyapatite nanoparticles on proliferation and apoptosis of osteoblast-like cells. Acta Biomater 2009;5:338–345.
53. Li XM, Feng QL, Cui FZ. In vitro degradation of porous nano-hydroxyapatite/collagen/PLLA scaffold reinforced by chitin fibres. Mater Sci Eng C 2006;26:716–720.
54. Yang D, Jin Y, Ma G, Chen X, Lu F, Nie J. Fabrication and characterization of chitosan/PVA with hydroxyapatite biocomposite nanoscaffolds. J Appl Polym Sci 2008;110:3328–3335.
55. Bose S, Darsell J, Kintner M, Hosick H, Bandyopadhyay A. Pore size and pore volume effects on alumina and TCP ceramic scaffolds. Mater Sci Eng C 2003;23:479–486.

56. Banerjee SS, Bandyopadhyay A, Bose S. Biphasic resorbable calcium phosphate ceramic for bone implants and local alendronate delivery. Adv Biomater (Adv Eng Mater) 2010;12:B148–B155.
57. Buehler MJ. Molecular nanomechanics of nascent bone: Fibrillar toughening by mineralization. Nanotechnology 2007;18:295102.
58. Li XM, Liu XH, Zhang GP, Dong W, Sha ZY, Feng QL, Cui FZ, Watari F. Repairing 25mm bone defect using fibres reinforced scaffolds as well as autograft bone. Bone 2008;43:S94–S94.
59. Li XM, Feng QL, Jiao YF, Cui FZ. Collagen-based scaffolds reinforced by chitosan fibres for bone tissue engineering. Polym Int 2005;54:1034–1040.
60. Zhang R, Ma PX. Synthetic nano-fibrillar extracellular matrices with prede-signed macroporous architectures. J Biomed Mater Res 2000;52:430–438.
61. Li WJ, Laurencin CT, Caterson EJ, Tuan RS, Ko FK. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. J Biomed Mater Res 2002;60:613–621.
62. Holzwarth JM, Ma PX. Biomimetic nanofibrous scaffolds for bone tissue engineering. Biomaterials 2011;32:9622–9629.
63. Liu X, Jin X, Ma PX. Nanofibrous hollow microspheres self-assembled from star-shaped polymers as injectable cell carriers for knee repair. Nat Mater 2011;10:389–406.
64. Peng F, Yu XH, Wei M. In vitro cell performance on hydroxyapatite particles/poly(L-lactic acid) nanofibrous scaffolds with an excellent particle along nanofiber orientation. Acta Biomater 2011; 7:2585–2592.
65. Hu J, Sun X, Ma HY, Xie CQ, Chen YE, Ma PX. Porous nanofibrous PLLA scaffolds for vascular tissue engineering. Biomaterials 2010;31:7971–7977.
66. Park JE, Todo M. Development and characterization of reinforced poly (L-lactide) scaffolds for bone tissue engineering. J Mater Sci Mater M 2011;22:1171–1182.
67. Ren J, Zhao P, Ren TB, Gu SY, Pan KF. Poly (D,L-lactide)/nano-hydroxyapatite composite scaffolds for bone tissue engineering and biocompatibility evaluation. J Mater Sci Mater M 2008;19: 1075–1082.
68. Li XM, Feng QL, Wang WJ, Cui FZ. Chemical characteristics and cytocompatibility of collagen-based scaffold reinforced by chitin fibers for bone tissue engineering. J Biomed Mater Res B 2006; 77B:219–226.
69. Martel-Estrada SA, Martínez-Pérez CA, Chacón-Nava JG, García-Casillas PE, Olivas-Armendáriz I. In vitro bioactivity of chitosan/poly (D,L-lactide-*co*-glycolide) composites. Mater Lett 2011;65: 137–141.
70. Chen C, Dong LS, Cheung MK. Preparation and characterization of biodegradable poly (L-lactide)/chitosan blends. Eur Polym J 2005;41:958–966.
71. Jayakumar R, Menon D, Manzoor K, Naira SV, Tamurab H. Biomedical applications of chitin and chitosan based nanomaterials—A short review. Carbohydr Polym 2010;82:227–232.
72. Zhao JH, Han WQ, Chen HD, Tu M, Zeng R, Shi YF, Cha ZG, Zhou CR. Preparation, structure and crystallinity of chitosan nano-fibers by a solid-liquid phase separation technique. Carbohydr Polym 2011;83:1541–1546.
73. Zhao JH, Han WQ, Tu M, Huan SW, Zeng R, Wu H, Cha ZG, Zhou CG. Preparation and properties of biomimetic porous nanofibrous poly(L-lactide) scaffold with chitosan nanofibers network by a dual thermal induced phase separation technique. Mater Sci Eng C 2012;32:1496–1502.
74. Smart SK, Cassady AI, Lu GQ, Martin DJ. The biocompatibility of carbon nanotubes. Carbon 2006;44:1034–1047.
75. Zhang L, Ercan B, Webster TJ. nanotubes and nanofibers for tissue engineering applications. In: Liu C, editor. The Area of "Carbon,." Trivandrum: Research Signpost; 2009.
76. Price RL, Waid MC, Haberstroh KM, Webster TJ. Selective bone cell adhesion on formulations containing carbon nanofibers. Biomaterials 2003;24:1877–1887.
77. Zanello LP, Zhao B, Hu H, Haddon RC. Bone cell proliferation on carbon nanotubes. Nano Lett 2006;6:562–567.
78. Li XM, Gao H, Uo M, Sato Y, Akasaka T, Abe S, Feng QL, Cui FZ, Watari F. Maturation of osteoblast-like $SaoS_2$ induced by carbon nanotubes. Biomed Mater 2009;4:015005.
79. Li XM, Gao H, Uo M, Sato Y, Akasaka T, Feng QL, Cui FZ, Liu XH, Watari F. Effect of carbon nanotubes on cellular functions in vitro. J Biomed Mater Res A 2009;91A:132–139.
80. Li XM, Liu HF, Niu XF, Yu B, Fan YB, Feng QL, Cui FZ. The use of carbon nanotubes to induce osteogenic differentiation of human adipose-derived MSCs in vitro and ectopic bone formation in vivo. Biomaterials 2012;33:4818–4827.
81. Sitharaman B, Shi X, Walboomers XF, Liao H, Cuijpers V, Wilson LJ, Mikos AG, Jansen JA. In vivo biocompatibility of ultra-short single walled carbon nanotube/biodegradable polymer nanocomposites for bone tissue engineering. Bone 2008;43:362–370.
82. Supronowicz PR, Ajayan PM, Ullmann KR, Arulanandam BP, Metzger DW, Bizios R. Novel current-conducting composite substrates for exposing osteoblasts to alternating current stimulation. J Biomed Mater Res 2002;59:499–506.
83. Venkatesan J, Ryu BM, Sudha PN. Preparation and characterization of chitosan-carbon nanotube scaffolds for bone tissue engineering. Int J Biol Macromol 2012;50:393–402.
84. Ehrlich H, Ilan M, Maldonado M, Muricy G, Bavestrello G, Kljajic Z, Carballo JL, Schiaparelli S, Ereskovsky A, Schupp P, Born R, Worch H, Bazhenov VV, Kurek D, Varlamov V, Vyalikh D, Kummer K, Sivkov VV, Molodtsov SL, Meissner H, Richter G, Steck E, Richter W, Hunoldt S, Kammer M, Paasch S, Krasokhin V, Patzke G, Brunner E. Three-dimensional chitin-based scaffolds from Verongida sponges (Demospongiae: Porifera). Part I. Isolation and identification of chitin. Int J Biol Macromol 2010;47:132–140.
85. Dvir T, Timko BP, Kohane DS, Langer R. Nanotechnological strategies for engineering complex tissues. Nat Nanotechnol 2011;6: 13–22.
86. Kolambkar YM, Dupont KM, Boerckel JD, Huebsch N, Mooney DJ, Hutmacher DW, Guldberg RE. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials 2011;32:65–74.
87. Kisiday J, Jin M, Kurz B, Hung H, Semino C, Zhang S, Grodzinsky AJ. Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: Implications for cartilage tissue repair. Proc Natl Acad Sci USA 2002;99:9996–10001.
88. Frisman I, Seliktar D, Bianco-Peled H. Nanostructuring of PEG-fibrinogen polymeric scaffolds. Acta Biomater 2009;6:2518–2524.
89. Li Y, Yang C, Khan M, Liu S, Hedrick JL, Yang YY, Ee PLR. Nanostructured PEG-based hydrogels with tunable physical properties for gene delivery to human mesenchymal stem cells. Biomaterials 2012;33:6533–6541.
90. Kumar PTS, Srinivasan S, Lakshmanan VK, Tamura H, Nair SV, Jayakumar R. β-chitin hydrogel/nano hydroxyapatite composite scaffolds for tissue engineering applications. Carbohydr Polym 2011;85:584–591.