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Nanotechnology controlled drug delivery for treating bone diseases

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Rapid developments at the intersection of nanotechnology and controlled drug delivery have triggered exceptional growth in treating various bone diseases. As a result, over the past decade, nanotechnology has contributed tremendously to controlling drug delivery for treating various bone diseases, and in many cases, has led to increased bone regeneration. In this review paper, the recent experimental progress towards using nanotechnology to treat bone-specific diseases is reviewed. Novel applications of different types of nanomaterials (from nanoparticles to 3D nanostructured scaffolds) for treating bone diseases are summarized. In addition, fundamental principles for utilizing nanomaterials to create better drug delivery systems, especially for treating bone diseases and regenerating bone, are emphasized.

Keywords: bone diseases, bone regeneration, drug delivery, implants, nanotechnology, tissue engineering

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1. Introduction

Currently, nanotechnology is being exploited to promote and control biological interactions because nanoscale (1 ~ 100 nm, i.e., 10^{-9} m ~ 10^{-7} m) materials are ubiquitous in nature. For example, all human tissues have hierarchical structures fundamentally based at the nanoscale. The interdisciplinary fields of nanobiotechnology, including nanomedicine, are revolutionizing the traditional disciplines of science. As a perfect example, research in the area of controlled drug delivery has experienced tremendous progress as of late thanks to the emergence of nanotechnology. Novel applications of nanomaterials are enabling site-specific drug delivery and controlled release of traditional pharmaceuticals, recombinant proteins, vaccines and nuclei acids [1]. Novel drug delivery systems have been devised and fabricated by nanotechnology. These systems are approaching multifunctional 'smart' entities with the ability to sense, diagnose, image and cure numerous ailments [1-3]. Owing to these advancements, interest in nanotoxicity has also been soaring because it is widely unknown how the body reacts to particulate drug-delivering materials at this size range. While it may be true that the initial vision of nanotechnology towards enhancing drug delivery was the everlasting idea of a "nano-robot" or "nano-doctor" in which "nano-machines" continuously survey the body treating diseases on-the-spot, more practical applications of nanomaterials in drug delivery have emerged.

Among the broad spectrum of nanotechnology-based drug delivery applications, treating bone diseases is one of the most active and urgent areas. There are several kinds of diseases associated with bone, most commonly osteoporosis, osteoarthritis, Paget's disease and bone cancer [4]. In the US today, 10 million people have osteoporosis and almost 34 million more have low enough bone mass to place them at an increased risk for developing osteoporosis [5]. Moreover, according to the American Academy of Orthopaedic Surgeons, 19 million people

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visited a physician for osteoarthritic problems in 2006 [6]. It has also been estimated that 3% or 8.2 million people have Paget's disease currently in the US [7]. In addition, the American Cancer Society reported that about 2380 new cases of bone and joint cancer were diagnosed in 2008 while according to projections, 1470 deaths from these cancers are expected [8].

Current medications and clinic therapies towards treating these bone diseases are summarized in **Table 1**. Most of the listed medications have been approved by the FDA. Although these medications and therapies are effective in preventing and treating bone disease symptoms, most of these bone diseases listed in **Table 1** are considered largely uncurable [5,9]. Thus, it is clear that our current drug regimes for treating various orthopedic diseases need improvement. Besides the inherent side effects associated with all of the medications in **Table 1**, delivering and retaining drugs specifically in bone (to increase their efficacy) is challenging owing to the specific composition, architecture and structure of bone tissue limiting delivery of such drugs. Differing from other tissues, bone consists of 70% (dry weight) inorganic constituents (including hydroxyapatite (HA) and tricalcium phosphate) and related bone matrix proteins. Bone possesses one of the most delicate hierarchical micro- to nano-structures in the human body. These unique characteristics call for the proper selection of drug carriers that can target specific diseased parts of bone [10,11]. Similar to other fields, applying nanomaterials as effective and efficient drug delivery systems has provided a lot of promise to orthopedic applications. This is in large part due to the ability of nanoparticles to traverse the nanostructure of bone, attach to a site of bone disease and prolong drug release [12]. Such efforts started with creating nanostructured ceramic-polymer composites as bone substitutes (without drug carriers) to mimic the exact constituent nanometer bone structure. Because these materials accelerated bone tissue regeneration, an obvious next step was the exploration of nanoparticles for drug delivery orthopedic applications [10-12].

The engagement of nanotechnology in drug delivery for treating various orthopedic diseases is new, but becomes important when considering bone regeneration using bone implants and replacement strategies. The material forms used for these drug delivery purposes span a wide range of nanoscale chemistries including (but not limited to) polymers, metals, ceramics, semiconductors, sol-gels and self-assembled molecular complexes. Diverse nanomaterial geometries have also been fabricated including particles, fibers, capsules, tubes, whiskers, dendrimers and so on [1,13]. Such nanomaterials have been used to deliver both small molecular drugs and also various classes of biomolecules tailored to specific release kinetics and biodistribution [1], enabling effective remedies for first treating and, then second, enhancing bone regeneration by promoting bone cell growth. In many circumstances it is the orthopedic drug carrier itself that promotes new bone growth [10-12]. This is in extreme contrast to traditional drug delivery in which the function of the carrier is completed once the drug is released.

In some orthopedic nanotechnology drug delivery applications, the drug is released from the carrier to treat the disease but the carrier itself remains to stimulate new bone growth. Moreover, the drug carrier can be designed to provide immediate bone strength to the diseased bone before the drug is even released. In this review, the current developments and novel applications of orthopedic-related nano drug delivery are summarized and the trends of current studies and future directions outlined. However, it is necessary to emphasize that most nanomaterial-based drug delivery systems are still in the early phases of research and development, and the understanding of their toxicity and safety is still lacking, thus, limiting clinical evaluation.

2. Nanomaterials as drug delivery systems: fundamentals and principles

A successful drug delivery system should be able to overcome the inherent limitation associated with biomacromolecular therapeutics (such as short plasma half-lifes, poor stability and potential immunogenicity) to maximize therapeutic activity while minimizing toxic side effects of the drug [1,14]. The key issues towards achieving these requirements include site-specific targeting, transportation of drug carriers and the controlled release of drugs. Traditional drug delivery systems have difficulties in drug stability during transportation, prolonging drug release and releasing drugs into individual cells. These limitations (often times leading to an unhealthy initial burst release of drugs) can be more serious for drug delivery to bone than to other tissues. This is because when dealing with bone implants, a time-delay and stable drug delivery with little initial burst release is desirable while the drug carrier maneuvers the complex intricacies of bone structures to get to the diseased site [15].

The inherent properties of nanoscale materials (such as physical, chemical, mechanical, electrical, magnetic and optical properties) can be utilized to strengthen the performance of drug delivery systems. Specifically, the nanometer size of a drug carrier provides numerous advantages for drug delivery purposes, such as: i) enhanced transport across cell membranes, thus, reducing clearance from the body and providing for more targeted drug delivery [1,14]; ii) greater surface area-to-volume ratios and subsequently more surface reactivity, thus, increasing drug loading ability, providing controlled dissolution rates and drug bioavailability [16]; iii) increased dispersibility for homogeneous drug loading and release of drug molecules; iv) promoted mechanical properties (such as matching the strength and ductility of natural bone), thus, serving as a strong immediate matrix backbone; and v) size similarity to natural tissue components (e.g., HA crystals in natural bone are 50×25 nm [17]), thus, enabling better tissue acceptance by biomimicking tissue architecture.

Table 1. Current medications and clinic therapies for bone diseases.

Bone diseases	Current medications or therapies	Ref.
Osteoporosis	Antiresorptive medications: bisphosphonates, calcitonin, estrogen and estrogen agonists/antagonists; anabolic (bone forming) medications: parathyroid hormone; surgery or bone replacement	[5]
Osteoarthritis	Nonsteroidal anti-inflammatory drugs, COX-2 inhibitors, corticosteroids, and artificial joint fluid; surgery or bone replacement	[110]
Paget's disease	Risedronate, pamidronate, alendronate, zoledronic acid, etidronate, tiludronate, and calcitonin; surgery or bone replacement	[9]
Bone cancer	Surgery, radiation therapy and chemotherapy	[111]

Several studies have reported that the size variation of drug carriers in the nanoscale range strongly affects drug bioavailability and blood circulation times [1,18,19]. For example, particles with diameters < 10 nm can be rapidly removed through extravasation and renal clearance after systemic administration, while particles with sizes in the range of 70 – 200 nm revealed prolonged circulation times [1,18]. A wide size distribution of nanoparticulates can also vary gene transfection efficiency in gene delivery therapies [13]. Therefore, a preferred size range of 10 – 100 nm for nanomaterial drug carriers has been suggested [1].

Besides the influence of nanometer particle size, a variety of geometries and architectures of drug carriers have been studied. As an example, nanoscale hollow structures (specifically, tubes, cages, shells, etc.) have demonstrated great control over drug loading amounts and time of release [20]. Specifically, by controlling tube length, diameter and wall thickness, one can control many aspects of drug delivery (such as loading amounts and time of release) [20]. Another study proposed a drug delivery system in which drugs were conjugating on gold nanocages and the nanocages simultaneously served as optical imaging contrast agents to tag cancer sites and release drugs [21]. In addition, distinctive magnetic, electrical and optical properties of nanomaterials can also benefit controlled drug delivery processes by assisting in the external control of drug transportation, targeting, and even simultaneously sensing, diagnosing and treating the disease [22-24]. Conventional drug delivery processes often only treat diseases, but do not play a role in diagnosing the disease.

To construct better drug delivery systems through nanotechnology, surface properties of nanomaterials have been carefully tailored through physical or chemical adsorption methods. Usually, the physical or chemical adsorption of drugs to nanoparticles has been governed by the inherent chemistry or physical properties of the materials, which has also influenced drug loading, particle transportation and drug release as well as biocompatibility and degradability properties. Surface hydrophilicity of the drug carriers has also been a key surface property. Specifically, this property can determine the construction and assembly of nanomaterials *in situ*, affecting the adsorption/desorption of drug and proteins, cell and tissue responses as well as clearance from the body. For example, several types of proteins (such as fibronectin and vitronectin) that promote osteoblast (bone forming cells) functions are hydrophilic and, thus, prefer to adsorb on hydrophilic surfaces [25]. If these proteins adsorb in high quantities (as they have been shown to do on nanoparticles of ceramics, metals and polymers), bone growth ensues. Electrical charges on drug carriers have also been shown to be effective for drug loading. Specifically, electrical properties of surfaces interact strongly with drugs or biological systems to promote an even greater impact on drug biological activity, drug release kinetics, conjugation to targeting moieties and transport in bone. For instance, cationic nanoparticles can localize in the cytoplasma and within mitochondria, while anionic nanoparticles remain in lysosomes [1,26]. Last, for bone regeneration, the porosity of nanomaterial drug carriers is another key factor that can be tailored to promote drug delivery [27]. Specifically, porosity of particles controls degradation, material solubility and drug release profiles. Particle porosity also affects material biocompatibility properties and bone cell growth potential.

3. Nanoparticulate drug delivery systems

Owing to simple synthesis, high reproducibility and feasibility of less invasive administration, particulate materials have been widely investigated in numerous areas of drug delivery for treating bone diseases and reconstructing bone tissue (**Figure 1**). Differing from larger size drug-delivery particles which release high local drug concentrations, nanoparticulate drug-delivery systems can be endocytosed directly and, thus, can release drugs either outside or inside target cells (allowing for smaller amounts of drug delivery to achieve a desired effect [1]). Nanoparticulates also have benefits towards gene delivery therapy owing to their sizes, which are small enough to be internalized into the cell, thus, providing the

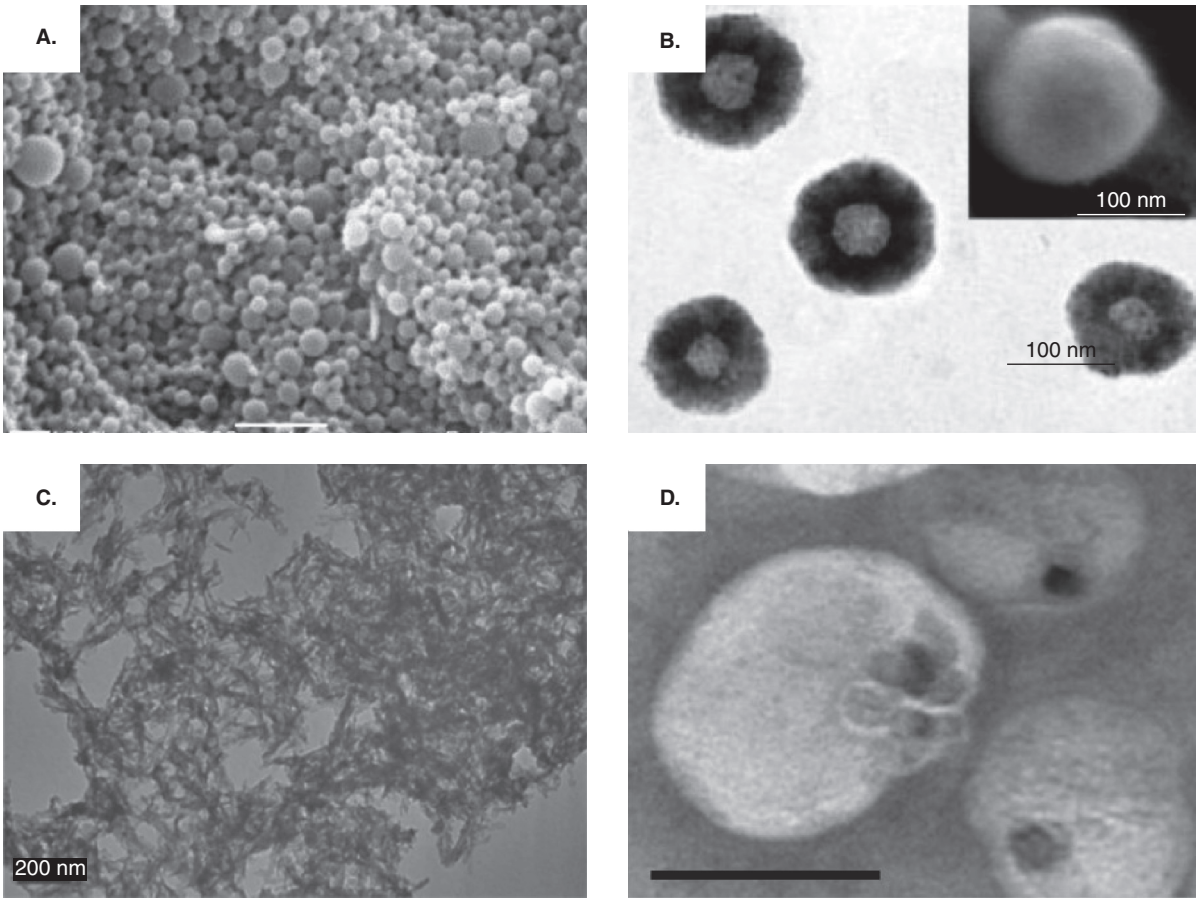

Figure 1. Representative nanoparticulate drug-delivery systems for treating bone diseases. A. Poly (lactic-co-glycolic acid) nanoparticles, scale bar = 1 μm; **B.** Hollow calcium phosphate nanospheres, scale bar = 100 nm; **C.** Calcium phosphate coated γ-Fe_2O_3, scale bar = 200 nm and **D.** Magnetic liposomes, scale bar = 100 nm.
Figure 1A, B, C and D reprinted from [112], [44], [49] and [55], respectively, with permission.

capability of escaping endosomes/lysomes (which protect DNA) until they reach the target [28]. In addition to size benefits, nanoparticles also have various properties that can be tailored for specific applications owning to their exceptional bulk or surface properties. For example, in addition to their ability to bind peptides and proteins in a much more biologically active manner than micron particles, optical and photothermal properties of gold nanorods can be used to specifically target and image cancer cells by scattering light, and finally lysing cancer cells by converting light energy into heat [23]. The following sections provide more specific examples of the use of polymer, inorganic and composite nanoparticles in orthopedics.

3.1 Polymeric nanoparticles

Polymeric nanoparticles are probably the largest category of nanomaterials used in drug delivery owing to their easily tailored biodegradability properties. Drug loading and release profiles of polymeric nanoparticles can be easily adjusted by altering molecular mass (MM), surface hydrophilicity/charge and free functional groups. Poly (D,L-lactic-co-glycolic acid) (PLGA) is a good example of such a common drug delivery nanoparticle and has been modified for bone-specific drug-delivery applications (**Figure 1A**). PLGA has a highly hydrophilic surface with numerous carboxylate end groups. A method of controlling the density of reactive carboxyl groups on PLGA to prolong drug release was recently reported [29]. This study revealed that adjusting the combination of high MM encapped and low MM non-encapped PLGA could change the carboxyl density with a higher proportion of high-MM PLGA contributing to prolonged celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene-sulfonamide, commonly used for treating osteoarthritis and rheumatoid arthritis) release. Moreover, modified PLGA nanoparticles have exhibited great potential for orthopedic drug delivery [30]. For example, alendronate, a type of bisphosphonate, which has a strong affinity for binding to calcium phosphate in bone and has been used clinically for treating osteoporosis, can be conjugated to PLGA and collectively these structures are expected to have a great ability to target bone and release drugs at specific bone sites [31]. Besides albumin microparticles, micro- or nanoparticulate PLGA is

also a possible candidate for intra-articular drug delivery for osteoarthritis [32]. The efficacy of intra-articular PLGA nanoparticles (300 – 490 nm) loaded with betamethasone phosphate can last about 6 weeks. However, issues such as fast cellular uptake and reproducibility still need to be investigated before launching the nanoparticulate PLGA-based drug systems for intra-articular applications.

Copolymers also exhibit great potential for bone-specific drug delivery because they can combine advantages of different polymers. This is also clearly true at the nanometer level [13,33]. One drug-delivery strategy has been to create copolymers of different hydrophilicity properties to achieve self-assembled structures such as polymeric micelles (sizes about 50 – 100 nm) with a hydrophobic core and hydrophilic outer shell. Through physical or covalent bonding, hydrophobic cores can be used to contain hydrophobic drugs (such as antiphlogistic and anticancer drugs, DNA or other proteins [34-36]). As another example, a novel heparin-conjugated micelle has been shown to incorporate and deliver both basic fibroblast growth factor (used as a model growth factor) and hydrophobic indomethacin (used to treat rheumatoid arthritis and gout) [37]. The micelle used Tetronic®-poly(ε-caprolactone) (PCL)-heparin as a copolymer and *in vitro* drug release profiles showed that indomethacin slowly released over 3 weeks while basic fibroblast growth factor released over 2 months in a controlled manner. Coupling hydrophobic and electrostatic interactions, more complex micelle like core-shell structures were obtained. The detail and properties of these structures have been discussed in several recent reviews [38,39].

Another strategy centered on co-polymerization is to use PEG to increase blood circulation times of the drug carrier and mask bounded biomolecules (proteins, DNA, etc.), also known as PEGylation, until the carrier gets to its destination [13,40]. For example, PEG-PLGA nanoparticles have enhanced protein delivery and biodistribution in a rat by extending the half-life of bovine serum albumin 20 times compared to bovine serum albumin loaded in PLGA alone [41]. PEGylated copolymers (such as methxy poly(ethylene glycol)/PCL), amine-terminated methxy poly(ethylene glycol)/PCL and PEG-cationized gelatin) have also been used as non-viral gene delivery carriers and for modulating DNA transfection efficiency, which is not discussed in detail here due to space but can be found in several recent literature articles [42,43].

3.2 Inorganic nanoparticles

Owing to chemical similarities to bone, nanoparticulate inorganic materials have also been extensively studied for orthopedic drug delivery. In contrast to polymeric nanoparticles, inorganic particles have much longer biodegradation times or are even undegradable, which clearly alters drug release mechanisms and has a greater impact on subsequent drug release kinetics. In addition, inorganic nanoparticles exhibit electrical, mechanical, magnetic and optical properties that are rarely seen in polymeric materials. These distinct properties have led to several novel applications in bone-specific drug delivery as reviewed here.

Calcium phosphate nanoparticles (including HA, tricalcium phosphate, calcium tetraphosphate and their derivatives) have been widely studied and used in bone tissue engineering applications and bone-specific drug delivery owing to their biocompatibility, bio-absorbability and biological activity. In addition, owing to their chemical, structural and size similarity to the inorganic components of natural bones, calcium phosphate nanoparticles are perfect for bone regeneration purposes. Moreover, rich hydroxyl groups on HA as well as the hydrophilic property of calcium phosphates readily enable the conjugation of both small- and large-molecule drugs. Recently, hollow-structured calcium phosphate nanospheres were designed and fabricated to achieve an "on-off" release of drugs using ultrasound (**Figure 1B**) [44]. The novel HA nanospheres were fabricated by an ultrasonic-assisted wet chemical reaction with hexadecyl (cetyl) trimethylammonium bromide as a modifier. These uniform and mono-dispersed hollow HA structures can collapse and transfer into pin-shaped HA nanocrystallites under ultrasonic treatment. In this manner, the drug release of encapsulated compounds can be triggered by ultrasound and regulated by altering ultrasound power density. An experiment using amylose indicated that the drug released amounts could be consistently controlled by ultrasound application time [44,45]. Calcium phosphate nanoparticles are also potential candidates for bone-specific gene delivery. Numerous studies have revealed that nano-calcium phosphates possess a higher penetration rate into cell membranes and their transfection efficiency can be up to 25-fold higher than that of conventional particles. In addition, nano-HA can load greater amounts of drugs because of their notoriously high adsorption affinity and high area to volume ratio [45].

Magnetic nanoparticles have also attracted increasing interest in orthopedic drug delivery. The most widely studied magnetic nanoparticles are nanoscale iron oxides including magnetite γ-Fe_2O_3, magnetite Fe_3O_4 and associated compounds (also know as superparamagnetic iron oxide nanoparticles; SPION), which have been actively used for MRI, hyperthermic treatment and non-bone drug delivery [46,47]. Recently, a strategy of using SPION to treat bone diseases (such as osteoporosis, osteoarthritis and bone cancer) was proposed using biocompatible SPION conjugated with specific drug molecules, which were delivered to osteoporosis sites or diseased tissue in the presence of a directional magnetic field [48,49]. The drug molecules ranged from bone growth factors (such as bone morphogenetic protein-2; BMP-2) to those for treating bone diseases (such as bis- and di-phosphonates and estrogen modulators). A preliminary investigation aimed at treating osteoporosis demonstrated that iron oxide nanoparticles could be coated with calcium phosphate and then modified with biologically active agents (YRGDSPC) to promote bone

growth while still retaining magnetic properties [48]. Another preliminary study revealed that γ-Fe_2O_3 nanoparticles and γ-Fe_2O_3 coated with calcium phosphate (**Figure 1C**) in the presence of bovine serum albumin significantly increased osteoblast (bone-forming cell) proliferation compared to controls without nanoparticles [49]. Such promise in the use of inorganic nanoparticles at promoting bone growth without using more drugs is unprecedented. Coupled with the successful applications of SPION in biomedical imaging, SPION render drug delivery systems multi-functional for purposes of imaging, diagnosis and therapy.

More specifically for bone cancer, novel selenium nanoparticles have been developed (again without the use of drugs) [50]. Selenium naturally exists in the human body and is known to reduce various types of cancers (including pancreatic, gastric, lung, nasopharyngeal, breast, uterine, respiratory, digestive, hematological and gynecological) [51]. Biocompatibilty of selenium and the anti-carcinogenic effects of selenium nanoparticles synthesized through a colloidal technique have been evaluated [50,52,53]. Results showed that nanoparticles of selenium have bone-specific biocompatibility and when grown on titanium have significantly inhibited the competitive growth of cancerous osteoblasts while increasing the growth of healthy osteoblasts. Clearly, these new findings support the feasibility of using inorganic nanoparticles (as drug carriers) for bone cancer treatment.

3.3 Nanoparticle composites

As mentioned in the previous section, polymeric nanoparticles are biodegradable and biocompatible, while inorganic nanoparticles possess many distinctive physical properties. Harnessing these properties into a single composite drug delivery system enables a synergic and multi-purpose therapy for treating bone diseases. As an example, nano magnetic liposomes (**Figure 1D**) fabricated by combining magnetic moieties with phospholipids have been used as carriers for recombinant human BMP-2 (rhBMP-2) for bone regeneration in a rat-bone defect [54,55]. The results showed that a single topical application of magnetic liposomes with an appropriate amount of rhBMP-2 (3 μg) incorporated under magnetic induction immediately after surgery was effective at promoting new bone formation. Moreover, the combined treatment of topical magnetic rhBMP-2 liposomes and magnetic force assisted implantation was effective for healing bone defects [55]. A similar rationale of achieving desirable properties by combining corresponding nanoparticles has been widely applied in orthopedics and other examples are not reviewed here owing to space limitations but can be found in several recent reviews [13,22,56].

4. Nanostructured scaffolds as controlled release matrices

Nanostructured scaffolds can serve several purposes including supporting cellular functions by mimicking biological micro- and nano-environments (such as the bone extracellular matrix) and controlling drug release. Scaffold-based drug delivery can be achieved by the incorporation of signaling molecules into scaffolds through simple dispersion or through stronger immobilization to nanoparticles by electrostatic or covalent bonding [57]. In such molecule-incorporated and nanoparticle-derived scaffolds, the architecture (such as hierarchical structures, alignment, assembly) of the building block materials and associated porosity have been adjusted to play key roles in drug release kinetics, drug stability, drug transportation, scaffold degradation and biocompatibility. Additionally, because of their structure and volume, scaffolds are typically delivered by invasive measures, which significantly differ from the less invasive routes of administration for particulate drug delivery systems.

4.1 2D nanostructured surfaces

2D nanostructured surfaces refer to coatings, films or other layers with nanoscale features and topographies, which have much larger scales in 2D than 3D (**Figure 2**). These surfaces are usually attached or coated on substrates or other bulk materials such as orthopedic implants to provide biologically active surfaces for drug delivery. 2D nanostructured surfaces usually receive less attention than 3D scaffolds for tissue engineering but could play a very important role for currently used metallic or ceramic implant efficacy. In contrast to nanoparticles, 2D nanostructured surfaces (including surface roughness, topography, surface energy and surface chemistry) have a much more profound impact on controlling protein adsorption, subsequent cellular events and, of course, drug release directly to adjacent bone [58-61].

For example, the selenium nanoparticles mentioned in the previous section have been coated on titanium and stainless steel implants to provide an anti-carcinogenic coating (**Figure 2A**) [62]. This study showed that selenium nanoclusters could be firmly bonded and well dispersed on titanium and stainless steel implant surfaces through a simple colloidal technique. The payload of selenium on implants can be controlled by the concentration of selenium in the synthesis solution. Results revealed an increase in non-cancerous osteoblast density on selenium coated titanium compared to uncoated samples and a similar osteoblast density on both coated and uncoated stainless steel (both after 4 h and 1 day). These results suggested that selenium coated implants are cytocompatible, but more importantly, the function of cancerous osteoblasts on these selenium-coated surfaces was significantly inhibited compared to results obtained on uncoated materials for up to 3 days. This inhibiting effect was also enhanced as the selenium concentration on the implants increased.

Besides treating bone cancer, many 2D nanostructured drug carriers have been designed to promote bone regeneration. Specifically, nanotubular anodized titania surfaces with a tube width of tens of nanometers and a tube length of a few hundred nanometers have been promising bone-growing

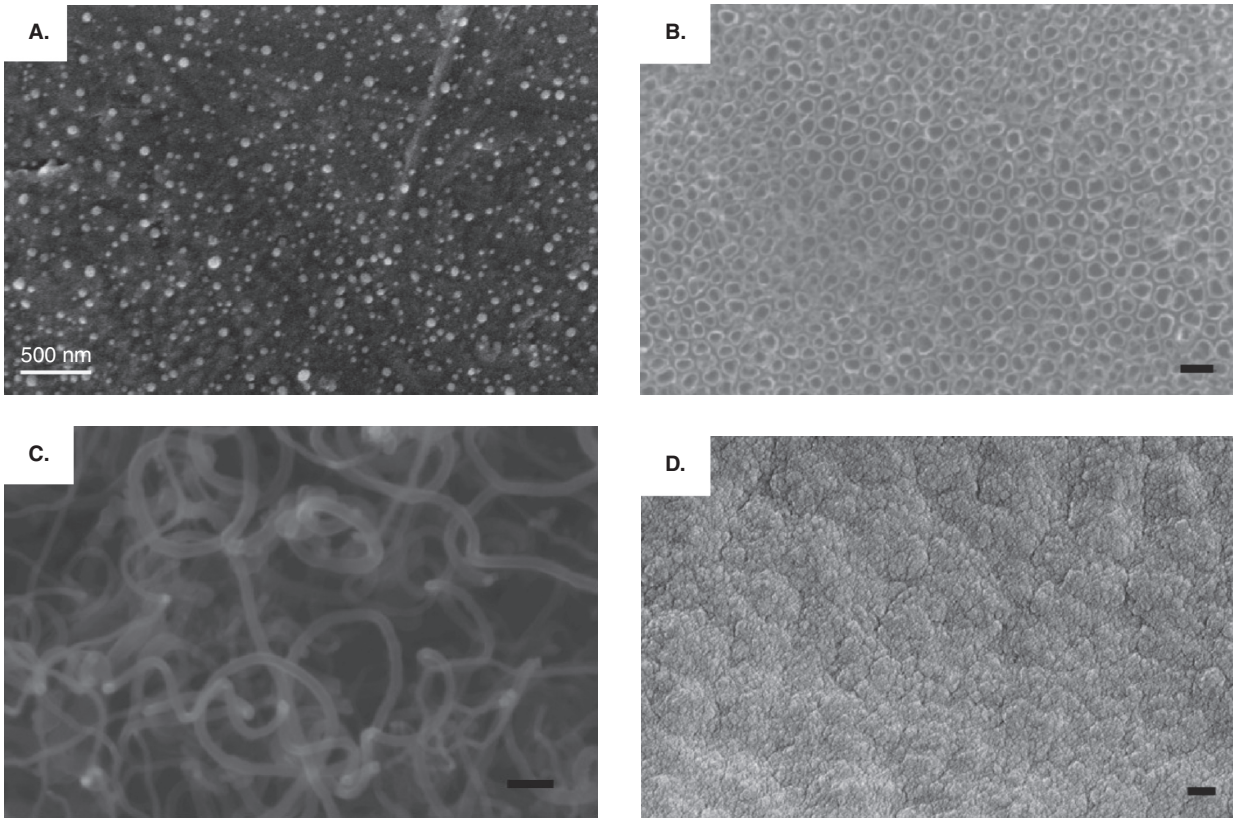

Figure 2. Representative 2D nanostructured surfaces for bone-specific drug-delivery systems. A. Selenium nanoparticle coated titanium surface, scale bar = 500 nm; **B.** Nanotubular anodized titanium surface, scale bar = 100 nm; **C.** Carbon nanotubes grown out of an anodized nanotubular titanium surface, scale bar = 200 nm and **D.** Nanocrystalline diamond surface, scale bar = 200 nm.
Figure 2A, B and C reprinted from [24], with permission.

candidates (**Figure 2B**). Nanotubular titania surfaces can be readily fabricated by direct anodization of titanium implants [63,64]. Anodization is a technique to grow an *in situ* metallic oxide layer on metal surfaces in an electrochemical cell, which uses the metal as an anode and frequently uses platinum as a cathode (as well as a water-based electrolyte solution). To create nanoscale tubular or porous topographies on newly formed metallic oxide layers, a voltage between 10 and 40 V and fluorine-based electrolytes are often used. The mechanisms of forming nanometer features through anodization are still under investigation, but usually, the local electrical sparking at defect sites on the dielectric metallic oxide layer and electric field-assisted dissolution of the metallic oxide layer are believed to account for the formation of nanoscale features [64,65]. Theoretically, anodization can be applied to any metal that is stable to oxide formation to fabricate nanoscale tubular or porous surfaces. The unique tubular structures in metals can serve as a source of loading bone growth factors or antibiotics to promote bone growth or suppress bacterial infection when released [64-67]. This is a novel application for metals because anodization can transform a metal into a drug delivery device. Importantly, metals are the most common orthopedic implant material, and yet, few metallic drug delivery strategies exist from metals.

A recent study has shown that immobilizing the amino-acid peptide sequence of a segment of BMP-2 (the knuckle epitope, CKIPKASSVPTELSAISTLYL) into anodized titanium nanotubes (~ 70 nm in diameter and 200 nm in depth) can promote osteoblast adhesion [67]. Another study modified anodized titanium nanotubes to increase drug-loading efficiency and prolong drug release by attaching amine or methyl groups to the anodized nanotubular titanium surfaces through a silanization process [68]. Results of that study revealed a prolonged continuous release of antibiotics and anti-infection agents decreasing *Staphylococcus Epidermis* colonization [68]. A similar result of incorporating titania nanotubes with gentamicin (an aminoglycoside antibiotic particularly useful for treating Gram-negative infection) also significantly reduced initial *S. Epidermis* adhesion on orthopedic implants [68]. In addition, immersing nanotubular titania into a mixture of simulated body fluid and penicillin G created a drug loaded calcium phosphate coating, which prolonged drug release for up to 21 days. These results clearly indicate the promise of using metallic nanotubular surfaces for controlling drug release and improving bone implant efficacy.

More promise exists for the controlled drug release from orthopedic implants by using novel self-assembled material coatings. Specifically, helical rosette nanotubes (HRN), which are soft organic materials obtained through the self-assembly of low MM synthetic DNA molecules, have been developed to mimic the natural nanostructure of collagen and other components in bone [69]. HRN have demonstrated an exceptional potential to enhance osteoblast adhesion when either coated on titanium or incorporated into HA [70]. One recent study focused on conjugating arginine-glycine-aspartic acid (RGD) peptides (a cell adhesive peptide) or lysine on HRN and placing this new material on solidified polymerized 2-hydroxyethyl methacrylate hydrogels [71]. Results indicated up to a twofold enhancement in osteoblast adhesion on 10% HRN-RGD-K (HRN loaded with RGD and lysine) coated hydrogels than on uncoated hydrogels and 73% more than on collagen-coated hydrogels of similar concentration. Fibronectin adsorption tests also revealed increased protein adsorption on RGD or lysine conjugated HRN. A recent study also showed that HRN loaded with dexamethasone prolonged drug release for up to 28 days due to the penetration of the drug down the long (up to several microns) interior of the HRN tube. These results highlight the promise that self-assembled biologically active systems have to deliver specific protein segments or molecules to promote bone growth.

Carbon nanotubes (CNT) or carbon nanofibers have also been studied as a local drug-delivery system to promote and guide bone-tissue regeneration [72,73]. CNT have been successfully coated or patterned on titanium, anodized nanotubular titanium (**Figure 2C**) and polycarbonate urethane [74,75]. A recent animal study revealed that multi-walled CNT combined with collagen and rhBMP-2 accelerated bone formation after implantation in a mouse muscle [76]. This finding is important toward the development of new drug delivery systems or scaffold materials for bone regeneration using multi-walled CNT as it also demonstrated no toxicity. Furthermore, rhBMP-2 loaded multi-walled CNT can be coated on implants (such as plates and screws) to form 2D nanostructured surfaces for rapid bone healing.

Lastly, nanocrystalline diamond (NCD) has been studied as a versatile 2D nanostructured surface for orthopedic drug release purposes (**Figure 2D**). Specifically, BMP-2 has been immobilized on oxygen-terminated NCD by physisorption and the drug-coated implant enhanced *in vivo* osseointegration when inserted into sheep calvaria [69,77]. More excitingly, this BMP-2 coated NCD system strongly activated the expression of osteogenic markers in mesenchymal stromal cells cultured on this surface. In addition to superior anti-wear properties and the knowledge that the topography of NCD exhibits greater cytocompatibility properties than diamond films of larger grain sizes (i.e., submicro- or micro-crystalline diamond) [78,79], an atypical multifunctional drug delivery platform to promote or inhibit bone growth depending on what events are desired per implant location can be created.

4.2 3D nanostructured scaffolds

3D drug-delivery scaffolds possess a greater resemblance to bone compared to the aforementioned 1D or 2D structures. Different from most 2D surfaces that serve as coatings on implants, most 3D scaffolds are biodegradable or biologically active and possess cell-inductive architectures (such high porosity), enabling the enhanced recruitment and migration of peripheral host cells into the scaffolds. Therefore, 3D nanostructured scaffolds are more versatile and ready for controlled drug delivery to promote cellular events or guide morphogenetic processes [57,80,81]. In this case, 3D scaffolds serve both as a temporary scaffold for promoting cell activity and as a delivery vehicle for controlling drug release, which is more suitable for bone regeneration after removal of diseased bone tissue [82]. Here, the recent progress of bone-specific drug delivery using 3D scaffolds is reviewed, focusing on those inspired by nanotechnology (**Figure 3**).

Electrospun nanostructured scaffolds have been intensively investigated as 3D scaffolds for bone tissue engineering. The principle of electrospinning is to apply high voltages on a polymer solution (or polymer melt) to overcome surface tension and draw the charged solution through an electric field from a nozzle onto a collector plate to form 3D (or 2D) structures. Due to the ease of fabrication, ability to control final product structure and extreme versatility in terms of the types of materials that can be used (mainly polymers), electrospinning has been widely used to prepare porous fibrous scaffolds with tunable drug release profiles and biodegradabilities. Advantages of electrospun scaffolds include high porosity, high surface to volume ratio, and a structural similarity to fibrous proteins in native extracellular matrices or collagen fibrils in bone [1,13]. As drug-loaded platforms, electrospun polycaprolactone (PCL) 3D scaffolds carrying the drug simvastain were recently studied for regenerating bone. A rat study recently revealed significant osseous integration in the simvastain loaded PCL scaffolds and better *in vivo* bone mineralization compared to the PCL scaffolds alone after 6 months [83]. Natural polymers (such as silk) have also been electrospun into scaffolds and loaded with molecules such as BMP-2 to promote human bone marrow-derived mesenchymal stem cell growth and differentiation toward osteogenic outcomes (**Figure 3A**) [84]. *In vitro* results showed that the BMP-2 loaded silk scaffolds supported higher calcium deposition and enhanced transcript levels of bone-specific markers than the silk scaffolds alone, indicating that the silk scaffolds were excellent vehicles for BMP-2.

Injectable nano-apatite scaffolds have also been developed for cell/growth factor delivery [85]. Tetracalcium phosphate ($Ca_4(PO_4)_2O$) and dicalcium phosphate ($CaHPO_4$) have been fabricated into self-setting calcium phosphate cements (CPC) and CPC have been further synthesized into strong nanostructured scaffolds using chitosan and various porogens (such as absorbable fibers and mannitol). In particular, water-soluble mannitol crystals which dissolve in a day and absorbable fibers which degrade after weeks, even months,

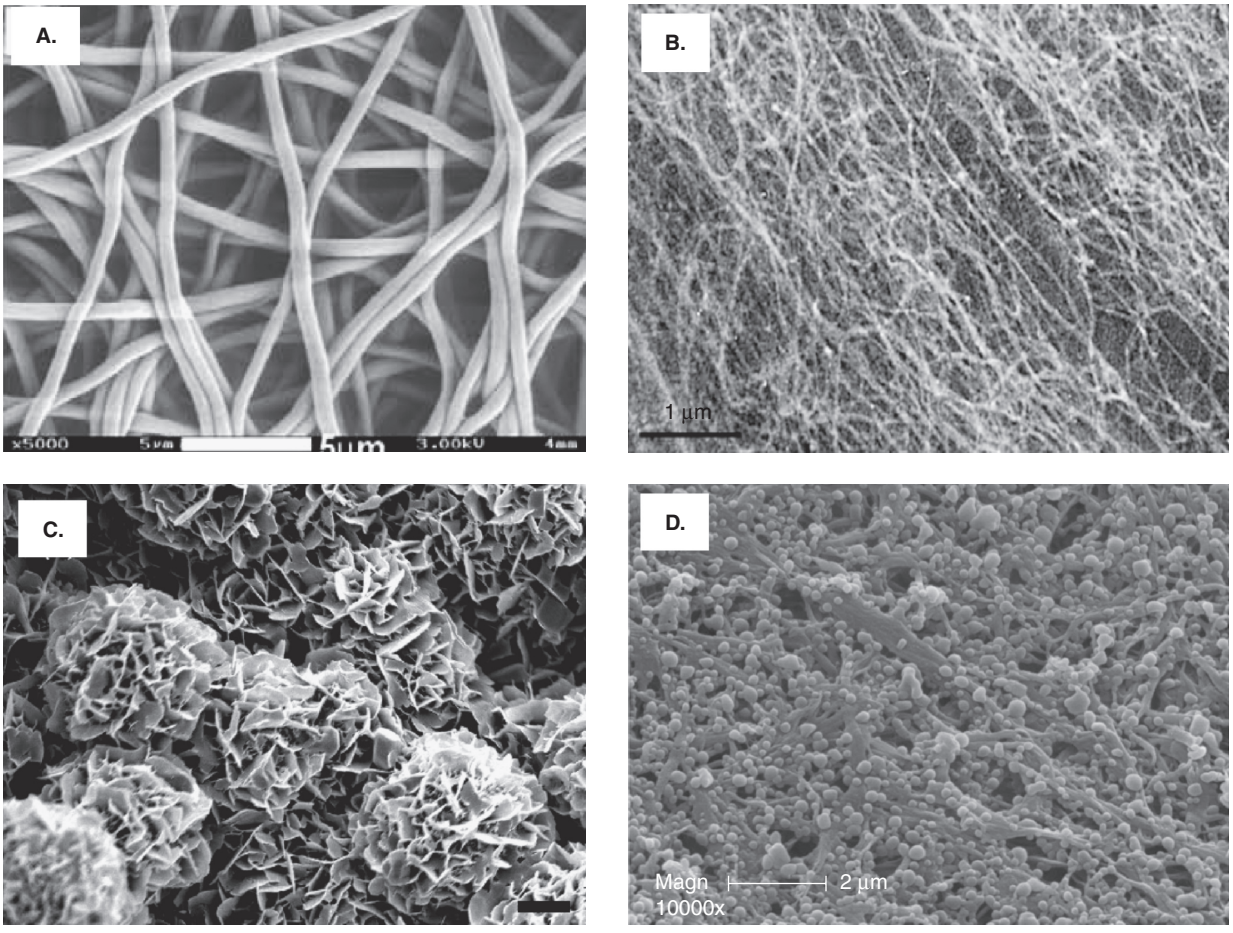

Figure 3. Representative 3D nanostructured scaffolds for bone-specific drug-delivery systems. A. Electrospun silk scaffold with bone morphogenetic protein-2 loaded, scale bar = 5 µm; **B.** Self-assembled peptide-amphiphile nanofibers network, scale bar = 1 µm; **C.** Nanocrystalline apatite modified poly (lactide-co-glycolide) microsphere scaffolds, scale bar = 2 µm and **D.** Poly (L-lactic acid) nano-fibrous scaffolds incorporated with poly (lactic-co-glycolic acid) nanospheres, scale bar = 2 µm.
Figure 3A, B, C and D reprinted from [84], [86], [87] and [92] with permission.

have been used to create macropores in CPC. Clearly, it has been hypothesized that new bone could gradually grow into the macropores formed sequentially by mannitol and fibers after implantation, thus, maintaining the strength of scaffolds during bone growth. Studies have shown good attachment and proliferation of MC3T3-E1 osteoblast-like cells on CPC infiltrating into the macropores and anchoring into nanostructured apatite crystals on the pore walls. This novel scaffold was also modified to control the release of growth factors by adjusting liquid and chitosan ratios.

Bone regeneration through the controlled release of BMP-2 from an injectable peptide amphilphile (PA) nanofibrous scaffold has also been observed [86]. 3D nanofibrous scaffolds were formed from the self-assembly of PA in the presence of BMP-2 (**Figure 3B**). *In vivo*, the BMP-2 release profile showed a 24-day release from PA nanofibrous scaffolds after the scaffolds were implanted into the back subcutis of a rat, compared to a 2-day duration of direct administration. This elongated release of BMP-2 induced significant homogeneous ectopic bone formation around the injected site.

Several 3D nanocomposites have been designed to impart biological activity, controlled drug loading and drug release. For example, poly (lactide-co-glycolide) sintered microsphere scaffolds were modified by growing nanocrystalline apatite on their surfaces to enhance drug adsorption and slow drug release (**Figure 3C**) [87]. Heparin-conjugated PLGA scaffolds have been fabricated through techniques such as gas-foaming/salt-leaching and have been used to sustain drug delivery of growth factors such as BMP-2 to enhance bone formation [88,89].

Lastly, several recent studies have focused on the subtle tuning of bone-specific drug release profiles rather than simply prolonging release time. For example, sequential growth factor delivery from 3D scaffolds was realized using microspheres of polyelectrolyte complexes of alginic acid and poly (4-vinyl pyridine) [90]. The microspheres were loaded into PLGA scaffolds in the presence of growth factors (BMP-2

and BMP-7) and their effects on bone marrow stem cell growth studied. Results indicated that the sequential release of BMP-2 and BMP-7 enhanced osteogenic differentiation. To control drug release in a temporal fashion, a novel system of incorporating nanospheres into prefabricated nanofibrous scaffolds (NS-scaffolds) was developed (**Figure 3D**) [91,92]. *In vitro* release kinetics indicated that NS-scaffolds could release BMP-7 in a temporally controlled manner, exhibiting tunable release phases (especially, a controlled initial burst release) depending on chemical and degradation properties of the nanospheres. *In vivo* test results showed that the NS-scaffolds actively induced new bone formation throughout the scaffold as a result of BMP-7 release.

5. Conclusions

Recent research has demonstrated that nanotechnology can assist drug delivery efforts and have exceptional potential towards treating various bone diseases owing to their ability to modulate drug release kinetics, incorporate multifunctional molecules, target-specific bone sites or cells and respond to various external signaling sources (whether biological, electric, magnetic or mechanical). These novel drug delivery systems involve an extensive spectrum of materials (from polymers to ceramics and from 1D to 3D) and a plethora of fabricating techniques, which will continuously revolutionize the orthopedic drug delivery area. Although most of the *in vitro* or *in vivo* studies reviewed here are still distinct from necessary clinical trials and toxicity investigations of nanomaterials (which is still an increasing concern), the extraordinary properties of nanomaterials mentioned here and the continual understanding of physical and biological properties of bone at the nanometer scale will open exciting and promising avenues to prevent, diagnose and treat numerous bone diseases.

6. Expert opinion

Highly controllable drug delivery systems, first introduced in early 1970s [93], and nanotechnology, first conceptualized by Richard Feyman and first defined in a scientific publication in 1974 [94,95], complement each other and allow researchers to fabricate numerous exciting materials to diagnose and treat diseases. As an interdisciplinary field, nanotechnology-based drug delivery has experienced a tremendous revolution over the past decade, which has impacted every aspect of orthopedic drug delivery (from concept to material fabrication to drug transportation pathways [1,12,96]).

In this review paper, the focus is placed on the latest experimental progression in controlling drug release for treating bone diseases and regenerating new bone in areas specifically desired. In every form of bone-specific drug delivery discussed (for example, in applications involving nanoparticles, 2D or 3D nanostructured drug carriers), novel drug species, drug delivery kinetics, biocompatibility and so forth have been optimized and improved using nanotechnology. Strong advances have been made by combining the advantageous properties of different forms of nanomaterials and by fabricating composites. This progress has allowed better drug delivery systems with tunable release profiles, adjustable degradability, promoted biocompatibility and drug biological activity with several functionalities. In addition to creating composites discussed in a previous section, there are several continuing critical topics in nanotechnology-derived controlled drug delivery for orthopedics:

- Understanding of metabolism, toxicity and elimination routes of nanotechnology-derived drug delivery systems. Not just for bone diseases, but the whole nanotechnology-based drug delivery field lacks a fundamental understanding of how tissues and cells function in the presence of nanomaterials. This has been a significant hurdle translating promising *in vitro* and animal studies to human clinic trials. An understanding of metabolism, toxicity and safety issues (such as manufacturing of nanomaterials) can provide essential guidance for the chemical and structural design, biodegradability, release kinetics and biocompatibility properties of nano drug delivery systems.
- Refining drug delivery systems to achieve controllable multi-phase drug release kinetics. In addition to the previous examples of delayed drug delivery, sequential delivery and temporally controlled drug delivery, more complicated release mechanisms are being developed. These multi-phasic drug deliveries include pulsed drug delivery, self-regulated drug delivery and externally-regulated drug delivery [57]. Pulsed drug delivery, which releases drugs over specific periods of time, has been used in PLGA-based systems but has not been used for treating bone diseases [97,98]. Self-regulated drug delivery is one in which drugs are released as modulated in response to feedback information without external intervention. The biological feedback information which triggers release includes pH, bio-molecule concentration or more specific molecular presence [99-101]. In contrast, externally-regulated drug delivery systems release drugs by an external stimulus including temperature difference, electrical and magnetic stimuli as well as ultrasound [44,100-103]. These 'smart' release mechanisms have not been widely used for orthopedic-specific treatments, but would be clearly beneficial for treating bone diseases and stimulating new bone growth. One recent attempt towards this approach is incorporating drugs into multiwalled CNT grown out of nanotubular titania and controlling drug release from these tubes through an electrical field.
- Gene delivery and multi-factor delivery. Until now, drug molecules used for bone-specific treatments are mostly forms of BMP for bone growth and antibiotics for fighting bacteria infection. However, gene delivery is also a potent candidate for bone-specific drug delivery [104,105]. Furthermore, multi-factor delivery in one drug system could be an optimal solution for treating bone diseases and regulating

tissue generation during different stages of bone repair [106,107]. One approach to achieving multi-factor delivery is incorporating nanospheres into scaffolds [91]. Nanospheres would allow for a wider number of drugs and release profiles. Several drugs can be immobilized on different nanospheres of varied degradation rates, and in this manner, nanosphere-scaffold complexes can simultaneously deliver a variety of factors with varied release profiles.

- Multifunctional scaffolds. As discussed before, 2D and 3D scaffolds can serve both as a cell/tissue support and drug carrier. Recent research has attempted to extend the scope of scaffold functions and fabricate 'smart' scaffolds that can simultaneously incorporate disease sensing, diagnosis, imaging and treatment. To realize this, numerous nanomaterials can be used to fulfill these functions and be immobilized into/onto scaffolds. For example, multiwalled CNT grown out of nanotubular titania have revealed the feasibility to support and sense new bone growth, scar tissue and biofilm formation by measuring tissue conductivity [24]. Together with gene delivery [76,108], a "smart system" can be designed to stimulate new bone growth in response to an electrical feedback signal of the lack of bone formation. Similarly, such materials can sense whether infection or inflammation is occurring by measuring such tissue conductivity and drugs can then be released on demand. Nanotechnology plays a key role in this process by measuring the conductivity of individual cells and proteins. As another example, magnetic nanostructures (e.g., SPION) with their ability to be used in MRI and thermal ablation can be devised to image, target and lyse bone cancer cells while at the same time promoting healthy new bone growth through growth factor delivery [109].

Declaration of interest

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