
Chapter

6

APPLICATIONS OF NANOPARTICLE-BASED DRUG DELIVERY SYSTEMS IN BONE TISSUE ENGINEERING

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6.1. INTRODUCTION

Large bone defects caused by trauma, infection, tumour, or other factors are still a big challenge for surgeons to repair. Autologous bone and allograft bone grafts are widely used for the clinical treatment of bone defects; however, there are many drawbacks including limited supply, bone graft resorption, instability in large bone defects, high risk of infection, high failure rates in difficult *in vivo* environments and immunological rejection, all of which impede clinical success [1-6]. Therefore, the urgent need to repair large bone defects has prompted the rapid development of biomaterials and bone tissue engineering. There are three basic elements for bone tissue engineering: biomaterial scaffolds, seed cells and bioactive factors [7]. By combining biomaterials, cells and bioactive factors, tissue engineered bone grafts can provide a native template for promoting the regeneration of bone tissue and finally achieve repair the bone defect. Although lots of basic and clinical researches have shown the feasibility and effectiveness of tissue engineered bone grafts to repair bone defects, there are still many limitations which greatly impede its wider use clinically, such as the uncontrolled release of bioactive factors and insufficient bone formation. Controlled drug delivery of bioactive factors at the bone defect site is essential for triggering and enhancing angiogenesis and osteogenesis of the biomaterial scaffolds and seed cells [8-10]. However, these bioactive factors such as bone morphogenetic protein (BMP), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), and platelet-derived growth factor (PDGF) generally have a short biological half-life, and a rapid inactivation *in vivo*. Repeated administration of these bioactive factors may lead to unexpected side effects including carcinogenicity and toxicity because of uncontrolled drug distribution and accumulation in other tissues or organs [11-13]. An ideal tissue engineered bone graft should have biological properties with a biomimetic local microenvironment and the controlled release of bioactive factors. Thus, a drug delivery system with targeted and controlled release of bioactive factors to the bone defect site is critical to obtain a satisfying therapeutic effect of tissue engineered bone.

To get a controlled and targeted drug delivery system, it should be able to overcome the related limitations to maximise bioactivity while minimising the side effects of bioactive factors [14,15]. Traditional drug delivery systems have difficulty achieving long-term targeted drug release and retaining the stability of bioactive factors *in vivo*. The burst release of drugs makes it hard to achieve continuous and stable drug delivery to simulate osteogenesis by mimicking the natural process [16]. In order to overcome these drawbacks, novel drug delivery systems have been developed with the development of nanotechnology and nanoscale materials. A novel nano-based drug delivery system can achieve site-specific drug delivery and the controlled release of

bioactive factors in the bone defect sites to enhance the bone regeneration. Among these nano-based drug delivery systems, nanoparticles are the most widely used drug carriers, which can control and target the release of drugs into the bone defect sites [17]. The small size of nanoparticles enables better biocompatibility *in vivo*, and its high surface to volume ratio increases drug loading ability and provides better drug bioavailability [18]; meanwhile, the biomaterial property of the nanoparticle can serve as a temporary matrix with which to enhance the mechanical properties of tissue engineered bone. In many researches, the nanoparticle-based drug carrier itself promotes bone regeneration [19,20]. These nanoparticle-based drug delivery systems have been widely used to carry and release bioactive factors to stimulate bone formation in the bone defect site.

With the development of nanomaterials and nanotechnology, this opens up new opportunities in bone tissue engineering with a targeted and controlled drug delivery system to induce and promote bone regeneration. In this chapter, we try to introduce the current developments and applications of nanoparticle-based drug delivery systems used in bone tissue engineering.

6.2. THE PRINCIPLES OF NANOPARTICLE-BASED DRUG DELIVERY SYSTEM

Nanoparticles are usually defined as submicron-sized particles between 1–100 nm in size. These are the most widely used drug carrier vectors, are simply made and have high reproducibility. Many kinds of materials and technological methods can be used to synthesise nanoparticle-based drug delivery systems. Several critical principles need to be considered in order to make intelligent use of a nanoparticle-based drug delivery strategy in bone regeneration.

The first principle is to choose the most suitable material according to the actual situation of clinical application. The inherent physical or chemical properties of the nanoparticle, including the material, size and surface properties, will influence the loading and release of drugs to nanoparticles, as well as their biocompatibility and degradability [21]. There are many kinds of nanoparticle-based drug delivery systems according to different materials which have respective advantages and disadvantages. Liposome nanoparticle-based drug delivery systems have a high drug loading capacity, but their release behaviour is difficult to control [22]. Polymer nanoparticle-based drug delivery systems can be synthesised to generate specific molecular weights and compositions, but their drug loading capacity is low [23]. Particle size is also very important to the biological properties of loaded bioactive factors [24]. Nanoparticle size variation within the nano-scale range can exhibit distinctive physical, mechanical and bioavailability properties, which are very different from in the macroscopic size. Surface properties such as

hydrophobicity also have a significant influence on drug delivery. Surface properties can be modified to improve the loading and release behaviour of drugs, cells and tissue responses *in vivo* [25]. Besides, suitable materials for constructing nanoparticles should have good biocompatible properties, no immunogenicity or toxicity, and suitable biodegradable properties [26].

The second principle is that it is essential to make sure the nanoparticle-based drug delivery system with optimal and controlled drug release behaviour meets the temporal and spatial demands during the process of bone regeneration. Because of the longer period of bone formation compared with other tissues, it needs to make sure that the released bioactive factors can be maintained at the local bone defect area within therapeutic concentrations for a long time as well as the temporal and spatial optimal distribution. The release behaviour of drugs from nanoparticles may be affected by several factors, such as the degradation rate of the nanoparticle, the physiological diffusivity of the loaded drug, the methods used to load the drug and other factors. Biodegradable properties are critical for the release of bioactive factors and should ensure the slow release of bioactive factors *in vivo* to accommodate to the long duration of the bone regeneration process [27].

The third principle is that when the nanoparticle-based drug delivery system is prepared, it needs to ensure the biological activity of the drug when loaded onto the nanoparticle. The conditions of preparation should be mild without harsh solvents, high temperatures or pressures, and extreme pH. After the drug is incorporated into the nanoparticle, the bioactivity of the drug should be examined carefully to avoid any undesirable changes [28].

To achieve better therapeutic outcomes and bone formation, these basic principles should be carefully considered when designing and preparing nanoparticle-based drug delivery systems in tissue engineered bone. Also, we will introduce different kinds of nanoparticle-based drug delivery systems which are used in bone regeneration.

6.3. POLYMER NANOPARTICLE-BASED SYSTEM

Many synthetic polymers have good biodegradability and biocompatibility, and the property of drug loading and release behaviour can be easily improved by changing the molecular mass and surface functional groups. Thus, polymeric nanoparticles are widely used as the best candidates for drug delivery vectors used in bone tissue engineering.

Among these polymeric materials, poly(D,L-lactic-*co*-glycolic acid) (PLGA) exhibits good drug loading property because of its high molecular weight, biologically compatible degradation and free carboxylate end-groups. Therefore, PLGA nanoparticles have been widely used for the sustained release of encapsulated drugs or genes. PLGA nanoparticles have shown a great

therapeutic effect as a drug delivery system in bone tissue engineering [29]. For example, a bone scaffold with controlled releasing recombinant human bone morphogenetic protein-7 (rhBMP-7) was developed to enhance bone regeneration. The rhBMP-7-containing PLGA nanospheres were loaded onto nano-fibrous poly(L-lactic acid) (PLLA) bone scaffolds. *In vitro* results showed that this PLGA nanosphere-immobilised bone scaffold could release rhBMP-7 in a controlled manner. Also, *in vivo* results showed rhBMP-7 delivered from PLGA nanosphere scaffolds induced significant ectopic bone formation, while the passive adsorption of rhBMP-7 into the PLLA bone scaffold without PLGA nanospheres resulted in the failure of bone regeneration at 6 weeks [30].

Besides loading the proteins of growth factors, PLGA nanoparticles can also be used to load bioactive molecules which can enhance bone regeneration. For example, the PLGA nanoparticles were loaded with dexamethasone (DEX) which was a bioactive molecule for bone regeneration. These DEX-loaded PLGA nanoparticles were prepared using the method of water-in-oil standard emulsion and then immobilised onto the surface of a collagen membrane; the release behaviour of DEX from PLGA nanoparticles showed a sustained release of DEX. Also, the *in vivo* results showed this collagen membrane with DEX-loaded PLGA could repair the calvarial bone defects of rats and result in significantly more new bone formation compared with other bone defects that were unfilled or filled with collagen membrane alone [31].

Owing to the good biodegradability of PLGA nanoparticles, it can also be used as a favourable vector for non-viral gene delivery in bone tissue engineering applications. PLGA nanoparticles can offer the protection of genes to nuclease degradation and increase DNA uptake with the sustained release of encapsulated DNA. For example, PLGA containing alkaline phosphatase (ALP) plasmid DNA (pDNA) exhibited high encapsulation efficiency and sustained release behaviour. *In vivo* results of transfection in a rat tibial muscle showed that this gene delivery system based on PLGA nanoparticles allowed 28 days of sustained gene transfection with increased ALP expression levels [32]. Blood vessel growth is necessary for bone regeneration and polymeric nanoparticles have also been used for gene delivery to promote vascularisation. For example, VEGF pDNA-loaded PLGA nanoparticles were prepared and used for *in vitro* cell transfection and *in vivo* gene transfer. The result showed that it could enhance *in vivo* angiogenesis and increase the density of new capillaries [33].

Surface modification of polymer nanoparticles has also been reported to improve the targeting effect of drug delivery in bone regeneration. For example, tetracycline has good adsorption to calcium phosphate and can be used as a targeting adjunct for bone tissue drug delivery. Thus, tetracycline-modified PLGA nanoparticles were prepared and showed a great affinity with natural bone tissue. This surface modification of PLGA nanoparticles can be used as a targeting drug carrier for bone regeneration [34]. Alendronate is also a targeting moiety that has a strong affinity for bone, and other PLGA nanoparticles modified with both alendronate and poly(ethylene glycol) (PEG)

were prepared by the dialysis method. The results showed that this alendronate-modified PLGA nanoparticle had a strong and specific adsorption to hydroxyapatite (HA) which was the main content in natural bone tissue [35]. Besides surface modification of polymer nanoparticles with bone-specific moiety to improve targeting drug delivery in bone, other materials and methods were also used to modify the surface properties of polymer nanoparticles to improve drug release behaviour. For example, heparin was used to modify the surface of nanoparticles to sustain growth factor release. Several growth factors were known to bind heparin tightly, such as bone morphogenetic protein 2 (BMP-2), and transforming growth factor β (TGF- β) [36]. A heparin-functionalised nanoparticle combined with fibrin gel was prepared and used as a bone scaffold with the sustained release of BMP-2. The formation of new bone was significantly enhanced and more mature bone was obtained by using heparin-functionalised nanoparticles in a rat calvarial bone defect model [37]. Other heparin-conjugated PLGA nanoparticle-loaded BMP-2 were prepared and co-cultured with undifferentiated bone marrow-derived mesenchymal stem cells (BMMSCs). *In vitro* and *in vivo* testing found that these heparin-conjugated PLGA nanoparticles loaded with BMP-2 could induce significantly more new bone formation than control group [38].

6.4. LIPOSOME NANOPARTICLE-BASED SYSTEM

Liposome nanoparticles are also widely used as drug-delivery vehicles to load the bioactive factors. Since liposome nanoparticle-based drug delivery systems can be prepared with phospholipids, which form the natural structure of cell membranes, they are regarded as biocompatible and non-toxic. Because of their phospholipid bilayer membrane, they can also pass through the cell membranes and get into the cells [39]. There are many reports about using this liposome nanoparticle-based drug delivery system for bone tissue engineering.

For example, BMP-2 complementary DNA (cDNA) plasmids were loaded with the liposome nanoparticles and used in the repair of cranial defects of a rabbit model. In this study, the BMP-2 Liposome nanoparticle-loaded system showed great bone repair effect. After 6 weeks, the cranial defects of rabbits were filled with new bone after using BMP-2-loaded liposome nanoparticles [40]. Another study showed that the liposome vector could also be effective for *ex vivo* cell-mediated BMP-2 gene transfer. After pre-treatment with BMP-2 cDNA-loaded liposome vehicles, the bone marrow stromal cells were transplanted into critical bone defects in rats. After 6 weeks, the bone defect area was completely repaired with new bone formation [41].

When combined with magnetic particles, the magnetic liposomes can be modified to increase retention of the drug at the target site under magnetic force. For example, magnetic egg phosphatidylcholine liposomes were prepared with the addition of magnetite particles for TGF- β 1 delivery to

stimulate new bone formation in animal models [42]. The magnetic liposomes system loaded with recombinant human bone morphogenetic protein 2 (rhBMP-2) has also been used to treat bone defects in rats [43]. The bone defect was filled with complete bone bridge formation when treated with rhBMP-2 magnetic liposomes. These magnetic liposomes have better bone formation than conventional liposomes due to the longer persistence of BMP at the bone defect site under magnetic force. It may provide a new method for bone defect treatment with the method of using topical magnetic force to control magnetic drug-loaded liposomes at the injured site.

However, there are also some limitations when liposomes are used as drug delivery vectors in bone repair; the drug loading and release behaviour of liposome nanoparticles is relatively difficult to control. Other limitations include low dissociation efficiency, quick degradation of the drug, and instability of the injected liposome drug complex in solvent [44].

6.5. INORGANIC NANOPARTICLE-BASED SYSTEM

Besides the polymer and liposome nanomaterials, ceramic nanomaterials such as calcium phosphate, HA, and bioactive glass are also used for drug delivery and provide mechanical support in bone tissue engineering. There are chemical similarities to natural bone which provide suitable mechanical strength. These inorganic nanomaterials have much longer biodegradation times and special properties such as electrical, mechanical and magnetic functions. These distinct nanomaterials can be used for specific drug delivery systems in bone repair.

For example, a calcium phosphate nanoparticle was prepared to load BMP-2 and then encapsulated in PLGA microspheres. The controlled release of BMP-2 was obtained for over 7 weeks and higher osteocalcin was expressed when using this calcium phosphate nanoparticle [45]. Another plasmid DNA-loaded calcium phosphate nanoparticle was also proven to be an effective non-viral vector for gene delivery and functioned well for odontogenic differentiation through BMP-2 transfection [46]. HA nanocrystals were also used and cross-linked with collagen to control the release of BMP-2. The animal result showed both good mechanical strength and the formation of new bone using these HA nanocrystals [47]. Another bioactive glass nanoparticle (BGn) with loading of ampicillin or siRNA has been prepared and gained potential application in bone regeneration. The results showed that these bioactive glass nanoparticles had good cell viability and excellent apatite-forming ability. While the ampicillin released relatively rapidly, the loaded siRNA could be released for 3 days with almost zero-order kinetics. The siRNA-nanoparticles were also easily taken up by the cells with a transfection efficiency of up to 80 %. It may be a promising drug release system in bone tissue regeneration [48].

6.6. COMPOSITE NANOPARTICLE-BASED SYSTEM

Organic nanoparticles such as polymeric and liposome nanoparticles have good biodegradability and biocompatibility, while inorganic nanoparticles have many other special properties. Combining different organic and inorganic materials into a composite nanoparticle can provide a synergic function to benefit bone regeneration.

For example, a kind of magnetic liposome with incorporated rhBMP-2 was prepared, and the efficiency for bone formation after topical injection was evaluated in a rat bone-defect model. The results showed that the combined treatment of topical magnetic rhBMP-2 liposomes and magnetic implantation at the injury site was effective for the treatment of bone defects [43]. A novel bone cement pellet with sustained release of vancomycin was prepared by combining mesoporous silica nanoparticle and calcium sulphate alpha-hemihydrate. This composite pellet showed a strongly drug-sustained release effect and *in vitro* cell assays showed high biocompatibility and suitability to be used as bone cement in the treatment of open fractures [49]. Another study showed a new tigecycline-loaded calcium-phosphate/PLGA nanoparticles for controlled drug delivery with a double effect. In the first step, a drug was released from PLGA nanoparticles; in the second stage, after the resorption of PLGA nanoparticles, non-bioresorbable calcium phosphate remained the chief part of the particle and took the role of a filler, filling a bone defect. The average size was from 65–95 nm. This composited nanoparticles proved to be an adequate system for local and controlled drug release [50]. Another group of novel composite nanoparticles combining glycidyl methacrylate derivatised dextrans with gelatine was reported. Also, *in vitro* drug release studies showed that the efficient BMP release from this nanoparticle was maintained for more than 12 days under degradation conditions, and more than 90 % of the loaded BMP was released. No obvious cytotoxicity was found in this composite nanoparticle system [51].

6.7. OTHER NANOSTRUCTURE MATERIALS-BASED SYSTEMS

Although the above-mentioned nanoparticles are generally considered the spherical particulate, other nanostructures, such as dendrimers, nanofibres, nanogels and nanotubes, can also be considered generalised nanoparticles [14]. As a result of the different nanostructures, they may offer unique interfacial and functional advantages compared with spherical nanoparticles when used as drug delivery vectors in bone tissue engineering.

Dendrimers have a highly branched dendritic architecture which can be greatly controlled, as can their shapes, sizes, densities and surface properties. The drug can be physically entrapped by the dendritic architecture or

chemically attached to the surface by electrostatic interactions [52]. These advantages make the dendrimers attractive drug-delivery systems used in bone tissue engineering. For example, folate-poly(amidoamine) (PAMAM) dendrimer was used to carry the human bone morphogenetic protein-2 (hBMP-2) gene-containing plasmid for *in vitro* transfection of mesenchymal stem cells (MSCs). All osteogenic markers such as alkaline phosphatase activity, osteocalcin secretion and calcium deposition were significantly stronger in transfected cells. This study showed the possibility of PAMAM dendrimers used for inducing *in vitro* differentiation of MSCs to osteoblast phenotype [53]. Another dexamethasone-loaded carboxymethylchitosan / PAMAM dendrimer was also used to prompt the proliferation and osteogenic differentiation of rat bone marrow stromal cells *in vitro*. The results showed that this drug loaded dendrimer was a promising drug delivery system in bone tissue engineering [54]. The dexamethasone-loaded dendrimer was also evaluated *in vivo* by being implanted subcutaneously on the back of rats and the results showed that it could promote superior ectopic *de novo* bone formation *in vivo* [55].

Nanofibres are defined as fibres with diameters less than 100 nm. Nanofibres can be used to enhance the mechanical strength for tissue engineered bone and mimic the architecture of natural bone tissue [56]. Besides, the high surface-to-volume ratio and high porosity of the nanofibres combined with their nanostructure make them suitable drug carriers, and the drug release rate can be controlled by changing the morphology, porosity and composition of the nanofibres [57]. For example, BMP-2 was immobilised on a membrane surface made of chitosan nanofibres and half of the initial BMP-2 was attached to the membrane surface. This BMP-2-conjugated chitosan nanofibre membrane significantly promoted cell proliferation, alkaline phosphatase activity, as well as calcium deposition, indicating significant and localised bone formation [58]. DEX was also loaded into PLLA nanofibrous scaffolds by electrospinning. This drug-loaded nanofibre not only increased the mechanical strength in comparison with pure PLLA nanofibres, but also showed a sustained release profile for over 2 months. The cell proliferation and osteogenic differentiation of human mesenchymal stem cells cultured with these drug-loaded nanofibres were both improved compared to the scaffolds without drugs [59]. Rifampicin was also reported to be loaded onto the nanofibre meshes by depositing rifampicin-containing PLGA micro-patterns onto the PCL/chitosan biomimetic nanofibre meshes *via* ink-jet printing. This drug-loaded nanofibre mesh not only prevented the biofilm formation, but also favoured the attachment, spreading and osteogenic differentiation of pre-osteoblasts by up-regulating the gene expression of bone markers. This drug-loaded nanofibre mesh provides a feasible multifunctional surface for enhancing bone tissue formation while controlling infection [60].

A nanogel is a nanoparticle composed of a nano-scale hydrogel which presents a cross-linked hydrophilic polymer network with tens to hundreds of

nanometres in diameter. Like hydrogels, the pores in nanogels can be filled with drugs with a high drug-loading capacity for its high surface-to-volume ratio and heterogeneous nanostructure [61]. With regard to their properties of swelling, degradation and chemical functionality can be controlled when chemically or physically cross-linked. As drug vectors, nanogels display extended stability, sustained release and low cytotoxicity when used in bone tissue engineering [62]. For example, prostaglandin E2 (PGE2), a bone anabolic agent, was loaded onto a nanogel of cholesterol-bearing pullulan (CHP) and injected on to the calvariae of mice. This PGE2-loaded CHP nanogel induced new bone formation, while PGE2 alone or CHP alone did not induce any new bone formation. The results showed that this nanogel-based delivery system is efficient for promoting bone regeneration [63]. Another cholesterol-bearing pullulan nanogel-crosslinking hydrogel (CHPA/Hydrogel) was used to deliver BMP and implanted into the calvarial defects. The results showed that BMP loaded nanogel could induce osteoblastic activation and new bone formation *in vivo* [64]. Cholesteryl group- and acryloyl group-bearing pullulan (CHPOA) nanogels were aggregated to form fast-degradable hydrogels by cross-linking with thiol-bearing PEG. Also, two distinct growth factors, BMP-2 and recombinant human fibroblast growth factor 18 (FGF18), were loaded onto the nanogels and then implanted into a critical-size skull bone defect. The results showed that the drug-loaded hydrogel treatment strongly enhanced and stabilised the BMP-2-dependent bone repair by inducing osteoprogenitor cell infiltration inside and around the hydrogel. This report showed the successful delivery of two different proteins to the bone defect to induce effective bone repair by nanogel-based drug delivery systems [65].

A nanotube is a nanometre-scale tube-like nanostructure nanoparticle which can offer advantages over spherical nanoparticles for some applications. Many kinds of materials such as polymers, metals and inorganic materials can be used to fabricate nanotubes [66]. For its special nanostructure, it has large inner volumes which can be filled with kinds of drugs with different sizes, and the open-mouthed structure of nanotubes makes the drug loading process much simpler [67]. The nanotube can be used as a suitable drug vector in bone tissue engineering with its biocompatibility, low cytotoxicity and ability to promote bone formation. For example, DEX was used to be loaded onto the rosette nanotubes and the results showed for the first time that the drug could be easily encapsulated into nanotubes and released for a long time to promote osteoblast function [68]. Another TiO₂ nanotube was also used as a drug vector for loading bone morphogenetic protein 2 and then constructed on titanium substrates covered with gelatine and chitosan. The result showed that BMP-2-loaded nanotube was able to stimulate proliferation and promote the osteoblastic differentiation of mesenchymal stem cells [69]. Another *N*-acetyl cysteine (NAC)-loaded nanotube titanium (NLN-Ti) implant was also prepared as a potential drug delivery system to promote bone formation. *In vitro*, NAC was released in a sustained manner from NLN-Ti implants. Cell viability was

increased and inflammatory responses were decreased when mouse osteoblastic cell line (MC 3T3-E1) cells were co-cultured with the drug-loaded implant. *In vivo* results also showed increased newly formed bone volume and bone mineral density when the drug-loaded nanotubes implanted in the mandibles of rats [70].

6.8. SUMMARY AND FUTURE CHALLENGES

Nanoparticle-based drug delivery systems have generally been used in bone tissue engineering and bone regeneration with its outstanding characteristics. It provides a more effective and efficient method with which to deliver growth factors, genes or other bioactive factors to induce and enhance the process of bone regeneration. Different materials and methods used to prepare the nanoparticles have different properties with respective advantages or disadvantages. Combining different materials and methods to obtain composite nanoparticles as a drug delivery system can have a synergic function and benefit in the form of a better effect of bone regeneration. With the development of nanomaterials and nanotechnology, this drug delivery strategy may be a promising approach with which to overcome the previous limitations of bone tissue engineering when used in the clinic.

However, it is necessary to realise that most nanoparticle-based drug delivery systems are still in the early phases of laboratory research, and their toxicity and safety when used in patients are still lacking. A precise understanding about how different bioactive factors influence the bone regeneration process is still unclear. Thus, the wide use of nanoparticle-based drug delivery systems in clinical application is faced with more challenges.

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