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Review

Recent advances of PLGA micro/nanoparticles for the delivery of biomacromolecular therapeutics

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ABSTRACT

Recent advancements in biopharmaceutical industry have facilitated the development of novel bioactive macromolecular therapeutics. One of the challenges towards the clinical use of these biomacromolecules lies in the selection of appropriate carriers to protect, deliver and release them *in vivo* to maximize their pharmacological effects. Micro/nanoparticles made from biodegradable poly (D,L-lactic-*co*-glycolic acid) (PLGA) have been explored as delivery vehicles for therapeutics. Due to their excellent biocompatibility and controllable biodegradability, PLGA micro/nanoparticles could protect macromolecules from instant degradation *in vivo* while allowing tunable release rate and profile. In this review, recent progress in the design, fabrication/formulation and application of PLGA based micro/nanoparticles for the controlled delivery of biomacromolecules are discussed. Special focuses will be on the novel loading methods and releasing mechanisms of macromolecules as well as the *in vivo* applications of therapeutic macromolecule-loaded PLGA micro/nanoparticles.

1. Introduction

Selection of an appropriate delivery strategy for a therapeutic agent is crucial to the efficacy of disease treatments [1–3]. Conventional drug delivery systems, such as capsules, tablets and direct injection of free drugs intravenously or subcutaneously, were proved effective but mainly for small molecular drugs [4]. The recent rise of biomacromolecular therapeutics, such as proteins, peptides and nucleic acids, has raised the demand of new strategies for drug delivery, as free biomacromolecules usually suffer from short half-life, tendency to degrade and denature in physiological environment [5–7]. Polymeric micro/nanoparticles (MPs/NPs), especially those made with biodegradable polymer, are promising carriers of macromolecular drugs due to their advantages over conventional drug delivery vehicles [8,9]. (I) These particles could provide protection of encapsulated macromolecular drugs from chemical and enzymatic degradation and increase their half-life *in vivo* [10–12]; (II) the small size of particles could help to bypass physiological barrier such as the brain-blood barrier and cell membrane [13–16]; (III) the biodegradability of these particles endow them with sustained and controlled release [12,17–19]; (IV) The availability of various methods for surface modification could enable the particles to target specific organ, tissue, group of cells or the focal area which could result in more efficient delivery or reduced side effects and dosages of drug administration [20–23].

Poly(D,L-lactic-*co*-glycolic acid) (PLGA) is one of the most commonly used biodegradable polymer which is usually synthesized by the ring-open co-polymerization of lactide and glycolide [24]. As the polyester of two α-hydroxyacids which could be metabolized into its constituent monomers, PLGA was initially used as clinical suture materials as they could be completely absorbed by the body after the surgery [25,26]. Due to its good biocompatibility, excellent safety profile and tunable rate of biodegradation *in vivo*, PLGA has been approved by FDA as effective carriers for drug delivery and as scaffolds for tissue engineering [19,27–30]. Formulation of PLGA into micro/nanoparticles, which embraces the advantages of both the biodegradable polymer and miniaturized delivery vehicles, is particularly intriguing for the design of novel drug delivery systems [31]. PLGA-based particles have been used as carriers for over fifteen FDA-approved small molecular drugs and many of them have been reviewed elsewhere [32,33]. Nevertheless, efforts are still needed towards the development and commercialization of PLGA MPs/NPs for the delivery of macromolecular therapeutics. In this review, we aim to overview the recent progress towards this goal. The review will be separated into four parts with an overview on preparation methods of PLGA-based MPs/NPs, an introduction to the current strategies for the loading of biomacromolecular drugs including proteins and nucleic acids into these particles, an explanation of the mechanism used for the controlled release of macromolecules and finally, and the recent examples using PLGA-based particle system for the

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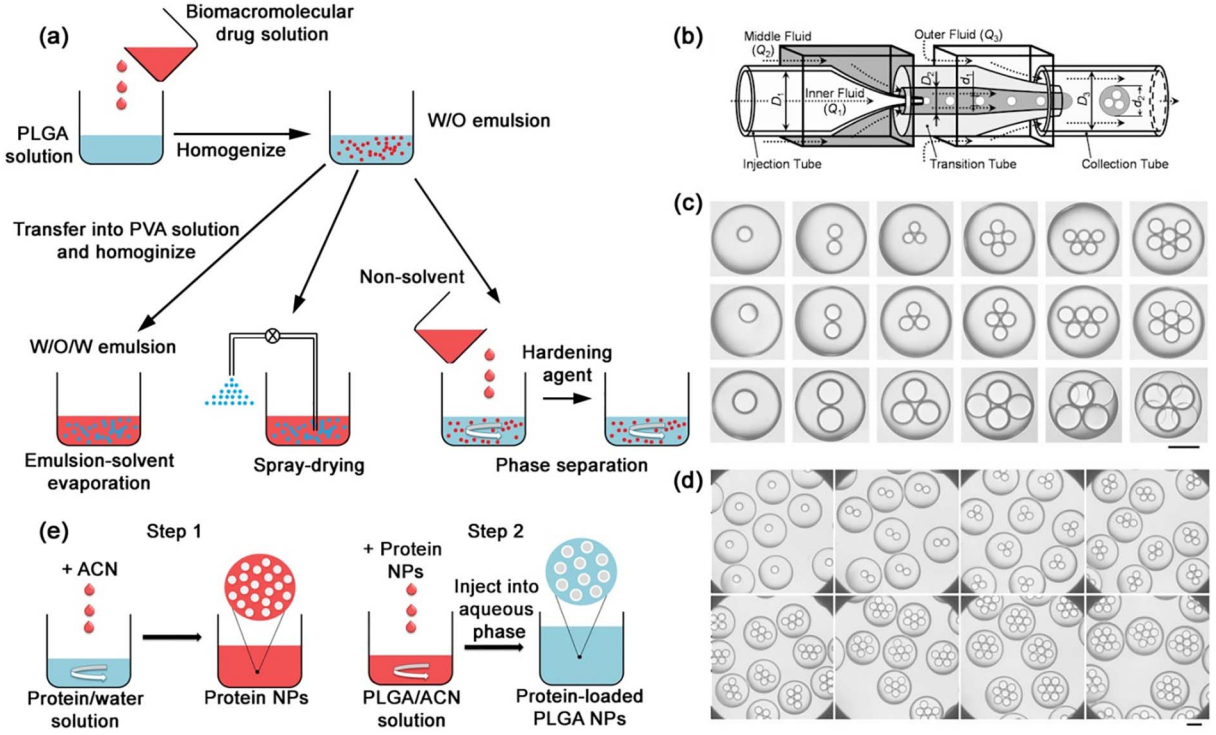

Fig. 1. Preparation of PLGA MPs/NPs. (a) Schematic illustration of preparation methods for PLGA MPs, including emulsification-solvent evaporation, spray-drying and phase separation. (b) Schematic of a capillary device that combines two sequential co-flow emulsion generators. (c) Optical micrographs of monodisperse double emulsions showing controlled increase in the diameter of the inner droplets in each column, while the number of inner droplets is constant. (d) Optical images of monodisperse double emulsions containing a controlled number of monodisperse single emulsions. Both scale bars represent 200 μm. (e) Schematic illustration of the encapsulation of proteins into PLGA NPs by two-step nanoprecipitation. Figures (b)–(d) are reproduced with permission from Ref. [39]. Copyright 2007 WILEY-VCH Verlag GmbH & Co. Figure (e) is reproduced with permission from Ref. [42]. Copyright 2009 Elsevier Ltd.

delivery of various macromolecules with a special focus on *in vivo* efficacies. For the clarification, a size boundary of 1000 nm in diameter is used for the distinguishment of MPs from NPs in this review.

2. Preparation methods of PLGA MPs and NPs

A wide range of techniques have been utilized to fabricate PLGA MPs/NPs for biomacromolecules delivery according to the intrinsic feature of polymer, physicochemical properties of loaded drugs as well as their usages [34]. Most of the methods rely on a preliminary dispersion of aqueous phase in the organic polymer solution which is further processed by specific strategies to prepare biomacromolecules–loaded particles [35] (Fig. 1a). These strategies will be discussed individually and their advantages and drawbacks are summarized in Table 1.

2.1. Preparation of PLGA MPs

2.1.1. Emulsification-solvent evaporation

Emulsion-solvent evaporation technique has become the most frequently used method to prepare biomacromolecules-loaded PLGA MPs. In this technique, PLGA is first dissolved in a water immiscible, volatile organic solvent (*e.g.*, dichloromethane (DCM)). The organic phase is then emulsified in a large volume of water phase by using high speed homogenizers or ultrasound [35] in the presence of emulsifier or surfactant, usually poly vinyl alcohol (PVA). The organic solvent is then removed to form particles under applicable conditions, either by evaporation at reduced or atmospheric pressure or dilution with a large

Table 1
Advantages and drawbacks of techniques for PLGA particles preparation.

Method	Advantages	Drawbacks	Reference
Emulsification-solvent evaporation	Easy of scaling-up; Certain ability of controlling particle size;	Biomacromolecule instability at the interface or under shear stress; Batch-to-batch variance; Polydispersity of particle size.	[36,37]
Microfluidics	Precise of processing parameters; Monodispersity; Ease of fabricating double, triple and even higher order emulsions.	Instrument dependent; Relatively low yield.	[38,39]
Spray-drying	Fast and convenient; Suitable for industrial scaling-up; Less harsh conditions for proteins.	Adhesion of the MPs to the inner walls of the spray-dryer; Difficulty in control of size.	[27,40]
Phase separation	Tunable size with a wide range of processing parameters.	Negative effect of organic solvent on protein/nucleic acid function;	[27]
Nanoprecipitation	Ease of processing; Reproducibility.	Originally developed for hydrophobic drugs; Negative effect of organic solvent on protein/nucleic acid function.	[34,41]

quantity of water or other quenching medium to diffuse the solvent out [27]. The resultant solid particles are then washed to remove PVA and dried by freeze-drying before the final formulation is achieved [38]. Single emulsion is not an appropriate method to prepare biomacromolecules-loaded PLGA particles due to the hydrophilic nature of therapeutic proteins and nucleic acids which make them diffuse into aqueous phase before the polymer solidification. Thus, double emulsion-solvent evaporation technique, usually water-in-oil-in-water (w/o/w) emulsions, has been developed for biomacromolecule encapsulation where a protein/nucleic acid-containing aqueous solution is first emulsified in PLGA-containing organic phase, followed by the second emulsification of the w/o emulsion into a second aqueous phase [36]. There are also exceptions where w/o/o technique is employed for the preparation of protein-loaded PLGA particles [43].

Emulsion-solvent evaporation methods are advantageous due to their relative control of particle size and release rate to certain level. However, they are also associated with inherent drawbacks and problems, especially the biomacromolecule instability [36]. Proteins are susceptible to destabilization at the aqueous-organic interphase and shear stress during homogenization which might cause them to unfold or aggregate [37]. Thereby, excipients including bovine serum albumin (BSA) [44], sugars [45] and polyethylene glycol (PEG) [46] have been employed to stabilize proteins in the formation of emulsion based PLGA particles. In addition, bulk emulsion-solvent evaporation methods are also lack of accurate control over the resultant PLGA particles, such as poor control of size homogeneity, inaccurate loading/dosing, batch-to-batch variance, and unpredictable release kinetics [47]. Microfluidic techniques, on the other side, allow the fabrication of polymeric MPs with precisely controlled size and morphology. In contrast to bulk emulsification methods, emulsions in a microfluidic device are made by precisely fabricating one drop at a time between two immiscible liquid phases. This process results in highly monodisperse emulsion droplets, thus leading to precise control over the size of final products [48]. Another attractive advantage of microfluidic techniques is that they allow the fabrication of double, triple, and even higher order of emulsions, where the size and number of the inner droplets can be accurately tuned [39]. Similarly to bulk double emulsion, microfluidic-based double emulsions could be used for fabrication of microparticles for protein or DNA delivery. In a typical example (Fig. 1b), the device employs two sequential co-flow emulsion generators: an injection tube with a tapered end is inserted into a transition tube, whose other end is also tapered and inserted into a third, coaxially aligned cylindrical capillary tube. The innermost fluid containing the payloads and flowing through the injection tube is emulsified in the transition tube by coaxial flow of the middle fluid and the primary emulsion is subsequently emulsified again in the third tube by coaxial flow of the outermost fluid, which is finally injected into the outer stream through the square capillary. The size and number of both the outer and inner drops can be precisely controlled by tuning the sizes of the orifices and the flow rates of the three fluids [49] (Fig. 1c and d).

2.1.2. Spray drying

Spray drying is a rapid, convenient way to prepare PLGA MPs with mild conditions and very few processing parameters, thereby making it suitable for industrial scaling-up [27]. In this process, drug-loaded microspheres are prepared by spraying a solid-in-oil dispersion or water-in-oil emulsion in a stream of heated air. The type of drug (hydrophobic or hydrophilic) decides the choice of solvent to be used in the process. The nature of solvent used, temperature of the solvent evaporation and feed rate affects the morphology of the final product. This method is known to encapsulate most drugs/proteins into microparticles without significant loss in their biological activity [27]. But the main disadvantage of this technique is the adhesion of the microparticles to the inner walls of the spray-dryer. In addition, control of the particle size is difficult, while yields for small batches are moderate [40]. Various spray drying techniques have been developed for the preparation of PLGA particles for the delivery of proteins or peptides [50–52] and DNA [53].

2.1.3. Phase separation

Phase separation methods are developed to fabricate drug loaded PLGA MPs using liquid-liquid phase separation. Hydrophilic drugs such as protein or peptides are either dispersed in an organic solution of PLGA or preferably processed in an aqueous solution of water-in-oil (w/o) emulsion [35]. Then a non-solvent agent (*e.g.*, silicon oil) that decreases the solubility of PLGA in its solvent, is gradually added to emulsion while stirring, extracting the polymer solvent and resulting in phase separation of polymer by forming a soft coacervate of drug containing droplets. Cargos are encapsulated in PLGA-rich liquid phase (coacervate) and newly formed microspheres (MSs) are immersed into a quenching bath to quench and solidify micro droplets [54]. The PLGA particles are harvested by washing, sieving, filtration, centrifugation or freeze drying. A wide range of processing parameters including polymer concentration, quenching temperature, quenching time and solvent composition could be controlled to tailor the morphology and size of resultant particles [27].

2.2. Preparation of PLGA NPs

All the methods mentioned above for MPs preparation are universal to prepare PLGA NPs by tailoring the processing parameters including the volume ratio of organic phase to aqueous phase and the rate of mixing [27]. Particularly, the most widely used method for the preparation of solid, polymeric nanoparticles is the emulsification-solvent evaporation technique, especially double-emulsion for biomacromolecules-loaded NPs. Another common technique for preparation of PLGA NPs is nanoprecipitation. In this method, polymers and drugs are dissolved in a polar, water-miscible solvent (DMSO, acetone, or ethanol). The solution is added in a drop-by-drop manner into an aqueous solution with surfactant. NPs are instantaneously formed by rapid solvent diffusion. Main advantages of the method are the ease of processing, reproducibility [34] and mild processing condition, as the drugs can be encapsulated without exposure to shearing stress and elevated temperatures [36]. A major problem is that the method was originally developed for encapsulation of hydrophobic molecules [41]. Thus, modifications of nanoprecipitation have been established for the delivery of hydrophilic biomacromolecules. For instance, a two-step nanoprecipitation method has been developed to encapsulate enzymes including lysozyme and α-chymotrypsin into PLGA NPs, where a protein nanoprecipitation step is followed by a second polymer nanoprecipitation step (Fig. 1e) [42]. An encapsulation efficiencies > 70% and a high residual enzyme activity > 90% can be achieved in this method. Moreover, lysozyme was also hydrophobically ion-paired with dextran sulfate and then the complex was encapsulated into PLGA NPs by nanoprecipitation, which also endowed the proteins with high encapsulation efficiency and retention of the enzyme's biological functionality [55]. Moreover, PLGA is also synthesized into block copolymers with polycations and engineered into nanoparticles with a core-shell structure that act as a robust vector for the encapsulation and delivery of negatively charged proteins through electrostatic interactions [56].

3. Loading of biomacromolecular therapeutics into PLGA microparticles and nanoparticles

The loading of biomacromolecules into PLGA MPs/NPs has evolved into diverse directions based on the methods of particle preparation and the attributes of loaded drugs. Proteins could be either absorbed on the particle surface or encapsulated inside the particles, while nucleic acids are more favorable of being loaded through the electrostatic interactions due to their negative charges. The drug loading efficiencies of PLGA particles are influenced by a variety of parameters, including the

type of solvents and surfactants, PLGA concentration, surfactant concentration, and the preparation methods [38].

3.1. Protein encapsulation

For a majority of the fabrication methods discussed above, the encapsulation of cargo proteins into the particles is accomplished simultaneously with the preparation of particles. Proteins are encapsulated into PLGA particles by their dispersion either into organic solvents dissolving PLGA or in an aqueous solution involved in the primary emulsion that could be further processed by w/o/w double emulsion, spraying drying or coacervation [35]. Nanoprecipitation method, on the other hand, involves minor differences in the process to load proteins. Proteins could either be solely precipitated in the first-step nanoprecipitation [42], complexed into hydrophobic ion pair before nanoprecipitation [55] or absorbed into the particles through electrostatic interactions after the nanoprecipitation [56].

3.2. Protein adsorption

Compared to protein encapsulation, less examples of protein adsorption have been established. Protein adsorption on the surface of blank MPs/NPs is conducted by the incubation of PLGA MPs/NPs with protein at desired conditions, including the temperature, buffer composition and pH value, as well as weight ratio of proteins to PLGA [57,58]. Afterwards, excessive proteins could be removed from the particles by ultracentrifugation or gel filtration. Examples of protein adsorption include proteinaceous antigen, enzyme, and BSA [57].

3.3. Nucleic acid loading

Loading of nucleic acids by positively charged polymers or oligomers has been widely established, either by forming polyplexes with polycations [59,60] or by condensing with polycation-functionalized NPs [61–63]. On the other hand, direct adsorption/condensation of DNA or RNA molecules into PLGA MPs/NPs is rather difficult due to the negative charges of both entities. Similarly to the loading of proteins, DNA has been encapsulated into PLGA NPs by the dispersion in the first aqueous phase of w/o/w emulsion [64–66]. However, due to the labile nature of nucleic acids as a major limiting factor, conditions used in the NPs preparation, such as sonication, may create shear stresses, leading to their degradation [64,67]. Thereby, another way of loading nucleic acids is the conjugation of PLGA with other polymers, especially cationic polymers, for instance polyethylene glycol (PEG), polyethylenimine (PEI) [68–70] and poly-L-lysine [71], before the loading of DNA or RNA. These polymers could complex with nucleic acid molecules, enhance cellular uptake, and endosomal escape of PLGA NPs. PEGylation of PLGA NPs also increases their solubility and stability, blood circulation half-life, but decreases their immunogenicity, reduces the intermolecular aggregation and finally avoids recognition of NPs by reticuloendothelial system (RES) [72,73]. Moreover, recent studies are evolving into the blending and coating/surface modification of PLGA NPs with positively charged polymers and surfactants *via* a variety of methods that enable the complexation with nucleic acids in the next step, which include PEI [74–77], cationic lipids [78], chitosan [79,80] and carbosilane dendrons with protonable amine groups [81]. For blending, one strategy is to mix the cationic polymers with PLGA during particle preparation to endow them with positive surface charges before the loading of nucleic acids [82]. Moreover, PLGA is also physically blended with cationic polymers for instant DNA loading, where plasmid DNA is incorporated into PLGA NPs as a result of electrostatic interactions with poly(amino ester) [83]. The loading efficacy is positively dependent on the context of poly (amino ester) in NPs. For surface modification, a straightforward way is the direct decoration of cationic polymers or lipids on the as-prepared NPs' surface by electrostatic interactions [74,75,84], or hydrophobic interactions between lipids and PLGA [78]. But the PEI was associated with increased cytotoxicity compared to non-modified PLGA NPs [74]. Moreover, surface functionalization with PEI only gives rise to moderate transfection of 293 T cells, while a further conjugation with PEG cross-linkers is needed for a greater transfection efficacy [77]. Besides the decoration of as-prepared NPs, another simpler method is to coat the PLGA NPs with cationic polymers (*e.g.* chitosan) during solvent evaporation and formation of PLGA NPs [80,85], which is advantageous in reducing the complexity of processing. This protocol or polymer also allows the NPs a high DNA loading (up to 100 wt%, DNA/NP) [80]. In addition, in the case of co-delivered small molecular drugs with nucleic acids, such chitosan coating also changes the release profile of co-delivered drugs [85]. Apart from the non-covalent modifications, covalent conjugations have also been developed for the loading of nucleic acids onto PLGA NPs. For instance, PLGA NPs have been conjugated with cationic carbosilane dendrons through the carbodiimide reaction which link the free carboxylic groups of the polymer along with the amine focal group of the dendrons, thus allowing the loading of oligonucleotides before a further coating of PEG for the sake of protecting the payload [81]. These methods suffer from complex chemical synthesis, but allow the customization of modified polymers, *e.g.* the dendrons could be further extent in length to modulate the complexation ratio with nucleic acids. In summary, diverse loading methods of nucleic acids into or onto PLGA NPs are correlated to a variety of attributes for the drug delivery systems, such as the complexity of preparation, the loading efficiency, the transfection efficacy, cytotoxicity and release of co-delivered drugs. One would be able to choose the proper modification polymer and method in accordance with their specific purpose.

4. Drug release from PLGA microparticles and nanoparticles

Generally, drug release from PLGA particles is achieved through a combination of diffusion and erosion both at the surface and in the bulk, which can be tailored by the physicochemical attributes of PLGA polymers [86]. Water absorption by the PLGA polymer happens immediately upon the contact of PLGA particles with water or administration *in vivo*. This water-filling process creates pores inside the polymer matrix, which increase in size and number as a function of time, resulting in a porous inter-connected network which enables the drug diffusion from the polymer matrix [87]. In addition, the desorption of bound protein molecules from particle surfaces has been reported as another pathway of drug release [20]. PLGA undergoes degradation immediately by hydrolysis through the cleavage of backbone ester bonds and subsequent decrease in Mw, upon the exposure to water. Hydrolysis of PLGA produces carboxylic groups and locally further catalyzes hydrolysis of PLGA [88]. This auto-catalytic phenomenon is reported to cause heterogeneous degradation inside PLGA matrices, for example faster degradation at the center of the PLGA matrix than at the surface. The hydrophobicity of polymers decreases with decreased Mw, which finally become water-soluble at a Mw of ~1.1 kDa with continuing hydrolysis [86] and diffuse into the release medium, resulting in erosion, the mass loss of polymer of PLGA particles. The degradation of PLGA copolymer is the collective contribution of bulk diffusion, surface diffusion, bulk erosion and surface erosion [27]. Generally, drug release from PLGA particles exhibits a classic tri-phasic pattern [86]. It starts from an initial burst (phase I) due to non-encapsulated drug on the particle surface or drug molecules close to the surface that is easily accessible by hydration [89]. Afterwards, there is a slow release phase (phase II), during which the drug diffuses either through the relatively dense polymer or through the limited existing pores as polymer hydration and degradation take place. It is followed by a faster release (phase III) attributed to polymer erosion, which is sometimes called a second burst release. It's also noticed that not all release profiles from PLGA particles follow the classic tri-phasic pattern. For example, if the second phase is fast, there may be a slower release in phase III [90,91].

One of the most distinguishing features of PLGA polymers as drug delivery systems is the possibility of tuning the physicochemical properties of PLGA and their particles in order to achieve the desired release profile [35]. Polymer related factors including the molecular weight and lactide/glycolide ratio, particle size and morphology and release medium conditions are all import parameters that could be modulated to control drug release profiles. Particularly, “smart” polymeric particles that are responsive to environmental stimuli are emerging as advanced formulations in PLGA based drug delivery systems in recently years [92,93].

4.1. Physicochemical features of PLGA

4.1.1. Lactide/glycolide (L/G) ratio

Polymer composition is the major factor impacting particle degradation and drug release rate from PLGA particles. PLGA polymer composition (lactide/glycolide ratio) is correlated to the hydrophilicity, crystallinity, and hydration rate of the PLGA polymers [35]. The polyglycolide acid is more hydrophilic attributed to the absence of methyl side group than poly-lactide [94]. Thus a higher content of glycolic acid in the copolymer is linked to more water uptake and consequently faster degradation and erosion. For example, PLGA 65:35 (L/G) shows faster degradation than PLGA 75:25 and PLGA 85:15 [95]. It's also reported that the protein antigen (HBsAg) release from PLGA microspheres was associated with the polymer composition (L/G ratio) [96]. The L/G ratio also influences the crystallinity of polymers, which indirectly affects the degradation rate [27]. It has been demonstrated that a decrease in lactide proportion is corresponded to a decline in the degree of PLGA crystallinity [54], whereas decayed degradation rate was discovered with increased crystallinity [97]. In one word, the L/G ratio is a critical parameter in modulating the degradation, thus the drug release rate from PLGA MPs/NPs.

4.1.2. Molecular weight and polymer end functionality

Drug release from PLGA particles is also relied on the average molecular weight of polymers. Polymers with higher molecular weights have longer polymer chains, which deserve longer durations to degrade than shorter polymer chains, thereby causing slower release rate [34,98]. On the other side, end moieties of PLGA polymers affect the water absorption and subsequently the degradation rate of particles. It's reported that modification with hydroxyl end groups resulted in slower degradation than unmodified carboxylic group [99]. In general, low L/G ratio, low Mw and uncapped PLGA polymers endow them with increased water uptake, accelerated hydrolysis and erosion, and thus accelerated drug release [98].

4.2. Particle size, morphology and surface modification

Various studies have also demonstrated the effects of particle size and surface morphology on drug release from PLGA particles [100]. Larger particles have a less burst release than smaller counterparts [20], since they have a longer diffusion path length. It's also shown that bulk degradation is faster than pure surface degradation for PLGA, which allows a faster release of drugs from smaller particles [27]. Moreover, it's reported that a higher L/G ratio is associated with higher surface roughness [94], but decreased surface porosity [101]. These multiple factors also work collectively to determine the drug release profile from PLGA particles [34]. As particles become larger, there is an increase in diffusion path length which negatively impacts the release rate. On the other hand, larger particles undergo more autocatalysis in hydrolysis [88]. Furthermore, surface modification also directly changes the drug release profile of PLGA particles. For instance, surface coating with chitosan strikingly resulted in an improvement in the release kinetic of the model protein BSA from PLGA MPs by reducing initial burst release and extending continuous release, where chitosan performed as a diffusion barrier [102].

4.3. Properties of loaded drugs

Besides the factors coming from PLGA polymers and the particles made thereof, the loaded biomacromolecules also contribute to the control of their release profile, in terms of their molecular weight, solubility in the release medium, polymer-drug interactions and drug loading efficiency [20,34]. Diffusion of proteins in the water-filled pores depends on their solubility and size. Regarding polymer-drug interactions, the presence of the drug might frustrate the rate of hydrolysis, which becomes more serious for the high-Mw and lactide-rich polymers [103]. Moreover, Sandor et al. investigated the effect of protein molecular weight and loading amount on release kinetics. In low protein loading efficiency, release of larger proteins was dominated by diffusion through pores, while the release for smaller proteins initially relied on diffusion through pores and on degradation at later stages. At higher protein loading, however, there was no obvious transition from diffusion-based to polymer degradation-based release due to the presence of more interconnecting channels [104].

4.4. Release conditions

The conditions of release medium, such as pH, ion strength, sink condition and surfactants also greatly impact the release of drugs from PLGA particles [34,86]. The pH of release medium plays a key role in controlling protein release from PLGA particles by affecting the polymer degradation rate and protein stability [105]. For example, an accelerated release at lower pH (2.4) after the initial burst release from PLGA microspheres was observed compared to physiological pH [106]. In addition, ionic strength in the release medium tunes the release rates through regulating the swelling status of the polymer matrix which could promote or hinder the protein diffusion from the particles [101].

4.5. Stimuli-responsive release

In recent years, stimuli-responsive “smart” materials-based drug delivery systems (DDS) have attracted extensive and continuously increasing attentions due to their capability to react in a specific and predictable way upon their exposure to certain external/internal environmental stimuli [107–110]. These environmental triggers could be physical (*e.g.* temperature, light, electric field, magnetic field), mechanical (*e.g.* ultrasound), chemical (pH and certain molecules *e.g.* glucose) or could be biological factors such as specific enzymes [92,111,112]. “Smart” materials are able to change their conformational structure, chemical or optical features in response to one or more of the above-mentioned stimuli [113]. These “smart” capabilities endow the drug delivery systems with controlled-release of the cargos, thus leading to overall mitigation of their side-effects and improved treatment efficacy [111]. In other words, PLGA-based drug delivery systems could be sophisticatedly designed to control the release of drugs with desirable pharmacokinetics at their specific targeting sites.

A majority of recent works for the development stimuli-responsive PLGA-based MPs/NPs have focused on the delivery and controlled release of small molecules including docetaxel [114], doxorubicin (DOX) [115–117], paclitaxel [118], 5-FU [119], and busulfan [120]. On the other side, “smart” stimuli-sensitive PLGA particles engineered for the delivery of biomacromolecular therapeutics were also reported in a few studies, especially those with pH or ultrasound responsiveness, which will be further discussed in the following section.

4.5.1. pH-responsive release

It is well-known that there are significant pH gradients between healthy organs within human body, especially in the gastrointestinal tract (organ level). Additionally, there is a lower pH in tumors compared to corresponding normal tissues (*i.e.* 7.4) (tissue level) and considerable pH variances inside cells (*e.g.* lysosomes (4.5–5), endosomes (5.5–6), and cytosol (7.4)) (cell level) [111,121]. Stimuli-responsive

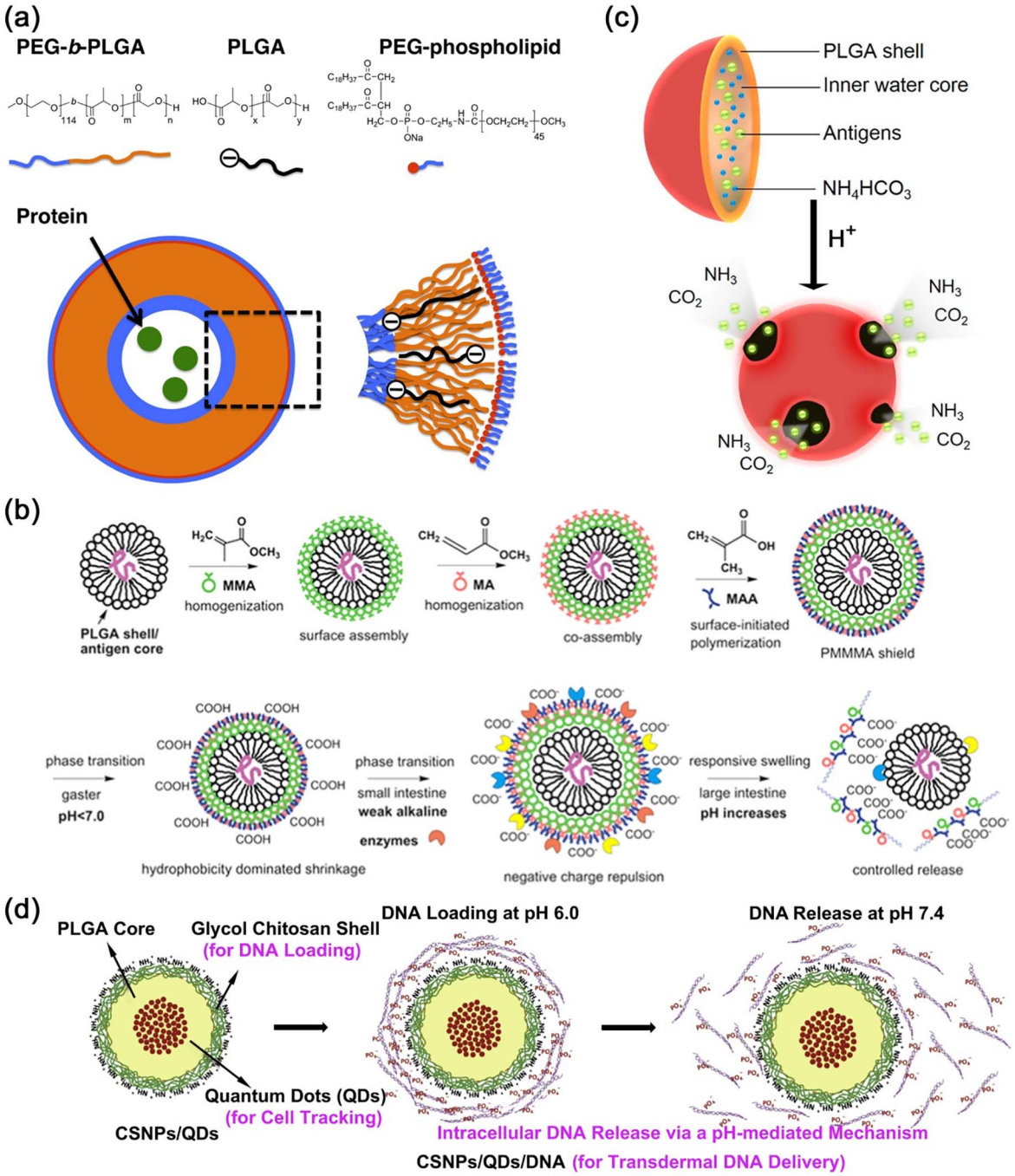

Fig. 2. Examples of pH-responsive release of proteins and DNA from PLGA particles. (a) Schematic illustration of the reverse polymer micelle made of PLGA, PEG-*b*-PLGA and PEG-phospholipid. The system released the proteins under neutral and basic conditions, while retaining them inside particles at acidic pH. Reproduced with permission from Ref. [133]. Copyright 2014 Elsevier Ltd. (b) Schematic illustration of synthesis and controlled release property bilayer (PTRBL) PMMMA-PLGA particles sensitive to the pH and trypsin for oral vaccination. The pH-dependent phase-transition resulted in large intestine targeted release through small intestine escaping mechanism owing to the surface negative charge repulsion. Reproduced with permission from Ref. [134]. Copyright 2016 Elsevier Ltd. (c) Schematic illustration of the composition and structure of gas-generating pH-responsive PLGA NPs for antigen delivery and their working mechanism. Reproduced with permission from Ref. [132]. Copyright 2015 ACS. (d) Schematic illustrations of the multifunctional core-shell PLGA nanoparticles designed for transdermal DNA delivery and its pH-mediated DNA release mechanism. Reproduced with permission from Ref. [137]. Copyright 2010 Elsevier Ltd.

DDS have been engineered to possess specific pH-sensitive functionalities in order to be capable of targeting specific sites according to the physiological pH gradients/variances mentioned above in specific organs, intracellular organelles or even pathological sites such as cancers and inflammatory tissues [122–127]. There are 3 major strategies to develop pH-sensitive DDS, either by utilizing polymers with ionizable moieties [128], by coupling acid-cleavable bonds into the polymers [129–131], or by incorporating gas-generating entities into the particles [132].

Compared to other stimuli, pH is more utilized in the design of responsive PLGA particles for modulated drug release. Koyamatsu et al. reported reverse polymer micelles made from an amphiphilic diblock copolymer of PLGA and PEG and PLGA molecules with a terminal carboxyl moiety [133] (Fig. 2a). The micelles loaded with BSA and streptavidin released the proteins under neutral and basic conditions, whereas the proteins remained in the particles at acidic pH. Zhang et al. fabricated poly [(methyl methacrylate)-*co*-(methyl acrylate)-*co*-(methacrylic acid)]-PLGA (PMMMA-PLGA) NPs with a pH-switchable

phase-transitional coating layer for oral delivery of vaccines [134] (Fig. 2b). The PMMMA shells with pH regulated swelling and phase transition protected PLGA NPs and drugs from harsh conditions and digestion in the stomach and small intestine (acidic and weak alkaline conditions), avoided selective cellular uptake of the NPs in the small intestine, and subsequently allowed targeted release of PLGA/antigen NPs due to increased pH in the large intestine for cell uptake and vaccination, where a high level of specific antibody IgM was produced in the *in vivo* studies. Similarly, NPs composed of block copolymers of PLGA and PEG [135] and hydrophobic ion pairing (HIP) technique-based PLGA particles [136] were used for the oral delivery of insulin, which exhibited a pH-dependent release profile: a low total release inside stomach (pH ~ 2) in the beginning, followed by a slower release at pH 6.6/6.8 and a much faster release at pH 7.4. It could be concluded that these PLGA particles were relatively stable in the stomach, gradually dissolved in small intestine and then almost completely dissolved in colon, acting as promising candidates for oral delivery of insulin.

In addition to the pH-sensitive shielding or polymer modification, PLGA particles with gas-generation capability have also been utilized in the regulation of protein release upon the exposure to pH stimuli. Liu et al. fabricated pH-dependent PLGA NPs with rapid intracellular release of antigen [132] (Fig. 2c). The PLGA NPs had large inner space for the loading of antigens and ammonium bicarbonate (NH_4HCO_3), which performed as an antigen release trigger in dendritic cells (DCs). Lower pH in endosomes and lysosomes (~5.0 and 6.5) allowed the reaction between hydrogen ions (H^+) and NH_4HCO_3 to generate NH_3 and CO_2, which broke NPs, released antigens and induced greater lymphocyte activation, stronger cytotoxic capacity and enhanced production of antigen-specific $CD8^+$ T cells and specific IgG antibodies.

Besides proteins, DNA could also be incorporated into pH-switchable PLGA NPs for controlled release. For example, positively-charged glycol chitosan (GC) was employed to coat hydrophobic PLGA NPs where fluorescent quantum dots (QDs) were encapsulated [137] (Fig. 2d). DNA was loaded into the chitosan coating at lower pH due to the electrostatic interactions and released in a pH-mediated mechanism for transdermal DNA delivery: a lower release rate at pH 6.0 which was largely accelerated at pH 7.4 that mimicked the pH environments in the cytoplasm and nuclei of cells. Gaspar et al. reported the formulation of gas-generating pH-responsive D-α-tocopherol PEG succinate (TPGS-PLGA) hollow microspheres loaded with both DOX and minicircle DNA (mcDNA) nanoparticles as a strategy for the co-delivery of drug and DNA [138]. Microcarriers incubated at acidic pH enabled a CO_2 gas-mediated payload release, demonstrating the stimuli-switching characteristics of these carriers.

4.5.2. Ultrasound-responsive release

In addition to pH, there have been also a few studies investigating "smart" PLGA particles sensitive to ultrasound for controlled release of biomacromolecules. Ultrasound is of great importance in modern medicine since they play important roles in many medical applications such as diagnosis (*e.g.* imaging at low frequencies) and treatment (*e.g.* the removal of tumors at high frequencies) [111]. It has been involved recently as a physical stimulus in drug delivery owing to its simplicity, security, availability and real time applications [139].

Di et al. have fabricated insulin-loaded injectable polymeric nano-network based on PLGA NPs which was sensitive to ultrasound [140] (Fig. 3a). The nano-network (NN) is obtained by mixing oppositely charged nanoparticles together: PLGA NPs coated with positively charged chitosan or negatively charged alginate. The insulin release was triggered by ultrasound due to cavitation induced by focused ultrasound system (FUS). In the *in vivo* study using chemically-induced adult diabetic mice, a rapid decrease of blood glucose level was observed when the nano-network treated animal was exposed to FUS for 30 s, suggesting the pulsatile release profile of insulin resulting from ultrasound. Importantly, repeated FUS treatment at time intervals allows this ultrasound-triggered insulin delivery system to regulate insulin delivery. Bao et al. developed chitosan functionalized PLGA microspheres for the delivery of lysozyme. High-intensity focused ultrasound (HIFU) was used as a trigger to enable shape memory and lysozyme release of cylindrical rod made of these microparticles [141] (Fig. 3b). The incorporation of chitosan into PLGA particles could tailor the transition temperature of the material from 45 to 50 °C, affect shape memory ratio of the fabricated cylindrical rod, and more importantly, synchronize the release kinetics of the encapsulated enzyme in the rod in a switchable manner. Furthermore, the two processes could be modulated by adjusting the acoustic power and ultrasound treatment duration.

5. Macromolecular therapeutics delivered by PLGA MPs/NPs

Similar to other types of polyesters such as PLA and PHA, the good biocompatibility of PLGA makes it a promising material for drug delivery [142–145]. PLGA based pharmaceutical microspheres have been developed for over 30 years [146,147]. The small size of PLGA MPs/NPs as well as facile methods for surface modification makes it possible to circumvent physiological barriers and to release the drug at target tissue/organ/cell [35]. Thus, the dose of the drug can be reduced and the side effect can be alleviated. Although most of the current commercialized PLGA particle-based drug products are loaded with small molecular weight drugs, the increased number of protein/peptide based drugs on the market and the maturation of the gene therapy boosts the research on the development of PLGA based micro/nano-vehicles for macromolecular therapeutics.

5.1. PLGA-based MPs/NPs for protein drug delivery

The enzymatic and chemical degradation of protein drugs *in vivo* makes it challenging for therapeutic delivery. The formulation of these drugs into polymeric MPs/NPs, however, could possibly protect them from degradation and provides more efficient and sustained delivery [148–150]. MPs/NPs made from PLGA and their derivatives are among the most frequently used carriers for protein delivery and recent publications have shown efforts towards effective delivery of a variety of proteinaceous therapeutics. They are summarized in Table 2 and discussed below.

5.1.1. Insulin

The management of type I diabetes mellitus requires the routine administration of insulin for glycaemic control. However, the current standard administration method is still through invasive subcutaneous or intravenous injection which requires daily injection and is painful. Therefore, PLGA-based MPs/NPs have been engineered to provide non-invasive solutions for insulin delivery. An early study showed that PLGA nanospheres with mean diameter of 400 nm could be used as vehicles for pulmonary delivery of insulin [151]. The ultrasonic nebulized droplets of insulin-loaded PLGA nanospheres can be easily inhaled through a respirator. As a result, the blood sugar level of the guinea pig was reduced immediately and the hypoglycaemia was prolonged to over 48 h. In another work, PLGA-cyclodextrin porous MPs were formulated for aerosol based pulmonary delivery of insulin [152]. The insulin was efficiently delivered to the pulmonary alveoli of the rats and an insulin dose as low as 0.5 IU/kg was able to trigger prolonged hypoglycaemic effect in type I diabetic rats (Fig. 4). Nevertheless, the aerosol delivery requires specialized nebulizer or inhaler and the different lung condition of individuals could dramatically influence the effect of the delivery [153], both of which could limit the effect of insulin delivery. As an alternative, PLGA-based NPs were also designed to bypass the physiological barriers (chemical, enzymatic and absorption barriers [154]) for oral delivery of insulin. As an example, PLGA-phospholipid complexing NPs were fabricated for oral delivery of insulin [155]. The introduction of phospholipid increased the liposolubility of insulin and enhanced the loading efficiency. The *in vivo*

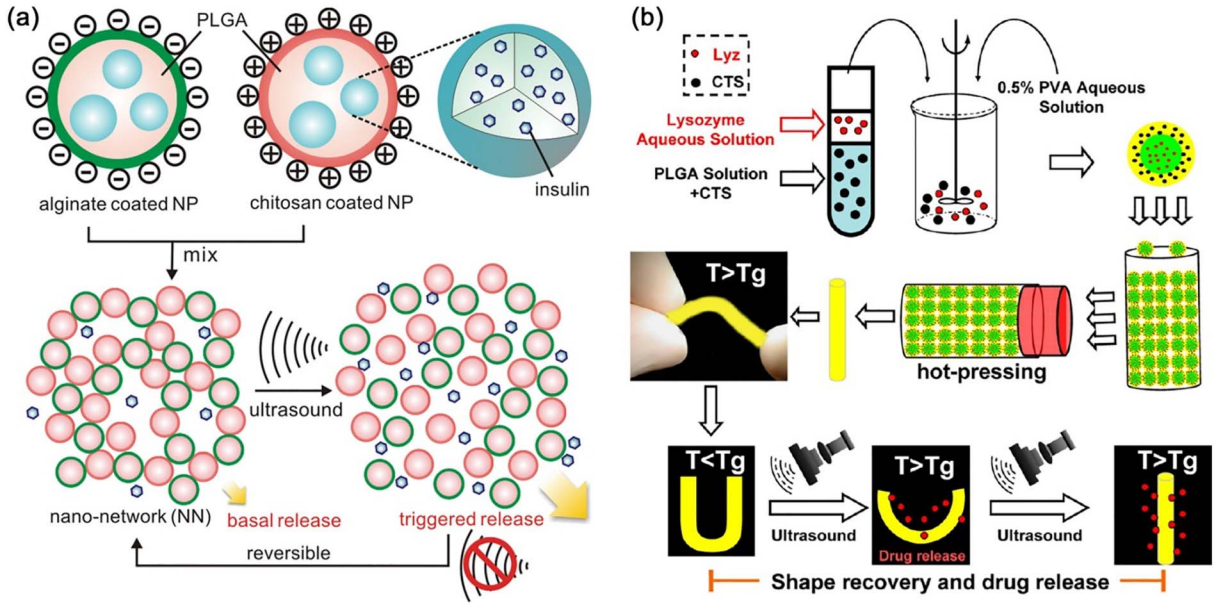

Fig. 3. Examples of ultrasound-responsive PLGA-based drug delivery systems. (a) Schematic of the FUS-introduced insulin delivery using nano-network (NN) made of oppositely charged PLGA NPs which were loaded with insulin and coated with chitosan and alginate respectively. The FUS triggered the dissociation of NN and promoted insulin release from the formulation. Reproduced with permission from Ref. [140]. Copyright 2014 WILEY-VCH Verlag GmbH & Co. (b) Schematic illustration of the preparation and ultrasound-responsive shape recovery and drug release from lysozyme-load PLGA MPs which comprised cylindrical rod. Reproduced with permission from Ref. [141]. Copyright 2013 ACS.

experiment results showed a reduction of blood sugar level in diabetic rats peaked at 6 h after administration and the hypoglycaemic effect continued for > 12 h. Nevertheless, the bioavailability was found to be 7.7%, lower than the clinical requirement (> 15%) which indicated the protection of insulin functionality with PLGA-phospholipid matrix could not eliminate the degradation imposed by gastrointestinal fluids. Indeed, PLGA particles based oral insulin delivery systems reported elsewhere all fall short of bioavailability (mostly between 5 and 10%) [156–159]. Towards more effective oral insulin delivery, Sun et al. recently developed pH-responsive microcapsules encapsulating PLGA NPs as insulin carrying vehicle [136]. To render a pH-responsive releasing profile, copolymer of methyl acrylate, methyl methacrylate and methacrylic acid (PMMMA) was used to encapsulate PLGA NPs to form core-shell microcapsules. As PMMMA is water soluble only in neutral and basic environment [160], the PMMMA shell preventing insulin from a burst release at pH 1.2 (stomach) and ensured a sustained release of insulin at pH > 6.8. A bioavailability of 15.6% was achieved in the subsequent *in vivo* release test using the insulin-loaded microcapsules as compared to that of only 0.5% with naked insulin administration. The results indicated the effective protection of insulin-loaded PLGA core from gastric degradation by pH-responsive out-shell and could lead to an insulin delivery system with applicable clinical applications.

5.1.2. Growth factors

Growth factors represent a group of functional proteins which stimulate cell growth, proliferation and differentiation and are crucial in the regulation of multiple cellular processes. They have been intensively used as therapeutics for the treatment of various kinds of diseases including cancer [161], cardiac disease [162], skin wound [163], and intervertebral disc degeneration [164]. Biocompatible PLGA-based MPs/NPs were proved to be advantageous for the delivery of these growth factors. Vascular endothelial growth factor (VEGF) is a signal protein with major role in angiogenesis [165]. It has been used as a therapeutic to accelerate the cardiovascular, muscle, bone and neuron repair [166–168]. PLGA MPs/NPs have been employed for efficient VEGF delivery with potential clinical applications, such as the treatment of avascular necrosis [169]. Golub et al. fabricated 50:50 PLGA NPs loaded with VEGF for limb treatment [170]. The release of VEGF was found to last for 2 weeks with the majority released in 4 days. A mouse femoral artery ischemia model was used to evaluate the effect of treatment and a significant increase in total vessel volume was observed in mice treated with VEGF-loaded PLGA NPs compared to mice treated with naked VEGF. Besides, VEGF-loaded PLGA MPs were injected into myocardial infarcted mice for cardiovascular recovery [171]. One month after the treatment, angiogenesis as well as arteriogenesis were observed in the mice injected with VEGF-loaded MPs and more new capillaries and arterioles were formed compared to the mice administrated with free VEGF. These results substantiated the efficient protection of VEGF by PLGA and the sustained release profile due to the biodegradability of PLGA.

Fibroblast growth factors (FGFs) are a family of growth factors which bind to their specific cell-surface receptors (FGFRs) to trigger various signalling pathways and are crucial players to regulate cell proliferation and differentiation [172,173]. They have been used as therapeutics for treatment of cardiovascular disorder, cancer and skin wound [174]. PLGA based MPs/NPs could enable sustained release and could also increase the half-life of the degradation-prone proteinaceous growth factors. Jeon et al. fabricated heparin-conjugated PLGA nanospheres with surface attached FGF2 [175]. The nanoshperes were embedded inside a fabrin gel for the controlled delivery of FGF2. The sustained release of FGF2 lasting up to 21 days was achieved and more importantly, the released FGF2 remained bioactive after 15 days which is much longer than the half-life of free FGF2 in physiological condition (7.6 h) [176]. With a mouse limb ischemia model, more neovascularization was observed in mice treated with the NP-loaded fabrin gel as compared to the mice treated with daily injection of FGF2. Moreover, FGF2 could also be loaded into PLGA-PEG NPs for delivery to brain with intranasal administration [177]. Due to their small size, the NPs can pass the blood-brain barrier and bioactive FGF2 can be found in the central neuron system of the rats. The Morris water maze test showed an improved learning and memorizing ability of the Alzheimer's disease model rats after the administration, making it a potential treatment strategy for the disease. Despite these two examples, FGF-loaded PLGA MPs/NPs were also employed to affiliate bone transplantation [178], to promote arteriogenesis [179] and to repair the spin cord injury [180],

Table 2
Summary of PLGA based MP/NP systems for the delivery of protein drugs *in vivo* as covered in this review.

PLGA formulation	Type of protein	Particle type and size	Delivery target	Disease	Animal model	Administration route	References
Insulin							
75:25 PLGA	Insulin	NP[a]; 400 nm[b]	Lung	Diabetes	Guinea pig	Inhale	[151]
50:50 PLGA, β-cyclodextrin	Insulin	MP; 26 μm	Lung	Diabetes	Rat	Intratracheally	[152]
50:50 PLGA, phospholipid	Insulin	NP; 100–400 nm	GI tract	Diabetes	Rat	Oral	[155]
50:50 PLGA, hypromellose phthalate	Insulin	NP; 150–170 nm	GI tract	Diabetes	Rat	Oral	[156]
50:50 PLGA, *N*-trimethyl chitosan	Insulin	NP; 250 nm	GI tract	Diabetes	Rat	Oral	[157]
50:50 PLGA, chitosan	Insulin	NP; 134 nm	GI tract	Diabetes	Rat	Oral	[158]
50:50 PLGA, chitosan, folic acid	Insulin	NP; 170–250 nm	GI tract	Diabetes	Rat	Oral	[159]
75:25 PLGA, PMMMA[c]	Insulin	NP; 213 nm; microcapsule; 1–5 μm	GI tract	Diabetes	Rat	Oral	[136]
Growth factors							
50:50 PLGA	VEGF[d]	NP; 200–600 nm	Femoral artery	Hindlimb ischemia	Mouse	Local intramuscular injection	[170]
50:50 PLGA	VEGF	MP[e]; 5 μm	Heart	Myocardial infarction	Rat	Intramyocardial injection	[171]
50:50 PLGA, heparin	bFGF[f]	NP; < 250 nm	Femoral artery	Hindlimb ischemia	Mouse	Local intramuscular injection	[175]
50:50 PLGA-co-PEG-maleimide	bFGF	NP; 105–120 nm	Brain	Alzheimer's disease	Rat	Local injection (hippocampu)	
50:50 PLGA	bFGF and VEGF	MP; size not mentioned	Bone	bone Transplantation	Rat	Incorporation in bone graft	[178]
85:15 PLGA	bFGF	NP; 100 nm	Femoral artery	Hindlimb ischemia	Mouse	Artery catheterization	[179]
50:50 PLGA, heparin	bFGF	NP	Spin cord	Spin cord injury	Rat	Intrathecal injection	[180]
Growth factors							
50:50 and 75:25 PLGA	IL-1α[g]	MP; 50–70 μm	Limb	Tumor	Mouse	Local injection (tumor)	[186]
50:50 PLGA, PLA and dextran	IL-2[h]	MP; 50 μm	Dorsal cervical	Colon carcinoma	Mouse	Local injection (tumor)	[187]
50:50 PLGA	TGF-β[i]	NP; 100–300 nm	Lymphoid organ	Autoimmune disease	Mouse	Intraperitoneal injection	[188]
50:50 PLGA	NRG1[j], FGF1	MP; 5.1 μm	Heart	Myocardial infarction	Rat	Local injection (infarct)	[189]
PLGA	NRG1	MP; size not mentioned	Spin cord	Spin cord injury	Rat	Intrathecal injection	[190]
50:50 PLGA	BMP-2[k]	MP; 1–10 μm	Bone	Bone implantation	Rat	Incorporation in implant	[194]
85:15 and 50:50 PLGA	PDGF[l]	MP; 40 μm	Skin	Skin wound	Rat	Administration at wound	[195]
50:50 PLGA, alginate	EGF[m]	MP; 11–17 μm	Muscle	Diabetic foot ulcers	Rat	Intralesional injection	[196]
Antibodies							
50:50 PLGA, PLA	Bevacizumab	MP; 12 μm	Eye	Ocular disease	Rat	Intravitreal injection	[201]
PLGA	Bevacizumab	NP; 819 nm	Eye	Choroidal neovascularization	Rat	Intravitreal injection	[202]
PLGA-co-PEG	Cetuximab	NP; 80 nm	Lung	Lung cancer	Mouse	Intravenous injection	[204]
Vaccine (antigen)							
PLGA	HBcAg[n]	NP; 266–278 nm	Immune system	Hepatitis B	Mouse	Subcutaneous injection	[208]
50:50 PLGA, chitosan	BmpB[o]	MP; 3 μm	Immune system	Swine dysentery	Mouse	Oral	[211]
75:25 PLGA, NH_4HCO_3	Ovalbumin	NP; 1000 nm	Immune system	Infectious diseases	Mouse	Subcutaneous injection	[132]
PLGA, PMMMA	SIP[p]	NP; 40 nm	Immune system	Bacteria infection	Tilapia	Oral	[134]

[a] NP: nanoparticle.
[b] mean diameter unless mentioned.
[c] PMMMA: poly (methyl methacrylate)-*co*-(methyl acrylate)-*co*-(methacrylic acid).
[d] VEGF: vascular endothelial growth factor.
[e] MP: microparticle.
[f] bFGF: basic fibroblast growth factor.
[g] IL-1α: interleukin-1α.
[h] IL-2: interleukin-2.
[i] TGFβ: transforming growth factor β.
[j] NRG1: neuregulin-1.
[k] BMP-2: bone morphogenetic protein-2.
[l] PDGF: platelet-derived growth factor.
[m] EGF: epidermal growth factor.
[n] HBcAg: hepatitis B core antigen.
[o] BmpB: basic membrane protein B.
[p] SIP: surface immunogenic protein.

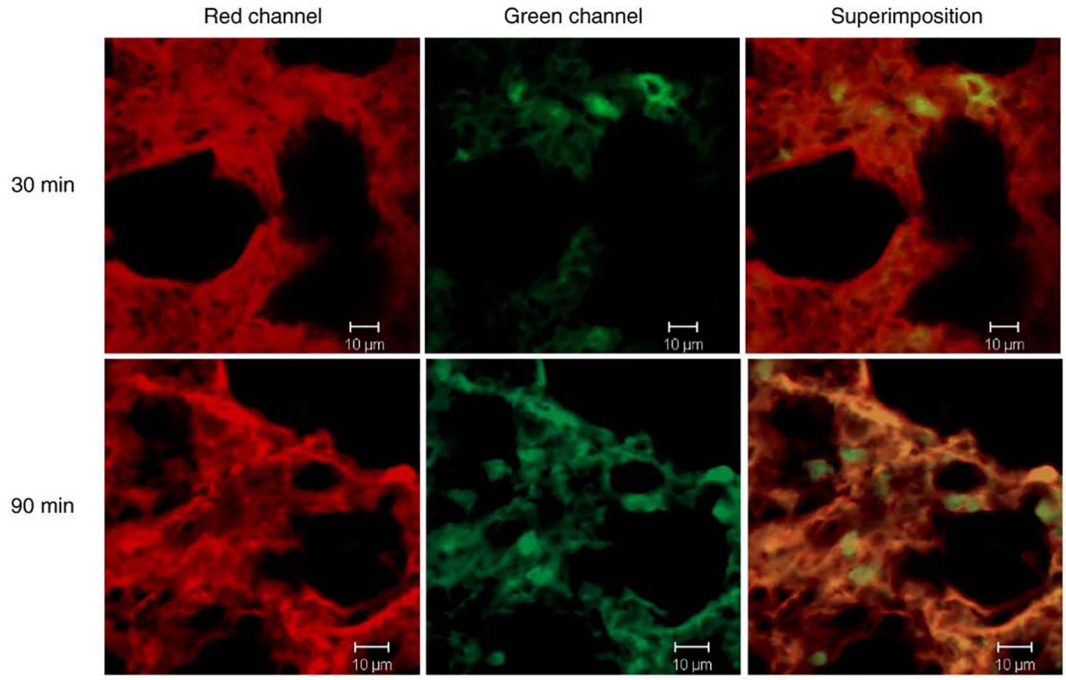

Fig. 4. Confocal laser scanning images of rat pulmonary alveoli after aerosol-based delivery of FITC-insulin loaded PLGA-cyclodextrin porous MPs. Red fluorescence: sulforhodamine-labeled alveoli membrane. Green fluorescence: FITC-insulin. Reprinted with permission from Ref. [152] Copyright 2009 Elsevier Ltd. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

all of which have shown great potentials in FGF-based therapeutics.

Interleukins (ILs) are a family of cytokines mainly secreted by immune cells and they modulate the activation, proliferation and differentiation of immune cells [181]. They have been administrated for the immune therapies of manifold diseases such as cancer [182,183], psoriasis [184] and neuropathy [185]. In an early study, 50:50 and 75:25 PLGA MPs were fabricated to co-encapsulation of IL-1α and BSA, with the latter served as an accelerator for the interleukin release [186]. Although the degradation of IL-1α was observed in the *in vitro* releasing test due to the drop of local pH in the PLGA MPs during the hydrolytic degradation, *in vivo* test showed an increase in survival rate of the tumor model mice compared to the mice injected with placebo or with free IL-1α. In another study, PLGA-PLA was utilized as a coating material onto IL-2 loaded dextran MPs for sustained delivery of IL-2 from the core-shell structure [187]. While the PLGA-PLA provided the sustained releasing capability, a more significant tumor shrink was observed in tumor model mice administrated with the MPs compared to those treated with free IL-2.

PLGA based MP/NPs were also chosen as delivery vehicles for other types therapeutic growth factors. McHugh et al. reported the co-delivery of transforming growth factor beta (TGF-β) with IL-2 with avidin-coated PLGA NPs [188]. These particles are administrated through intraperitoneal injection into mice and a significant increase in the generation of regulatory T cells was achieved, indicating its potential application in autoimmune therapy. In addition, PLGA MPs were loaded with Neuregulin-1 (NRG1) and FGF1 were used for the treatment of myocardial infarction [189]. The co-delivery of the two growth factors was found to inhibit cardiac remolding which resulted in the less infarct area compared to the control group after three months of treatment (Fig. 5). Despite the application in cardiac repair, NRG1-loaded PLGA MPs were also delivered to the mice to treat induced spin cord injury [190]. The controlled release of NRG1 from these particles resulted in the neuroprotective effect *in vivo*, with reduced glial scar formation and increased oligodendrocyte and axonal preservation. Bone morphogenetic factor (BMP) is an important growth factor in bone generation and repairing [191,192] and recombinant BMP-2 (rhBMP-2) is a FDA-approved therapeutic for spin fusion treatment [193]. Quinlan et al. developed collagen-hydroxyapatite scaffold embedded with PLGA MPs for localized delivery of rhBMP-2 for bond regeneration [194]. It was found that PLGA MPs allowed more sustained release of rhBMP-2 than alginate MPs and resulted in a higher pro-osteogenic effect *in vitro*. When transplanted into the mice with bone defect, the scaffold with growth factor-loaded PLGA particles rendered a significant higher degree of bone generation after 8 weeks, demonstrating their potential in bone engineering (Fig. 6). Despite the aforementioned examples, PLGA-based MP/NPs were also tailored for the delivery of other growth factors including platelet-derived growth factor (PDGF) [195], epidermal growth factor (EGF) [196] and insulin-like growth factor (IGF) [197].

5.1.3. Therapeutic antibodies

Since the first monoclonal antibody drug, Muromonab-CD3, was approved by FDA in 1985 for the treatment of transplantation rejection, there are over 70 antibody-based drugs approved by FDA till 2017 which make them the largest biopharmaceutical industry [198]. These antibodies target either certain antigens or cell surface receptors to either block the antigen function or to prevent the signalling molecule to bind to the receptors, both of which could have therapeutic effects [199]. The controlled release of these antibodies could reduce the injection times and the dosages, given the high cost of these drugs. Therefore, PLGA based MPs/NPs could be an ideal carrier for these antibodies. Bevacizumab is anti-VEGF monoclonal antibody used to treat age-related macular degeneration [200]. The ocular delivery of Bevacizumab requires prolonged releasing for effective therapy. Yandrapu et al. developed a novel process using supercritical (CO_2) fusion and pressure quench to fabricate PLGA-based MPs encapsulated with bevacizumab-loaded PLA NPs [201]. After intravitreal injection of these MPs into rat eyes, the unique structure of NP-encapsulated MPs enabled maintenance of antibody concentration for two months in vitreous body which is longer than the injection period of one month in present standard therapy. Furthermore, bevacizumab-loaded PLGA NPs were delivered to the rats with ruptured Bruch's membrane induced by laser photocoagulation [202]. After two weeks of drug releasing, the suppression of choroidal neovascularization can be observed which also proved the therapeutic potential of PLGA MP/NP-based delivery for eye

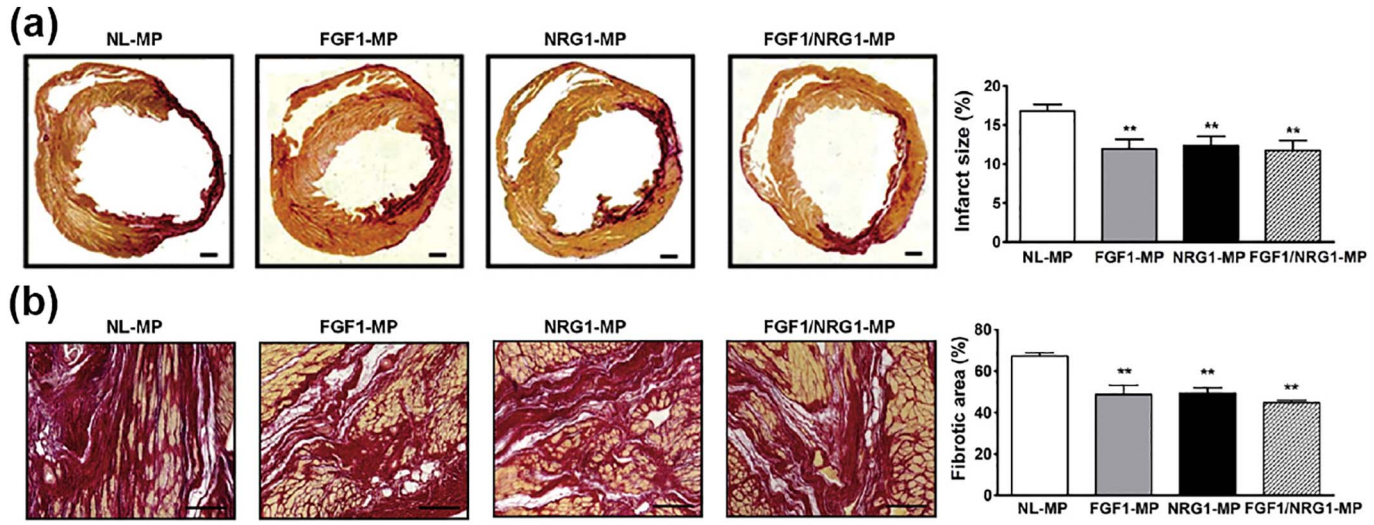

Fig. 5. PLGA MPs loaded with FGF1 and NRG1 inhibited cardiac remolding with less infarction size and fibrosis area after delivery. (a) The infarction size of left ventricular after treatment. (b) The fibrosis area after treatment. NL-MP: no MPs (control); FGF1-MP: FGF1-loaded MPs; NRG1-MP: NRG1-loaded MPs; FGF1/NRG1-MP: FGF1 and NRG1-loaded MPs. Reprinted with permission from Ref. [189]. Copyright 2014 Elsevier Ltd.

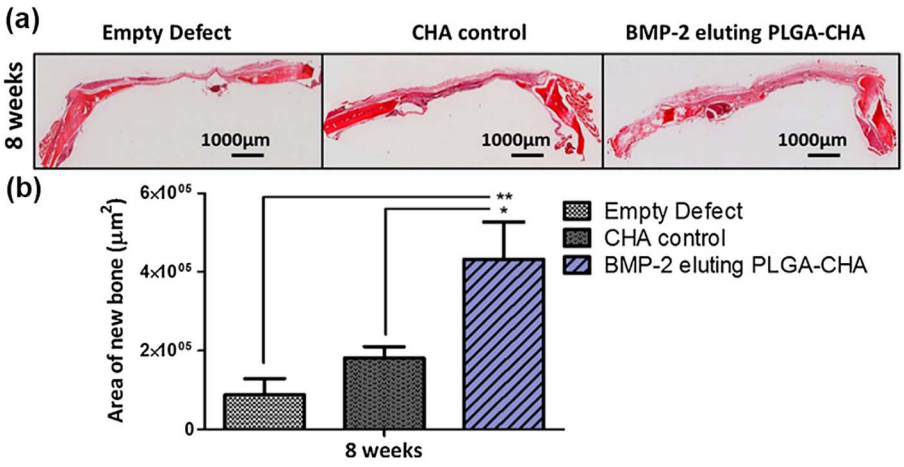

Fig. 6. Representative images of sections of explants from 7 mm calvarial defects in Wistar rats left empty or treated with collagen-hydroxyapatite (CHA) scaffolds or BMP2-loaded PLGA MPs in CHA scaffolds during 8 weeks and quantification of new area of bone. (a) Qualitative observation of bone healing. (b) Quantitative data of the area of new bone. Enhanced bone healing is observed in defects where the implant scaffold was loaded with BMP-encapsulated PLGA MPs. Reprinted with permission from Ref. [194]. Copyright 2015 Elsevier Ltd.

disease therapy. Foong et al. fabricated 75:25 PLGA MPs for the encapsulation of infliximab, which is a monoclonal anti-tumor necrosis factor-alpha (TNF-α) antibody [203]. The *in vitro* cell test showed the higher survival rate of fibroblasts in the presence of TNF-α and a higher migration rate of cells to the wound region in a cell wound model. Although *in vivo* results were not shown, this preliminary study may lead to a PLGA MPs based delivery method for therapy of Crohn Disease. While most monoclonal antibodies are incorporated into PLGA MPs/NPs as direct therapeutics, Karra et al. reported the surface coating of cetuximab, an antibody targeting EGF receptor (EGFR), onto PLGA NPs to guide the targeted delivery of paclitaxel to EGFR-overexpressed cancer cells [204]. While *in vitro* study showed a strong binding affinity to EGFR on cancer cells, NP administration into mice with induced lung cancer effectively slowed down the tumor progression and prolonged the animal survival time.

5.1.4. Vaccine (antigen)

Vaccines are antigenic reagents used to stimulate the immune system to recognize and to eliminate certain pathogens, thus protecting the body from certain diseases. Traditional antigen vaccines such as attenuated and inactive microbes, free surface antigen protein and recombinant protein are effective for disease prevention but are still frustrated by limitations including the requirement of repeated administration to boost immune response and the potential side effects of inflammation. The motivation using polymeric MP/NP-based for vaccine delivery lies on the fact that these particles could offer protection of vaccine antigens for long-lasting sustained release, the capability to co-encapsulate adjuvant or immune modulators for enhanced immune response and more precise targeting to the specific group of immune cells [205,206]. PLGA MPs/NPs are among the mostly used polymeric antigen carriers to aiming for safer and more efficient vaccine delivery [207]. Chong et al. developed hepatitis B core antigen (HBcAg) loaded PLGA NPs for hepatitis B vaccination in mice [208]. Interestingly, stronger *in vivo* immune responses from T helper cell type 1(Th1) were observed in the mice subjected to PLGA NPs co-delivering both HBcAg and the adjuvant monophospholipid A (MPLA) in contrast to those injected with free HBcAg or with single component-loaded PLGA NPs, either HBcAg or MPLA alone. This result indicated the advantage of using PLGA NPs as vaccine vehicles, with a co-delivery of both antigen and adjuvant to enhance immunity. In another study, PEGylated PLGA NPs were prepared for the delivery of ovalbumin (OVA), a model antigen [209]. The use of PLGA NPs allowed the anchoring of integrin-binding RGD peptide onto the particle surface for targeted delivery towards microfold cells (M cells) which were the major group of cells responsible for mucosal immunity [210]. *In vitro* cell experiment showed an immune response with much reduced amount of antigen required (5 μg *vs* 100 μg in common assay), proving the elevated efficiency of using target-aiming PLGA NPs. The similar strategy was later

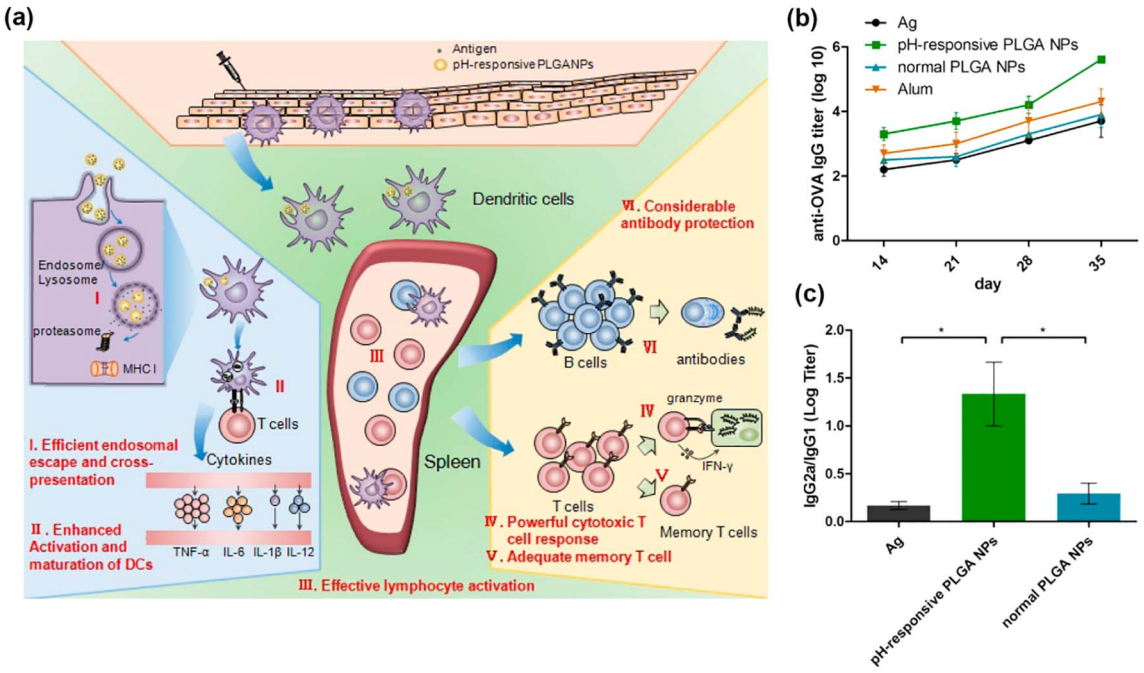

Fig. 7. pH-responsive PLGA NPs as vaccine delivery vehicles to dendritic cells. (a) The antigen releasing, delivery and immunization mechanism. (b) The serum titer of anti-OVA IgG in mice immunized with different antigen/antigen loaded NPs. (c) The serum ratio of IgG2a to IgG1 (IgG2a/IgG1) after immunization. The significant higher serum IgG2a/IgG1 in pH-responsive NP immunized mice indicates an increase in Th1 cell polarization. Reprinted with permission from Ref. [132]. Copyright 2015 ACS.

taken by Jiang et al. to deliver a membrane protein from *Brachyspira hyodysenteriae* (BmpB) to mice as a vaccine [211]. Porous PLGA MPs coated with M cell homing peptide (CKS9) were used to aim for M cell and to promote the presentation of antigen to immune cells. With these targeting MPs, *in vivo* results in mice model revealed the elevated response from Th1 and Th2 cells in contrast to mice administrated with free BmpB or non-targeting MPs. These two examples represent a common strategy to engineer PLGA MPs/NPs for vaccination, *i.e.* with surface coating of receptor-aiming molecules and with internal loading of immune antigen. Such strategy was also employed for the aiming of dendritic cells for vaccination [212]. As dendritic cells act as intracellular antigen processing and presenting machinery, an ideal dendritic targeting antigen vaccine carrier should allow maximum and fast intracellular release of antigen while minimizing antigen loss before entering the cells [213]. To this end, Liu et al. designed PLGA-shelled NPs incorporating NH_4HCO_3 as a pH responsive substance to achieve fast intracellular release of antigen in dendritic cells (Fig. 7) [132]. As aforementioned (Fig. 2c), these “smart” NPs can release antigens in acidic organelles such as lysosomes and endosomes while protecting these antigens from releasing or degradation in other physiological environment with nearly neutral pH (Fig. 7a). Mice immunized with pH-responsive NPs loaded with OVA showed higher serum titer of anti-OVA IgG as well as a higher ratio of serum IgG2a to IgG1 (Fig. 7b, c). These results suggested that antigen vaccine delivered by these NPs rendered a more efficient stimulation to the immune system and the stimulation was through the induction of Th1 cell polarization. In another scenario, Zhang et al. developed PLGA based NPs with PMMMA shell for the oral delivery of pathogenic microbial surface immunogenic protein (SIP) in tilapia [134]. The unique PMMMA shell could protect the antigen-loaded PLGA core from chemical and enzyme-mediated degradation in stomach and small intestine while it decomposed only in large intestine with elevated pH. All the tilapia immunized with these pH-responsive NPs loaded of SIP from *Streptococcus agalactiae* were protected from the infection of the bacteria while the control group immunized with unprotected antigen showed an infection rate > 60% in 5 months, proving the effectiveness of the strategy.

5.2. PLGA-based MPs/NPs for the delivery of therapeutic DNA/RNA

Therapeutics based on the delivery of gene has a boosted development in the past 20 years and recently, Tisagenlecleucel (commercial name Kymirah) licensed by Novartis, has become the first FDA approved gene therapy product. Compared to protein drugs and small molecular drugs, DNA molecules are more stable in physiological environment and have less cytoxicity, bringing more efficient and safer drug administration [214]. Besides viruses, non-viral vectors made from lipids, polymers and peptides have been investigated for gene delivery in the past decades, among which cationic polymers have been considered as the most promising candidates with intriguing potentials and advantages compared to their counterparts including their unique capacity to form polyelectrolyte complexes with genes and protect them from enzymatic degradation, low toxicity, cost effectiveness, ease of production, and versatility for different applications [215,216]. The negative charge on PLGA could result in low encapsulation efficiency for direct incorporation of DNA or RNA molecule. Therefore, PLGA modified with cationic molecules or PLGA formulated cationic copolymers were usually used for the fabrication of MPs/NPs for gene delivery [217]. Due to their good biocompatibility, PLGA MPs/NPs has been used for the delivery of diverse types of therapeutic DNA/RNA molecules including plasmid DNA, oligonucleotide and small interfering RNA (siRNA), which are listed in Table 3 and will be reviewed below.

5.2.1. Plasmid DNA

Plasmid DNAs (pDNAs) are circular double-strained DNA molecules encoding specific proteins which are commonly found in bacteria cells [218–220]. As a therapeutic agent, pDNA can be delivered into eukaryotic cells, triggering the intracellular DNA transcription and translation machinery and resulting in the expression of either a functional protein for disease treatment (as a pro-drug) [221,222] or an antigen protein for the stimulation of immune system (as a vaccine) [223,224]. PLGA MPs/NPs embrace advantages as a plasmid carrier as they are able to escape endo-lysosomal degradation [225] and they can also be effectively uptaken by immune cells for intracellular

Table 3
Summary of PLGA based MP/NP systems for the delivery of nucleic acid drug *in vivo* as covered in this review.

PLGA formulation	Type of nucleic acid	Particle type and size	Delivery target	Disease	Animal model	Administration route	References
50:50 PLGA, PEI	Plasmid DNA (vaccine)	MP[a]; < 10 μm	Immune system	Lymphoma	Mouse	Subcutaneous	[227]
53:47 PLGA, PEI	Plasmid DNA (vaccine)	NP[b]; 235–275 nm	Immune system	Bacterial infection	Mouse	Intramuscular or endotracheal	[230]
50:50 PLGA, PEI	Plasmid DNA	NP; 200 nm	Tumor cells	Prostate tumor	Mouse	Intravenous	[231]
PLGA, PEI	Plasmid DNA	NP; 40–50 nm	Stem cells	Cartilage injury	Mouse	Subcutaneous	[74]
50:50 PLGA, PEI	Plasmid DNA	NP; 250 nm	Stem cells	Limb ischemia	Mouse	Intramuscular	[76]
PLGA, chitosan	Plasmid DNA (vaccine)	NP; 700 nm	Immune system	Viral infection	Chicken	Intranasal	[234]
50:50 and 75:25 PLGA, PEI	Antisense oligonucleotide	MP; 10–20 μm	Viral gene	Viral infection	Mouse	Subcutaneous	[237]
50:50 PLGA	Antisense oligonucleotide	MP; 6 μm	Brain	Social recognition	Mouse	Local injection (brain)	[238]
PLGA, calcium phosphate, PEI	siRNA	NP; 150 nm	Colon	Intestinal inflammation	Mouse	Intrarectal	[244]
50:50 PLGA, PEG, PLL	siRNA	NP; 150 nm	Tumor cells	Tumor	Mouse	Intravenous	[245]
PLGA, PEI	siRNA, plasmid	NP; 80–180 nm	Stem cells	Cartilage injury	Mouse	Subcutaneous	[75]

[a] MP: microparticles.
[b] NP: nanoparticles.

transcription [226]. To efficiently incorporate negatively charged plasmid into PLGA based MPs/NPs, PLGA is usually conjugated or co-polymerized with other cationic molecule/moieties such as polyethylenimine (PEI) [227] and chitosan [228]. Ksaturi et al. were among the first to conjugate PEI onto PLGA MPs for efficient plasmid loading and delivery [229]. With PEI modification, the loading capacity of PLGA MPs increased for more than five times with a high loading efficiency of 50 to 90%. *In vitro* experiment showed higher mRNA level transcribed from plasmid gene in murine macrophage cells compared to cells subjected to plasmid delivered by unmodified PLGA MPs. Later work from other research groups proved that the PLGA-PEI particles were also effective in the delivery of plasmid *in vivo* [74,227,230,231]. As an example, Kim et al. developed PLGA-PEI NPs for the delivery of pDNA encoding SOX9, a collagen II-inducing protein which promotes chondrogenesis, into mesenchymal stem cells (MSCs) [74]. Results from *in vivo* experiment revealed that, when pDNA was transfected into MSCs with PLGA-PEI NPs, there was a significant increase in the expression of chondrogenesis protein markers such as SOX9, collagen II and aggrecan and the production of polysaccharide markers such as glycosaminoglycan (GAG), as compared to the MSCs transfected with other nanocarriers. These results demonstrate the potential of using these particles for tissue engineering applications. In another recent study, Park et al. fabricated PEI-coated PLGA NPs loaded with both pDNA expressing VEGF and apelin peptide for neoangiogenesis of mesenchymal stem cells (MSC) (Fig. 8a, b) [76]. The delivery of angiogenesis-promoting pDNA and peptide together triggered the signalling pathway for the expression of a series of angiogenesis-relating factors (VEGF, VEGF receptor, apelin receptor, bFGF, PECAM-1 *etc.*) and resulted in the induction of MSC differentiation in to endothelial cells (ECs) with tube (vessel) formation in the *in vitro* culture (Fig. 8c). Once MSCs with uptaken NPs were injected into the mouse with induced hind limb ischemia, faster neovascularization and the recovery of blood flow were observed, while the ischemic limbs were detached from the mouse due to the insufficient blood flow in the control groups injected with PBS or NPs loaded with only apelin (Fig. 8d). These results could be promising for the MSC-based therapies in regenerative medicine [232]. Despite PEI, chitosan was also used as a coating/blend materials in PLGA MPs/NPs for plasmid delivery [233]. Tahara et al. fabricated chitosan-coated PLGA MPs in a W/O/W emulsion method for loading and releasing of plasmid DNA [79]. The coating of chitosan not only increased the loading capacity but also reduce the burst release of plasmid from particles with a more sustained releasing profile over five days. This strategy was later utilized by Zhao et al. to deliver plasmid vaccine against Newcastle disease virus in chicken [234]. In this study, steady release of plasmid from PLGA-chitosan NPs was maintained for > 10 days *in vitro*. The administration of vaccine-loaded NPs to chicken significantly reduced the mortality after challenged with high virulent virus which proved the *in vivo* effectiveness. While most studies utilized chitosan as coating material for PLGA MP/NPs for plasmid delivery, Gasper et al. made PLGA-Chitosan MPs in an opposite manner, *i.e.* with DNA-loaded chitosan NPs loaded inside PLGA-$NaHCO_3$ MPs, for the delivery of minicircle DNA (mcDNA), a pDNA derivative freed from all prokaryotic vector parts [138]. The bulk PLGA-$NaHCO_3$ provided pH-responsive release of mcDNA in acidic environment through the gas generating reaction of $NaHCO_3$ with hydrogen ions, which created pores on the MP surface and initiated a fast release of encapsulated mcDNA.

5.2.2. Oligonucleotide

Antisense single strand DNAs can be used as therapeutics to block the biosynthesis of a certain protein through hybridization with target pre-RNA or mRNA and subsequent recruitment of RNase H for degradation [235,236]. The good biocompatibility and effective cellular uptake of PLGA based MPs/NPs encouraged their use in the delivery of antisense oligonucleotide for gene therapy. In an early study, Khan et al. entrapped antisense oligonucleotides inside PLGA MPs to monitor their releasing profile and biodistribution [237]. MPs made with 75:25 PLGA allowed sustained release of oligonucleotide for over 56 days *in vitro* and *in vivo* test displayed the active release of oligonucleotide after 7 days of administration, whereas the clearance of oligonucleotide was found in mice injected with free drug. Choleris et al. employed the similar PLGA MPs to delivery antisense oligonucleotide against the cell receptor of oxytocin which is a neuropeptide associated with social recognition in rodents [238]. When the MPs were locally delivered into the brain, the release of oligonucleotide decreased the expression of oxytocin receptor and eventually influenced the social behaviour of mice. This result is a clear proof that PLGA MPs are effective delivery carriers of antisense oligonucleotide which are able to not only act on the molecular level to reduce the expression certain proteins but also influence the phenotype (behaviour) of the animal.

5.2.3. siRNA

While antisense oligonucleotide based therapy was demonstrated effective in animal models many of them failed to reach the final FDA approval for clinical applications [239]. An alternative way to render the silence of protein expression is *via* the RNA interference by the

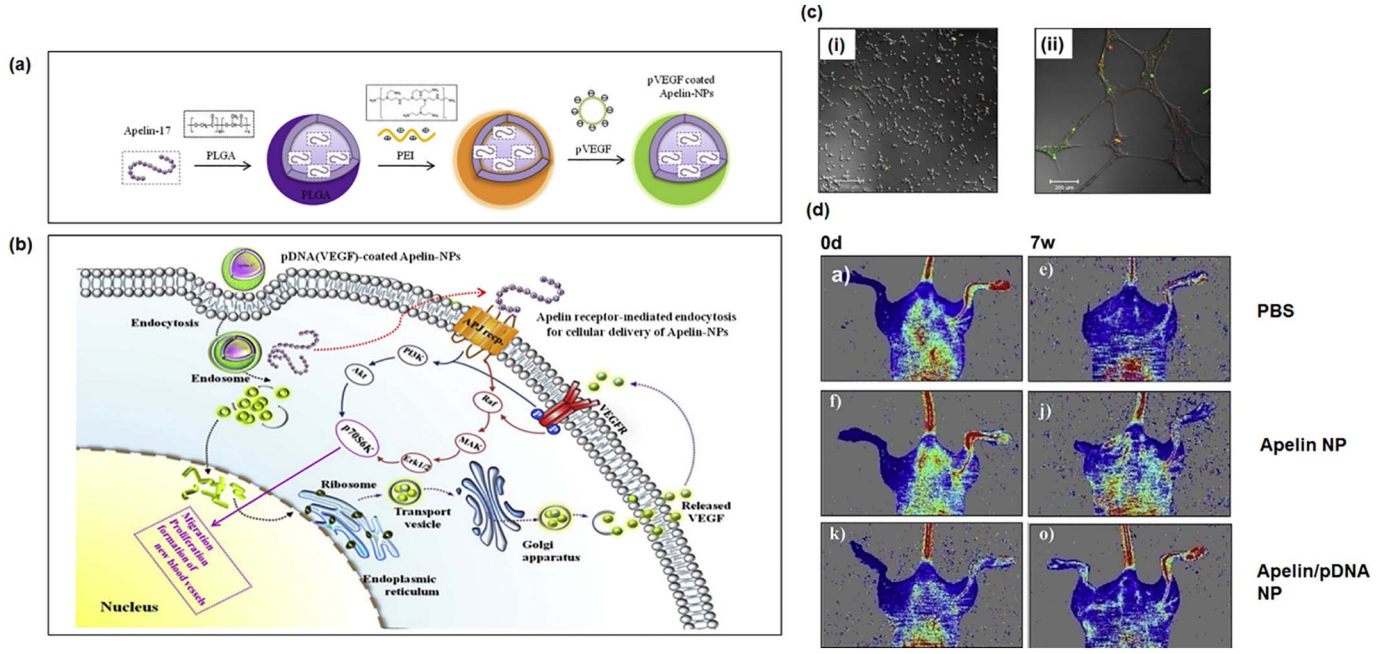

Fig. 8. PLGA NP-based co-delivery of VEGF-encoding pDNA and apelin promoted MSCs to ECs differentiation and (a) fabrication process of NPs. (b) Schematic process of pDNA/apelin co-delivery induced signalling in MSCs to promote neovascularization. (c) Tube (vessel) formation in cell culture of MSCs after uptaken of pDNA/apelin loaded PLGA NPs. (i) At 0 min; (ii) after 24 h. (d) Laser Doppler perfusion imageing showing the neovascularization in the mouse limb ischemia model. PBS: mouse injected with PBS saline (control); Apelin NP: mouse injected with apelin loaded NPs; Apelin/pDNA NP: mouse injected with apelin/pDNA loaded NPs. Red, yellow and blue color represents high, medium and low blood flow, respectively. Reprinted with permission from Ref. [76]. Copyright 2016 Elsevier Ltd. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

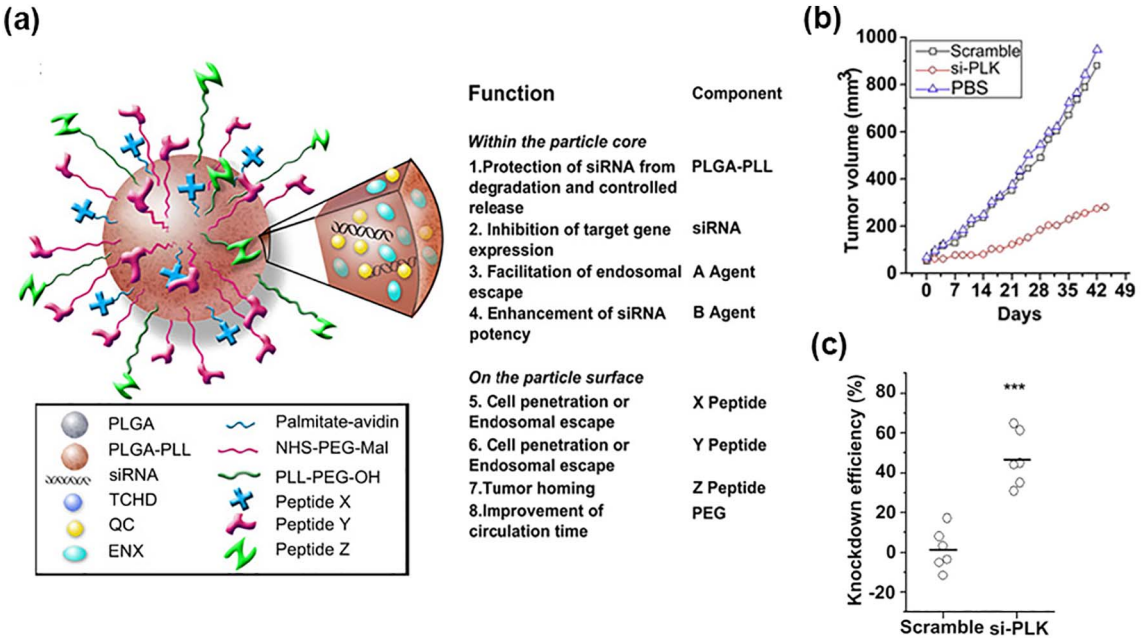

Fig. 9. Rational design of functional molecules-incorporated PLGA NPs for efficient siRNA delivery. (a) Incorporation of different molecules and their functions in the promotion of siRNA delivery. (b) The improved efficacy of siRNA-loaded NPs (siPLK) in tumor suppression as compared to the control group (PBS) and the scramble group. (c) The improved efficiency of siRNA-loaded NPs to knock down the target gene compared to the scramble group. Reprinted with permission from Ref. [245].Copyright 2012 Elsevier Ltd.

delivery of small interference RNA (siRNA) [240]. siRNA is a group of double-stranded RNA containing 20–25 base pairs, which can be assembled into RNA-induced silencing complexes (RISCs), subsequently bind to complementary mRNA and trigger the intracellular mRNA degradation [241]. siRNA guided gene silencing could be more potent than antisense oligonucleotide based therapy as the RISC could facilitate the binding between siRNA with mRNA while no such facilitator is found so far in hybridization between antisense oligonucleotide and target RNA, *i.e.* the binding is through simple diffusion [236]. Therefore, numerous studies have been focused on the development of carrier for siRNA delivery, among which PLGA based MP/NP is a promising candidate [242]. Patil et al. incorporated PEI into PLGA NPs to encapsulate siRNA for gene silencing *in vitro* [243]. While it was expected that the cationic PEI increase the loading capacity of siRNA, the PLGA-PEI blending also demonstrated an increase in the uptake of particle into the cell as well as a drastic reduction of cytoxicity owing to PEI,

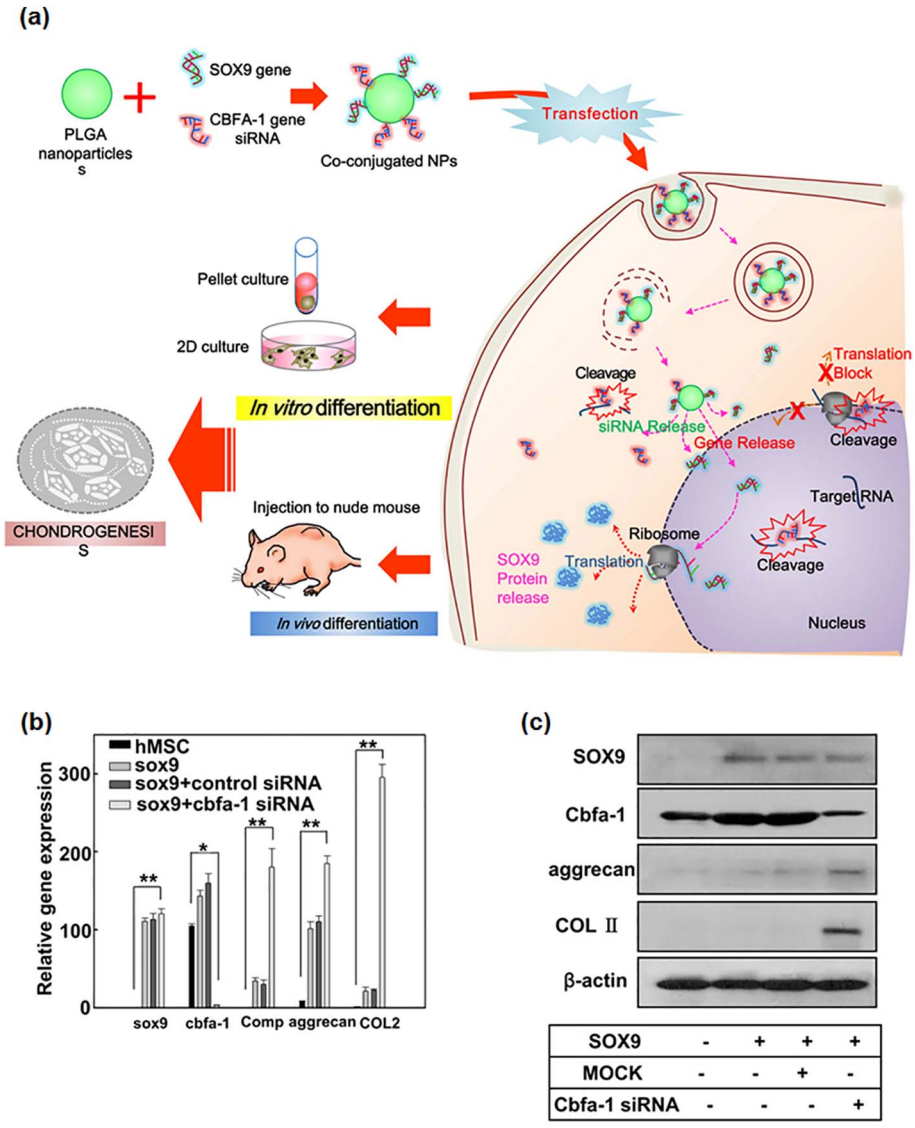

Fig. 10. PLGA NP-based co-delivery of pDNA and siRNA for chondrogenesis of MSCs. (a) Schematic illustration of the transfection process with *SOX9* gene plasmid and *Cbsa-1* targeting siRNA-loaded NPs and the subsequent induced chondrogenesis in MSCs. (b) The gene expression level (mRNA level) of chondrogenesis-related genes in MSCs from *in vivo* experiment. (c) Western blotting results for MSCs from *in vivo* experiment. These results displayed a significant increase in the expression of two chondrogensis markers, aggrecan and COL2 (collagen II), both in mRNA and protein level, which was only observed in the MSCs transfected with both plasmid and siRNA. Reprinted with permission from Ref. [75]. Copy right 2012 Elsevier Ltd.

which made the hybrid blending a more superior strategy for siRNA delivery. Towards *in vivo* applications, Frede et al. recently fabricated PEI-coated PLGA-calcium phosphate hybrid NPs to deliver siRNA to intestine for treatment of colonic inflammation [244]. These NPs were found to effectively protect siRNA from nuclease-mediated degradation in *ex vivo* experiment conducted in mice colonic fluid. Moreover, the intrarectal delivery of NPs, which were loaded with siRNAs against cytokine genes, significantly relieved the symptom in mice with induced colitis, suggesting the beneficial therapeutic effects using PLGA particle based delivery system. In order to achieve more efficient delivery of siRNA, Zhou et al. designed a PLGA-poly-L-lysine based NP delivery system incorporating multiple functional molecules to synergistically promote siRNA delivery *in vivo* [245]. These functional molecules could either facilitate endosomal escape of RNA (quinacrine) and increase siRNA potency (enoxacin) or enhance particle penetration to cells (TAT and iRGD peptides) and improve circulation time of particle in blood (PEG) (Fig. 9a). These elaborately designed PLGA-based NPs were shown to efficiently deliver siRNA which targeted cancer-related genes to suppress tumor progression in mice (Fig. 9b and c). The capability of PLGA-based MPs/NPs to encapsulate multiple reagents could also be utilized to co-delivery siRNA with other therapeutics to enhance efficacy. For example, Jeon et al. fabricated PLGA NPs for co-delivery of siRNA and plasmid DNA for enhanced induction of MSC chondrogenesis (Fig. 10a) [75]. Plasmid containing *SOX9* gene, which encoded aggrecan expression-inducing SOX9 transcription factors, and siRNA against *Cbfa-1* gene, which expressed Cbfa-1 protein to retard cartilage regeneration, were co-delivered to MSCs. After the delivery of *SOX9* gene and siRNA-loaded NPs to MSCs, *in vivo* studies displayed an increased expression of SOX9 and a sharp decrease in Cbfa-1 expression (Fig. 10b). Moreover, the expression of chondrogenesis markers, collagen II and aggrecan, was observed only when NPs containing both siRNA and plasmid DNA were used, indicating the crucial synergistic effect of both to the induction of MSC differentiation into chondrocytes (Fig. 10c).

6. Conclusions

The recent boost in the development of novel biomacromolecular drug reflects the increasing market demands for therapeutics with higher potency, efficiency and specificity. Thus, it is of utmost importance to choose appropriate carrier systems to effectively and efficiently deliver these macromolecules to the targeted site, to release them in a programmable profile while retaining their biofunctionality throughout the entire delivery process. The good biocompatibility, biosafety and biodegradability of PLGA has made it a promising candidate as drug carrier material and MPs/NPs composed of PLGA have

proven their success in the pharmaceutical market as delivery vehicles for small molecular drugs. In this review, we have focused on the recent progress in the development of PLGA MPs/NPs to incorporate and to delivery therapeutic biomacromolecules which are more vulnerable to both manufacturing and physiological environment and require more precision for targeted delivery. We have seen a number of novel recipes and processes to maximize the loading efficiency of macromolecules. Moreover, careful modulation of the particle structure and formulation has led to sustained, and more controlled release profiles for biomacromolecules *in vivo*. Numerous preliminary animal studies have displayed the great potential of these biomacromolecule-loaded PLGA MPs/NPs in the treatment of various diseases including diabetes, cancer, cardiac disorder, bacterial/viral infection, autoimmune diseases and cartilage damage.

On the other hand, there are still challenges towards the clinical application of PLGA based MPs/NPs. First, the acidic micro-environment resulted from the hydrolysis of PLGA could degrade the biomacromolecules, especially for proteinaceous therapeutics whose tertiary structures could collapse at acidic pH [246], and it could dramatically reduce the bioactivity of the therapeutics. Although efforts have been made to co-formulate PLGA with pH neutralizing agents [247] or hydrolysis-suppressing materials [248] to alleviate the adverse effect of high acidity, few studies have reported the improved functionality of bioactive macromolecular therapeutics *in vivo*. Second, more effective design strategies for “smart” stimuli-sensitive PLGA MPs/NPs should be developed to enable “on-demand” release of biomacromolecules. Despite the aforementioned pH and ultrasound responsiveness, PLGA MPs/NPs may also be endowed with responsiveness to other stimuli such as temperature [249] and redox environment [114] to take advantage of a variety of physiological or pathological conditions. Last but not least, site-specific delivery system could be formulated to guide the PLGA MPs/NPs to target disease sites or organs for the accumulation of macromolecular drugs with enhanced therapeutic efficacy. Recent developments in the ultrasound or magnetic field-based guidance system could be promising for this purpose [250,251]. With the advancement of material engineering and the drug delivery technology, we envision the explicit tackle of aforementioned challenges and PLGA particulate systems will be more frequently investigated in the clinical trials as delivery vehicles for biomacromolecular therapeutics, which will potentially open the avenue to the final clinical applications.

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