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Review

Sustained Release Systems for Delivery of Therapeutic Peptide/Protein

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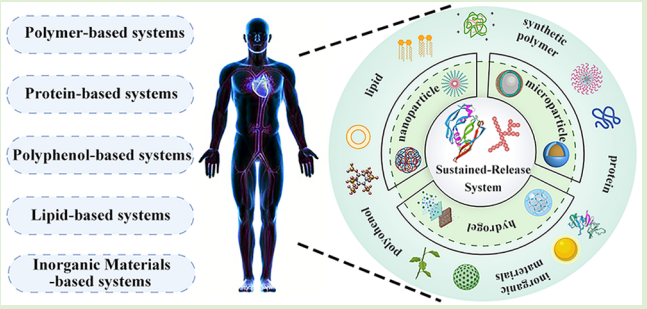
ABSTRACT: Peptide/protein therapeutics have been significantly applied in the clinical treatment of various diseases such as cancer, diabetes, *etc.* owing to their high biocompatibility, specificity, and therapeutic efficacy. However, due to their immunogenicity, instability stemming from its complex tertiary and quaternary structure, vulnerability to enzyme degradation, and rapid renal clearance, the clinical application of protein/peptide therapeutics is significantly confined. Though nanotechnology has been demonstrated to prevent enzyme degradation of the protein therapeutics and thus enhance the half-life, issues such as initial burst release and uncontrollable release kinetics are still unsolved. Moreover, the traditional administration method results in poor patient compliance, limiting the clinical application of protein/peptide therapeutics. Exploiting the sustained-release formulations for more controllable delivery of protein/peptide therapeutics to decrease the frequency of injection and enhance patient compliance is thus greatly meaningful. In this review, we comprehensively summarize the substantial advancements of protein/peptide sustained-release systems in the past decades. In addition, the advantages and disadvantages of all these sustained-release systems in clinical application together with their future challenges are also discussed in this review.



1. INTRODUCTION

Since the first peptide therapeutic, human insulin, was approved by the Food & Drug Administration (FDA) in 1982, diverse protein/peptide therapeutics such as glucagon like peptide-1 (GLP-1), monoclonal antibodies, chimeric proteins, recombinant human growth factors, or hormones for a myriad of diseases such as diabetes, cancer, inflammatory diseases, or infection have been exploited and developed in the past few decades.[1−3] According to the statistical data provided by Business Communications Company (BCC) Research, the global market for bioengineered protein drugs is expected to reach $228.4 billion by 2021 from $172.5 billion in 2016, rising at a compound annual growth rate (CAGR) of 5.8% from 2016 through 2021.[2,4]

Compared to the dominating small-molecular drugs in the pharmaceutical market, protein/peptide therapeutics gradually exhibit their huge potential in disease treatment owing to their higher specificity, higher bioactivity, and lower toxicity.[1,2,5] Hampered by the physiological environment in the gastrointestinal tract such as an acidic pH microenvironment, enzyme degradation, a mucus layer, and the presence of accompanying food, protein/peptide therapeutics oral bioavailability is low (<1%); hence, they are mostly administrated *via* parenteral routes such as intravenous, intramuscular, or subcutaneous injection.[6−8] However, enzyme degradation such as that due to metalloproteinase, rapid renal clearance, and *in vivo* instability in terms of their complex tertiary and quaternary structures is still inevitable even *via* parenteral administration. This thus leads to the short half-life of these therapeutics, which need frequent injection to maintain the steady-state concentration, consequently decreasing the patient compliance and significantly confining their clinical application.[5,9] According to the data provided by the World Health Organization (WHO), only half of the patients diagnosed with chronic diseases stick to the treatment regime in developed countries. In developing countries, this number is even lower.[10−12]

Though strategies of encapsulation within micro-/nanoparticles, chemical modification with hydrophobic polymers, and recombinant protein engineering have been demonstrated to prevent the protein therapeutics from enzyme degradation

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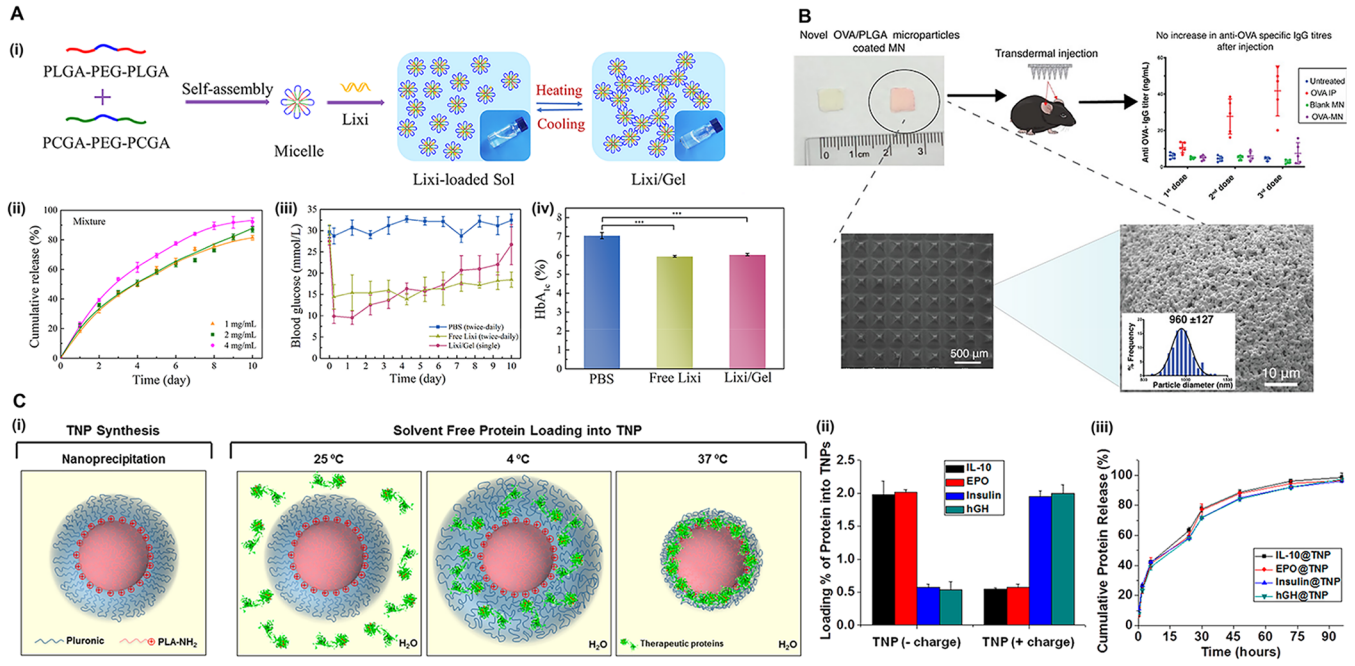

Figure 1. (A) Lixisenatide-loaded thermosensitive hydrogel systems for long-term hypoglycemic control. (i) Prepration of a lixisenatide-loaded thermosensitive hydrogel system. (ii) *In vitro* release profiles from the thermosensitive hydrogel system with the different concentrations of lixisenatide. (iii) Plot of blood glucose level versus time after subcutaneous injection of different formulations into db/db mice. (iv) HbA1c levels in plasma of db/db mice after treatment with lixisenatide-loaded thermosensitive hydrogel for a month. Published with permission from ref 51. Copyright 2019 American Chemical Society. (B) Transdermal protein delivery of OVA *via* electrohydrodynamic coating of PLGA microparticles onto microneedles. Published with permission from ref 55. Copyright 2020 American Chemical Society. (C) (i) Schematic illustration of a thermosponge nanoparticle (TNP) for protein loading without usage of organic solvent. (ii) Loading contents of therapeutic proteins (IL-10, EPO, insulin, and hGH) into TNPs. (iii) *In vitro* release of therapeutic proteins from TNPs. Published with permission from ref 60. Copyright 2014 American Chemical Society.

and enhancing the circulation time, issues in terms of initial burst release and uncontrollable release kinetics remain unsolved.[13−15] Therefore, it is urgent and necessary to exploit long-acting formulations by sustained protein/peptide delivery systems, aiming for higher stability against enzyme degradation, more controllable and sustainable pharmacokinetics, reduction in dosage and dosing frequency, fewer side effects, higher patient compliance, and better adherence to long-term treatment especially for chronic diseases such as diabetes.[16−18] An ideal sustained protein/peptide delivery system should meet the following requirements to realize long-term treatment of chronic diseases with minimized dosage and frequency: (1) provide high loading efficacy of target therapeutics, (2) protect the loaded therapeutics from enzyme degradation, hydrolysis, or oxidation, and (3) control drug release to maintain a steady-state concentration for an extended duration in the target tissues or organs.[13,14] Besides, materials for construction of the sustained-release system shall be biocompatible, biodegradable, and injectable with low immunogenicity and low toxicity and preferably FDA approval.[13,19,20]

Substantial advancements based on the formulation design and delivery strategies have been made in the development of protein/peptide sustained delivery. A number of injectable sustained-release formulations have been approved by the FDA in the past few decades.[21,22] However, the underlying problems, such as biocompatibility of new emerging materials, complicated preparation methods, denaturization of protein, initial burst drug release, and uncontrollable release kinetics, are still of concern. Hence in this review, recent progress in long-acting protein/peptide formulations including polymer-based, protein-based, polyphenol-based, lipid-based, and inorganic materials-based delivery systems is summarized and discussed comprehensively. Critical issues of current sustained-release systems are also included.

2. POLYMER-BASED SYSTEMS

Polymer is a kind of macromolecular compound synthesized through the addition or condensation polymerization of monomers with various reactive groups, which is now being widely applied in the exploitation and development of drug delivery systems owing to its good biocompatibility, ease of chemical modification, controllable molecular weight and structure, *etc.*[22−24] In the past decades, synthetic polymers such as poly(lactic-*co*-glycolic acid) (PLGA) and polyethylene glycol (PEG) have been developed and approved by the FDA as long-acting formulations for delivery of protein/peptide therapeutics such as Adagen, Eligard, Bydureon, *etc.*, which are now vastly applied in the clinical treatment of various diseases such as severe combined immunodeficiency (SCID), cancer, and diabetes.[25−27] Natural polymers such as various kinds of polysaccharide are now being investigated extensively as carriers for loading biomacromolecules such as proteins attributed to their abundant sources, low price, and good biocompatibility and biodegradability, holding great potential in the future for resolving the issues previously mentioned.[28−30]

2.1. Synthetic Polymers. Synthetic polymer-based long-acting formulations are usually constructed into a gel system or a micro-/nanoparticle system. Phase inversion/solvent extraction and exogenous trigger are the two main methods for

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preparation of gel systems.[1,31] In the case of phase inversion/solvent extraction, protein/peptide therapeutics are suspended in the water miscible organic solvent such as *N*-methylpyrrolidone (NMP), DMSO, ethanol, *etc*. where the polymers are dispersed. After injection, the organic solvent diffuses out in the aqueous environment, which consequently leads to the precipitation of hydrophobic polymers and thus encapsulation of the protein/peptide therapeutics inside the matrix.[32,33] The kinetic release of laden therapeutics is then controlled and tuned based on the type, concentration, molecular weight, and crystallinity of the polymer, solvent type, and additives. In other cases, stimuli-responsive polymers such as thermosensitive or pH-sensitive polymer solutions containing protein/peptide therapeutics could transform into gels under certain triggers such as physiologically temperature (37 °C) or pH (7.2−7.4) and do not need the participation of organic solvent, thus decreasing the toxicity of these formulations.[32−36] Owing to the controllable amphiphilic property of synthetic polymers, synthetic polymers could also be prepared as micro-/nanoparticle formulations *via* double emulsion methods (also known as microencapsulation methods) to load proteins or peptides.[37] Briefly, an aqueous protein solution is dispersed in the organic solvent ethanol or dichloromethane with synthetic polymers dissolved. Then, the primary emulsion is further dispersed into an emulsifier-dissolved aquatic phase following dialysis or rotation evaporation to remove the organic solvent.[38−40] In all, the methodology used in the preparation of the synthetic polymer long-acting formulation is mainly based on the hydrophobic interaction between polymers and proteins (or polymers and solvent), which makes organic solvents an essential element in the whole preparation process and hence may decrease the bioactivity of the loaded protein therapeutics.

2.1.1. Polyesters. Polyesters are synthetic polymers with more than one ester group in their molecular structure, which endow them with good biodegradability and biocompatibility.[22,41] PLGA, as the most widely used and classic polyester for developing sustained-release formulations, has been developed into injectable, long-acting PLGA microparticles or *in situ* gel/implant formulations loaded with various peptides. Nineteen of them have been approved by the FDA such as Lupron Depot, Lupaneta Pack, Zoladex Depot, Somatuline Depot, Eligard, and Bydureon, to name a few, which significantly improve patient compliance and extend the release of target peptide therapeutics to several weeks or even months.[33,42−45]

Thereafter, more and more long-acting protein therapeutic formulations on the basis of PLGA have been studied and developed. Through conjugation with hydrophilic PEG, PLGA−PEG copolymer exhibits better biocompatibility, longer circulation time, and distinctive thermosensitive property which allows the *in situ* transformation of PLGA−PEG copolymer into hydrogel under physiological temperature (37 °C).[46,47] Along with its good biodegradability, PLGA−PEG−PLGA has been successfully applied to load various proteins/peptides such as lysozyme, porcine growth hormone, insulin, lixisenatide, human calcitonin, or recombinant hepatitis B antigen.[48−50] For instance, Yu et al. designed lixisenatide loaded hydrogel based on the thermosensitive property of PLGA−PEG−PLGA (sol−gel transition temperature = 35 °C) for synchronous treatment of type II diabetes (T2D) and associated complications (Figure 1A).[51] The as-prepared hydrogel with uniform micelle (∼35 nm) formed with lixisenatide exhibited high aqueous stability for 1 week. The *in vitro* release profile (Figure 1A-ii) indicated that this long-acting lixisenatide formulation efficiently enhanced the half-life of lixisenatide from 2.2 to 30.3 h and realized the long-term release of lixisenatide for more than 9 days. Moreover, based on the *in vivo* imaging results, the fluorescence signal of Cy5.5-labeled lixisenatide could still be detected at the injection site after 15 days of the subcutaneous (*s.c.*) administration. In the results of hypoglycemic efficacy (Figure 1A-iii, iv), after a single dose, this lixisenatide long-acting formulation efficiently maintained the blood glucose level (BGL) under 15 mmol/L on a T2D murine model (db/db mice) for 4 days as the HbA1c (glycosylated hemoglobin) was reduced for about 15%. Similarly, Ding et al. also successfully loaded human calcitonin into this thermosensitive PLGA−PEG−PLGA hydrogel with uniform size ∼20 nm and loading capacity ∼0.48%, while the low loading capacity probably resulted from the substantial physical encapsulation of human calcitonin instead of other noncovalent or covalent interactions.[48] Besides, calcitonin loaded PLGA−PEG−PLGA hydrogel exhibited good biodegradability as 70% mass *in vitro* degradation was detected in 35 days. From the *in vitro* release profile, the release of human calcitonin was extended up to 3 weeks in the 20 wt % hydrogel with the initial burst release (∼10%) on day 1.

Aiming for resolving the burst release derived from hydrogel formulations, researchers introduced the double emulsion method to fabricate the polyesters into micro-/nanoparticles and tried to realize better controllable release kinetics and prevention of target protein therapeutics from denaturation. To give as an example, Igartua et al. prepared PLGA microparticles loaded with recombinant human epidermal growth factor (rhEGF) based on a W/O/W double emulsion/solvent evaporation method. The as-prepared PLGA microparticles (∼15 μm) with loading capacity of rhEGF at ∼1% efficiently reduced the initial burst release to around 20% and extended the release of target protein for 40 days.[52] Likewise, proteins/peptides such as interleukin 10 (IL-10), MOG, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) were also reported to be loaded into PLGA nanoparticles successfully *via* a double emulsion method, which efficiently extended the target protein/peptide release period up to several weeks.[34,53−55] Moreover, apart from traditional intravenous or subcutaneous injection, a new administration strategy such as transdermal administration was also developed for long-term release of proteins synergistically. For instance, Williams et al. introduced electrohydrodynamic atomization to coat ovalbumin (OVA) loaded PLGA nanoparticles (∼780 nm) onto microneedle arrays with 30% coating efficiency (Figure 1B) and successfully extended the release period of OVA up to 25 days *via* transdermal administration without obvious immunogenicity *in vivo* (Figure 1B-ii, iii), providing us a new strategy for protein/peptide therapeutics delivery.[55]

In recent years, new technologies such as spray drying, spray freeze-drying, ultrasonic atomization, electrospray, and the microfluidic method have emerged and exhibited advantages on size distribution control and scalable and continuous fabrication.[56−59] However, the usage of organic solvent during the preparation is still unavoidable, which significantly confines the development of polyester-based long-acting formulations. In order to get rid of the organic solvent usage in construction of polymer-based long-acting formulations, Farokhzad et al. designed poloxamer-coated polylactic acid (PLA) nanoparticle

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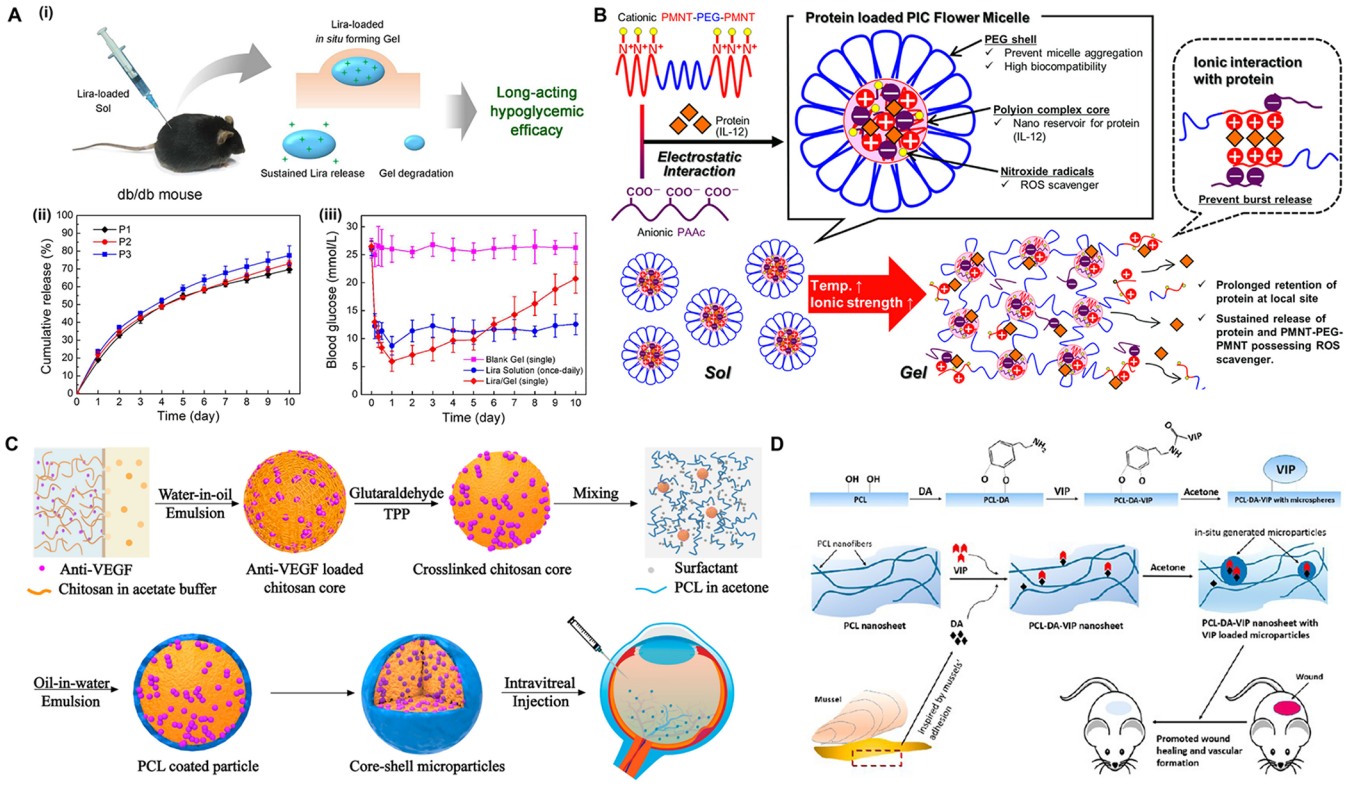

Figure 2. (A) (i) PEG−PGA-based *in situ* thermosensitive gel loaded with liraglutide for long-term hypoglycemic control. Published with permission from ref 73. Copyright 2016 American Chemical Society. (ii) *In vitro* release profiles of Liraglutide from PEG−PGA-based gel. (iii) Blood glucose levels of nonfasted db/db mice after injection of the different formulations. (B) Preparation of long-acting, redox-active polyion complex flower micelles *via* electrostatic interaction. Published with permission from ref 70. Copyright 2018 Elsevier Ltd. (C) Preparation of chitosan-PCL core−shell microparticles for sustained delivery of Bevacizumab. Published with permission from ref 80. Copyright 2020 American Chemical Society. (D) PCL nanosheet for sustained release of VIP peptide in wound treatment and vascular tissue engineering. Published with permission from ref 81. Copyright 2016 American Chemical Society.

(∼50 nm) (Figure 1C-i) and successfully loaded IL-10, erythropoietin (EPO), insulin, and human growth hormone (hGH) *via* electrostatic interaction. The volume expansion of the poloxamer shell at low temperature with loading content for all achieved around 2% (Figure 1C-ii). This facile single-step nanoprecipitation method without the usage of organic solvent efficiently extended the release of target protein therapeutics up to 90 h (Figure 1C-iii), decreased the *in vivo* toxicity induced by organic solvent, and greatly preserved the bioactivity of protein/peptide therapeutics.[60]

Overall, PLGA as a highly biocompatible and biodegradable polyester exhibits great potential in the protein/peptide delivery for treatment of diseases such as diabetes or cancer. However, its shortage of a cellular recognition site leads to relatively low affinity to cells and thus confines its application in the delivery of cytosolic protein such as Cas9 protein. Besides that, owing to its low mechanical strength and bad film-forming property, PLGA-based long-acting formulations are rarely applied in the tissue engineering of bone regeneration, vascular grafts, *etc.*

2.1.2. Polyethers. Polyethers are another group of polymers with an ether group as functional group in their backbone. The most widely investigated polyethers for developing long-acting protein/peptide therapeutic formulations are PEG and polycaprolactone (PCL).[61−64]

PEG, owing to its low immunogenicity, high hydrophilicity, and biocompatibility, is an important polymer that is widely applied in protein/peptide delivery for preventing enzyme degradation, decreasing protein immunogenicity, and prolonging half-life *via* conjugating to protein/peptide therapeutics such as PEGylated tumor necrosis factor-related apoptosis inducing ligand (TRAIL) or PEGylated ciliary neurotrophic factor (CNTF).[65,66] For now, 15 kinds of PEGylation protein therapeutics such as Adagen, JIVI, *etc.* with protein molecular weights ranging from 5,000 to 60,000 were approved by the FDA.[67−69] But the circulation time of the PEGylated protein still does not meet the needs for long-term treatment of chronic diseases such as diabetes and cerebral degenerative diseases. So synthetic polymers such as PLGA, poly(ε-caprolactone-*co*-glycolic acid) (PCGA), poly(L-lactide-*co*-caprolactone) (PELCL), and polyamines, PLA, among others, are designed and synthesized to form copolymers with PEG for construction of protein/peptide long-acting formulations.[70−76] For instance, in the work of Boury et al., based on the phase separation method, PLGA−PEG self-assembled into nanoparticles (∼255 nm) and successfully loaded stromal cell-derived factor-1α (SDF-1α) precipitates with loading capacity for ∼0.03%. The *in vitro* release profile suggested that the initial burst release of SDF-1α was high, up to ∼40% on day 1, and the total release period of this chemokine was extended to approximately 3 days.[70] In another work, taking advantage of the thermosensitive property of PEG−PCGA, liraglutide was efficiently encapsulated into this thermosensitive hydrogel (Figure 2A). The *in vivo* degradation assay suggested that the degradation rate of PEG−PCGA hydrogel could be tuned through adjusting the content of GA from 3 weeks to 8 weeks.

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In addition, the release of liraglutide *in vitro* after encapsulation can be extended to 10 days with 20% initial burst release on day 1 (Figure 2A-ii). A hypoglycemic efficacy study on T2D mice (Figure 2A-iii) verified that this liraglutide-loaded thermosensitive hydrogel maintained the BGL of db/db mice in a normal range for 5 days with single dosage, and even after 7 days, the BGL was still 50% lower than that from the control group.[73] Likewise, Nagasaki et al. designed and synthesized poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl)-aminomethylstyrene]-*b*-poly(ethylene glycol)-*b*-poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl)aminomethylstyrene] (PMNT−PEG−PMNT) triblock copolymer and applied it to construct thermosensitive injectable gel with poly(acrylic acid) (PAAc) for loading of IL-12 under the temperature 30 °C (Figure 2B).[72] This thermosensitive injectable gel with the micelle size formed between IL-12 and the copolymer of 50 nm significantly extended the *in vitro* release period of laden therapeutics to 2 weeks with ∼30% initial burst release on day 1. As expected, IL-12 loaded hydrogel demonstrated remarkable antitumor efficacy on colon-26 tumor-bearing mice with multiple subcutaneous injection (once every 4 days for 5 dosages in total) without evident adverse effects compared to the free form of IL-12.[72]

PCL, a highly hydrophobic polymer with good biocompatibility, biodegradability, and mechanical properties, is often conjugated with PEG for better controllable amphiphilicity and biodegradability.[77,78] For instance, Kok et al. prepared PCL−PEG microparticles (∼50 μm) *via* a membrane emulsification-based double emulsion method and successfully loaded VEGF with ∼0.8% loading capacity. These PCL−PEG microparticles effectively extended the *in vitro* release of VEGF to approximately 4 weeks with only 20% drug release on day 7 and successfully extended the release period of VEGF up to 28 days thanks to the low degradability rate of PCL.[79] In another work of Yang et al., a six-arm amphiphilic PCL−PEG (6S−PCL−PEG) copolymer was synthesized and loaded with ovalbumin (OVA), forming 6S−PCL−PEG nanoparticles (∼190 nm) *via* a W/O/W double emulsion method with high drug loading capacity of ∼28.5% benefiting from its star-shaped structure.[71] The *in vitro* release curve indicated that the release of OVA was up to 28 days profiting from the conformational cloud formed by 6S−PCL−PEG copolymer in aqueous solution. Apart from formulating into particles for controllable delivery of protein therapeutics, PCL was also applied in the surface coating of other sustained-release formulations for further controllable release of target therapeutics due to its moderate film-forming property. For example, Swindle-Reilly et al. used chitosan to encapsulate bevacizumab and further coat PCL through a W/O emulsion method (Figure 2C). The as-prepared core−shell microparticles (∼10 μm) efficiently reduced the initial burst release on week 1 from ∼60% to ∼40% compared to the chitosan microparticles without PCL coating and successfully extended the release of bevacizumab for 3 months. Unfortunately, only 50% of the protein therapeutics were released with the other half wasted.[80] In other cases, due to its good mechanical strength, PCL was also constructed as a sheet or membrane to load protein therapeutics in the application of tissue engineering such as vessel regeneration or wound healing (Figure 2D).[81,82] For instance, in the work of Yuan et al.,[82] a double-layered electrospun vascular graft loaded with two different growth factors in inner and outer layers was designed and prepared *via* an electrospinning technique with a coaxial electrospun membrane of chitosan hydrogel/poly(ethylene glycol)-*b*-poly(L-lactide-*co*-caprolactone) (PELCL) loaded VEGF as the inner layer and a coaxial electrospun membrane of emulsion/PELCL loaded platelet-derived growth factor (PDGF) as the outer layer. The average diameter of the fiber was 754 ± 385 nm, and the encapsulation efficiency for both proteins was a mere ∼17%. The release curve of these double-layered vascular grafts showed the release periods of both protein therapeutics were extended up to 25 days, which is critical for blood vessel regeneration. It is worth mentioning that 4 weeks of *in vivo* implantation in a rabbit carotid artery indicated this vascular graft effectively upregulated the expression of endothelial markers CD31 and smMHC-I in the first 2 weeks and demonstrated the promoted proliferation of vascular endothelial cells and vascular smooth muscle cells without thrombus, exhibiting great potential in facilitating revascularization and regeneration of other damaged tissues.

In general, PCL-based long-acting protein formulations, thanks to their biodegradability, good mechanical strength, and film-forming properties, have been vastly investigated in the application of ocular disease treatment and tissue engineering of blood vessel regeneration. However, in the application of protein delivery, issues in terms of its solubility with degradation, hydrophilic−hydrophobic composition, targeting features, surface texture, or environment responsiveness need to be balanced, while in the application of tissue engineering, parameters including hydrophobic composition with degradation, porosity with mechanical strength, hydrophilic composition with plasticity and elongation, and degradation and surface hydrophilicity with cell adhesion are critical to be taken into account.

2.1.3. Poloxamers. Poloxamers, also known as pluronics, are nonionic triblock copolymers with poly(propylene oxide) (PPO) as the central hydrophobic part and poly(ethylene oxide) (PEO) as the hydrophilic part.[22,83,84] Due to their thermosensitive property, poloxamers could transform into hydrogel under physiological temperature (37 °C), and they hence have been significantly investigated for developing protein/peptide therapeutics long-acting formulations. Also, they are inert in nature and could protect the loaded protein/peptide therapeutics against oxidation or hydrolysis thus preserving their bioactivity and prolonging their half-life.[22,85] The most commonly used poloxamer is pluronic F127 (PF127), which has been approved by the FDA as a functional material for protein/peptide therapeutics delivery. For now, protein/peptide therapeutics such as insulin, interleukin-2, recombinant hirudin variant-2, interleukin-1Ra, and various hormones (geslorelin, recombinant epithelial growth factor, and recombinant human growth hormone), to name a few, have been reported to be encapsulated in a poloxamer-based delivery system and realized sustainable release *via* different administration methods.[85,86] For example, Wang et al. prepared goserelin acetate (GOS) loaded poloxamer hydrogel as the inner W_1 phase and then further coated with PLGA to form core−shell didepot *via* a W/O/W method. Remarkably, the as-prepared GOS long-acting formulation (∼67 μm) effectively extended the release period of GOS to approximate 2 months with only 2% initial burst release on day 1.[86] Pharmacokinetic investigation further verified that the relative bioavailability of GOS *via s.c.* injection increased up to 9.36-fold compared to the commercial Zoladex implant, a classic clinical medication for prostatic cancer, which reduced the dosage and relieved the pain caused by Zoladex, making it a

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promising sustained-release system for delivery of triptorin and leuprolide with similar physical−chemical properties to GOS.[86]

Besides, poloxamer was also able to be conjugated with other polymers such as polysaccharide for better immobilization of protein/peptide therapeutics and synergistic prevention against enzyme degradation, hydrolysis, or oxidation.[87−89] For instance, Xiao et al. conjugated heparin with PF127 *via* EDC/NHS reaction for better protection and more effective drug loading of high-affinity heparin-bound aFGF through thermosensitive gelation (21−26 °C). The as-synthesized heparin-PF127 hydrogel with ∼17% loading capacity of aFGF efficiently reduced the initial burst release of laden therapeutics from 18% to 9% in the first 24 h compared to the hydrogel without heparin conjugation and extended the release period to approximate 28 days.[87] Moreover, on a spinal cord injury (SCI) mice model, single dosage of aFGF loading PF127-heparin hydrogel effectively reduced neuronal apoptosis and reactive astrogliosis and improved the rehabilitation of neuron and axonal, providing a new strategy for SCI protection and recovery.[87] After confirming its sustainable release behavior, Xiao et al. also applied it for the delivery of bFGF and nerve growth factor (NGF) for peripheral nerve regeneration in diabetic rats. The results revealed that the motor ability, axon and myelin rehabilitation and interaction, microtubule stabilization, and Schwann cell proliferation in diabetic rats with sciatic nerve injury were all effectively promoted after 30 days of treatment with one single injection.[88]

All these cases strongly supported significant clinical application and translation potency of poloxamer-based or poloxamer conjugated polymer-based long-acting protein formulations as promising candidates in the fields of tissue engineering and regeneration medicine, among others. However, weak mechanical properties and short residence time due to the rapid degradation in physiological conditions lead to relatively fast drug release kinetics. Therefore, poloxamers are now more prone to be combined with other microparticle systems or conjugated with other polymers for more sustainable release and longer release period.

2.1.4. Poly(N-isopropylacrylamide-co-propylacrylic acid) Copolymers (pNIPAAM). pNIPAAM is another kind of thermosensitive polymer that exhibits a lower critical solution temperature (LCST) at around 33 °C, which thus has been applied to construct hydrogel-based long-acting protein formulations. The distinctive thermosensitive property of pNIPAAM is owed to its pendant isopropylamide groups, which induces a change in the hydrophilic−hydrophobic balance as the temperature changes.[22,90,91] Mazzola et al. prepared bromelain loaded pNIPAAM hydrogel *via* redox polymerization with a high drug loading capacity of ∼30%.[92] The *in vitro* release profile suggested that the encapsulation of pNIPAAM hydrogel efficiently extended the release period of bromelain up to 96 h with ∼16% laden proteins released in the first 24 h. Along with its mucoadhesive property, the pNIPAAM hydrogel may be applicable in wound healing for controlled release of growth factors such as VEGF, EGF, *etc.* For resolving the nondegradable property stem from pNIPAAM, in the work of Awwad et al., biodegradable hyaluronic acid was incorporated to the pNIPAAM hydrogel matrix *via* cross-linking with poly(ethylene glycol) diacrylate (PEGDA) and loaded with infliximab (INF), allowing pNIPAAM oligomers to be soluble and excreted.[93] From the release curve of an *in vitro* PK-Eye model, it could be noted that the release of INF was prolonged to 12 days with initial burst release less than 20% on day 1. However, only half of the INF could be released from the hydrogel which is a great waste of drugs especially for the antibodies, whereas the controllable and sustainable delivery of IFN makes it a promising long-acting formulation for treatment of ocular diseases. Concluding from these cases, pNIPAAM would be a potential candidate for long-term release of proteins in application of tissue engineering based on its low LCST, mucoadhesive property, and film-forming property. Challenges of slow gelation response to the surrounding temperature, nonbiodegradability, and underlying toxicity resulting from long-term accumulation still need to be overcome before the clinical translation in the future.

2.1.5. Arginine-Based Polymers. Arginine-based polymers as a kind of polyester amide consist of arginine, diols, and diacids, and they are synthesized by solution polycondensation of monomers with various arginine ratios.[94,95] Arginine-based polymers with cationic property derived from arginine could form nanocomplexes with different proteins or peptides *via* electrostatic interaction. For instance, Wu et al. designed and successfully synthesized a diblock copolymer of PLGA and arginine-based polymer (PLGA-*b*-PC). Through the electrostatic interaction, proteins/peptides including BSA, OVA, TNF-α, insulin, and Ac2-26 peptide were loaded into PLGA−PC nanoparticles by simply aqueous mixing, achieving high encapsulation efficiency (>95%) and loading capacity (>20%). For higher size stability, DSPE-PEG_{2000} was coated onto the surface of PLGA−PC nanoparticles to form an ∼200 nm sphere. The *in vitro* release profile of BSA from nanoparticles observed burst release (∼20%) on day 1 and a moderate release from day 6 with a total release period for approximate 3 weeks.[96] Based on this work, Huang et al. prepared PEG-coated PLGA−PC nanoparticles with core−shell structure *via* a nanoprecipitation method instead of electrostatic interaction for oral delivery of insulin. The as-prepared nanoparticles with 43 nm in diameter exhibited great mucus penetration and transepithelial transport, which efficiently decreased the BGL for more than 50% in 3 h after single *s.c.* injection without causing hypoglycemia in comparison with the free insulin group.[97]

Besides that, Wu et al. also investigated the arginine-based polymer hydrogel for sustainable release of peptides *via* physical cross-link (*e.g.* UV light). Through the photo-cross-linking between cationic arginine-based unsaturated poly(ester amides) (Arg-UPEAs) and polyethylene glycol diacrylamide (PEG-DA), insulin, and transdermal peptide 1 (TD-1), coloaded hydrogel (PEG-DA/Arg-PEA/insulin/TD-1) was successfully prepared for transdermal delivery of insulin. The as-prepared hydrogels showed the initial burst release of insulin on the first 9 h with cumulative release of insulin for ∼37.58%. After that, the release rate gradually slowed down owing to the depletion of insulin near the hydrogel surface, and the whole release period lasted for more than 3 days. On the T1D murine model, PEG-DA/Arg-PEA/insulin/TD-1 efficiently reduced the mice BGL from 23.63 mmol/mL to 12.63 mmol/mL in 4 h, indicating the potential to be translated for clinical diabetes treatment.[98]

Overall, as an essential amino acid for humans, arginine-based polymer exhibits high biocompatibility, water solubility, and good biodegradability. Thanks to its cationic property, the electrostatic interaction between polymer and various proteins or peptides efficiently enhances the encapsulation efficiency and also highly preserves the bioactivity of loaded drugs. Moreover, unlike those cationic block copolymers (*e.g.*,

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polylactide−poly-L-lysine block copolymer) with a cationic group dispersed in one block of the copolymer, the cationic charge unit (arginine) in arginine-based polymers could be evenly dispersed in the backbone of the polymer. Thus, the positive charge density of the polymers could be easily tuned to meet the needs for loading different proteins/peptides. Though exhibiting great potential in delivering protein/peptide therapeutics, the investigations of most arginine-based sustained-release systems remain at the cellular level (except for the insulin delivery) and its suitability for other diseases needs to be further investigated.

Concluding from all these synthetic polymer-based long-acting formulations (Table 1), we could confirm that the encapsulation of such protein/peptide therapeutics with biocompatible and biodegradable polymers efficiently prevents the oxidation, hydrolysis, or enzyme degradation and hence preserves the bioactivity of these therapeutics. Meanwhile, different construction materials and dosage forms (*in situ* implant/gel, micro-/nanoparticles) efficiently alter the pharmacokinetics and prolong the release period of drugs. However, the usage of organic solvent and shearing stress during the preparation based on the double emulsion method would possibly reduce the bioactivity of protein/peptide therapeutics. Besides that, severe side effects owing to the initial burst release resulted from the structure of hydrogel/particle long-acting formulations; immune reactions induced by acidic hydrolytic products (*e.g.* lactate of polymers) are other issues of concern in the development of synthetic polymer-based systems in the future.[99,100]

2.2. Natural Polymers. Natural polymers constructed for long-term delivery of proteins or peptides are mainly referred to polysaccharides such as chitosan, dextran, and alginate, among others. Hydrogels and micro-/nanoparticles are the main strategies that are being used to develop natural polymer-based long-acting formulations.[101−103] Hydrogel systems are mostly prepared *via* physical or chemical cross-linking by UV light, formaldehyde, glutaraldehyde, *etc.* based on the reactive groups such as the amine group and carboxyl group existing in the structure of these polysaccharides.[104,105] It is difficult for polysaccharides owing to their hydrophilicity to form into micro-/nanoparticles without the participation of other substances. Therefore, based on the natural-born highly charged property derived from amine or carboxyl groups, the noncovalent interaction such as the electrostatic interaction between polysaccharides such as chitosan (positively charged) or hyaluronic acid (negatively charged) and proteins is the most used mechanism to load target drugs.[106,107] Besides that, through the chemical conjugation of a hydrophobic segment for improved amphiphilicity, modified polysaccharides could form into micelles in aquatic conditions and hence load target protein/peptide therapeutics *via* hydrophobic interaction.

2.2.1. Chitosan. Chitosan, obtained by the deacetylation process of chitin, is a linear copolymer including N-acetyl-D-glucosamine and D-glucosamine linked by a β-1,4 glycosidic bond.[108−110] As a natural cationic polymer with high biocompatibility approved by the FDA, chitosan is an excellent candidate for cosmetic or biomedical applications owing to its admirable biological properties such as biodegradability, mucoadhesivity, and antimicrobial capacity.[111−113] Chitosan is generally constructed into formulations including micro-/nanoparticles, hydrogels, or nanofibers, and it has been successfully applied into loading various protein/peptide therapeutics of IgG antibody, insulin, Avastin, and VEGF

Table 1. Synthetic Polymers for Protein/Peptide Delivery and Its Application

Carrier	Formulation	Preparation method	Loading capacity (%)	Release period	Protein/peptide	Application
PLGA	Hydrogel	Cross-linking under physiological temperature	~0.48	9−21 days	lysozyme, porcine growth hormone, insulin, lixisenatide. human calcitonin or recombinant hepatitis B antigen	Treatment of diabetes, bone resorption, Noonan syndrome, hepatitis B.[48−51]
	MPs/MPs	double emulsion, noncovalent interaction	~1%	15−40 days	OVA, EGF, IL-10, VEGF, bFGF, MOG35-55	Wound healing. Treatment of autoimmune encephalomyelitis.[53−55]
PCL	MPs/NPs	double emulsion	0.8−28.5%	25−90 days	OVA, bevacizumab	Treatment of posterior ophthalmic diseases, revascularization.[71,79]
	Film/sheet	electrospinning technique	~17%	25 days	VEGF, PDGF	Vessel regeneration, wound healing.[82]
PEG	Hydiogel	Cross-linking under physiological temperature	~0.03%	3−14 days	SDF-1α, liraglutide, IL-12, aFGF, bFGF	Treatment of diabetes and cancer.[70,72,73]
PF127	Hydrogel	Cross-linking under physiological temperature	~17%	5−28 days	recombinant hirudin variant-2, EGF, NGF, recombinant human growth hormone	Treatment ot diabetes, spinal cord injury and cancer. Peripheral nerve regeneration, Wound healing.[87,88]
	MPs/NPs	double emulsion cross-linking under	~15%	~60 days	Geslorelin acetate	Treatment of cancer.[86]
pNIPAAM	Hydrogel	Cross-linking under physiological temperature	~16%	4−12 days	EGF, VEGF, bromelain	Treatment of ocular disease, Wound healing[92,93]
Aiginine	Hydiogel	photocross-linking	>20%	~3 days	insulin, TD-1 peptide	Treatment of diabetes.[98]
	MPs/NPs	nanopericipitation	>20%	~21 days	BSA, OVA, TNF-α, insulin, and Ac2-26 peptide	Treatment of diabetes, cancer and inflammatory diseases.[96,97]

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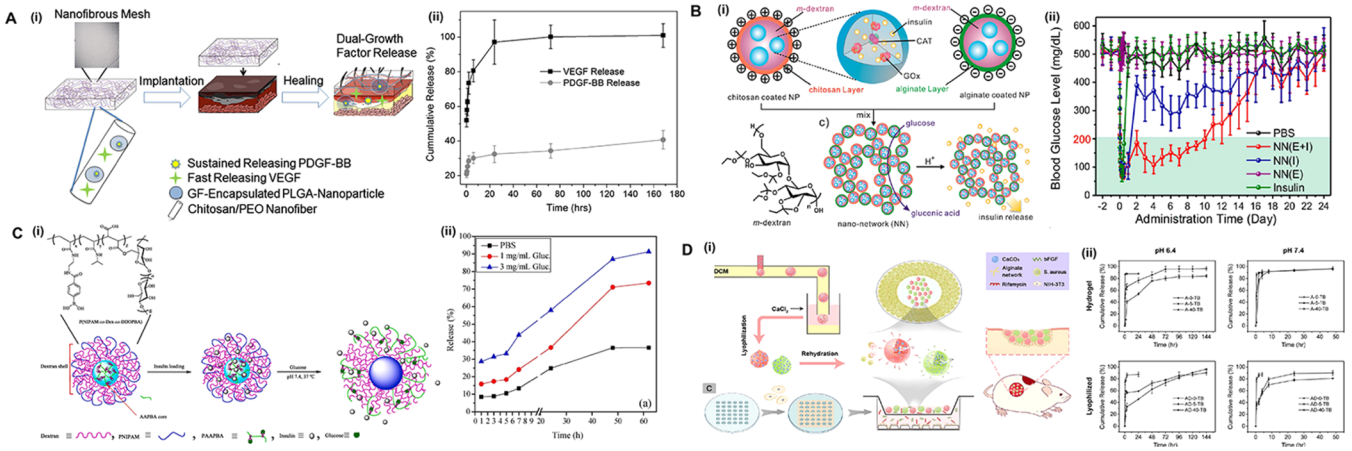

Figure 3. (A) (i) Chitosan/PEO nanofibrous mesh for dual-growth factor (PDGF-BB and VEGF) release in treatment of wound healing. (ii) *In vitro* release of PDGF-BB and VEGF from chitosan/PEO nanofibrous mesh. Published with permission from ref 117. Copyright 2013 Acta Materialia Inc. (B) (i) Injectable glucose-responsive dextran-based nanonetwork loaded with insulin for hypoglycemic control of Type I diabetes. (ii) Blood glucose levels in diabetic mice after single *s.c.* injection with the nanonetwork. Published with permission from ref 120. Copyright 2013 American Chemical Society. (C) (i) Dextran as the cross-linker for construction of glucose-responsive poly(NIPAM-*co*-Dex-*co*-DDOPBA) hydrogel in the treatment of Type I diabetes. (ii) *In vitro* release of insulin from the glucose-responsive hydrogel. Published with permission from ref 121. Copyright 2020 Elsevier Ltd. (D) (i) Schematic illustration of alginate/$CaCO_3$ composite microparticles preparation and their applications in wound healing. (ii) *In vitro* release profile of alginate/$CaCO_3$ composite microparticles at hydrogel state or after lyophilization. Published with permission from ref 131. Copyright 2019 American Chemical Society.

and dramatically prolongs the release of the target drugs to several days or even weeks.[114−117] For instance, in the work of Fletcher et al., alginate-chitosan hydrogels prepared through the cross-link between alginate and calcium sulfate slurry solution were applied for prolonging release of IgG model antibodies and Fab antibody fragments from PLGA microparticles (∼15 μm).[114] The *in vitro* release profiles of IgG suggested that the addition of chitosan decreased the release amount of IgG antibodies from hydrogels from 10% to 0.3% on day 1 and effectively extended the release period up to 7 weeks. Similarly, in the work of Xu et al.,[115] an injectable chitosan-alginate hydrogel was synthesized *via* a vial tilting method and successfully loaded with Avastin, the first choice drug for the treatment of age related macular degeneration yet being clinically confined due to its short half-life in intraocular. *In vitro* release study suggested that the initial burst release of Avastin could be reduced from 55% to 30% on day 1 with the increase of alginate content (from 0.5 wt % to 2 wt %), and the release period could be extended maximally to 3 days. Moreover, *in vitro* degradation study suggested that through the adjustment of alginate content, the degradation period of the hydrogel could be up to 18 days. Apart from the traditional subcutaneous injectable form of the hydrogel, in the work of Nazar et al., an N-trimethyl chitosan chloride (TMC)-based thermosensitive semirigid gel was designed and fabricated through the gelation of glycerophosphate and efficiently encapsulated insulin for nasal mucosa delivery.[116] The *in vitro* release profile indicated that the initial burst release of insulin was up to 60% in the first 10 min and the whole release period only lasted for 60 min. A hypoglycemic efficacy study revealed that this semirigid hydrogel *via* nasal administration is capable of reducing blood glucose yet in the abnormal range over 24 h on the diabetic-rat model, indicating its limitation in stable glycemic control on clinical diabetes patients since its high burst release may induced undesirable and severe side effects such as hypoglycemia.

Assisted by an electrospinning technique, chitosan could also be fabricated into nanofibers to effectively load protein therapeutics for applications in tissue engineering. For instance, Xie and co-workers prepared nanofiber composites containing chitosan (CS) and poly(ethylene oxide) (PEO) to encapsulate VEGF using an electrospinning method and PLGA nanoparticles (∼153 nm) loaded with platelet-derived growth factor-BB (PDGF-BB) and embedded within nanofibers.[117] Thus, the nanoparticle-in-fiber system as an alternative strategy that could endow a dual release was formed (Figure 3A-i). The *in vitro* release profiles revealed that VEGF releasing from CS/PEO nanofibers displayed an initial burst release of 63% in 1 h and reached nearly 100% within day 1 while PDGF-BB exhibited a more sustainable manner with initial burst release about 30% on day 1. Fast release of VEGF is essential for promoting the angiogenesis at the wound while the sustained release of PDGF-BB is needed to proliferate fibroblast growth in the long term for more effective wound healing (Figure 3A-ii). Four weeks of treatment with one single dosage on a rat skin wound model indicated longer epithelial tongues in the CS/PEO-NPs group at week 1 in contrast with the control group, which is attributed to the function of the fast release VEGF. At week 2, granulation tissue thickness from the CS/PEO-NPs group was effectively reduced compared to those of week 1 and control, which demonstrate these CS/PEO nanofibers with fast and slow biphasic release for VEGF and PDGF-BB hold a great potential for treatment of chronic wound healing.

On the whole, cationic chitosan could efficiently load various proteins/peptides with different isoelectric points (pI) through electrostatic interaction. Thanks to its natural-born anti-inflammation, mucoadhesive property, and film-forming property, chitosan-based long-acting formulations are now significantly investigated in the field of tissue engineering such as chronic wound healing. However, chitosan is only soluble in acidic solution, which greatly confines its application for delivery protein therapeutics with pI > 7. Chemical modifications of chitosan are urgently needed to enhance its solubility and hydrophilicity. Besides that, the allergenicity is

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another issue that significantly limits its wide clinical application.

2.2.2. Dextran. Dextran is a highly water-soluble polysaccharide with predominant composition of linear α-1,6-linked glucopyranose units and some degree of 1,3-branching, which is vastly applied in plasma volume expansion, peripheral blood flow enhancement, thrombosis prophylaxis, and artificial tears benefiting from its high biocompatibility, low toxicity, and immunogenicity.[118,119] To date, numerous therapeutic proteins such as insulin, EGF, VEGF, and OVA have been successfully loaded to a dextran-based carrier with an extended release period up to several days or even weeks.

For instance, Gu et al. developed an injectable and acid-degradable polymeric network, in which insulin and glucose-specific enzymes (GOx) were loaded into pendant acetal modified dextran nanoparticles *via* a W/O/W double emulsion method and then coated with cationic chitosan or anionic alginate to form a nanonetwork *via* electrostatic interaction (Figure 3B-i).[120] The as-prepared chitosan-coated and alginate-coated dextran nanoparticles were 340 and 293 nm in diameter with loading capacity of 7.9% and 11.4%, respectively. Interestingly, under a simulated hyperglycemic level (400 mg/dL glucose), this novel nanonetwork rapidly released ∼1400 μg/mL insulin in 7 h because GOx encapsulated in the nanonetwork catalyzed glucose into gluconic acid, leading to a local pH decrease from 7.4 to 4.2, and thus triggered the dissociation of acid-degradable pendant acetal modified dextran to release the laden peptide. Moreover, the results of the hypoglycemic efficacy investigation suggested that this nanonetwork significantly improved the glucose control of the type 1 diabetic mice model and maintained the BGL in the normal range for up to 10 days after one single injection without induction of hypoglycemia (Figure 3B-ii). Likewise, Elshaarania et al. designed and synthesized a glucose-responsive copolymer namely poly(NIPAM-*co*-Dex-*co*-DDOP-BA) with dextran-grafted maleic acid (Dex-MA) as a biodegradable cross-linker and phenylboronic acid (DDOP-BA) as the glucose responder and prepared an insulin-loaded hydrogel (160 nm) *via* EDC chemical cross-linking (Figure 3C-i).[121] The *in vitro* release profile suggested that insulin loaded hydrogel exhibited a glucose-dependent release of the payload. Under simulated physiological conditions, the release of insulin in the first 24 h was ∼20% while under 3 mg/mL glucose, this release could be up to ∼55% (Figure 3C-ii), which provides a new strategy to smart control of blood glucose in diabetes patients.

Besides that, polymer-based long-acting formulations were also embedded into dextran-based hydrogel to enhance the prolonged release of target drugs. For instance, Gan et al. embedded TGF-β3-loaded PLGA nanoparticles (∼218 nm) into dextran-alginate hydrogel cross-linked *via* Schiff base between oxidative dextran (Oxi-Dex) and amino gelatin. The *in vitro* release kinetics indicated that the TGF-β3 loaded carrier system remained a stably sustained-release profile for more than 28 days with a low release percentage of ∼7% on day 1.[122] Likewise, dextran-based hydrogel encapsulated chitosan microparticles (∼255 μm) loaded with EGF and VEGF were prepared *via* the cross-link of adipic acid dihydrazide. A therapeutic efficacy study on a wound skin rat model emphasized that the controlled released system (1 dosage *per* week) performed more efficiently in terms of reducing the wound area than free EGF + VEGF application every 2 days, indicating great potency in the treatment of wound healing.[123]

After being modified with a hydrophobic segment such as PLGA or 2-ethoxypropene, dextran could also be constructed into micro-/nanoparticles *via* double emulsion methods. For instance, Chen et al. successfully encapsulated the ovalbumin (OVA) into 2-ethoxypropene modified dextran microparticles (∼1 μm) and realized the controlled antigen delivery. Pharmacodynamic investigation suggested that acetalated (ace) dextran microparticles loaded with OVA induced greater cytokine and antibody production with a duration of 42 days compared with the free form OVA, suggesting it is a promising candidate for developing long-acting and robust nano-vaccines.[124] Similarly, in the work of Suarez et al., ace-Dextran microparticles (∼30 μm) were prepared *via* a W/O/W double emulsion method and successfully loaded with an engineered hepatocyte growth factor fragment (HGF-f). The release period of HGF-f in a heart failure postmyocardial infarction model was tuned from 3 days to 20 days through the adjustment of acetalated content (from 43% to 57%). An *in vivo* therapeutic efficacy investigation indicated that HGF-f laden ace-Dextran microparticles significantly increased the diameter of arteriole from ∼37 μm to ∼45 μm, alleviating the apoptosis of cardiomyocyte and decreasing scar size on an infarcted heart rat model, which may be applicable in the future for the treatment of cardiovascular diseases.[125] Aiming for more controllable and sustainable release of protein therapeutics, Zhang and co-workers constructed PLGA-coated VEGF loaded-dextran microparticles (40−100 μm) *via* an S/O/W method for therapeutic neovascularization.[126] The *in vitro* release profile suggested that the release of VEGF from PLGA-coated dextran microspheres followed a sustained manner lasting for more than 1 month with initial burst release of ∼10% during the first day. Moreover, on a rat hind-limb ischemic model, VEGF loading dextran microparticles coated with PLGA efficiently promoted the capillaries and smooth muscle α-actin positive vessel formation, indicating their great potential in the treatment of limb ischemia.[126]

Benefiting from their hypoglycemic efficacy, antifungal property, and acceleration on wound enclosure, dextran-based long-acting formulations are hence widely applied in the treatment of diabetes and tissue engineering of diabetic wound healing or tissue regeneration. Owing to its cell adhesive property, dextran-based hydrogels loaded with growth factors are mostly applied in wound healing, whereas in the treatment of diabetes and cardiovascular diseases, dextran-based nanoparticles are mostly employed due to their hypoglycemic efficacy. However, its industrial extraction methods are currently confined to acidic, basic, or enzymatic hydrolysis of yeast, which exhibits issues of complicated procedures, low purity, and low yield. Though the microwave method has been prevalently exploited, it is still immature and confined to lab applications due to the limits of the microwave instrument for scalable extraction of dextran.

2.2.3. Alginates. Alginates as natural polymers are widely used in biomedical research owing to their attractive properties including wide sources, inertness, low cost, and good biocompatibility and biodegradability.[127−129] Alginate-based long-acting formulations have been successfully employed to load various protein/peptide therapeutics such as growth factors.[130,131] For example, Zhu et al. constructed an alginate microparticle-embedded thermosensitive chitosan/dextran-polylactide/glycerophosphate hydrogel in which bone mor-

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Table 2. Natural Polymers for Protein/Peptide Delivery and Its Application

Carrier	Formulation	Preparation method	Loading capacity (%)	Release period	Protein/peptide	Application
Chitosan	Hydrogel	Chemical cross-linking	∼1%	3−49 days	IgG antibody, insulin, Avastin	Treatment of diabetes, macular degeneration[114−116]
	Nanofibers	electrospinning technique	<1%	∼28 days	VEGF, PDGF-BB	Wound healing [117]
Dextran	MPs/NPs	double emulsion	1.7% ∼ 11.4%	10−40 days	insulin, HGF-f, VEGF and OVA	Treatment of diabetes, cardiovascular diseases and limb ischemia.[120,124,125]
	Hydrogel	Chemical cross-linking	14.9% ∼ 17.9%	3−14 days	insulin, TGF-β3, EGF and VEGF	Treatment of diabetes; Wound healing[121−123]
Alginate	Hydrogel	Cross-linking under physiological temperature	<1%	∼21 days	bFGF	Wound healing.[131]
	MPs	Ca^{2+} cross-linking	<1%	∼28 days	BMP-2	Bone repair and regeneration.[130]
Pectin	MPs	spray drying technique. thin-film hydration method	7.2% ∼ 14%	∼1 days	AGP peptide, nisin	antimicrobial, dietary supplement[133,134]

phogenetic protein-2 (BMP-2) was encapsulated into alginate microspheres *via* the cross-linking of calcium chloride. The as-prepared BMP-2 laden alginate microspheres (∼20 μm) embedded into hydrogel efficiently extended the release period of loading drugs to more than 4 weeks, and the initial burst release of BMP-2 was reduced from ∼37% to ∼7% on day 1 compared to microspheres without alginate hydrogel embedment. The result indicated the significant role of alginate hydrogel on controlling release of BMP-2 to prevent the side effects of bone cyst-like formation derived from its burst release. Pharmacodynamic investigation revealed that the BMP-2 long-acting formulation efficiently promoted the calcium content in muscle tissue of rats from day 7 to day 14 after single dosage and exhibited ectopic osteogenesis of where it was injected, indicating significant potency in bone repair and regeneration.[130] Besides its application in the polymer-based long-acting formulations, alginate-based hydrogel has also been reported to combine with inorganic materials for long-term delivery of protein therapeutics. In the research of Shi et al., alginate/$CaCO_3$ composite microparticles (∼430 μm) were fabricated *via* a microfluidic technique to encapsulate bFGF (Figure 3D-i).[131] The *in vitro* release profile indicated that the integration of $CaCO_3$ composites in the whole system efficiently prolonged the release of bFGF up to 144 h and reduced the initial burst release of bFGF from 90% to 40% in the first 24 h (Figure 3D-ii). On a full-thickness skin wound rat model, alginate/$CaCO_3$ composite microparticles greatly accelerated wound healing with higher granulation tissue thickness and better bioactivity to stimulate angiogenesis followed by a sustained release of bFGF for more than 21 days, suggesting a great potency to be translated as dressing materials for clinical wound healing.

Through the cross-link of Ca^{2+}, alginate hydrogel exhibits the advantage of better preserving loaded protein therapeutics without usage of other chemical cross-linkers, whereas the fast cross-link between Ca^{2+} and alginate also results in uncontrollable preparation and leads to great differences from batch-to-batch. Besides that, the high swell ratio of the as-prepared alginate hydrogel is another critical reason for initial burst release of alginate-based long-acting formulations, which requires integration of other materials to better control the drug release kinetics.

2.2.4. Other Polysaccharides. Pectin is an anionic polysaccharide, comprising a linear backbone composed by galacturonic acid units linked by α-1,4 bonds. It is an emerging material for protein/peptide therapeutics owing to the high biocompatibility, biodegradability, and low toxicity.[132] For instance, in the work of Novaa et al., a combination of Surelease (ethylcellulose aqueous dispersion) and pectin was used to prepare microparticles (1.8−2.5 μm) *via* a spray drying technique and successfully loaded with AGP (L-alanyl-L-glutamine dipeptide), while the *in vitro* release of AGP did not extend distinctively probably due to the high hydrophilicity of the dipeptide.[133] In order to prolong the release of the laden proteins, Brandelli et al. designed and prepared pectin and polygalacturonic acid-coated liposomes (∼120 nm) *via* the thin-film hydration method for more controllable and sustainable release of nisin. As expected, the *in vitro* release results indicated a reduced initial release of nisin from liposomes on the first 24 h from ∼80% to ∼40% owing to the coating of pectin and polygalacturonic acid, which provides a new long-acting formulation for antifungal application.[134]

Cyclodextrins (CDs) owing to their amphiphilic properties have been widely used for delivery of hydrophilic or lipophilic drugs. Due to the highly porous structure, cyclodextrin-based nanosponges (CD-NS), prepared *via* melt, solvent, ultrasound-assisted synthesis, and microwave-assisted synthesis, can offer the unique advantage of controlled release as an innovative carrier in protein/peptide delivery. Model proteins such as bovine serum albumin (BSA) and lysozyme have been reported to be loaded onto the newly forming nanosponges through freeze-drying or solvent evaporation for long-term release.[135]

Overall, apart from preservation of protein/peptide therapeutics from enzyme degradation, hydrolysis, or denaturization, natural polymers exhibit higher biocompatibility and biodegradability compared to synthetic ones (Table 2). Its natural-born physiological functions of anti-inflammation, antibacteria, *etc.* make natural polymers widely applicable in the fields of disease treatment of diabetes or tissue engineering of diabetic wound healing. In addition, unlike the case of the degradation of synthetic polymers may induce an undesirable immune reaction, the degradation products of natural polymers such as polysaccharide are mostly safe and inert to the immune system. Therefore, natural polymers are a safer, more suitable, and effective protein delivery material for anti-inflammation, antibacterial, or antifungal applications compared to synthetic polymers. However, the molecular weight distribution of the natural substance is usually wide, and it is difficult to control the quality of natural polymer-based long-acting formulations, which may significantly influence the consistency of clinical trials in the future. Moreover, the usage of chemical cross-linker during the preparation may result in the denaturation of protein/peptide, reducing its therapeutic efficacy. Also, undesirable side effects induced by the severe initial burst release from the formulation type (mainly

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hydrogel) are another issue to be addressed. Investigation of strategies using microspheres or macroscopic devices based on more hydrophobic synthetic polymers (*e.g.* PLGA, PCL) becomes more prevalent in the research field.

3. PROTEIN-BASED SYSTEMS

Protein is a kind of special polymer through the condensation polymerization of various amino acids, which has also been investigated significantly as a carrier for controllable and sustainable delivery of protein/peptide therapeutics due to their abundance, ease of modification, amphiphilicity, low toxicity, moderate mechanical property, and good biocompatibility and biodegradability. Proteins such as silk-like protein, elastin-like protein, collagen, gelatin, and albumin have been investigated and developed into long-acting formulations for controllable release of protein/peptide therapeutics.

Unlike traditional preparation methods for producing polymer micro-/nanoparticles, preparation methods for protein-based particle long-acting formulations are mainly based on physical methods such as solution-enhanced dispersion, desolvation, self-aggregation, or the microinjection pump method, which do not need the participation of organic solvent and hence better preserve the bioactivity of protein/peptide therapeutics.[136−139] Thanks to the abundant active radical groups such as amino group, carboxyl group, *etc.*, long-acting protein-based hydrogels are mainly fabricated through physical (acidification, temperature change, or shearing forces induced by sonication) or chemical (like EDC/NHS) cross-linking. Also, with the development of new techniques, protein-based sustained-release systems for protein/peptide therapeutics delivery were also designed and integrated into films, wafers, reservoirs, discs, foams, and microneedles for more controllable release kinetics or better mechanical strength to meet various needs from different disease treatments.[140−142]

3.1. Silk-like Proteins (SLPs). SLPs due to their good biocompatibility, biodegradability, moderate mechanical strength, controllable structure, and self-assembly properties have been vastly exploited in long-term release of protein/peptide therapeutics.[143,144] The most investigated SLPs are recombinant forms of natural silk fibroin domains from cocoons of silkworm (*Bombyx mori*) or dragline of spider (*Nephila clavipes*). The heavy chain of SLPs with amphiphilic property consists of 12 crystallizable hydrophobic segments and 11 amorphous hydrophilic segments, which can be tuned to realize the controllable drug release kinetics and degradation. Besides that, SLPs can be sterilized by autoclave, γ radiation, and ethylene oxide, which is a critical step for the successful clinical translation.[145,146]

For now, various peptides, antibodies, growth factors, or cytokines have been reported to be loaded onto SLP through absorption or chemical conjugation like disulfide bond for higher drug loading and better preservation of bioactivity. For instance, in the work of Reeves et al., SLP films were prepared *via* a water vapor annealing method to entrap interferon gamma (IFN-γ) or interleukin-4 (IL-4) for macrophage polarization. The crystalline content of SLP was tuned to optimize the solubility of the films and hence control the pharmacokinetics of loaded drugs. The result showed that the cytokine release from soluble films with low crystalline contents was extended up to 10 days while IFN-γ or IL-4 was hardly released from insoluble films with high crystalline contents.[142] Similarly, Engert et al. prepared the SLP films (2.5 × 3 cm; 3.33 mg/cm^2) with further coating of three layers of silk protein *via* a water-based method without any usage of organic solvent to form a "sandwich" structure, which could highly preserve the bioactivity of laden proteins. The *in vitro* release profile suggested that bovine serum albumin (BSA) loaded sandwich-like SLP films efficiently extended the release period of BSA to ∼41 days with only ∼10% initial release on day 1. Besides that, SLP could also be incorporated or constructed into hydrogels to load peptide or protein therapeutics.[147] For instance, in the work of Kaplan et al., SLP hydrogel was prepared by sonication and a subsequent lyophilization method, in which the sonication power was tailored from 20% to 65% amplitude to achieve the sol−gel transition based on the SLP concentration. By increasing the SLP content (from 3.1% to 6.7%) in the gel, the initial burst release of antibody IgG1 was reduced from ∼70% to ∼10% on day 1, and the release profile was up to more than 40 days, exhibiting great potential in developing long-acting antibody formulations.[148]

Moreover, new techniques were also introduced in exploiting large foam systems like SLP discs for more controllable and sustainable release of protein therapeutics. In the work of Yavuz et al., SLP discs were prepared by a water vapor annealing method and successfully loaded with 5P12-RANTES (human immunodeficiency virus, HIV inhibitors). The *in vitro* release profile of 5P12-RANTES loaded SLP discs exhibited a tunable release period of the HIV inhibitors up to 31 days with adjustment of NaCl content in the formulation. The sustainable released inhibitors exhibited prophylactic efficacy on HIV infection in both blood and colorectal tissues *ex vivo*, indicating a great clinical translation potential in the prevention of HIV infection.[149]

Overall, the preparation methods for SLP long-acting formulations could better preserve the loading protein/peptide from denaturization of organic solvent, shearing stress, *etc.* which widely existed in preparation of polymer-based long-acting formulations. However, the release of SLP-based long-acting formulations is uncontrollable considering the sustained behavior is mainly realized through the enzyme degradation of protease *in vivo*. Besides, SLP may induce an inflammatory response *in vivo* for 1 to 3 weeks, which may increase the degradation rate and thus accelerate the release of loaded drugs. Therefore, a comprehensive understanding of the underlying structure−function relationship between SLP and target proteins needs to be further investigated for rational design of SLP-based long-acting formulations in the future.

3.2. Elastin-like Proteins (ELPs). ELPs are thermosensitive proteins derived from a human extracellular matrix with excellent mechanical properties and are widely applied in drug delivery and tissue engineering. The most investigated motif of ELPs is the replicates of the VPGXG sequence, in which "X" represents any amino acid except proline.[144,150] The transition temperature of ELPs could be tuned to enable the solubility of ELPs at room temperature and form insoluble coacervates under physiologically relevant temperatures.[151,152]

Therefore, in most research related to ELPs-based long-acting formulations, ELPs were usually fused with target protein or peptide therapeutics *via* biosynthesis of *Escherichia coli* with the aid of plasmid vector to construct fusion proteins, which may transform into gel under physiological relevant temperature spontaneously and further control the release of fused proteins/peptides. For instance, in the work of Chilkoti et al., glucagon-like peptide 1 (GLP-1) was fused with ELPs

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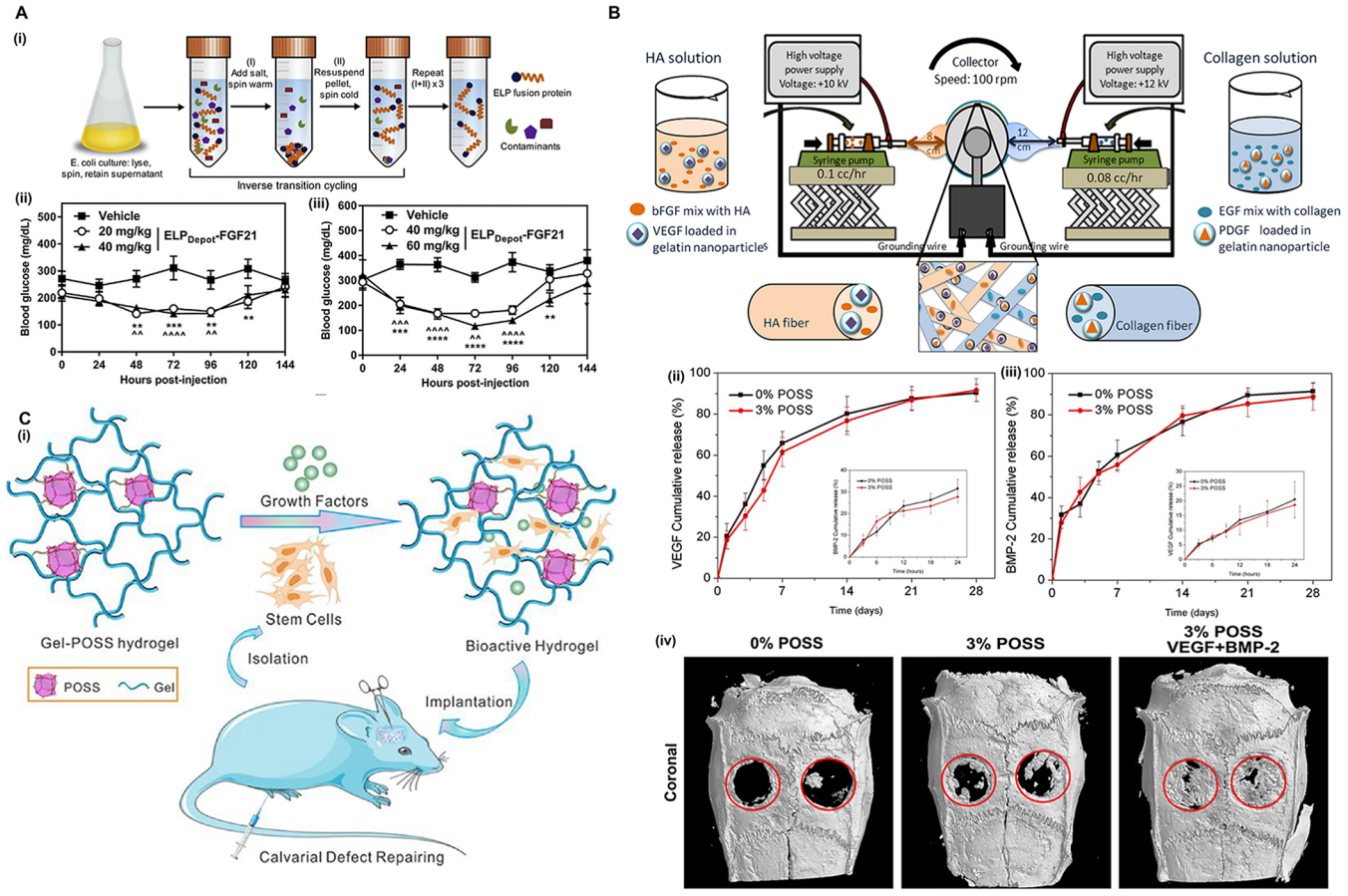

Figure 4. (A) Fusion of FGF21 to ELP (ELP$_{depot}$-FGF21) forms an injectable depot for sustained hypoglycemic control. (i) Preparation and purification of ELP fused protein *via* inverse transition cycling. (ii) (iii) Hypoglycemic efficacy of ELP$_{depot}$-FGF21 with different dosages. Published with permission from ref 154. Copyright 2018 Elsevier B.V. (B) Preparation of collagen and hyaluronic acid interstacking nanofibrous for delivery of bFGF, EGF, and PDGF. Published with permission from ref 174. Copyright 2014 Acta Materialia Inc. (C) (i) Polyhedral oligomeric silsesquioxane (POSS)-modified porous gelatin hydrogels for large bone defect repair. *In vitro* release of (ii) VEGF and (iii) BMP-2 from POSS modified gelatin hydrogels. (iv) Representative coronal and sagittal images of calvarial bone defects after implantation with POSS modified gelatin hydrogels for 8 weeks. Reproduced with permission from ref 184. Copyright 2020 American Chemical Society.

and demonstrated stable hypoglycemic efficacy on three diabetic murine models for 10 days and monkey models for 17 days after single subcutaneous (*s.c.*) injection. Besides that, glycosylated hemoglobin levels (HbA1c) and weight gain in ob/ob mice were evidently reduced and maintained in the normal range for 8 weeks with one dosage a week.[153] Based on this work, Chilkoti et al. also constructed fibroblast growth factor 21 (FGF 21) fused ELPs depot for sustained hypoglycemic control on a T2D murine model (Figure 4A-i).[154] Hypoglycemic efficacy study revealed that mice BGL were maintained in the normal range for 5 days after a single *s.c.* injection (Figure 4A-ii, iii), suggesting that FGF 21 fused ELPs depot is a promising candidate of genetically engineered drug for hypoglycemic control of T2D. By cross-linking ELPs depots through chemical or photoirradiation cross-link methods, the degradation rate could be reduced and hence extend the release time of loaded protein/peptide therapeutics. Chilkoti et al. cross-linked the ELPs with a disulfide bond *via* oxidation of H_2O_2, which did not decrease the bioactivity of laden proteins due to the mild oxidant H_2O_2. This chemical cross-linking efficiently extended the release of model protein BSA for more than 5 days with ∼45% initial burst release on day 1, suggesting its potential application in drug delivery, tissue engineering, and regenerative medicine in the future.[155]

Moreover, owing to its distinctive mechanical property, therapeutic proteins or growth factors such as SDF-1α, αB Crystallin, lacritin, or recombinant human bone morphogenetic protein-2 (rhBMP-2) were reported to be fused with ELPs and applied in tissue engineering of wound healing, ocular diseases, or bone regeneration.[156−159] For instance, in the work of Yeboah et al., SDF-1α fused ELPs were prepared through biosynthesis of *Escherichia coli* with the aid of a plasmid vector and applied in the treatment of diabetic wound healing. The Western blot analysis of SDF-1α-ELPs incubated with elastase for 12 days did not detect any SDF-1α release indicating the great stability of the hydrogel, providing good protection of the loaded proteins. Moreover, a therapeutic efficacy study on a diabetic skin wound mice model suggested that SDF-1α fused ELPs significantly promoted the skin wound healing as 95% wound enclosure was achieved on day 21 in contrast with 80% achieved by free form SDF-1α, confirming its great potential for the treatment of chronic wound healing.[156]

Taking advantage of genetic engineering, ELPs could be fused with various kinds of proteins, which hence is a promising sustained-release formulation applying for disease treatment such as diabetes or tissue engineering clinically. Currently, most investigations on ELPs are typically based on

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bacterial expression systems to produce ELPs fused proteins. However, this method limited the laden peptides or proteins to those that do not require glycosylation and can properly fold in the bacterial cytoplasm. Thus it is urgent to exploit a mammalian expression system that could facilitate posttranslational modification and folding.

3.3. Collagen. Collagen is a natural protein with excellent biocompatibility, biodegradability, and low immunogenicity, and it is widely distributed in the extracellular matrixes of living organisms.[160−162] The characteristic structure of collagen is an elegant structural motif in which three parallel polypeptide strands in a left-hand, polyproline II-type (PPII) helical conformation coil about each other with a one-residue stagger to form a right-hand triple helix. Based on these triple helices, collagen can thus self-assemble into the macroscopic fibers and networks as observed in living organism tissues.[163−165]

Therefore, in collagen-based long-acting formulations for tissue engineering of wound healing, bone regeneration, and vascularization, fibers or hydrogels with moderate mechanical strength and high stability were often prepared by the freezing/thawing cycle method or chemical cross-linking such as EDC/NHS.[166−168] However, these collagen long-acting formulations exhibit issues of initial burst release *etc.* Thus strategies of conjugation with other polymers such as hyaluronic acid, heparin, or liposome are currently significantly investigated to enhance the mechanical strength, better protect protein/peptide therapeutics from hydrolysis or enzyme degradation, and better control the release kinetics of loaded drugs.[167,169−174]

For instance, collagen-HA hydrogels cross-linked *via* UV light induction have been reported to load epidermal growth factor (EGF) for wound healing and demonstrated an accelerated wound closure rate on mice models through a single dose of topical administration, triggering the re-epithelialization, dermal reconstruction, and formation of mature vasculature on the skin of STZ-induced diabetic rats.[169] Similarly, in the work of Mantovani et al., heparin-modified collagen hydrogels loaded with pleiotrophin (PTN) were prepared by adjusting the pH to initiate the polymerization process of collagen. The *in vitro* release profile of the as-prepared heparin/PTN-modified collagen hydrogels revealed that with the modification of heparin, the initial burst release of PTN was reduced from 35% to 15% on day 1 and the whole release period was extended to ∼2 weeks. Besides that, researchers fused the target protein/peptide therapeutics to collagen affinity peptide instead of collagen to enhance the interaction between drugs and collagen and thus better control the drug release kinetics.[173] For instance, Lu et al. fused SDF-1α with a collagen affinity peptide derived from the von Willebrand factor (C-SDF-1α) and then bonded that to a book-shaped acellular fibrocartilage scaffold (BAFS) with abundant collagen fibers collected from fibrocartilage tissues of rabbits for bone-tendon healing. In the *in vitro* release profile, the initial burst release of SDF-1α was reduced from ∼40% to ∼10% on day 1 owing to the high affinity between the recombinant SDF-1α and BAFS. Furthermore, only 50% cumulative release was detected on day 9 while nearly 100% cumulative release was detected in the nonrecombinant SDF-1α group, indicating effective controllable release kinetics in the recombinant SDF-1α group. On a rabbit partial patellectomy model, the C-SDF-1α/BAFS improved the bone-tendon healing efficiently as indicated by the larger cartilaginous metaplasia region, better fibrocartilage regeneration, additional bone formation, and improved biomechanical properties.[175]

In addition, collagen was also decorated on other nanoparticles such as liposomes or polymer nanoparticles and loaded with proteins as the outer matrix to further prolong the release of target therapeutics.[170,174] For instance, in the work of Wang et al., VEGF and PDGF were loaded into gelatin nanoparticles prepared *via* a EDC/NHS cross-link and further embedded into HA-collagen nanofibers (∼534 nm) fabricated by an electrospinning technique (Figure 4B). The *in vitro* initial releases of both growth factors were confined to ∼10% on day 1, and the release period was up to 30 days. The pharmacodynamic investigation revealed an increased wound closure rate, along with enhanced collagen deposition and elevated maturation of vessels on streptozotocin (STZ)-induced diabetic rat models with one single topical administration, suggesting the great potency of HA-collagen nanofibers in the treatment of chronic wound healing.[174]

On the whole, due to their high affinity with tissues, collagen-based long-acting formulations are significantly applied in wound dressings for chronic wound healing. However, the sources of collagen are varied from animal skin to marine organisms, which leads to a high batch-to-batch variance and thus hinders its further clinical translation. Also, chains of some recombinant collagen may not form a stable triple helical structure without sufficient enzyme activity. Besides that, the control of its degradation rate is merely confined to the conventional methods of adjusting the cross-linking degree or composite proportion, which could not meet the needs for different tissue regeneration and thus needs vast investigation on the mechanism of collagen degradation *in vivo*.

3.4. Gelatin. Gelatin is a denatured product from acidic and alkaline hydrolysis of collagen with advantages of facile modification, high biocompatibility, biodegradability, and thermosensitive property.[176,177] The hydrolysis affects the electrical property of collagen and thus endows gelatin with different isoelectric points.[178] The tunable charge of gelatin allows gelatin complexation with a wide variety of proteins of different charges, making it an ideal protein platform.[176,179] Various growth factors have been reported to be encapsulated into gelatin hydrogel *via* chemical cross-link and applied in the tissue engineering of peripheral nerve regeneration, neovascularization, or antifungal.[180,181] For instance, Perroteau et al. prepared VEGF-A165 loaded gelatin-agar hydrogel *via* genipin cross-linking and successfully extended its release up to 1 months but with only ∼27% loading drugs released from the hydrogel at the end, which is a great waste.[180] Conjugation with polymers such as HA, poly(vinyl alcohol) (PVA), chitosan, and gelatin could also be constructed into hydrogels *via* chemical cross-linking of EDC, genipin, and glutaraldehyde. For instance, Tsai et al. prepared recombinant thrombomodulin loaded-gelatin/HA hydrogel (porous diameter = 20−300 μm) *via* EDC/NHS chemical cross-linking for treatment of chronic wounds.[182] Unfortunately, the initial burst release of thrombomodulin laden-gelatin/HA hydrogel in the first 3 h was so high (∼ 40%) that the release profile was only up to 12 h. Therapeutic efficacy investigation on a diabetic wound mice model indicated that once every-3-day injection of the hydrogel efficiently enhanced granulation tissue formation, re-epithelialization, collagen deposition, and angiogenesis in wound repair. Likewise, in the work of Simchi et al., an electrospinning technique was used to fabricate gelatin/PVA nanofibers (average outer diameter = 180 nm, average inner

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Table 3. Proteins Carriers for Protein/Peptide Delivery and Its Application

Carrier	Formulation	Preparation method	Loading capacity (%)	Release period	Protein/peptide	Application
SLPs	Film/Disc	water vapor annealing method	<1%	10−40 days	IFN-γ, IL-4, BSA, 5P12-RANTES	Prevention of HIV infection. Treatment of cancer.[142,147,149]
	Hydrogel	sonication and lyophilization method	5−6%	∼40 days	IgGl	antibody fomulations.[148]
ELPs	Fused protein	plasmid transfection and expression		10−56 days	GLP-1, FGF 21, SDF-lα,αB Crystallin, lacritin	Wound healing, treatment of diabetes.[153,154,156−159]
	Hydrogel	Chemical cross-linking/ photocross-linking	1−5%	∼5 days	BSA	Not show.[155]
Collagen	Hydrogel	freezing/thawing cycle method or chemical cross-linking	<1%	10−15 days	EGF, PTN, SDF-lα,	Wound healing, bone regeneration and vascularization.[169,173,175]
	Nanofibers	electrospinning teclmique	<1%	∼30 days	VEGF, EGF	Wound healing.[174]
Gelatin	Hydrogel	Chemical cross-linking	<1%	1−30 days	VEGF-A165, thrombomodulin	Peripheral nerve regeneration, neovascularization, antifungal and wound healing[180,182]
	Nanofibers	electrospinning technique	∼2%	∼7 days	Lysozyme	Not shown.[183]
Albumin	NPs	Chemical crosslhikmg	<1%	25−28 days	VEGF.BMP-2	Bone defect repair and wound healing.[184]
	NPs	Desolvation combined with chemical cross-linking	∼3.7%	∼130 days	Cx43MP	Treatment of rerinal ischemia.[188]
	Fused protein	plasmid transfection and expression		5 h ∼ 4 days	FXIIa inhibitor, coagulation factor IX	Treatment of hemophilia.[191,192]

diameter = 106 nm) and cross-link *via* glutaraldehyde; the *in vitro* cumulative release of lysozyme on day 1 was also high, up to 50%, but the release period was extended to 166 h.[183]

Therefore, aiming for longer-term and more controllable drug release, researchers loaded the protein/peptide therapeutics into micro-/nanoparticles prior to the embedment into the gelatin hydrogel matrix. For instance, in the work of Li et al., polyhedral oligomeric silsesquioxane (POSS)-modified gelatin hydrogel loaded with VEGF or BMP-2 was designed and successfully prepared *via* EDC cross-linking (Figure 4C).[184] Compared with the free form VEGF and BMP-2, the initial burst releases of both proteins encapsulated in the gelatin hydrogel were reduced for ∼5% (VEGF ∼ 25%, BMP-2−18%), and the total *in vitro* release profiles were up to 28 days (Figure 4C-ii, iii). Moreover, in the repair of rat calvarial defects, POSS hydrogel loaded with VEGF and BMP-2 exhibited excellent efficacy on promoting angiogenesis and bone regeneration, indicating great potency in the clinical treatment of large bone defect repair (Figure 4C-iv). In another work, Vossoughi et al. prepared BSA loaded-PCL microparticles (∼5 μm) *via* a W/O/W double emulsion method and then added them into a gelatin/chitosan/PVA hydrogel fabricated *via* adjustment of pH and temperature to trigger the gelation. The embedment of PCL microspheres into the hydrogel successfully reduced the release of BSA from 25% to 15% on day 1 compared to the PCL microspheres alone. Moreover, through adjusting the content and ratio between PCL and PVA (from 1:0.5 to 3:1), the initial burst release of laden BSA was tuned from 10% to 20% on day 1. Moreover, the optimized hydrogel (PCL:PVA = 3:1) was loaded with bFGF to accelerate wound healing, which exhibited 50% wound closure in 4 days compared with the control group in 12 days, indicating great clinical potential in accelerated wound healing dressings.[185]

Overall, as the hydrolysis products of collagen, gelatin-based hydrogels loaded with various growth factors such as VEGF are widely studied in tissue engineering of peripheral nerve regeneration, wound healing, *etc*. Moreover, compared with collagen, the price of gelatin is much lower. However, it often exhibits batch-to-batch variance owing to the different extraction methods and different sources of collagen. Besides, further cross-linking may occur during the storage and transportation due to the existence of reactive groups—aldehyde group, imine group, or ketone group—in the structure, which may influence the release kinetics of loading therapeutics. In addition, its biodegradability rate is too high and can easily lead to the initial burst release of laden therapeutics, which need further chemical modification in the structure for more controllable and sustainable release behavior.

3.5. Albumin. Albumin is a natural protein derived from plasma with large abundance and low immunogenicity, which is generally available as ovalbumin (OVA), bovine serum albumin (BSA), and human serum albumin (HSA). All albumins are recognized by a unique structure of disulfide double loops that repeat as a series of triplets.[186,187] Due to its abundant availability, high affinity, various binding sites, and specific target potential, albumin has been significantly investigated as a protein/peptide sustained-release system for various disease treatments in cancer, ocular diseases, *etc*.[188−192] For instance, Rupenthal et al. developed HA-coated HSA nanoparticles (∼250 nm) and efficiently encapsulated Connexin43 mimetic peptide (Cx43MP) with an encapsulation efficiency of ∼71% for treatment of retinal ischemia. In contrast with HSA nanoparticles without an HA coating, the initial burst release of Cx43MP in HSA nanoparticles with an HA coating efficiently reduced from 50% to 10% on day 1 and the whole release period was extended up to 130 days since the hydrophilic HA decoration formed a further barrier to confine water diffusion into the inner matrix and thus slowed down the release process of Cx43MP. Pharmacodynamic investigation suggested that Cx43 MP loaded HSA nanoparticles significantly prevented the thinning of retinal layers and the disruption of retinal blood vessels, exhibiting great potency in the treatment of chronic retinal degenerative and inflammatory disorders.[188] Besides that, albumin fused proteins are widely applied in extending the circulation times of protein therapeutics. For instance, Heinis et al. developed an albumin-

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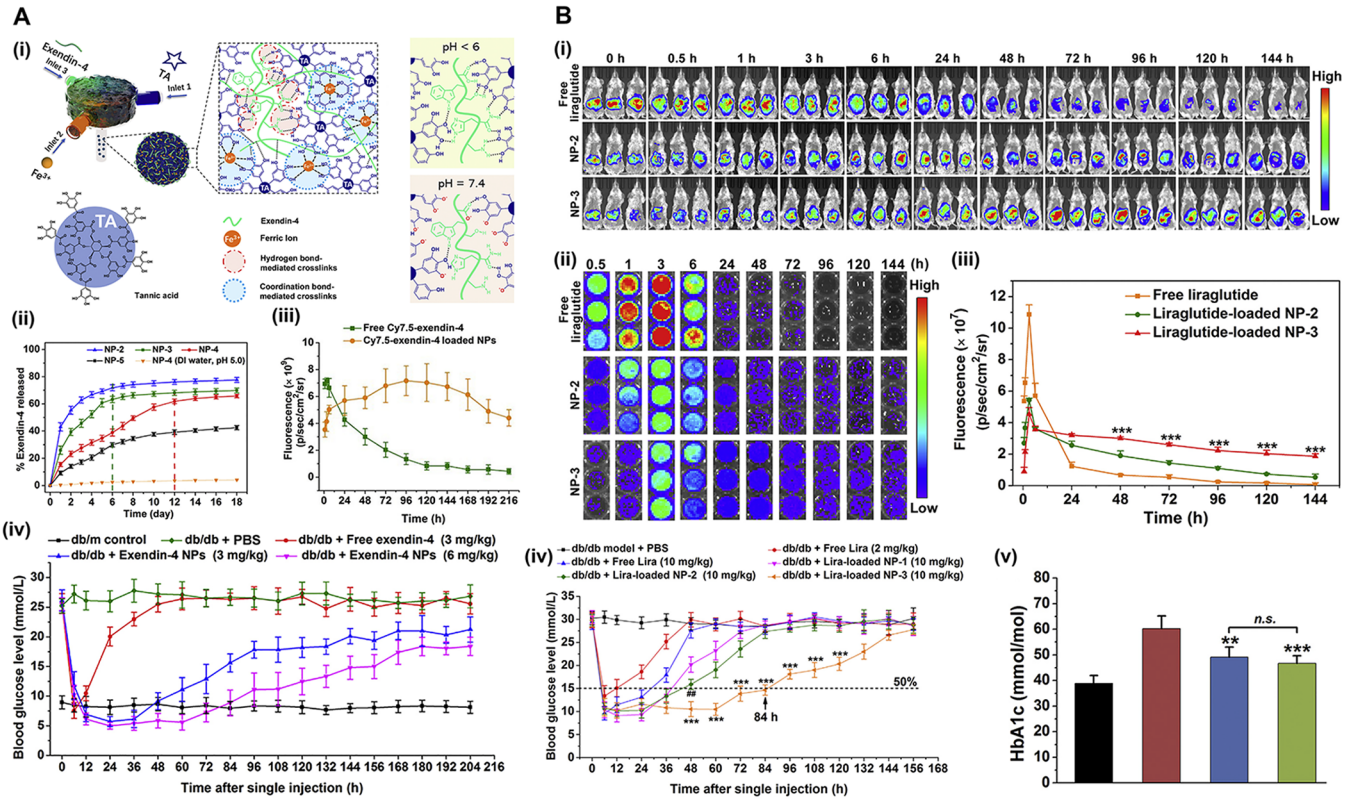

Figure 5. (A) (i) Schematic illustration of FNC preparation of TA/exendin-4/Fe^{3+} nanoparticles and cross-linking structures. (ii) *In vitro* release profile of TA/exendin-4/Fe^{3+} nanoparticles at different concentrations of TA. (iii) Fluorescence intensity of the Cy 7.5-labeled free exendin-4 and TA/exendin-4/Fe^{3+} nanoparticles in the peritoneal cavity given by IVIS images. (iv) Blood glucose levels of db/db mice after single *i.p.* administration of different formulations. Published with permission from ref 200. Copyright 2019 Elsevier B.V. (B) (i) *In vivo* fluorescence imaging of mice administered with TA/Lirag/Al^{3+} nanoparticles. (ii) Fluorescence imaging of blood samples collected at different time points. (iii) Semiquantification of fluorescence intensity of the Cy 7.5-labeled Lira in blood samples given by IVIS. (iv) BGL of db/db mice following single *i.p.* injection of TA/Lirag/Al^{3+} nanoparticles. (v) HbA1c level of db/db mice after treatment with TA/Lirag/Al^{3+} nanoparticles for a month. Published with permission from ref 201. Copyright 2019 Elsevier B.V.

binding ligand based on a peptide−fatty acid chimera with high affinity for HSA and successfully fused with FXIIa inhibitor, thus extending its half-life from 13 min to over 5 h.[191] Similarly, in the work of Jacobs et al., coagulation factor IX fused with albumin (rIX-FP) exhibited an extended terminal half-life of 102 h, which now is in Phase 3 trials for hemophilia treatment.[192]

On the whole, albumin as an endogenous protein exhibits great potential on prolonging half-life of protein/peptide therapeutics and thus is vastly applied in the disease treatment of cancer, ocular diseases, *etc.* However, though albumin itself is biocompatible and low immunogenic, its conjugation with target proteins may induce the conformational changes and possibly increase its immunogenicity risk.[187] In addition, the scalability of albumin-based sustained-release systems is a major challenge for commercialization considering its inherent batch-to-batch difference derived from natural polymers. There are still great challenges for albumin-based sustained-release systems to overcome before their final regulatory approval for commercialization.

Overall, protein-based systems owing to the high amphiphilicity, biocompatibility, biodegradability, and natural high affinity to protein weigh significantly in developing protein/peptide therapeutics long-term release formulations. The encapsulation or fusion of protein/peptide therapeutics in the protein matrix efficiently preserves their bioactivity under different physiological conditions and efficiently extends their release period up to several weeks or months. Moreover, compared to the previously mentioned synthetic or natural polymers, protein as a special kind of polymer delivery platform exhibits higher affinity with the target protein or peptides owing to its abundant conjugation sites (Table 3). In addition, the preparation methods of protein-based systems are more moderate compared with the phase inversion/solvent extraction method or double emulsification method vastly used in preparation of polymer-based systems. However, the protein denaturalization issue from physical/chemical cross-linking during the preparation of hydrogel formulations remains to be solved. Besides that, hydrogel-based long-acting formulations generally display high initial burst release (>20%) and hence induce undesirable and severe side effects. The conjugation with other polymers to increase its hydrophobicity may help to decrease the initial burst release. Besides, the sources of these natural protein carriers are varied, which may greatly enhance the differences of prepared formulations from batch to batch. It is thus hard to control the quality of protein-based sustained-release systems, which may hinder the clinical trials in the future.

4. POLYPHENOL-BASED SYSTEMS

Polyphenols with excellent antioxidative capability are a kind of phenolic compound that is usually extracted from plants or marine organisms and have been reported to reduce the

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incidence of diseases such as cardiovascular disease, diabetes, or cancer.[193−196] Four major groups including flavonoids, stilbenes, lignans, and phenolic acids are categorized depending on the number and binding structure of phenol units.[197] Owing to the abundant phenolic hydroxyl-rich moieties (gallol groups, catechol groups, *etc.*) in the structure, polyphenols could form conjugates with protein *via* noncovalent interaction including hydrogen bonding and hydrophobic interaction. This cross-link between polyphenols and protein/peptide, therefore, improves the thermal stability of the system and breaks the hydrophilic/hydrophobic balance of protein/peptide which may alter its solubility profile and other critical functional properties such as emulsifying, foaming, *etc.* Together with its gel property, polyphenols hold great potential in the development of long-acting protein/peptide formulations.[198,199] However, general noncovalent interaction between polyphenols and protein/peptides is transient and hard to control, which easily leads to the aggregation of conjugates and rapidly forms into flocculent precipitates, decimating its injectability. Therefore, quick and effective reaction control and termination are the most significant factors that should be taken into account during the exploit of a polyphenol-based sustained-release delivery system.

Aiming to resolve these issues, He et al. developed a flash technology-based self-assembly technology, namely flash nanocomplexation (FNC) technology, to realize the homogeneous mixture of exendin-4 and tannic acid (TA) with a confined impinging jet (CIJ).[200] Meanwhile, in order to terminate the interaction and prevent the further growing and aggregation of TA/exendin-4 conjugates, trivalent ironic ions were introduced to quench the excessive phenol groups *via* coordination interaction and thus halt the excessive growth of TA/exendin-4 conjugates (Figure 5A-i). The dynamic light scattering (DLS) characterization results showed that the as-prepared spherical TA/exendin-4/Fe^{3+} nanoparticles were ∼115 nm in diameter with astonishing encapsulation efficiency (∼100%). The *in vitro* release profile (Figure 5B-ii) showed that the overall exendin-4 release profile with high TA concentration (>3 mg/mL) was extended to 12 days with initial burst release ∼10% on day 1, suggesting that the concentration of TA played a critical role on sustainable release of exendin-4. This can probably be explained by the more intense interaction between TA and Fe^{3+}/exendin-4 with higher TA concentration, forming a more condensed structure and exhibiting slower and more controllable release kinetics. Moreover, this sustained-release behavior was pH-dependent since under physiological conditions (pH = 7.4), the partial ionization of phenolic groups weakened the cross-link system based on hydrogen bonding, triggered the decomplexation of TA/exendin-4/Fe^{3+} nanoparticles, and thus released the target peptide. Pharmacokinetic study (Figure 5A-iii) further confirmed the extended exendin-4 release from TA/exendin-4/Fe^{3+} nanoparticles (up to 6 days) in BALB/c mice following intraperitoneal injection (*i.p.*). Furthermore, a hypoglycemic efficacy study (Figure 5A-iv) on a type II diabetes (T2D) murine model demonstrated that TA/exendin-4/Fe^{3+} nanoparticles effectively reduced the BGL to the normal range in 6 h and maintained the BGL in the normal range for 72 h after a single intraperitoneal administration. Moreover, the BGL was still 50% lower than that of the original level even at 132 h postinjection, indicating great potency in long-term clinical hypoglycemic control for T2D patients.

After confirming the possibility of a TA/metal ion system for sustainable release of target peptide, He et al. further applied this system to deliver another peptide (Liraglutide, Lirag) for the treatment of T2D and its cardiovascular complications.[201] The same FNC technology was used to prepared the TA/Liraglutide/metal ions sustained-release system, while Al^{3+} instead of Fe^{3+} was used to quench the excessive phenolic groups since phenol-Fe^{3+} conjugates exhibited as a dark purple color, which is not ideal for subcutaneous injection in clinical application. The as-prepared TA/Lirag/Al^{3+} nanoparticles were ∼50 nm with a high encapsulation efficiency of ∼100%. *In vivo* imaging results (Figure 5B-i-ii) suggested that TA/Lirag/Al^{3+} nanoparticles extended the *in vivo* release of loading peptide to more than 96 h, and even after 144 h of the injection, fluorescence signals could still be detected at the injected region. Moreover, pharmacokinetic study (Figure 5B-iii) further verified that higher TA concentration (2 mg/mL) significantly reduced the blood fluorescence signal intensity (FSI) by 5-fold compared to the free Lirag group at 0.5 h-post injection and efficiently increased the blood FSI by 4-fold and 30-fold compared to 1 mg/mL TA groups and free Lirag groups at 144 h-post injection. Hypoglycemic study (Figure 5B-iv, v) on T2D murine models showed that after single *i.p.* injection of TA/Lirag/Al^{3+} nanoparticles, the mice BGL reduced to the normal range in 6 h and was maintained for more than 60 h; even after 84 h of the injection, the BGL was still lower than half of the original level, indicating a great long-acting hypoglycemic efficacy. Remarkably, in a long-term study (30 days treatment), the mice HbA1c, indicator of BGL fluctuation, reduced 20% compared to the model groups, indicating the steady and normal BGL during the long-term treatment profiting from the stable and sustainable release of liraglutide in T2D murine models. Besides that, cardiovascular function of T2D murine models was also improved after treatment for 30 days as TA/Lirag/Al^{3+} nanoparticles effectively reduced the cardiac triglyceride and diacylglycerol levels by 40% and 30%, respectively, compared to the model group, indicating a significant alleviation of lipid-overload in heart.

From the work of He. et al., it could be concluded that polyphenol-based long-acting formulations are a promising and potential carrier for peptide delivery in the treatment of the chronic disease diabetes and in particular that TA, as an abundant natural polyphenol approved by the FDA Generally Recognized as Safe (GRAS) list as a food supplement, was reported to exhibit beneficial antioxidative, anti-inflammation, and antithrombosis efficacy on health following injection.[202] Also, the one-step aquatic preparation method based on the FNC technology without usage of organic solvent could prevent the denaturation of protein/peptide and hence preserve the bioactivity, enhancing its potential in future clinical translation. Besides that, the high encapsulation efficiency and controllable pharmacokinetics of TA/metal ion systems ensure their applicability to various but also only peptide delivery and disease treatments. Whether proteins with higher molecular weight and delicate tertiary or quaternary structure are also suitable in this system needs to be studied and verified considering that the noncovalent interaction between polyphenol and protein may interfere with the spatial structure and thus reduce the bioactivity of protein therapeutics. In addition, as a natural substance, its sources may also lead to the high batch variance and thus hindered commercialization. Besides, the tendency to be oxidized easily

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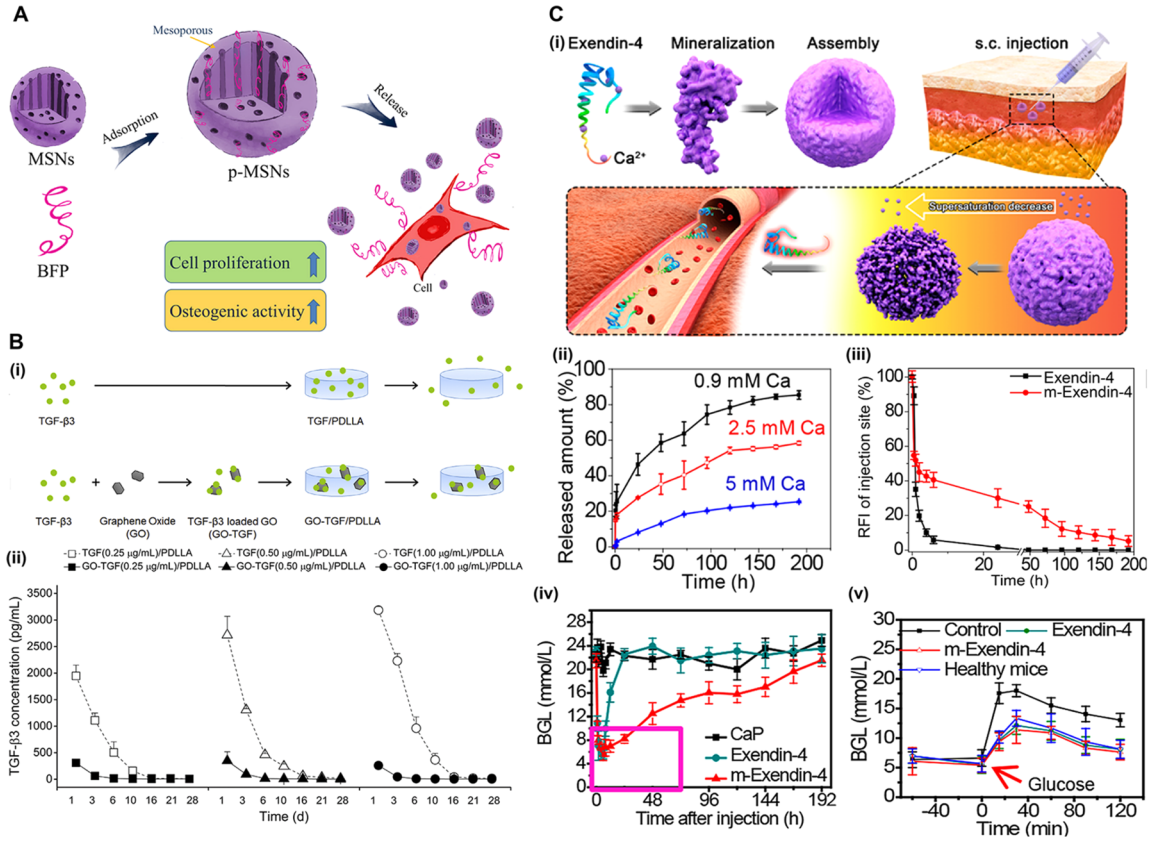

Figure 6. (A) Schematic illustration of BMP-7 loaded MSNs for osteodifferentiation. Published with permission from ref 211. Copyright 2015 Elsevier B.V. (B) TGF-β3 loaded GO/PDLLA hydrogel for accelerating chondrogenic differentiation of human mesenchymal stem cells. (i) Preparation of TGF/PDLLA and GO-TGF/PDLLA hydrogels. (ii) *In vitro* TGF- β3 release from d GO/PDLLA or PDLLA hydrogels. Published with permission from ref 212. Copyright 2020 Acta Materialia Inc. (C) (i) Schematic illustration of mineralized exendin-4 sustained-release formulation and its disassembly in response to physiological supersaturation. (ii) *In vitro* release profile of different kinds of encapsulated growth factors in the formulations. (iii) Quantitative analysis of the relative fluorescence intensity at the injection site of mice treated with exendin-4 or mineralized exendin-4 at different time points. (iv) Long-term hypoglycemic efficacy of mineralized exendin-4 on diabetic mice. (v) Intraperitoneal glucose tolerance test of diabetic mice at 1 h postadministration of exendin-4 and mineralized exendin-4. Published with permission from ref 210. Copyright 2017 American Chemical Society.

makes its transportation and storage challenging, which may consequently lead to the aggregation and thus decrease the bioactivity of loaded proteins/peptides.

5. LIPID-BASED SYSTEMS

Lipid-based drug delivery systems with high affinity to cell membrane due to its similar components are a classic delivery system applied in protein or peptide delivery for various disease treatments owing to their facile surface modification, good biocompatibility and biodegradability, and amphiphilicity. After encapsulation in the matrix of lipids, the loaded proteins will be protected by the lipid components from enzyme degradation, oxidation, or hydrolysis for enhanced half-life and improved bioavailability. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are the main two types of lipid-based drug delivery systems for sustainable release of protein/peptide therapeutics. The high-pressure homogenization method and microemulsion method are the two basic methods used in the fabrication of SLNs.[203,204] Unlike liquid oil used in the preparation of liposome, solid lipids such as saturated fatty acid, glycerides, stearic acid, *etc.* were introduced as the basic matrix for better protection of protein therapeutics from chemical degradation and more controllable release kinetics. However, low loading content and drug expulsion issued from crystallization of the solid lipids during preparation are inevitable. Therefore, in the development of NLC formulations, a blend of solid and liquid lipids was employed to construct the long-acting protein formulations to avoid the crystallization process existing in SLN preparations, which has been demonstrated to increase the loading capacity and reduce leakage of the encapsulated protein/peptide therapeutics during storage.[205,206] NLC formulations were also prepared through high-pressure/melt/cold homogenization methods based on the properties of laden drugs. To date, more than 20 cosmetic products have been demonstrated to contain SLNs or NLCs especially in the fields of pharmaceutical dermal products.[207]

For instance, in the work of Gainza et al., glycerides, Precirol ATO 5, or Miglyol182 was introduced to prepare SLN- and NLC-based formulations *via* a melt homogenization method and successfully applied to load with rhEGF for comparative study of these two long-acting rhEGF formulations in the treatment of chronic wound healing.[208] The as-prepared Precirol ATO 5-based SLN-rhEGF and Precirol ATO 5/Miglyol182-based NLC-rhEGF were 332.45 ± 16.62 and 348.35 ± 10.25 nm in diameter, respectively, with both loading capacity ~1% (w/w). The *in vitro* rhEGF release profiles displayed an initial burst release on day 1 related to the

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percentage of surface-associated protein (25.15% for SLN-rhEGF and 26.8% for NLC-rhEGF), and both release periods were effectively extended up to 72 h. Moreover, on a full-thickness excisional wound model, twice a week topical administration of both rhEGF nanoformualtions particularly NLC-rhEGF was demonstrated to induce faster and more effective wound closure and healing attributed to the more controllable and sustainable release of rhEGF on the wound area compared to the free form rhEGF. Based on the great therapeutic efficacy of NLC-rhEGF on the mice model, Gainza et al. further evaluated its efficacy on a porcine full-thickness excisional wound model.[209] Pharmacodynamics analysis suggested that after treatment of 25 days, twice a week topical administration of NLC-rhEGF (20 μg) significantly improved the wound closure rate and wound healing quality compared to the free rhEGF (75 μg) with the same administration frequency, suggesting its great translation potential for treatment of chronic wound healing clinically.

Overall, a lipid-based sustained-release system could effectively prevent the oxidation or hydrolysis of protein/peptide therapeutics under the physiological conditions and improve their stability and bioavailability. Besides that, SLN/NLC long-acting formulations efficiently increase the cellular uptake owing to the high affinity between lipid and cell membrane, increasing the bioavailability of loaded drugs. Compared with the aforementioned materials, the synthesis and preparation procedures of lipids are relatively mature and products such as Vansolin, Rifamsolin, *etc.* are already in preclinical studies. Along with their FDA approval, lipid-based sustained-release systems hold significant potential in clinical translation. However, in the exploit of lipid-based long-acting formulations, further investigations are needed to solve issues including the toxicity of the cationic lipid, denaturation of protein/peptide therapeutics during the preparation owing to the organic solvent usage, and extreme temperatures or pressure, low loading content (1−5%), or severe side effects induced by the initial burst and uncontrollable release of proteins.

6. INORGANIC MATERIALS-BASED SYSTEMS

Inorganic materials, *e.g.* gold nanoparticles, mesoporous silica nanoparticles (MSNs), or graphene oxide, with various intriguing physiochemical properties, such as anti-inflammation, anti-infection, *etc.*, attract researcher's great interest for biomedical applications in drug delivery, disease diagnosis, and bioimaging thanks to their high biocompatibility, controllable morphology, and size, among others.[2,210−213] Most inorganic-based long-acting formulations are relying on their inherent biodegradability to realize the long-term release of laden protein/peptide therapeutics.

MSNs have aroused considerable attention for biomedical applications attributed to their favorable biocompatibility and controllable morphology and have been developed as sustained-release protein systems owing to the porous structure for high loading efficiency of drugs.[214,215] For instance, in the work of Luo et al. (Figure 6A), bone-forming peptide (BFP) was laded to the 400 nm MSNs *via* electrostatic interaction with the drug loading capacity of 7.1×10^{-6} mol/g. The *in vitro* release curves of the peptide laded-MSNs demonstrated a sustained release of the BFP pattern for more than 6 days with an initial burst release ∼10% on day 1, probably attributed to the low degradability rate of silica in the physiological conditions. Moreover, BFP loaded-MSNs efficiently up-regulated the expression of osteogenesis-related proteins and bone matrix mineralization in human mesenchymal stem cells (hMSCs) and significantly promoted the osteogenic differentiation of hMSCs, indicating their potential for bone repair and regeneration.[211] Besides that, MSNs could also integrate with stimuli-responsive polymers to meet different delivery needs for the treatment of different diseases.[216,217] For instance, Cai et al. successfully coated phenylboronic acid-human serum albumin (PBA-HSA) onto the surfaces of MSNs through a matrix metalloproteinase 2 (MMP-2) responsive polypeptide linker. The as-prepared BSA loaded MSNs with diameter ∼140 nm efficiently contained the release of BSA for ∼10% in 5 days without the MMP-2 participation while greatly releasing the loaded proteins for ∼90% in 5 days under MMP-2 condition. This smart controllable release kinetics holds great potential in therapeutic protein/peptide delivery for cancer treatment.[218]

Gold nanomaterials are a kind of plasmonic materials with controllable morphology, strong local surface plasmon resonance (LSPR), and light absorption.[219,220] Therefore, gold nanomaterials are widely investigated and exploited for drug delivery, disease diagnosis, *etc.* Thanks to their natural-born cationic property, gold nanomaterials could also efficiently load proteins or peptides such as insulin through electrostatic interaction. For instance, in the work of Vilaboa et al., hollow gold nanoparticles (∼54 nm) were used to construct a near-infrared (NIR) responsive hydrogel based on the self-clot of bovine fibrinogen for controllable delivery of BMP-2 secreted from mesenchymal stem cells. The *in vitro* release study indicated an efficiently triggered release of BMP-2 under NIR irradiation for more than 12 days. Moreover, on a bone defect murine model, this NIR-responsive hydrogel effectively induced a large increase in new mineralized tissue, indicating its broad potential for bone regeneration.[221]

Graphene oxide (GO) as a kind of carbon-based two-dimensional material is widely applied in drug delivery owing to its higher biocompatibility.[222] Moreover, owing to the structure of the hexatomic ring, macromolecules such as protein/peptide therapeutics could be efficiently loaded onto GO through $\pi-\pi$ stacking, hydrophobic interaction, and electrostatic interaction. In the work of He et al., transforming growth factor-β3 (TGF-β3) was loaded onto GO, forming a GO/TGF-β3 complex, and further encapsulated in the photopolymerizable poly-D,L-lactic acid/polyethylene glycol (PDLLA) hydrogel triggered by visible light for a more controllable release (Figure 6B-i).[212] Remarkably, from the *in vitro* release profile (Figure 6B-ii), the initial release of TGF-β3 from GO-TGF/PDLLA hydrogel was only ∼1% on day 1 compared with ∼10% from the TGF-β3/PDLLA hydrogel, which indicated that the noncovalent interaction between GO and TGF-β3 may significantly reduce the initial burst release. In addition, the *in vitro* sustained release of TGF-β3 in GO/PDLLA hydrogel was extended up to 4 weeks. Similarly, in another work from Cao et al., antibacterial peptides, $G(IIKK)_4I\text{-}NH_2$, were loaded onto a GO sheet and layer-by-layer (LBL) film based on the GO@G(IIKK)4I-NH2 composite (as a positively charged unit), poly(acrylic acid) (PAA, polyanion), and poly(β-amino esters) (poly β, polycation) as three assembling units were further constructed.[213] This LBL film significantly extended the release of antibacterial peptide for ∼10 days with an initial burst release of ∼50 μg/cm^2 peptide on day 1, enabling the sustainable release of laden

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peptide in a more controllable manner for antibacteria function.

In general, protein/peptide therapeutics were loaded onto inorganic materials *via* physical absorption, which is uncontrollable and unstable. Interestingly, Chen et al. proposed a biomimetic mineralization strategy to prepare a long-acting exendin-4 formulation.[210] Similar to natural biomineralization, exendin-4 can be mineralized to form nanoparticles through the interaction between acidic amino acid residues and calcium ions in a supersaturated environment without influencing its bioactivity (Figure 6C-i). This mineralized exendin-4 (m-exendin-4) with a size around 50 nm prolonged the *in vitro* release of peptide to more than 1 week with only ∼10% initial burst release on day 1 (Figure 6C-ii). Moreover, from the fluorescence imaging of mice injected with m-exendin-4, approximately 5.1% of the initial fluorescence signal was detected at the injection site even after 192 h (Figure 6C-iii). On a db/db mice model, m-exendin-4 efficiently maintained the BGL in the normal range for at least 28 h compared to the free exendin-4 (∼6.5 h) (Figure 6C-iv) and exhibited excellent hypoglycemic control in the intraperitoneal glucose tolerance test (Figure 6C-v), indicating its potential for clinical treatment for T2D in the future.

Generally, compared to organic materials, inorganic materials with specific elements endow unique functions for protein delivery platforms, making them promising in the future considering their diverse health beneficial functions. However, unlike natural polymers or polyphenols with confirmed high biocompatibility and good biodegradability, large investigations of these inorganic materials on metabolism and toxicity are needed before their further clinical application. In addition, the synthesis or preparation procedures of inorganic materials are complicated and may need extreme conditions in terms of temperature, pH, or pressure, which makes scale-up difficult, decreases the bioactivity of protein/peptide therapeutics, and confines the loading method to only physical absorption. Moreover, the complicated preparation methods also lead to the differences between batches, namely poor product quality control, hindering the clinical translation. Although biomimetic mineralization effectively solves some of the previously mentioned issues, more evidence is needed to further confirm whether the addition of metal ions will affect the delicate tertiary or quarternary structure and thus the bioactivity of protein therapeutics with high molecular weight.

7. FUTURE PERSPECTIVES

Various kinds of delivery systems for long-term release of protein/peptide therapeutics have been designed and exploited in the past decades. While exhibiting significant potential in protein therapies for various diseases, the clinical translation of long-acting formulations remains at its early phase with the following issues to be resolved. (1) The preparation procedures need to be simplified and optimized to meet the scale-up production demands and reduce the batch-to-batch differences. With the advancement of flash technology-based self-assembly technology such as FNC, it could be expected in the future that sustained-release protein formulations may be prepared in a more simple, continuous, scalable, and reproducible manner through more homogeneous mixture techniques.[223,224] (2) The loading capacity is relatively low, which confines its application in the treatment of chronic diseases of diabetes, cerebral degenerative diseases, *etc*. which requires long-term medication. To date, self-assembly long-acting formulations based on the noncovalent interaction (electrostatic interaction, coordination bond, hydrogen bond, *etc*.) between carriers and proteins have emerged as a novel strategy to effectively enhance the loading content/encapsulation efficiency. This may inspire us to take advantage of natural molecular interaction to realize the efficient loading of protein/peptide therapeutics without additional chemical agents participation and preserve the bioactivity of these biomacromolecule therapeutics.[225,226] (3) Side effects induced by initial burst release are the major obstacle for the clinical translation of sustained-release systems. It has been found this is mainly because the parts of loaded protein/peptide therapeutics are dispersed on the surface of the systems instead of the matrix. Therefore, smart sustained-release systems designed with stimuli-responsive and disease-specific properties for a controlled release of therapeutics at the target sites are promising in personalized medication.[19,227] (4) Long-term toxicity, especially the unbiodegradable materials, should be comprehensively investigated and verified. With the continuous progress of clinically approved controlled/sustained-release techniques and protein/peptide delivery strategies, we believe that these issues will be explicitly addressed and a new generation of sustained-release systems will be exploited and applied in the clinical treatments of various diseases in a safer, more controllable, and effective manner.

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Notes

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