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Nanotechnology for protein delivery: Overview and perspectives

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ABSTRACT

Protein-based therapeutics have made a significant impact in the treatment of a variety of important human diseases. However, given their intrinsically vulnerable structure and susceptibility to enzymatic degradation, many therapeutic proteins such as enzymes, growth factors, hormones, and cytokines suffer from poor physicochemical/biological stability and immunogenicity that may limit their potential benefits, and in some cases limit their utility. Furthermore, when protein therapeutics are developed for intracellular targets, their internalization and biological activity may be limited by inefficient membrane permeability and/or endosomal escape. Development of effective protein delivery strategies is therefore essential to further enhance therapeutic outcomes to enable widespread medical applications. This review discusses the advantages and limitations of marketed and developmental-stage protein delivery strategies, and provides a focused overview of recent advances in nanotechnology platforms for the systemic delivery of therapeutic proteins. In addition, we also highlight nanoparticle-mediated non-invasive administration approaches (e.g., oral, nasal, pulmonary, and transdermal routes) for protein delivery.

1. Introduction

The development of diverse protein therapeutics has seen an enormous surge over the past 3 decades, such as fully human antibodies, chimeric proteins, and new protein scaffolds capable of binding to "undruggable" targets, which has resulted in effective therapies for a myriad of human diseases including diabetes, cancer, infection, and inflammatory diseases. Human insulin, approved by the US Food and Drug Administration (FDA) in 1982, was the first commercially available recombinant therapeutic protein, and has since become the major therapy for diabetes mellitus type I and type II [1]. Ever since, the protein market has been growing dramatically, and many other protein therapeutics, such as PEGINTRON (PegInterferon-α2b for hepatitis C), Fabrazyme (agalsidase beta for Fabry disease), Cotazym (pancrelipase for cystic fibrosis or pancreatic insufficiency), and others [1], have been approved for clinical use. Notably, monoclonal antibodies (mAbs) have represented a promising segment of the protein therapy field since the approval of Muromonab-CD3 in 1986 [2]. The recent clinical validations of immune checkpoint mAbs such as Nivolumab and Pembrolizumab, which target the programmed death-1 (PD-1) receptor, are considered one of the exciting advances in cancer immunotherapy [3]. A study by BCC Research indicates that the global market for bioengineered protein drugs was valued at $151.9 billion in 2013 and is expected to grow to about $222.7 billion in 2019 [4].

Compared with the conventional small-molecule drugs that continue to dominate the overall pharmaceutical market, protein therapeutics offer the advantages of higher specificity, greater activity, and less toxicity [5]. Nevertheless, the high specificity often requires maintaining the structural complexity of proteins, which can make them difficult to modify and/or formulate. Moreover, the susceptibility to enzymatic degradation, short circulation half-lives, and poor membrane permeability pose significant barriers for effective delivery of many therapeutic proteins (e.g., enzymes and cytokines) to targeted disease sites. To achieve high therapeutic performance, these unfavorable intrinsic characteristics of proteins need to be counterbalanced by designing appropriate delivery strategies or platforms. Improper design or formulation of protein drugs can cause degradation, denaturation, and/or aggregation of the protein molecules, potentially causing both immunogenic side effects after administration and loss of pharmacological activity. Approaches such as encapsulation within microparticles, chemical modification with hydrophilic polymers, and recombinant protein engineering have been clinically validated to enhance protein therapeutic efficacy. Despite the continuous launch of successful biological products into the market, the know-how and the technologies for the development of biologic drugs with optimal activity, stability, pharmacokinetics and lack of immunogenicity remain elusive today. Furthermore, while nearly all existing biologic drugs were developed against soluble or extracellular targets, the ability for biologic drugs to enter cells and

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intracellular compartments can significantly broaden their utility for a myriad of existing targets.

Nanotechnology has demonstrated tremendous promise for medical applications [6,7]. Thus far, dozens of nanomedicines have already been approved for clinical use, and many more are under clinical investigation [8,9]. In particular, nanoparticles such as liposomes, micelles, polymer nanoparticles, and inorganic nanomaterials, which are typically in the range of 10–150 nm in size, have considerable advantages as drug carriers. In protein delivery, nanoparticle technologies can: i) protect proteins from premature degradation or denaturation in biological environment; ii) enhance systemic circulation half-life of proteins with poor pharmacokinetic properties; iii) control sustained and/or tunable release which can maintain drug concentration in the therapeutic range; and iv) target diseased tissues, cells, and intracellular compartments, thus improving the safety and efficacy of biologic therapeutics. The considerable success of nanoparticle formulations of small-molecules such as doxorubicin (Doxil and Myocet), daunorubicin (DaunoXome), paclitaxel (Abraxane), and amphotericin B (Ambisome) has paved the way for the exploration of nanoparticle technologies for protein delivery [10]. This review summarizes marketed protein delivery strategies, recent progress in intracellular protein delivery, design considerations of nanoparticle technologies and their advancement on systemic protein delivery, and the application of nanotechnology to develop non-injectable protein therapeutics that can enhance patient satisfaction and compliance.

2. Marketed protein delivery strategies

2.1. Microparticle delivery

Biodegradable polymeric microparticles (1–1000 μm) are promising parenteral depot formulations for long-term protein drug release (from weeks to months), in particular when the maintaining of protein concentration in therapeutic range is required for more than 1 week. They enable sustained release of proteins by both diffusion from the polymer matrix and the degradation/erosion of the polymer [11,12]. One of the most widely used materials for the encapsulation of proteins is poly(lactic-co-glycolic acid) (PLGA), as it is biocompatible, biodegradable with favorable degradation rates, and already approved by the FDA for use in humans [13]. Encapsulation of proteins into polymeric microparticles can be achieved by several methods such as double emulsion (the most widely used technique), single emulsion, phase separation (coacervation), ultrasonic atomization, spray-drying, and microfluidics [13]. Once the proteins are encapsulated into microparticles, their release kinetics depend on the microparticle size, molecular mass of the polymer, degradation rate, charge property of the polymers, ratio of hydrophilicity to hydrophobicity, polydispersity of microparticle size, protein loading amount, as well as the surrounding microenvironment. Currently, there are a number of microparticle protein-delivery formulations (e.g., Trelstar depot) on the market, and various microparticles are under preclinical development for delivering therapeutic proteins such as bone morphogenetic protein-2 [14], insulin [15], recombinant human epidermal growth factor [16], and recombinant human erythropoietin (EPO) [17]. However, these clinically successful microparticle systems may cause blockage of the needle required for administration, and the bioactivity of the released proteins under physiological conditions need to be considered for long-term delivery. Extended protein stability is still challenging, and in addition, degradation and erosion of biodegradable polymers including PLGA can lower the pH inside the microparticles, which can further lead to denaturation of the protein as well as aggregate formation.

2.2. Chemical modification

Proteins smaller than 70 kDa are mostly cleared from the systemic circulation by glomerular filtration [18]. Chemical modification of proteins with hydrophilic polymers can reduce renal clearance by increasing their molecular weight and/or hydrodynamic radius. The covalent attachment of polyethylene glycol (PEG) chains to proteins (PEGylation), as a typical example, enhances protein stability and pharmacokinetic (PK) properties, and these benefits have allowed some PEGylated therapeutic proteins (e.g., Adagen, Somavert, Oncaspar, and Naloxegol) to reach the market, with many other examples in various stages of clinical development. [19,20]. Hyperglycosylation can also extend biological half-life and improve stability by increasing the solubility as well as reducing immunogenicity of proteins. The addition of sugar molecules to a protein is a more natural process than PEGylation, since it is already a part of the endogenous post-translational enzymatic process, and polysaccharides are readily degraded into native glucose molecules [21]. N-glycosylated EPO (Aranesp) has been marketed by Amgen since 2001, and other glycosylated protein drugs are under preclinical and clinical investigation such as polysialylated forms of EPO, granulocyte-colony stimulation factor (G-CSF), and insulin [22]. Although chemical modification prolongs the circulation half-life of proteins, this approach may require complicated synthesis and impart unfavorable conformational changes as well as loss of both biological activity and binding affinity to the target due to steric hindrance and heterogeneity [23]. Such alterations in physicochemical properties leads to the systemic exposure of proteins in order to reach sufficient pharmacological potency, but toxicities related to peak exposure can limit clinical use. Various efforts to maintain protein activity include site-specific modification. For example, chemical ligation of synthetic peptides including levulinyllysine to EPO elicited hematopoietic activity superior to native protein [24]. More recent advances in chemo-selective targeting show that the incorporation of canonical and noncanonical amino acids can enhance selectivity while improving PEG architecture [25].

2.3. Genetic engineering

In addition to chemical modification, genetic constructs and fusion technologies to elevate protein half-life and therapeutic efficacy have been intensively studied. Fc-based fusion proteins composed of an immunoglobin Fc domain genetically linked to the therapeutic protein represent a promising approach, as Fc-fusion can endow a protein with unique effector functions mediated by Fc receptor binding and complement fixation [26]. The neonatal Fc receptor (FcRn)–mediated recycling and transcytosis process extends half-life (e.g., IgG: up to 21 days); in addition, the increased molecular weight of fusion proteins through the size of the Fc-domain (~50 kDa) reduces renal clearance [27]. A number of therapeutic proteins based on fusion with the IgG Fc domain have come into clinical use since Fc-fused tumor necrosis factor (TNF) receptor-2 (Enbrel; Amgen/Pfizer) was approved for the treatment of rheumatoid arthritis and plaque psoriasis in 1998, and several other candidates are currently in clinical trials [28]. Recent work on the Fc-fusion technology also focuses on retaining biological activity and binding affinity, which are often decreased after the fusion process [29,30]. Jung et al. included a 'chaperone' protein in Toll-like receptor 4 Fc-fusion to stabilize the desired partner [31]. Newly developed heterodimeric Fc platforms, based on strand-exchange engineered-domain CH3 heterodimers consisting of alternating segments of human IgA and IgG CH3, show multiple specificities within the homodimeric Fc-fusion platform [32]. Utilizing alternative backbones, such as IgA, IgE, and IgM, may also benefit the activity of the fused partner [33–35]. However, concerns remain about the immunogenicity of Fc-fusion proteins, because interactions between the Fc domain and its receptors have multivariable immunological consequences, which might limit their usefulness in the treatment of chronic disease [36]. Other attempts to target FcRn, including albumin fusion (which has direct interaction with FcRn) and genetic engineering of Fc domains have also been reported. A glucagon-like peptide-1 (GLP-1) albumin fusion achieved ~5-day half-life and received FDA approval (Albiglutide; GSK) for the