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Future of sustained protein delivery

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Abstract

“Despite limitations associated with every approach, delivery of proteins for an extended duration in a controlled manner is achievable... a clinically acceptable formulation that delivers proteins over a period of months is largely an unmet need.”

Keywords

biodegradable polymers; controlled release; implants; protein delivery; protein stability; sustained delivery

Proteins as biotherapeutics

Proteins are promising biotherapeutics offering advantages such as selectivity, specificity and potency. A number of protein therapeutics are currently marketed and projected to acquire a major share of the pharmaceutical market in the future. Design, development and production of protein biologics has reached advanced stages due to advances in protein characterization[1, 2], process control [3] and manufacturing technology [4]. The pharmaceutical/biotechnology industry invested nearly US\$150 billion in the past 3 years for R&D of new therapeutic molecules including biopharmaceutical R&D. As a result, more than 907 biotherapeutics are currently in the various phases of clinical trials. Despite excellent therapeutic characteristics such as selectivity, specificity and potency, and fast-paced development and production, success with protein biotherapeutics in treating diseases is limited.

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Rational for sustained controlled delivery of protein biologics

Suboptimal therapeutics efficacy is as a result of complex tertiary (3°) and quaternary (4°) structures, high molecular weight, high hydrophilicity, short permeability across biological membranes, susceptibility to enzymatic degradation, and poor half-lives. These factors pose unique challenges in delivering proteins leading to multiple parenteral administrations to maintain therapeutic levels. According to WHO, the major reason for failure of treatment strategies in chronic ailments is poor patient adherence to therapy [5]. A formulation approach that delivers biologics for an extended duration (over a period of months) in a controlled manner may potentially overcome many of these obstacles. Several novel approaches have been examined for the sustained delivery of protein biologics. However, it has been acknowledged that development of clinically acceptable sustained-delivery formulations for proteins is a highly challenging task. Furthermore, proteins pose unique challenges due to their complex structure, which is prone to degradation. Moreover, high molecular weight/volume leads to slow diffusion. Hence, one should be cognizant of the fact that approaches applicable to peptide and small molecules may not work for proteins. For example, lactide and glycolide-based copolymers have been widely employed for sustained delivery. However, degradation products of PLGA can lead to an acidic microenvironment which is detrimental to the stability of protein. In addition, covalent modification of peptides with PLGA degradation products within acidic microclimate may result in irreversible loss of bioactivity.

Polymeric biodegradable formulations

In situ gel and particles have had partial to complete success in a few cases. Hence they are clinically available for various peptides and proteins. Method of encapsulation (use of organic solvents water/organic interface and scale up) protein loading stability (during encapsulation within formulation and after release) burst release release pattern, protein–polymer and protein–excipient interaction/compatibility and IVIVC (*in-vitro–in-vivo* correlation) for release profiles are important variables impacting the development of extended-release protein formulations.

Protein release from formulations is controlled by dissolution, diffusion, degradation, or a combination of these. Dissolution-controlled release cannot sustain protein release for a longer duration (>2 weeks) due to very high water solubility of proteins. Protein encapsulation in polymeric injectable implants and particles may provide long-term delivery. Protein stability is one of the major obstacles in the development of clinically acceptable delivery systems [6]. Protein may denature at many stages of process development, such as encapsulation and emulsion formation [7]. Exposure to organic solvent has been shown to alter protein structure. Protein denaturation is often due to exposure to aqueous/organic interface, cavitation and heat stresses due to ultrasonication, as reported by several studies [8,9]. Delivery systems should provide protection against enzymatic degradation *in vivo* and maintain structural integrity throughout the release. Aqueous-solution-based techniques have been investigated to avoid the use of organic solvents, with limited success. Antibody denaturation via irreversible aggregation (formation of trimers and tetramers) at high concentration in an aqueous solution is well reported [10, 11]. Hence, incomplete release of

proteins *in vitro* as well as *in vivo* has been reported for *in situ* gelling systems at high loading [12]. Interactions of protein with polymer and degraded products of polymer also affect the stability of protein within the formulation. One classic example is use of PLGA polymers for protein and peptide delivery. Hydrolysis of protein at acidic pH within a microenvironment of PLGA matrix has been indicated [13]. Peptides have been shown to be chemically modified in an acidic microenvironment of PLGA, which may also occur with proteins leading to loss of bioactivity. A variety of additives has been tested to prevent or minimize the denaturation of proteins during the process of micro-encapsulation [13]. Some of the widely investigated additives are salts (MgCO_3 , $\text{Mg}(\text{OH})_3$, NaCl), sugars (mannitol and trehalose), surfactants (PVA, Tween 20 and Tween 80), and ionic complexation of protein using polyelectrolytes (dextran sulfate, chondroitin sulfate and SDS). Solvent-free techniques, such as use of supercritical CO_2 , are under development and may provide answers to stability issues [14].

Burst release is another limitation of the polymeric delivery systems including *in situ* implants, micro-and nano-particles. Burst release is as a result of surface binding of protein in case of polymeric particles. Solvent polarity and rate of solvent diffusion are some of the factors, which influence burst release from injectable implants. Such rapid release results in loss of significant amount of therapeutics leading to dose-dependent toxicity and shortened duration of release. A number of strategies have been examined such as use of less polar organic solvent in the case of inject-able implants and a nanoparticle-in-gel approach to minimize the burst effect. In the case of matrix-type polymeric delivery systems, drug release is controlled by diffusion and erosion of polymer matrix. Polymer degradation can be controlled by manipulating composition, molecular weight and hydrophobicity. However, it is challenging to achieve complete control over the release pattern from these systems. Novel approaches such as nanoparticle-in-gel combination systems, where biotherapeutics-loaded nanoparticles are suspended in thermosensitive hydrogel, have shown promise in achieving close to zero-order release [15].

Non-biodegradable implants

An ideal sustained-release formulation should provide release at zero order. Non-biodegradable silicon-based polymer implants and osmotically driven implants have shown considerable promise in achieving sustained release at constant rates for several proteins and peptides [16, 17]. Although these implants are bio-compatible, a major limitation is the need for surgery to administer and remove the implants. However, these are the most successful systems to deliver proteins for an extended duration (months) at a constant zero-order rate. In the case of biodegradable polymer-based formulation, one major concern is poor IVIVC for release profiles, which may substantially hinder the progress of formulation development, as *in vitro* release rate may not correlate with *in vivo* performance. This can be attributed to variable degradation rates of polymer under *in vitro* and *in vivo* conditions. Faster degradation *in vivo* may lead to rapid breakdown of polymer matrix leading to rapid release. Generation of more degradation byproducts may adversely affect protein stability. IVIVC for release profile of leuprolide acetate peptide from osmotic implants Viadur® is excellent [18]. It may be due to excellent stability of peptide in the implant system (72 mg leuprolide acetate in 104 mg of DMSO) [18]. In addition, the driving force for drug release

is osmotic pressure that can be systemically optimized to achieve desired release rates and may not alter under *in vivo* conditions. These implants employ organic and aqueous solvent to hold the therapeutic agent in the drug chamber. Despite the success in delivering peptide for extended durations, these systems may have limited application in the case of large proteins since these biomolecules are known to have poor stability in organic solvents. In addition, following the release of the protein in organic solvent, an aqueous/organic interface may form depending on the miscibility of the solvent with water. Such interface is also responsible for denaturation of proteins. If an aqueous solution of proteins is used, the solution needs to be concentrated to accommodate a large dose in the limited space of the drug chamber (volume ~150µl). Susceptibility of proteins to form irreversible aggregates at higher concentration may limit applicability of such an approach. Hence, the success of osmotically driven implants in delivering proteins may be dependent on the inherent stability of proteins against stresses such as organic solvent, concentrated aqueous solution and aqueous/organic interface.

Conclusion

Despite limitations associated with every approach, delivery of proteins for an extended duration in a controlled manner is achievable. Significant advancements have been made in the last decade to achieve this goal. Several formulations based on non-biodegradable and biodegradable polymers (injectable implants and microparticles) are in clinical use for the delivery of peptides and proteins. However, a clinically acceptable formulation that delivers proteins over a period of months is largely an unmet need. Formulation approaches applicable to small molecules and low molecular peptides are generally ineffective due to unique challenges posed by large molecular weight proteins, which are prone to degradation due to a number of factors and process variables. A number of new protein molecules are under clinical trials and hence there is an urgent need to develop novel approaches to deliver these highly potent biologics.

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Biographies