Sustained Delivery of Proteins for Novel Therapeutic Products

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Recent developments in biotechnology have resulted in a number of powerful new protein therapies for many heretofore untreatable conditions, including hepatitis C, multiple sclerosis, hormonal disorders, and different cancers. In spite of this, the use of most protein drugs is limited by the inconvenient and invasive manner in which they must currently be administered. This involves either intravenous infusion or frequent subcutaneous or intramuscular injections throughout the therapy.

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Delivering proteins is a challenge because of their large size and fragile three-dimensional structure, which must be maintained for biological activity. As a result, proteins exhibit poor oral bioavailability, eliminating the route by which small molecular weight drugs are most often delivered. A variety of approaches for improved delivery of therapeutic proteins are being explored in academia, government labs, and industry. One interesting approach is an injectable, biodegradable system that provides a sustained release of the agent over time.

The development of effective systems for the sustained delivery of therapeutic proteins requires that several key obstacles are overcome. These include (i) processing and formulating the protein and delivery system so that the protein's fragile conformation and biological activity are maintained throughout processing and during prolonged release in the body, (ii) controlling the release so that therapeutic levels are maintained for the desired time, and (iii) developing a manufacturing process to produce quantities of sterile material for clinical trials and commercialization.

Recently, these hurdles have been overcome and clinical trials have been initiated with the ProLease biodegradable microsphere delivery system for proteins and peptides (*1–3*). The ProLease system is a dry powder composed of biodegradable polymeric microspheres containing a protein in a polymer matrix that can be administered by injection in an aqueous diluent through a narrow-gauge needle (Figs. 1 and 2).

A number of processes have been developed for the encapsulation of low molecular weight drugs in biodegradable microspheres by using phase separation, solvent evaporation, emulsion, or spray drying steps. However, the conditions typically used in these processes, such as elevated temperatures, high concentrations of surfactants, or organic and aqueous solvent mixtures, may result in accelerated protein degradation. Degradation can decrease potency and increase immunogenicity, which in turn may adversely affect the safety and efficacy of the drug.

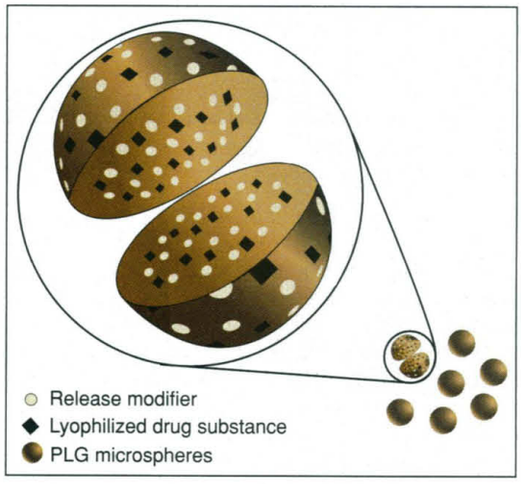

Fig. 1. ProLease microspheres. A cross section of a single microsphere containing protein and release modifier (such as zinc carbonate for hGH) encapsulated into the PLG polymer matrix.

Unlike the processes above, the ProLease microsphere fabrication process was specifically designed to achieve a high protein encapsulation efficiency while maintaining protein integrity (*4*). The process consists of (i) preparation of freeze-dried protein particles from bulk protein by spray freeze-drying the drug solution with stabilizing excipients, (ii) preparation of a drug-polymer suspension followed by sonication or homogenization to reduce the drug particle size, (iii) production of frozen drug-polymer microspheres by atomization into liquid nitrogen, (iv) extraction of the polymer solvent with ethanol, and (v) filtration and vacuum drying to produce the final dry-powder product. The resulting powder contains the solid form of the protein, which is homogeneously and rigidly dispersed within porous polymer particles. The polymer most commonly used in the process, poly(lactide-co-glycolide) (PLG), is both biocompatible and biodegradable.

The ProLease process has several advantages: Encapsulation occurs at low temperatures (>–40°C). During encapsulation, the protein is maintained in the solid state in the absence of water, thus minimizing water-induced conformational mobility of the protein, preventing protein degradation reactions that include water as a reactant, and avoiding organic-aqueous interfaces where proteins may undergo denaturation. The process uses solvents in which most proteins are insoluble, thus yielding encapsulation efficiencies of >95%.

Maintaining stability of the protein following injection of a sustained release formulation poses a considerable challenge because proteins in microsphere formulations remain in a concentrated, hydrated state at physiological temperatures for prolonged periods after injection. These conditions are conducive to protein degradation reactions, including aggregation (covalent and noncovalent), deamidation, and oxidation. Several stabilization strategies can be used to maintain protein integrity under these conditions (*5, 6*). The choice of one or more stabilizing agents is determined empirically. One effective approach is to form a complex with a divalent metal cation before encapsulation. Zinc has been employed in this manner to stabilize recombinant human growth hormone (rhGH) and recombinant α-interferon (α-IFN) in microspheres (*2, 7, 8*). Also, protein stability in hydrated microspheres can be improved by using certain salts. For example, ammonium sulfate has been shown to stabilize erythropoietin during release (*9*).

In addition to maintaining protein stability during processing and release, the microsphere formulation must display the release kinetics required to achieve a sustained therapeutic effect. Following injection of the microspheres into the body, the encapsulated protein is released by a complex process involving hydration of the particles, dissolution of the drug, drug diffusion through water-filled pores within the particles, and polymer erosion (*10–12*). Two primary considerations are minimizing how much protein is released immediately (called the burst) and achieving the desired duration and rate of protein release. The duration of

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release is governed by the type of PLG polymer used and the addition of release modifying excipients such as zinc carbonate (*13*).

The development of a sustained release system for a therapeutic protein begins with identifying a formulation with satisfactory stability characteristics and kinetics of release in animal models (*1*), toxicological and storage stability studies, and then human clinical testing.

In a collaboration between Alkermes and Genentech, rhGH has been formulated into microspheres (ProLease hGH). ProLease hGH was characterized with respect to protein stability and release kinetics in animals. hGH remained intact through the encapsulation process and during release (*2*). Toxicokinetic studies in primates and transgenic mice showed that microspheres were well tolerated and that ProLease hGH appeared to be no more immunogenic than rhGH administered in solution (*14*).

Clinical testing of ProLease hGH began with a phase I study in growth hormone–deficient adults, where hGH serum levels remained above baseline for a median of 23 days (*1*). The released hGH evoked an appropriate biological response (elevated insulin-like growth factor I and insulin-like growth factor–binding protein 3). The injections were well tolerated, and no antibodies to rhGH were detected (*1*). Advanced clinical testing of ProLease hGH in growth hormone–deficient children is under way.

Advantages inherent in sustained delivery of proteins are likely to include improved patient compliance (by reducing the need for self-injection), potentially lower costs (by reducing the frequency of visits to a caregiver's office), greater usage of a drug (through new indications and ease of use), and improved safety and efficacy (by reducing variability inherent in frequent injections). For certain proteins, it may also be possible to reduce the total dose per month, thereby reducing the cost to patients. Nevertheless, microsphere-based sustained delivery systems may be limited by the daily dose of protein needed for a therapeutic effect.

Alternative approaches for sustained delivery of therapeutic proteins are in various stages of development. An implantable osmotic pump system reportedly delivers peptide drugs at a constant rate for up to 1 year (*15*). Use of liposomes and other multivesicle systems to deliver proteins in a sustained manner and to reduce immunogenicity is also under development (*16*). Systems wherein therapeutic proteins are expressed and secreted from an implantable device containing recombinant cells are being explored (*17*), in certain instances for delivery to localized sites (*18*). In principle, gene therapy provides an alternative approach for sustained delivery of proteins to both localized and widely dispersed sites by reprogramming cells of the recipient to express the protein.

Improved immediate release technologies for therapeutic proteins are also being explored. For example, pulmonary delivery of

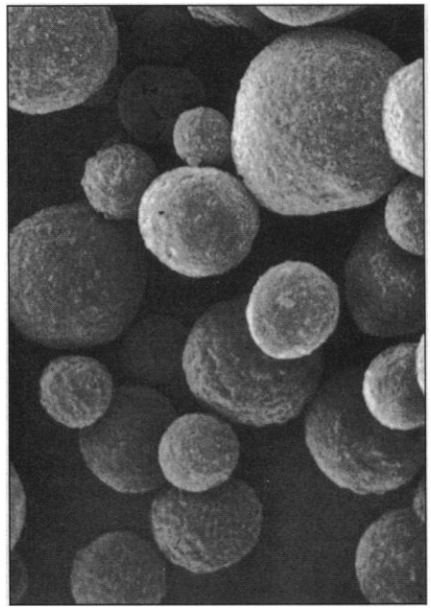

Fig. 2. Scanning electronic micrograph of ProLease microspheres.

proteins in the form of aerosols may provide a less invasive route of administration compared to injection (*19*). Alternatively, chemical modification with polyethylene glycol has been reported to extend the plasma half-life of therapeutic proteins such as α-IFN (*20*). This approach may reduce the injection frequency, albeit probably not to the extent possible with polymeric microspheres. Industrial and academic groups are also exploring new strategies for oral delivery of proteins (*21*). As these approaches advance toward commercialization, additional applications and technologies continue to emerge.

In addition to simple replacement therapy, exemplified by treatment of growth hormone deficiency with ProLease hGH, other applications of protein sustained-release systems are under investigation. For example, therapeutic antibodies, neurogrowth factors, and cytokines represent applications where sustained plasma levels of exogenous proteins may be effective. Injection of microspheres into or near the site of action may offer the opportunity to greatly increase the concentration of the protein at the target tissue, while decreasing systemic exposure of the protein. Sustained release of therapeutic proteins directly into certain tumors, as well as into the central nervous system, heart, eyes, or joints is an area for development.

Other potential applications may demand more sophisticated release profiles than those achievable with current microsphere systems, wherein drug release is primarily dictated by PLG degradation and erosion. Systems have been described where controlled release occurs in response to external stimuli such as an electric or magnetic field or to changes in the microspheres' biological milieu (*22*). Microspheres might be engineered to provide pulsatile drug release in response to relevant biofeedback (*22*) or to normal cyclical rhythms of the body. Additionally, formulations that contain multiple drugs and whose release profiles are tailored to changing physiological needs as treatment progresses represent another practical extension in this field. Examples of these indications are the dynamic cascade associated with wound healing and the degeneration, apoptosis, and regeneration sequence that occurs following spinal cord injury. This might be accomplished by developing more sophisticated microsphere formulations, or alternatively, by simply blending microspheres with different proteins and release characteristics. Finally, it is even conceivable that microelectronic chips might be interfaced with the injected polymer mass to provide programmed control of protein release, thus offering far greater moment-to-moment flexibility and precision in the release characteristics.

Improvements in protein delivery are just beginning to affect the manner in which diseases are treated. Advances in this technology, coupled with steady progress in biotechnology, will dramatically change the way medicine is practiced.

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