Delivering Tomorrow's Medicines

Demonstrating excellent control over release kinetics and the ability to preserve the protein's structural integrity and bioactivity, hydrophilic biodegradable multi-block copolymers offer an interesting solution.

Despite the approval of an increasing number of protein therapeutics, progress in developing sustained release formulations for proteins has been limited. Demonstrating the ability to control release kinetics and preserve bioactivity of proteins, hydrophilic biodegradable multi-block copolymers represent a unique platform for the advancement of sophisticated drug delivery systems for this rapidly expanding class of therapeutic compounds.

Therapeutic Proteins

The discovery of proteins and peptides for therapeutic purposes has been boosted by the progression of biotechnology and the completion of the Human Genome Project in 2003. Today, an increasing number of biotech-derived medicines are being studied in serious illnesses such as HIV/AIDS, Alzheimer’s disease, cancer, cardiovascular disease and autoimmune disorders.

Peptides and proteins are usually large molecules with a three-dimensional (3D) structure containing hydrophilic and charged groups. The bioactivity of these complex molecules is highly dependent on proper folding of the amino acid string. Their intricate 3D structure is stabilised by weak physical interactions and S-S bonds. They are intrinsically fragile and susceptible to shear forces, enzymes, and variations in pH and temperature. Furthermore, they show poor bioavailability when administered orally and, consequently, are usually delivered by subcutaneous, intramuscular, or even intravenous injection — but, due to their short half-life, frequent administration (daily or more) is required.

Sustained Release

It is therefore not surprising that growing attention is being paid to parenteral delivery of peptides and proteins using sustained release depot systems that provide a longer duration of action. In addition to lowering the frequency of administration — which improves patient comfort and therapy compliance — pharmacological therapies based on sustained release formulations effectively reduce side-effects and enhance efficacy due to lower and more constant drug concentrations in systemic circulation.

Poly(lactide-co-glycolide) (PLGA) copolymers have been successfully applied in microspheres, implants and injectable gels for the sustained release of various therapeutic peptides, especially for the treatment of prostate and breast cancer (1). Following the launch of Zoladex — a one-month releasing implant formulation of goserelin — in 1989 by AstraZeneca, and Lupron Depot — a one-month releasing microparticle formulation of leuprolide — in the same year by Takeda, multiple PLGA-based peptide formulations with one- to six-month sustained release have been launched. PLGA microspheres containing somatropin — a recombinant human growth hormone with a molecular weight of 22,125Da — were launched in 2000 by Genentech, under the name of Nutropin Depot, as a two-week releasing, subcutaneous injectable depot formulation for the treatment of paediatric growth hormone deficiency (2).

Shortcomings

However, since then — and despite an impressive amount of academic and industrial R&D effort — no new PLGA-based sustained release protein formulations...
Innovations in Pharmaceutical Technology | Issue 51

Based on hydrophobic PLGA polymers. Hydrophilic biodegradable polymers are interesting systems for the parenteral delivery of proteins since they are capable of accommodating large amounts of water, thereby forming a more protein-friendly, hydrogel-like environment.

Multi-Block Copolymers

Furthermore, during release, the internal pH in these microspheres tends to drop due to accumulation of acidic degradation products in the rigid PLGA matrix. The low pH catalyses degradation of the polymer, resulting in the irregular and biphasic degradation-controlled release profiles of proteins that are typically reported for PLGA. Finally, the acidic micro-environment may lead to protein aggregation, unfolding and the formation of covalent bonds – via acylation, for example – between protein and polymer degradation products, ultimately leading to incomplete protein release, loss of biological activity and immunogenic responses.

Hydrophilic Biodegradable Polymers

With the large number of protein therapeutics coming to the market, there is clearly a strong need for alternative drug delivery methods that overcome the shortcomings of the first-generation systems based on hydrophobic PLGA polymers. Hydrophilic biodegradable polymers are interesting systems for the parenteral delivery of proteins since they are capable of accommodating large amounts of water, thereby forming a more protein-friendly, hydrogel-like environment.

Release of proteins from these systems is typically governed by a combination of diffusion and polymer degradation. Due to their water-swollen character, acidic degradation products, when formed, do not accumulate in the polymer matrix. Instead, they are let go, thereby avoiding the formation of an acidic micro-environment during protein release.

SynBiosys® Pro represents a family of hydrophilic phase separated multi-block copolymers, composed of well-known and clinically acceptable monomers – lactide, glycolide, ε-caprolactone, polyethylene glycol (PEG), 1,4-butanediisocyanate and 1,4-butanediol. The polymers are prepared by a two-step synthesis process: in the first step, hydrophilic amorphous building blocks – such as poly(DL-lactide)-PEG-poly(DL-lactide) (PDLA-PEG-PDLA) or poly(ε-caprolactone)-PEG-poly(ε-caprolactone) (PCL-PEG-PCL) – and
hydrophobic semi-crystalline building blocks – such as poly(L-lactide) (PLLA) or poly(ε-caprolactone) (PCL) – are synthesised by standard tin octanoate-catalysed ring-opening polymerisation. In the second step, selected building blocks are then coupled together via chain-extension with 1,4-diisocyanate.

A family of multi-block copolymers of various block types, in different block ratios, with different molecular weights, and varying physicochemical degradation and release characteristics is obtained. The PEG-containing blocks absorb water, while the PCL or PLLA blocks form crystalline domains that act as physical crosslinks, stabilising the matrix and controlling its swelling degree.

By varying the molecular weight of PEG (600-5,000g/mole) and the block ratio, the PEG content – and thus the swelling degree of the multi-block copolymers – can be adjusted, allowing for fine-tuning of the release kinetics for proteins and peptides of various molecular sizes, such as lysozyme (see Figure 2, part A). Concomitant with drug release, the polymer starts to degrade due to hydrolysis of the ester and urethane bonds.

SynBiosys multi-block copolymers have been extensively tested for their biological safety, and have been safely used in thousands of patients as a biodegradable drug-eluting, coronary stent coating on the Combo Dual Therapy Stent (Orbusneich), which received CE mark approval in May 2013 in Europe, Hong Kong, Malaysia and a selection of other countries (3,4). Multi-block copolymers of a range of compositions are now being used as release-controlling excipients in parenteral and site-specific drug delivery systems. Microspheres and implants with long-term sustained release of up to six months are being developed for various peptides and proteins, for the treatment of – among others – ischemic heart disease (IHD), osteoarthritis, diabetes and various ocular diseases.

**Microsphere Preparation**

Protein-loaded SynBiosys Pro microspheres are typically prepared by a water-in-oil-in-water (W/O/W) emulsion process. Using a high speed homogeniser, an aqueous protein solution is emulsified with a solution of a polymer in a volatile organic solvent. Next, this primary emulsion of protein-containing droplets is emulsified in an aqueous extraction medium containing a surfactant (typically polyvinyl alcohol), thereby forming a suspension of microspheres. The microsphere suspension is stirred to extract and evaporate the organic solvent, then the protein-loaded microspheres are collected by filtration and, finally, lyophilised to obtain a dry powder (see Figure 3).

Insulin-like growth factor-1 (IGF-1) loaded microspheres have been prepared with hydrophilic (PCL-PEG-PCL)-b-(PLLA) multi-block copolymers via a W/O/W-based emulsification process, using membranes with...
Innovations in Pharmaceutical Technology   Issue 51

sulfate-polyacrylamide gel electrophoresis confirmed that the active site and structural integrity of IGF-1 released from microspheres were preserved. Preservation of the bioactivity was confirmed via activation of the signal transduction pathway using A431 as a reporter cell line.

Injectable Solid Implants

Besides microparticles, small diameter solid implants are being used as parenteral sustained release formulations. Examples include the aforementioned Zoladex goserelin implants and Ozurdex – a small-diameter, dexamethasone-releasing ocular implant, indicated for diabetic macular edema. Solid drug delivery implants are typically manufactured by processes such as hot melt extrusion or injection moulding, which offer the advantage of solvent-free processing. However, melt processing of most polymers, including PLGA, requires high processing temperatures which, in combination with shear stresses, may lead to degradation of fragile molecules like peptides and proteins.

Low-temperature, processable polymers – such as (PCL-PEG-PCL)-b-(PCL)-based SynBiosys multi-block copolymers with a crystalline PCL block – exhibit interesting properties to be used as long-term releasing implants for proteins. Due to the use of crystalline PCL blocks, such polymers have a low melting temperature which allows moulding or extrusion at temperatures as low as 50-55°C. The hydrophilic PEG-containing (PCL-PEG-PCL) blocks provide a favourable environment for proteins.

Besides microparticles, small diameter solid implants are being used as parenteral sustained release formulations. Examples include the aforementioned Zoladex goserelin implants and Ozurdex – a small-diameter, dexamethasone-releasing ocular implant, indicated for diabetic macular edema. An emerging approach to achieve effective site-specific delivery of GFs to the ischemic heart region is catheter-assisted, intra-coronary administration of GF-loaded microspheres of a uniform size of 15μm. Due to their size, the microspheres will become trapped in the small arteries of the ischemic region (see Figure 4), allowing localised release of GFs into the ischemic heart tissue. Locally released GFs will act directly on the resident endogenous cardiac stem cells, resulting in their activation and differentiation into cardiac myocytes, endothelial cells and smooth muscle vascular cells to regenerate the contractile tissue and the microvasculature.

Growth factor therapy for treatment of ischemic heart diseases

In the US and EU, 1.5 million acute myocardial infarctions (AMI) are treated annually. Current treatment by coronary angioplasty, combined with stent implantation, is successful in re-establishing the perfusion of the ischemic myocardium, but does not recover the injured tissue, leading to IHD and chronic heart failure. Local delivery of growth factors (GFs), such as vascular endothelial GF (VEGF), IGF-1 and epatocyte GF to regenerate ischemic cardiac tissue, following AMI, is considered a promising therapy to treat IHD.

An emerging approach to achieve effective site-specific delivery of GFs to the ischemic heart is catheter-assisted, intra-coronary administration of GF-loaded microspheres of a uniform size of 15μm. Due to their size, the microspheres will become trapped in the small arteries of the ischemic region (see Figure 4), allowing localised release of GFs into the ischemic heart tissue. Locally released GFs will act directly on the resident endogenous cardiac stem cells, resulting in their activation and differentiation into cardiac myocytes, endothelial cells and smooth muscle vascular cells to regenerate the contractile tissue and the microvasculature.

Figure 4: Schematic representation of IHD following AMI (left) and proposed site-specific GF delivery therapy by means of 15μm-sized microspheres (right)

11μm-sized pores (5). Spherical, uniformly-sized microspheres of 15μm, with a narrow particle size distribution containing up to 5 weight % IGF-1, were obtained. IGF-1 was released in vitro without any significant burst, according to diffusion-controlled kinetics. Figure 2, part B (see page 44) shows how a range of release rates can be obtained by varying the SynBiosys Pro composition and processing conditions. By optimising the permeability and degradation of the polymer matrix, even IGF microsphere formulations with an almost constant release rate could be obtained.

Detailed characterisation of released IGF-1 by enzyme-linked immunosorbsent assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis confirmed that the active site and structural integrity of IGF-1 released from microspheres were preserved. Preservation of the bioactivity was confirmed via activation of the signal transduction pathway using A431 as a reporter cell line.
Small-diameter, hot melt-extruded implants allow for sustained release of proteins of various sizes, such as goserelin (1,250Da), insulin (5.8kDa), lysozyme (14kDa), carbonic anhydrase (29kDa) and bovine serum albumin (66kDa), as shown in Figure 5 (6). In this example, all proteins fully preserved their structural integrity; protein release rate decreased with protein size and with decreasing PEG content of the polymers. By varying the molecular weight of PEG or PEG content (by adjusting the block ratio) of the (PCL-PEG-PCL)-b-(PCL) multi-block copolymers, the release of proteins of different sizes can be optimised, making them attractive candidate polymers for long-term, sustained release depot formulations for proteins.

**Future Progress**

Parenteral delivery of proteins is a rapidly evolving sector. However, to date, progress in developing sustained release formulations for proteins has been limited due to the technical shortcomings of currently available biodegradable, polymeric drug delivery systems.

A new class of hydrophilic, biodegradable multi-block copolymers has emerged that, when formulated into microspheres or implant-based sustained release formulations, offers control over protein release kinetics, and preserves the structural integrity and bioactivity of proteins. Their unique characteristics make these polymers excellent candidates for the development of sophisticated sustained release drug delivery formulations for an increasing number of pharmaceutical proteins. By improving pharmacokinetics, and reducing the side-effects and number of injections, not only will current protein-based pharmacotherapies be significantly advanced, but new therapies may be envisioned, especially in the field of site-specific protein delivery.

**References**

1. Stevensen CL, Rhodes CA and Prestrelski SJ, Delivery of peptides and proteins via long acting injections and implants, in Wright JC and Burgess DJ (Eds), Long Acting Injections and Implants, 2012
5. Hiemstra C et al, IGF-1 loaded monospheres for treating ischemic heart disease, 39th Ann Meet & Exp Controlled Rel Soc, Quebec City, Canada, 2012

---

**Figure 5:** Schematic representation of preparation of protein-loaded implants by hot melt extrusion (left) and cumulative release profiles of proteins from 50(PCL-PEG-PCL)-b-50(PCL) (right).

Source: Stanovic M et al (6)