New Biodegradable Polymers for Delivery of Bioactive Agents

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Summary: Biodegradable, thermosensitive triblock copolymer, PLGA-PEG-PLGA, can be easily fabricated into drug-loaded microspheres or injectable in situ hydrogel system for protein or water-insoluble drugs without use of organic solvent. Aqueous-based microsphere exhibited continuous release of intact insulin in vitro for 3 weeks while the microspheres prepared using dichloromethane showed initial burst and incomplete release. Confocal miscoscopy images of microspheres corroborated the release pattern. Next study with an injectable in situ hydrogel (ReGel^{TM}) exhibited zero-order insulin release in vitro and sustained plasma insulin level for 2 weeks in vivo upon single subcutaneous injection in SD rats.

Keywords: biodegradable polymers, drug delivery, hydrogels, microspheres, thermosensitive polymers

Introduction

Over the past two decades extensive research has been performed in the design area of polymeric drug delivery systems. Among them, the use of biodegradable polymers have been successfully carried out. They include polyesters, poly(orthoesters), polyanhydrides, polyamino acid, poly(alkyl-cyanoacrylates), polyphosphazenes, copolymers of (PLA/PGA) and aspartate or PEO.

Although these biodegradable polymers were used for drug delivery and some are successfully for human application, there remains fabrication problems, such as difficult processability and limited organic solvent and irreproducible drug release kinetics. In this presentation, new design of biodegradable polymers and their application for drug delivery will be discussed.

A series of thermoplastic biodegradable hydrogels (TBH) based on star-shaped poly(ether-ester) block copolymers have been synthesized in this laboratory.[1-4] Physically crosslinked TBH may

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present improved biocompatibility, mass transport, biodegradability, and processability, and thus can provide a better way of parenteral injectable drug delivery.

New star-shaped block copolymers, of which the typical molecular architecture is presented, results from their distinct solution properties, thermal properties and morphology. Their unique physical properties are due to the three-dimensional, hyperbranched molecular architecture and influence microsphere fabrication, drug release and degradation profiles.[1]

We recently synthesized thermosensitive biodegradable hydrogel consisting of polyethylene oxide and poly (L-lactic acid). Aqueous solution of these copolymers with proper combination of molecular weights exhibit temperature dependent reversible sol-gel transition. HPL-HPB-HPL (HPL=hydrophilic; HPB=hydrophobic) triblock copolymers (Hygel^{TM}) shows sol formation at an elevated temperature (~45 °C) and drug loaded sol forms gel upon rapid cooling to body temperature which acts as a sustained drug release matrix.[2-4] On the contrary, HPB-HPL-HPB molecular arrangements (ReGel^{TM}) provide unique behavior that sol (at low temperature) form gel (at body temperature). [5-6] The use of these two biodegradable polymers have great advantages for sustained injectable drug delivery systems. The formulation is simple, which is totally free of organic solvent and thus suitable for hydrophobic drug or protein drug loading.

Due to its amphiphilic nature, this polymer in sol or aqueous state can solubilize poorly water soluble drugs prior to forming gel matrix. ReGel^{TM} forms a controlled release drug depot with delivery times ranging from 1 to 6 weeks. ReGel^{TM} 's inherent ability to solubilize (400 to >2000-fold) and stabilize poorly soluble and sensitive drugs, inculding proteins is a substantial benefit. The gel provided excellent control of the release of paclitaxel for approximately 50 days. Direct intratumoral injection of ReGel /paclitaxel (Oncogel^{TM}) results in a slow clearance of paclitaxel from the injection site with minimal distribution into any organ. Efficacies equivalent to maximum tolerated systemic dosing were observed at Oncogel^{TM} doses that were 10-fold lower.[6]

Protein drugs can easily be loaded into the triblock copolymer system to yield an injectable depot system via microspheres as well as in situ gelling systems. We will give examples of insulin release from using both microsphere system and in situ gel forming device.

Preparation of Triblock Copolymer Microspheres

Fabrication of protein-loaded microspheres, either a water-in-oil-in-water (w/o/w) double-emulsion-solvent evaporation method or spray-dry method, usually involves the use of water-immiscible, volatile organic solvent such as dichloromethane (CH_2Cl_2). However, there are unresolved drawbacks associated with protein-loaded microspheres in the context of protein release and stability. Initial burst release followed by slow and incomplete release of proteins has often been observed. Also, residual level of organic solvent that is difficult to remove completely may bring about toxicity issues. The use of organic solvent as well as the harsh preparation condition are thought to be main reasons for these observed drawbacks that hamper controlled delivery of protein drugs due to the physical degradation of proteins at the interface of aqueous phase and the organic phase.

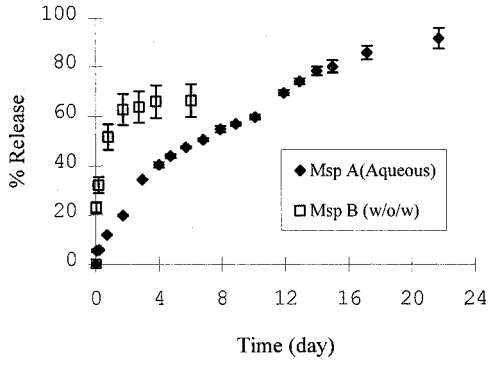

Fig. 1. In vitro release of human insulin from msp A (aqueous-based) and msp B (dichloromethane-based). (n = 3, mean ± S. D.).[7]

Microspheres of the biodegradable, triblock copolymer (PLGA-PEG-PLGA, Mw=4000, 1500-1000-1500 by NMR) was prepared in two methods: microspheres A (aqueous-based) and B (dichloromathane).[7] For both microspheres, same amount of Zn-insulin was loaded (~4% of polymer mass). In vitro release studies were carried out with both. As shown in Figure 1, microsphere A exhibited continuous and nearly complete release of insulin over 3 weeks. The first phase of insulin release (first 10 days) from msp A seems to be dependent more upon

diffusion since release rate was slightly decreasing. Then after day 10 the insulin release rate turned to an increasing mode and this is probably the degradation of the matrix, at this time point, begins to play more significant role in release than in the earlier phase. However, microsphere B showed initial burst release (~50 % in 1 day) and release was discontinued at ~ 60% afterwards. In preparing microsphere B, during the formation of primary emulsion where it involves high shear and heat generation to create a large water/organic solvent interfacial area, proteins can undergo rapid aggregation under this environment and thus the incomplete release of proteins from microspheres may be due to this trapped aggregates formed during microsphere fabrication. This accounts for slow and incomplete release after initial release phase with burst effect.

In the case of microsphere A, microsphere was prepared in a mild environment in that organic solvent and high shear was absent. As shown in Figure 2, circular dichroism (CD) spectrum of insulin released from microsphere at day 12 is virtually identical as that of freshly prepared native insulin solution. This means that released insulin preserved its secondary structure. In contrast, the CD spectrum for microsphere B indicates loss of secondary structure integrity due to the use of dichloromethane and the harsh preparation condition employed.

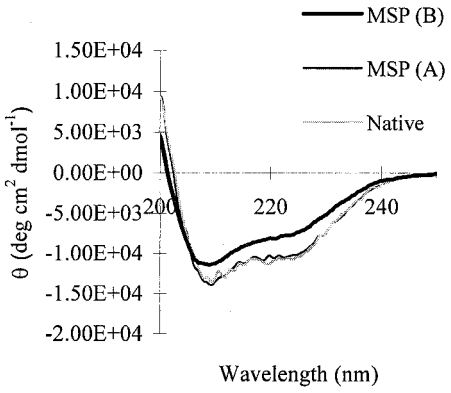

Fig. 2. Circular dichroism (CD) spectra of released insulin from msp A and msp B with respect to the native insulin solution.[7]

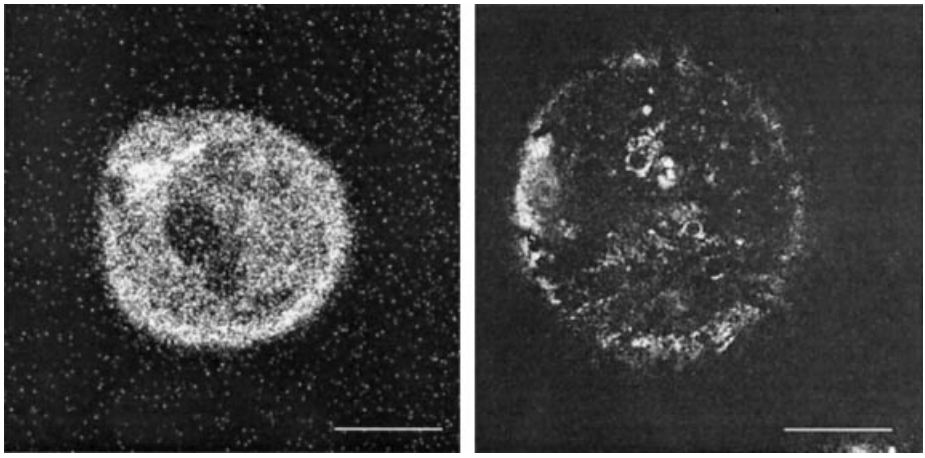

Fig. 3. Confocal microscopy images of FITC-insulin loaded microspheres. (Left) msp A. (right) msp B. Bar indicates 50 μm.

The observed release pattern from both types of microspheres lies in the distribution of the protein inside a microsphere, which is associated with the preparation method. In order to see this, FITC (fluorescein isothiocyanate)-insulin incorporated microspheres were observed under a confocal microscope. The fluorescence distribution is shown in Figure 3. For msp A, homogeneous distribution of fluorescence was observed while msp B exhibited rather heterogeneous distribution of FITC-insulin. In addition, msp B shows significant surface fluorescence. Hence, this is consistent with the observed initial burst from msp B and from constant insulin release from msp A over prolonged period of time. It is reported that the constant release of insulin from triblock copolymer hydrogel may be attributed to the hydrophilic/hydrophobic domain structure of the gel.[5] Low-molecular weight triblock copolymer of PEG and PLGA is known to form micelles at low concentrations and at higher concentrations, gel forms via packing of the micelles and interaction between hydrophobic phases of the micelles by partial overlap.[8] Hence the matrix possesses these microdomains throughout. Thus, significant fraction of insulin is incorporated in the hydrophobic domain that allows sustained release of insulin.

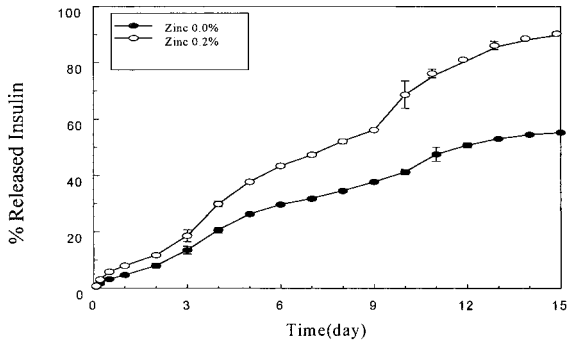

Fig. 4. In vitro release of human insulin from ReGel formulation (n=5).[5]

Insulin Release from Injectable in situ Hydrogel

Insulin release from triblock copolymer hydrogel both in vitro and in vivo was carried out. Aqueous solution of triblock copolymer (23% by mass) was prepared at 5 °C and mixed with insulin. This solution was transferred to 37 °C to form a hydrogel. In vitro release study was carried out at 37 °C by measuring released insulin by HPLC. Figure 4 shows the result of insulin release from the hydrogel (ReGel™) in vitro.[5] There was no initial burst effect of the insulin release from the ReGel™ formulations. This hydrogel system is thought to have a core-shell structure in an aqueous environment. The hydrophilic PEG occupies the shell region and hydrophobic PLGA hides into the core in order to decrease surface free energy. Assuming a domain structure of the hydrogel, the partitioning of drug between the hydrophilic domain and the hydrophobic domain was considered. Insulin can be hydrophobic (around isoelectric point) and may mostly be located inside the hydrogel network. Drug release from the hydrophilic domain can be described by diffusion and this is represented by the release profile till day 7. After day 7 the hydrogel network, especially hydrophobic PLGA started to degrade so that the diffusion and degradation governed the release profile of day 7 to day 15. The release profile of the insulin with zinc showed a constant (zero-order) release rate and almost 90% of the initial amount was released over 15 days.[5]

Animal studies using male Sprague-Dawley rats were performed with ReGel^{TM}/insulin. As shown in the in vitro release study, aqueous triblock copolymer solution containing insulin (10 IU/ml) at 5 °C was injected subcutaneously (s.c.). Upon injection, the polymer/insulin mixture formed a gel at body temperature. Figure 5 shows plasma insulin level at designated time points. There have been steady amount of insulin release from ReGel^{TM} formulation up to day 15 after an s.c. injection. Current protocol of insulin supplementation relies on daily or continous subcutaneous injection of insulin to meet the basal and postprandial requirements In this study, ReGel formulation maintains insulin injection twice per month for basal insulin requirements.[5]

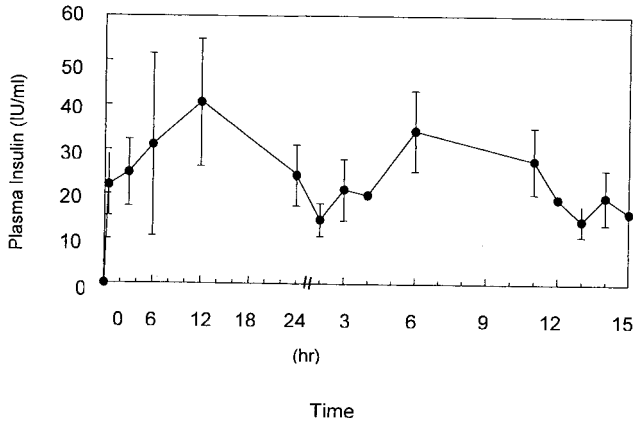

Fig. 5. Plasma insulin level in SD rats in vivo.[5]

Conclusion

Biodegradable, thermosensitive triblock copolymers are potentially useful drug delivery system for therapeutic protein drugs or hydrophobic drugs. As a model study, continuous insulin release for 2-3 weeks can be achieved using either microspheres of PLGA-PEG-PLGA or injectable in situ hydrogel device. While the insulin release from the typically prepared microspheres using CH_2Cl_2 exhibited initial burst release and incomplete release, continuous and nearly complete release (>90%) of insulin in vitro was achieved by aqueous-based microspheres and ReGel^{TM} system. In addition to in vitro data, constant insulin release for two

weeks in vivo after single s.c. injection of ReGel^{TM} loaded with insulin in rats makes the polymer a promising.candidtate for protein delivery.

Acknowlegments

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