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A long-acting formulation of a polypeptide drug exenatide in treatment of diabetes using an injectable block copolymer hydrogel

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ABSTRACT

This study is aimed to develop a long-acting injectable formulation in treatment of type II diabetes. A glucoregulatory polypeptide, exenatide (EXT), was chosen as the model drug, and an aqueous block copolymer system with a sol–gel transition upon the increase of temperature was selected as the delivery matrix of EXT. The thermoreversible hydrogel composed of poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers was found to slower the degradation of the polypeptide to a large extent. However, the initial formulation in this study exhibited a significant drug burst effect, which is a common problem to load a hydrophilic small or medium-size polypeptide into a hydrogel. Zinc acetate was then introduced to slow down the EXT release by formation of insoluble Zn-EXT complexes in the thermogel matrix. Yet an incomplete release became another crucial problem, which is also common for peptide and protein delivery. The synergistic effect of three excipients (zinc acetate, PEG, and sucrose) under an appropriate condition overcame these two problems simultaneously, and the sustained release of drug lasted for 1 week. *In vivo* experiments via mice oral glucose tolerance tests demonstrated an improved glucose tolerance for 1 week after a single subcutaneous injection of the optimal EXT formulation. As a result, a formulation of antidiabetic drugs was set up, and meanwhile a strategy using synergistic excipients to adjust release profiles of peptides from hydrogels was put forward.

1. Introduction

Diabetes has been a popular and serious chronic disease, and thus the development of antidiabetic drugs and associated formulations are very important [1–5]. Usually, an antidiabetic drug, for example, insulin, should be injected before meals, and thus it is rather inconvenient for patients to repeatedly inject every day. On the other hand, a sustained release of traditional antidiabetic drugs might lead to a hypoglycemic symptom during limosis and is thus very dangerous. Such a dilemma is hopefully resolved upon the emergence of the family of glucagon-like peptide-1 (GLP-1) analogs and their receptor agonists represented by exendin [6].

The most efficient and safe exendin drug is exendin-4, originally isolated from the salivary secretions of the lizard Heloderma Suspectum (Glia monster) [6]. It belongs to the group of incretin mimetics and has been established to enhance glucose-dependent insulin secretion, delay gastric emptying, and induce satiety [7,8]. In addition, exendin-4 promotes β-cell proliferation and islet neogenesis from precursor cells according to both *in vitro* and *in vivo* investigations [7,8]. Different from insulin, exendin-4 stimulates the secretion of insulin only in the case of high glucose level and thus the presence of exendin-4 does not lead to hypoglycemia. Consequently, exendin-4 is, rather than simply an antidiabetic drug, a glucoregulatory agent, which enables the development of sustained release formulations in treatment of type II diabetes.

The synthetic version of exendin-4 is called exenatide (EXT), a 39 residue polypeptide with the sequence presented in Fig. 1(a). Byetta®, the EXT solution for injection, has been approved by both United State (2005) and European Union (2006) for the treatment of type II diabetes. Bytta® is available in 2 doses and can be administered via twice-daily subcutaneous (SC) injections. The

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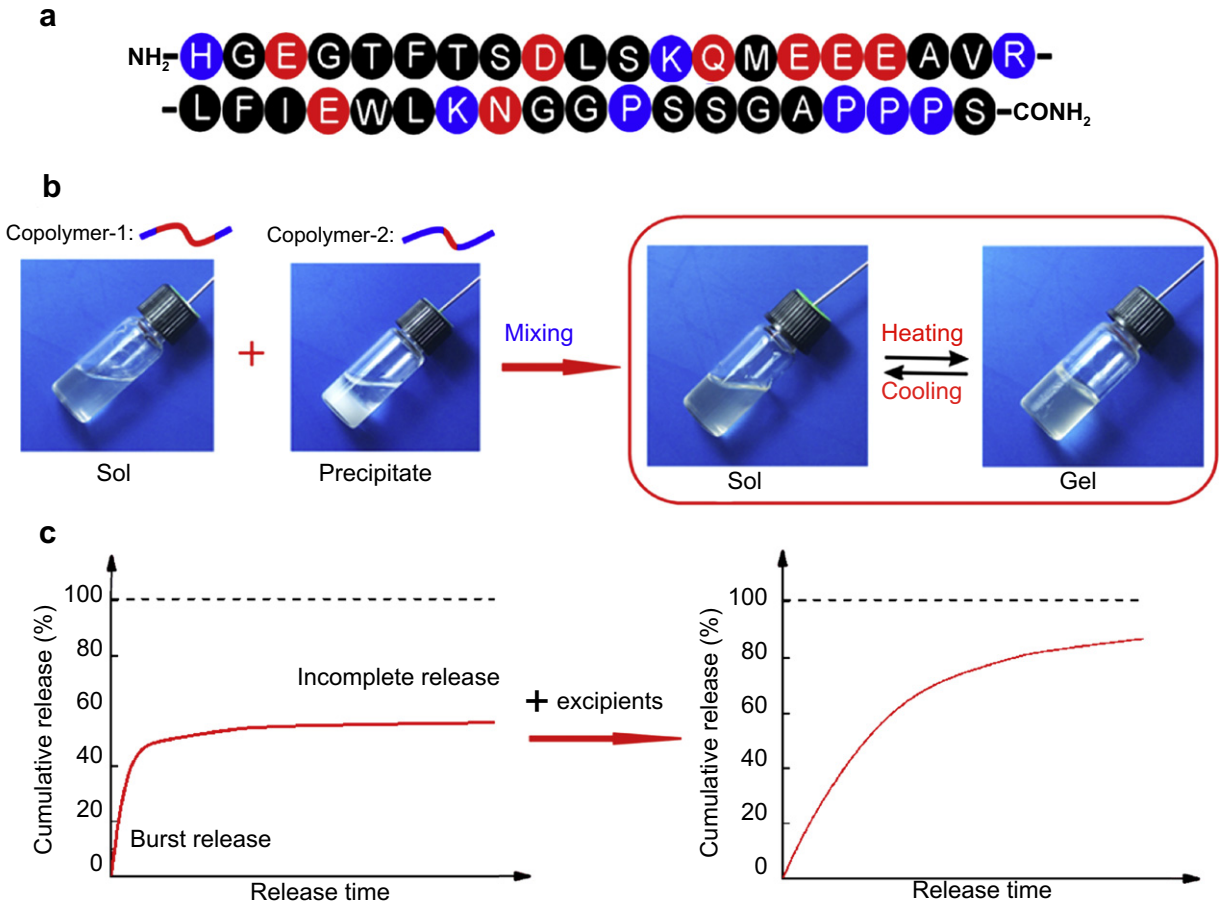

Fig. 1. (a) Amino acid sequence of EXT with acidic residues in red, alkaline residues in blue, and neutral residues in black. (b) Schematic presentation of a mixing approach to achieve a thermogel system; (c) Schematic presentation of the initial burst and incomplete release and a possible facile strategy to partially improve them simply by addition of some appropriate combination of excipients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

corresponding treatment usually begins with the dosage of 5 mcg, followed by the 10 mcg dosage in cases of no significant adverse effects of the low dosage. Such a repeated administration and complex treatment regimen is still not very convenient for patients. Thus, developing a long-acting drug delivery system (DDS) of EXT upon a single injection is much desired for clinical applications.

An appropriate injectable microsphere can significantly reduce frequency of administration of peptide or protein drugs [9–11]. The EXT-loading microsphere formulation (marketed as Bydureon®) has recently been approved by European Medicines Agency in June 2011 and US Food and Drug Administration (FDA) in January 2012. Bydureon® is the first once-weekly injection in treatment with type II diabetes, and the clinical trials have proved a better hypoglycemic effect and less side effects than Byetta® [11,12]. However, a microsphere formulation is always faced with multiple and complex preparation steps, difficulty to improve the drug entrapment ratio and remove residual organic solvents, high cost in sterilization etc. It is thus very meaningful to exploit more formulation ways of EXT. An oral administration of an antidiabetic polypeptide or protein is a very attractive way, yet faced with many difficulties to stabilize and absorb the drugs *in vivo*. A convenient implantation-type formulation by SC injections has thus been taken into consideration by us.

Injectable in situ gel-forming systems have been an alternative delivery system for peptides or proteins due to ease of manufacturing, avoidance of organic solvents, facile sterilization by filtration, and convenient medical application [13–22]. Generally, such a polymer system shows a liquid state at low temperatures and transforms into a gel state with increase of temperature [23–25]. The drugs can be easily entrapped into thermogels without any loss of drug content, simply by mixing drugs with the polymeric sol at a low temperature. A physical hydrogel was formed in situ at body temperature after injecting the drug-containing sol. Patient compliance can be significantly improved via a single injection with minimal surgical wounds. To date, various kinds of thermogelling polymers have been exploited, including copolymers of poly(ethylene glycol) (PEG) and biodegradable polyester [26–30], PEG/polypeptide [31,32], poly(phosphazenes) [33–35], and so on [36–38]. However, no thermogel has been extended into formulation of exendin or EXT yet, to the best of our knowledge.

Among the polymers exhibiting a thermoreversible sol–gel transition, poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers are the most popular biodegradable thermogel due to their tunable biodegradability and good safety profile [39–46]. While amphiphilic block copolymers could easily form micelles in water, the thermogelable composition range is, however, rather narrow [40,47]. Otherwise, the copolymers are just soluble into water or precipitate in water. Recently, Ding group has developed a facile method to prepare a thermogelling system by mixing a sol and a precipitate of PLGA-PEG-PLGA triblock copolymers (see Fig. 1(b)), which broadens the applicable window of pertinent polymers significantly [48,49].

In this study, the PLGA-PEG-PLGA mixture hydrogel will be tried as a sustained release carrier of EXT. If successful, a promising EXT-loading thermogel system would be achieved. However, because of the hydrophilicity and non-large sizes of EXT, such