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# Sustained intravitreal delivery of dexamethasone using an injectable and biodegradable thermogel

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ABSTRACT

Delivery of therapeutic agents to posterior segment of the eyes is challenging due to the anatomy and physiology of ocular barriers and thus long-acting implantable formulations are much desired. In this study, a thermogelling system composed of two poly(lactic acid-*co*-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) triblock copolymers was developed as an injectable matrix for intravitreal drug delivery. The thermogel was prepared by mixing a sol and a precipitate of PLGA–PEG–PLGA triblock copolymers with different block ratios, among which a hydrophobic glucocorticoid, dexamethasone (DEX), was incorporated. The DEX-loaded thermogel was a low-viscous liquid at low temperature and formed a non-flowing gel at body temperature. The *in vitro* release rate of DEX from the thermogel could be conveniently modulated by varying the mixing ratio of the two copolymers. The long-lasting intraocular residence of the thermogel was demonstrated by intravitreal injection of a fluorescence-labeled thermogel to rabbits. Compared with a DEX suspension, the intravitreal retention time of DEX increased from a dozen hours to over 1 week when being loaded in the thermogel. Additionally, intravitreal administration of the thermogel did not impair the morphology of retina and cornea. This study reveals that the injectable PLGA–PEG–PLGA thermogel is a biocompatible carrier for sustained delivery of bioactive agents into the eyes, and provides an alternative approach for treatment of posterior segment diseases.



## 1. Introduction

Eye is a relatively independent organ with high sensitivity and multiple protective mechanisms like corneal, scleral, and blood–retinal barriers, and thus it is very challenging to design and evaluate the drug delivery systems for ocular diseases [1,2]. Generally speaking, the ocular bioavailability of topically applied eyedrops is less than 7% [3]. It is undoubtedly more difficult to treat the posterior segment ocular diseases, such as chronic uveitis, because of the longer diffusional distance from ocular surface to posterior segment which retards significantly bioactive agents to reach the therapeutic concentration [4,5]. Therefore, development of novel intraocular delivery systems represents the trend of ophthalmic remedies, and a corresponding study is very meaningful.

Intravitreal injection is regarded as an important and effective approach to treat posterior segment ocular diseases, which not only delivers directly the bioactive agents into the eye but also avoids the potential side effects of systemic administration [6,7]. However, some issues associated with this administration route, such as repeated injections, the poor patient compliance, and the risks of serious complications [8], limited its application. To overcome these shortcomings, novel drug delivery systems are devised to reduce the frequency of intraocular injection, and thus ameliorate the suffering of patients and decrease the complications of repeated intravitreal administration [9,10]. To date, a few of long-acting formulations have been successfully utilized in ophthalmology clinic. For instance, the Surodex delivery system, a biodegradable depot composed of poly(lactic-*co*-glycolic acid) (PLGA), has been designed to put in the eye after cataract surgery, and a sustained release of dexamethasone (DEX) over a period of 7 days has been achieved [11]. Such implants also include Ozurdex, Retisert, and so on. However, a surgical procedure or a special device is required to implant these products into eyes

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[12]. To increase the convenience of administration and improve patient compliance, development of injectable intravitreal drug delivery systems using a conventional syringe is much desired.

Hydrogel is a kind of polymeric network containing plenty of water, which is physiologically similar to the vitreous, so it is suitable as a promising carrier for intraocular delivery of bioactive agents [13–15]. Recently, biodegradable thermogelling hydrogels which undergo a reversible sol–gel transition upon heating to body temperature have gained increased attention as a minimally invasive depot system for drug delivery, tissue engineering, etc. [16–22]. Chemical drugs, bioactive macromolecules, and even cells can be incorporated into the polymer aqueous solution at low or room temperature. The corresponding formulation can be injected into the target tissue, and then rapidly turns into a gel due to contacting with physiological heat at 37 °C to act as a slow releasing depot of drugs or a cell growing matrix. The use of organic solvents is thoroughly avoided during the whole process of application. So far, thermogelling PEG/polyester block copolymers [22–27], PEG/polypeptide block copolymers [28,29] and poly(phosphazenes) [30,31] have been reported.

As a typical example of thermogel, the poly(lactic acid-*co*-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) triblock copolymers have been investigated extensively due to their many advantages such as facile synthesis, adjustable gel performance, controllable biodegradation rate, versatile drug delivery, etc. [16,32–36]. However, the composition window of PLGA–PEG–PLGA triblock copolymers to form a thermogel is quite narrow [37,38]. Otherwise, the polymers are just soluble or precipitate in aqueous medium. Recently, our group has exploited a convenient approach to obtain a thermogelling system by simply mixing an aqueous sol of a PLGA–PEG–PLGA triblock copolymer and a precipitate of another triblock copolymer with different PEG/PLGA ratios. Thus, the applicable window of pertinent polymers has been broadened to a large extent [39,40]. Furthermore, the biodegradation and biocompatibility of these mixture hydrogels have been confirmed [40].

The initial attempt of the thermogel containing single PLGA–PEG–PLGA copolymer for intraocular application was to modulate the release of ganciclovir-loaded PLGA microspheres by dispersing the microspheres in the thermogel matrix [41]. Afterward, the thermoreversible PLGA–PEG–PLGA hydrogel was used for a topical administration of DEX acetate, a water-soluble form of DEX [42]. When the thermogel was instilled in the conjunctival sac, significantly increased bioavailability was observed compared with the normal eye drop. Nevertheless, the drug was rapidly eliminated from the anterior chamber in 8 h. Theoretically, the more advantages of the thermogel composed of PEG/PLGA copolymers, such as its biodegradability and the capacity of sustained drug delivery, will be well utilized when being injected directly in the vitreous. Unfortunately, whether the aqueous environment would affect the *in situ* formation of the PEG/PLGA hydrogel in the eye and how the incorporated bioactive agents should be released is still unknown. Herein, an injectable thermogel will be constructed based on the mixture of PEG/PLGA copolymers and be used as an intravitreal implant to perform prolonged drug delivery. Two PLGA–PEG–PLGA triblock copolymers with similar compositions but different block ratios were synthesized, in which one is soluble while the other precipitates in water. Their corresponding mixture thermogel was prepared, as presented in Fig. 1a and tried as an injectable depot for intravitreal delivery of DEX, a mainstay of uveitis treatment, as illustrated in Fig. 1b. Different from its water-soluble acetate and phosphate, DEX as a hydrophobic drug was used in the present study. The *in vivo* performance and biocompatibility of the mixture thermogel was evaluated after intravitreal injection. Finally, the intraocular pharmacokinetics of the DEX-loaded thermogel was examined, and correlation of the *in vivo* and *in vitro* DEX release from the thermogel was also discussed.

## 2. Materials and methods

### 2.1. Materials and animals

Poly(ethylene glycol) (PEG, molecular weight (MW): 1000 and 1500), stannous octoate ($Sn(Oct)_2$, 95%) and DEX (CAS: 50-02-2) were acquired from Sigma–Aldrich. D,L-Lactide (LA) and glycolide (GA) were purchased from Purac. Fluorescein sodium was supplied by Solarbio Corporation. Purified deionized water was prepared by the Milli-Q plus system from Millipore (Bedford, USA). All other chemicals used in this research were of analytical grade.

Male albino rabbits, weighing 1.5–2.0 kg, were provided by the Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and maintained individually in standard cages in a light-controlled room (12 h light and 12 h dark cycles) at 20–24 °C and 30–75% relative humidity, with access to food or water *ad libitum*. All animal experiments were performed in accordance with protocols evaluated and approved by the ethics committee of Fudan University.

### 2.2. Synthesis and characterization of PLGA–PEG–PLGA copolymers

PLGA–PEG–PLGA triblock copolymers were prepared by ring-opening copolymerization of LA and GA using PEG as the macroinitiator in the presence of $Sn(Oct)_2$. The detailed synthesis process has been described in previous publications concerning both ours and others' work [39,43]. In this study, two triblock copolymers with different PEG blocks (MW 1000 and 1500) but a fixed LA/GA ratio (4/1) were synthesized. For example, to synthesize Copolymer-1, 10 g of PEG 1000 was put into a three-neck flask and heated under vacuum at 130 °C for 3 h to remove the residual moisture of polymers. Next, LA (24.14 g) and GA (4.86 g) were added and heated under reduced pressure at 100 °C for 30 min. Then, $Sn(Oct)_2$ was transferred into the mixtures, and the reaction system was heated with continuous stirring under an argon atmosphere at 150 °C for 12 h. Then, the crude products were purified by washing with 80 °C water 4 times to remove soluble and low MW by-products. The residual water in the polymer was eliminated via freeze-drying and the final products were kept at −20 °C until further use. Another triblock polymer with PEG 1500 was synthesized using a similar procedure.

#### 2.2.1. $^1H$ NMR measurements

A 500-MHz NMR spectrometer (Bruker, DMX500 spectrometer) was used for $^1H$ NMR measurements to investigate the chemical structure and composition of the PLGA–PEG–PLGA triblock copolymers. $CDCl_3$ and tetramethylsilane (TMS) were used as the solvent and the internal standard, respectively.

#### 2.2.2. Gel permeation chromatography (GPC) characterization

The gel permeation chromatography system (Agilent 1100) with a differential refractometer was used to determine the MWs and their distributions of the PLGA–PEG–PLGA triblock copolymers. The measurements were performed at 35 °C and tetrahydrofuran was used as the eluent at a flow rate of 1.0 mL/min. Monodispersed polystyrene was used as the standard for MW calculation.

### 2.3. Characterization of sol–gel transition

#### 2.3.1. Determination of sol–gel transition temperature

The sol–gel transition of the aqueous polymer solutions was determined via the test tube inverting method with an increase