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In situ covalently cross-linked PEG hydrogel for ocular drug delivery applications

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ABSTRACT

Avastin® has been clinically proved to be effective in the treatment of intraocular neovascularization diseases. However, the short half-life of Avastin® need frequent administration to maintain its therapeutic efficiency. In this paper, we attempted to develop an in situ PEG hydrogels with great biocompatibility for sustained release of Avastin® to inhibit the corneal neovascularization. PEG hydrogels was formed via thiol-maleimide reaction using 4-arm PEG-Mal and 4-arm PEG-SH. The transparent hydrogel was rapidly formed under physiological conditions. By varying the concentration of 4-arm PEG-SH, PEG hydrogel with different gelling time, pore size, swelling ratio and mechanical property could be obtained. In vitro cytotoxicity indicated that the developed PEG hydrogel had no apparent cytotoxicity on L-929 cells after 7 days of incubation. In vitro release study showed the encapsulated Avastin® was sustained release from PEG hydrogels within a period of 14 days study. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis further confirmed that the released Avastin® did not undergo apparent hydrolysis within 14 days. As a conclusion, we could conclude that the developed PEG hydrogels as an injectable hydrogels might be suitable for extended Avastin® release to treat the corneal neovascularization.

1. Introduction

Corneal neovascularization represented the major cause of diminished corneal clarity and subsequently resulting in the significant reduction in visual function. Various diseases such as inflammation, infections and traumatic corneal disorders were involved in the corneal neovascularization (Kim et al., 2008; Perez-Santonja et al., 2010; Qazi et al., 2012). Up to now, numerous chemical compounds and drugs including steroids, methotrexate, heparin, etc. were proposed as inhibitor for corneal neovascularization (Folkman et al., 1988; Joussen et al., 1999; Hos et al., 2011). Steroids drug was still the first choice in clinical therapy. However, when the inflammation is not the cause of angiogenesis, anti-inflammatory steroids exhibited little or no effect on capillary growth. Furthermore, the great side effects of steroids such as glaucoma and cataract formation were also greatly limited its clinical applications (Nakao et al., 2007; Hoffart et al., 2010; Whitlock et al., 2010).

Angiogenesis was a complex process with a result of an up-regulation of angiogenic factors associated with down-regulation of antiangiogenic factors. Known angiogenic factors including vascular endothelilial growth factor (VEGF), basic fibroblast growth factor (bFGF) have been proved to be inducer of corneal neovascularization (Bao et al., 2009; Chang et al., 2010; Binder et al., 2012). Recent studies have demonstrated the overexpression of VEGF in inflamed and vascularized corneas (Binder et al., 2012; Qazi et al., 2012). Therefore, anti-VEGF substances are effective in slowing and inhibiting the growth of new blood vessels in corneal tissue. Avastin® (Bevacizumab) is chimeric anti-VEGF antibody that could specificity bind to and inhibit the biological activity of all human VEGF-A isoforms (Avery et al., 2006a,b; Arevalo et al., 2008). It was approved originally for the treatment of metastatic colorectal cancer and has been used in ophthalmology (off-label) for the treatment of age-related macular degeneration (AMD), iris rubeosis with promising results (Avery et al., 2006a,b). More recently, the inhibition of corneal

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neovascularization of Avastin® by topical instillation and subconjunctival injection have been evaluated in animal model and human clinical cases (Chen et al., 2009; You et al., 2009; Dastjerdi et al., 2010). Although topical administration of Avastin® is effective in neovascular inhibition, the short half-life of protein needs frequent administration to maintain its therapeutic efficiency. Therefore, there is a great need and desire to develop alternative strategies that are more effective and longer time lasting than current drug delivery system.

In past several decades, many strategies including micro/nanoparticles, implant, hydrogels, etc. have been developed for sustained drug delivery using polymeric materials (Jeong et al., 2002; Liu et al., 2009; Casolaro et al., 2012; Li et al., 2012; Stella et al., 2012; Elbialy et al., 2013; Swaminathan et al., 2013). Since the development of hydrogel in 1960s, numerous studies have been performed on adapting hydrogels as biomaterials for biomedical applications, especially in tissue engineering and drug delivery system (Jeong et al., 2002; Anumolu et al., 2010; Agrawal et al., 2012; Casolaro et al., 2012; Jung and Chauhan, 2012; Qiu and Park, 2012). In comparison to other drug delivery systems, the hydrogel exhibited numerous advantages owing to its great ability to swell in water solution and soft as well as rubbery consistency. Furthermore, the ability of drug molecules to diffuse into and out of hydrogels with desired release behavior will also be an appreciated delivery system. Among these hydrogels investigated over the years, covalently cross-linked hydrogel have been demonstrated to have great promise in drug delivery system owing to its excellent mechanical property and long-time survival of encapsulated islets (Schultz et al., 2009; Nimmo et al., 2011; Van Vlierberghe et al., 2011). Poly(ethylene glycol) (PEG) is a biocompatible materials that has been approved by Food and Drug Administration (FDA) for various biomedical application. Hydrogel systems made up of PEG and its derivatives have already been investigated as potential scaffold for bone and cartilage tissue engineering as well as wound healing applications (Lin and Anseth, 2009; Guarnieri et al., 2010; Tan et al., 2010).

Although extensive studies have been performed on smart hydrogel for ocular drug delivery, the development of solely PEG hydrogel for ocular drug delivery applications was limited. In this paper, we attempted to develop an in situ covalently cross-linked PEG hydrogels and evaluate its ability to sustain release of Avastin®.

2. Materials and methods

2.1. Materials

4-arm PEG-Mal (M_w ~10,000) and 4-arm PEG-SH (M_w ~20,000) were purchased from Shanghai Seebio Biotech, Inc. (Shanghai, China). Avastin® (Bevacizumab injection, Roche Pharma Ltd., Switzerland) was provided by Zhejiang eye hospital (WenZhou, China). All the materials used in the study were analytic reagent degree. Milli-Q grade water (Millipore, Bedford, MA, USA) was used for the preparation of solution.

2.2. Hydrogel formation

PEG hydrogels were synthesized by mixing the various weight ratios of 4-arm PEG-Mal and 4-arm PEG-SH (1:1; 1:2; 1:4 w/w). Briefly, 0.1 g 4-arm PEG-Mal was dissolved in 1 ml phosphate buffer solution (PBS, pH 7.4) to form 10% (w/v) 4-arm PEG-Mal aqueous solution. Thereafter, a 4-arm PEG-SH aqueous solution with various concentrations of 7.5%, 5% and 2.5% (w/v), were mixed with 4-arm PEG-Mal aqueous solution and placed at ambient temperature to form PEG hydrogel. For the preparation of avastin loaded PEG hydrogel, avastin (25 mg/ml) was prior mixed with 4-arm PEG-Mal aqueous solution and then mixed with 4-arm PEG-SH solution, thus obtaining the avastin loaded PEG hydrogel (final avastin concentration in hydrogel was 4.5 mg/ml).

2.3. Scanning electron microscopy (SEM) observation

The morphology of various PEG hydrogels was observed by a scanning electron microscopy (JSM-5900LV, JEOL, Japan). Lyophilized hydrogel samples (1 × 1 cm) were sputter-coated with gold for 60 s before the observation.

2.4. Rheological studies

Rheological studies were performed by a AR-2000 rheometer (TA instrument) using parallel plate (25 mm diameter) configuration at 37 °C in oscillatory mode. Samples were placed on the plate by co-injection of the separately dissolved 4-arm PEG-Mal and PEG-SH aqueous solution and the upper plate was immediately lower to the measurement gap size of 1.5 mm for measurement. To prevent water evaporation, a layer of oil was covered around the plate. The storage moduli (G′) and loss moduli (G″) as a function with time were monitored at a frequency of 1 Hz and a strain of 1%.

2.5. Swelling test

Lyophilized hydrogel samples (1 × 1 cm) were weighted (W_0) and then transferred into a 10 ml tube followed by the addition of 8 ml phosphate buffer solution (PBS, pH 7.4). After 7 days of incubation, the hydrated hydrogel samples were carefully removed from the medium and the resident water on the surface of hydrogels were cleared by filter paper, and weighted (W_{7d}). The swelling ratio of hydrogels was calculated by the equation:

$$\frac{W_{7d} - W_0}{W_0} \times 100\%.$$

2.6. Cell assay and cytotoxicity

The cytotoxicity of PEG hydrogel on contacting cells was evaluated by using L-929 fibroblast cells. PEG hydrogels were developed by the mixing 4-arm PEG-Mal aqueous solution (10%; w/v) and 4-arm PEG-SH aqueous solution (2.5%; w/v) in equivalent volume. Immediately after the mixing, the mixture was transferred into 24-well plate at 0.25 ml or 0.025 ml for completely or partly covering the bottom of the well, respectively. After 10 min of incubation at 37 °C, the formed hydrogel adhered at the bottom of well was rinsed with medium. L-929 cells suspended in the medium were seeded into each well at 5×10^3 cells/well. After 1, 3, 5 and 7 days of incubation, the morphology of cells were observed by optical microscope. To incorporate the L-929 cells, 0.025 ml of 4-arm PEG-Mal aqueous solution (10%; w/v) was mixed with 0.01 ml of cell suspensions followed by the addition of 0.025 ml of 4-arm PEG-SH aqueous solution (2.5%; w/v) to obtain cell/hydrogel constructs. The resultant cell/hydrogel constructs (5×10^3 cell/well) were cultured in medium for a period of study. On day 7, the cell/hydrogel constructs were rinsed with PBS and stained with calcein-AM/ethidium homodimer for fluorescent observation (λ_{ex} = 480nm; λ_{em} = 520nm).

2.7. In vitro release study

To incorporate the avastin into PEG hydrogel, 0.05 ml 4-arm PEG-Mal aqueous solution (10%; w/v) was prior mixed with 0.02 ml avastin solution (2.5%; w/v) in 5 ml test tube, and then mixed with 0.05 ml 4-arm PEG-SH aqueous solution (2.5%, 5% and 7.5%; w/v) to form the avastin loaded PEG hydrogel. Thereafter, 3 ml pre-warmed PBS (37 °C; pH 7.4) were added into the test tube for in vitro release study. At specific time intervals, 1 ml of aliquot release