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Gallic acid grafting effect on delivery performance and antiglaucoma efficacy of antioxidant-functionalized intracameral pilocarpine carriers

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ABSTRACT

Functionalization of therapeutic carrier biomaterials can potentially provide additional benefits in drug delivery for disease treatment. Given that this modification determines final therapeutic efficacy of drug carriers, here, we investigate systematically the role of grafting amount of antioxidant gallic acid (GA) onto GN in situ gelling copolymers made of biodegradable gelatin and thermo-responsive poly(*N*-isopropylacrylamide) for intracameral delivery of pilocarpine in antiglaucoma treatment. As expected, increasing redox reaction time increased total antioxidant activities and free radical scavenging abilities of synthesized carrier biomaterials. The hydrophilic nature of antioxidant molecules strongly affected physicochemical properties of carrier materials with varying GA grafting amounts, thereby dictating in vitro release behaviors and mechanisms of pilocarpine. In vitro oxidative stress challenges revealed that biocompatible carriers with high GA content alleviated lens epithelial cell damage and reduced reactive oxygen species. Intraocular pressure and pupil diameter in glaucomatous rabbits showed correlations with GA-mediated release of pilocarpine. Additionally, enhanced pharmacological treatment effects prevented corneal endothelial cell loss during disease progression. Increasing GA content increased total antioxidant level and decreased nitrite level in the aqueous humor, suggesting a much improved antioxidant status in glaucomatous eyes. This work significantly highlights the dependence of physicochemical properties, drug release behaviors, and bioactivities on intrinsic antioxidant capacities of therapeutic carrier biomaterials for glaucoma treatment.

Statement of Significance

Development of injectable biodegradable polymer depots and functionalization of carrier biomaterials with antioxidant can potentially provide benefits such as improved bioavailability, controlled release pattern, and increased therapeutic effect in intracameral pilocarpine administration for glaucoma treatment. For the first time, this study demonstrated that the biodegradable in situ gelling copolymers can incorporate different levels of antioxidant gallic acid to tailor the structure-property-function relationship of the intracameral drug delivery system. The systematic evaluation fully verified the dependence of phase transition, degradation behavior, drug release mechanism, and antiglaucoma efficacy on intrinsic antioxidant capacities of carrier biomaterials. The report highlights the significant role of grafting amount of gallic acid in optimizing performance of antioxidant-functionalized polymer therapeutics as new drug delivery platforms in disease treatment.

1. Introduction

It is known that high intraocular pressure (IOP) strongly involved in glaucomatous development and progression [1,2]. In addition, studies have described the effects of excessive oxidative

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stress on high IOP as a result of trabecular meshwork dysfunction leading to abnormal outflow of aqueous humor [3]. Currently, medical treatments for glaucoma patients involve in the use of prescribed drugs in the form of eye drops and gels to lower IOP. However, the need for drug molecules to diffuse and penetrate through the protective barrier of cornea often reduces their bioavailability and efficacy in ocular tissues, and thus frequent applications are associated with these types of formulations. Due to this, injections of drug-containing hydrogels appear to be an alternative approach to achieve improved effectiveness in glaucoma treatment. Specifically, we have demonstrated the synthesis of in situ gelling GN copolymeric hydrogels using biodegradable gelatin and thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm) for intracameral delivery of pilocarpine [4]. Furthermore, we compared differences in the molecular weights of PNIPAAm as a result of the physicochemical changes in GN copolymers and their effects in drug delivery characteristics [5]. Moreover, in situ gelling GN copolymers were further grafted with gallic acid (GA) molecules to provide additional antioxidant activities [6]. Supported by our previous studies, this work further examine the effects of different grafting amounts of GA onto GN copolymers on the physicochemical properties, drug release characteristics, and intracameral treatment of various antioxidative in situ gelling GNGA biomaterials.

Polyphenolics, including GA, have molecular structures consisting of aromatic rings and hydroxyl groups, which account for their outstanding antiviral, antibacterial, anti-inflammatory and antioxidant properties [7–10]. Traditionally, antioxidant small molecules were entrapped in the polymer carriers whereas the intrinsic properties of the matrix dictate the release rate of antioxidant [11,12]. Recently, the idea of grafting antioxidants onto biodegradable polymers has drawn many attentions since it functionalizes drug carriers. Theoretically, grafting antioxidants onto the backbone of polymeric structure allows a relatively high level of total antioxidant activity. Furthermore, continuous antioxidative stress ability can be achieved since the release of antioxidant depends on the degradation of the matrix, which is rather slow as compared to the diffusion-controlled mechanism in conventional encapsulation methods. Owing to these advantages, Yang et al. grafted antioxidative citric acid onto copolymers of poly(ethylene glycol) and PNIPAAm followed by the entrapping of chemokine [13]. Their results suggested that grafting of citric acid affected the physicochemical properties of the polymeric matrix, sustained the release of chemokine, and increased free radical scavenging ability and bioactivities of the drug carrier. In a similar study, van Lith et al. reported the grafting of citric acid and ascorbic acid onto 1,8-octanediol with excellent antioxidative ability in free radical scavenging, iron chelation, and inhibition of lipid peroxidation [14]. Of particular importance, studies in oxidative challenges using 200 μM hydrogen peroxide suggested that the drug carriers protected cells from attacks generated by reactive oxygen species (ROS) while antioxidative ability remained at a high level after degradation of the matrix materials. However, both studies only considered the changes in intrinsic antioxidative properties from grafting antioxidants onto biodegradable polymers rather than comparing the effects of different grafting amounts of antioxidant on their antioxidative abilities. Therefore, this work addresses the unknowns of different grafting amounts of antioxidant on drug release and bioactivities of the drug carriers.

Typical grafting procedures of GA onto polymer backbones involve in the use of a redox pair from ascorbic acid (AA) and hydrogen peroxide as the radical initiator [15,16]. It is a one step process with a relatively high yield. In addition, using AA redox reaction as a radical initiator allows the instantaneous functionalization of the polymer backbones without the production of toxic chemicals, which can seriously hinder the biocompatibility of the drug carriers. Furthermore, the hydroxyl groups on the aromatic ring of GA are preserved leading to strong antioxidant activities of GA for a prolonged time. Senevirathne et al. grafted GA onto chitosan using AA redox reaction as a radical initiator where the grafting amounts of GA were adjusted by the molar ratio of chitosan to GA [17]. These authors demonstrated that the antioxidant ability of GA-*g*-chitosan carriers against ROS increased with increasing grafting amounts of GA. Işıklan et al. demonstrated itaconic acid-grafted sodium alginate using cerium ammonium nitrate/nitric acid as a redox reaction initiator [18]. In addition, they studied the parameters in the redox reactions explicitly and suggested that a 5-h reaction time (with all other parameters set at optimal values) reached the highest grafting yield and efficiency. Others have reported the correlations between redox reaction times and grafting amounts of antioxidants onto polymer backbones using different polymer-redox systems [19–21]. These earlier findings motivate us to study the dependence of grafting amounts of GA onto GN copolymers on the AA redox reaction time. More importantly, we use AA redox reaction time to control the antioxidative performance of GNGA carrier materials designed for intracameral drug delivery application.

In current study, GNGA carrier materials were synthesized from three redox reaction times (i.e., 30 min, 90 min, and 180 min). This variation allows us to obtain in situ gelling carriers with different total antioxidant activities and free radical scavenging abilities. Since GA is a hydrophilic small molecule, increasing the grafting amount of GA (by increasing redox reaction time) alters the physicochemical characteristics of the GNGA carriers. In addition, we hypothesize that the amount of GA present in the GNGA biomaterials has a significant impact on their antiglaucomatous effects as well as their bioactivities as a drug carrier vehicle. Furthermore, pilocarpine, oldest and most frequently used medication to treat glaucoma, is encapsulated in GNGA injections to study their release rates associated with the grafting amount of the GA. However, it is noteworthy that the ability to lower oxidative stress in the anterior chamber from drug-containing polymer injections was independent of the presence of pilocarpine based on our previous results. By contrast, oxidative stress in the anterior chamber correlated highly with functionalization of the antioxidant GA molecules. Therefore, considering that pilocarpine and GA contribute to separate effects in the clinical responses of glaucoma, current research efforts mainly address on the effects of various grafting amounts of GA onto GN on the development of antiglaucomatous biomaterial carriers.

2. Materials and methods

2.1. Materials

Gelatin (type A; 300 Bloom), GA, AA, hydrogen peroxide, sulfuric acid, sodium phosphate, ammonium molybdate, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), matrix metalloproteinase-2 (MMP-2, EC 3.4.24.24), pilocarpine nitrate, and α-chymotrypsin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Before use, NIPAAm (Acros Organics, Geel, Belgium) was purified by recrystallization from n-hexane. Deionized water used was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Balanced salt solution (BSS, pH 7.4) was obtained from Alcon Laboratories (Fort Worth, TX, USA). Phosphate-buffered saline (PBS, pH 7.4) was acquired from Biochrom (Berlin, Germany). All the other chemicals were of reagent grade and used as received without further purification.

2.2. Synthesis of GA-functionalized gelatin-g-PNIPAAm (GNGA)

Detailed materials used in this work along with synthesis of GA-functionalized gelatin-*g*-PNIPAAm (GNGA) were described