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Systemic modulation of the stability of pluronic hydrogel by a small amount of graphene oxide

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ABSTRACT

Thermo-sensitive and injectable hydrogels have been widely investigated for drug delivery, tissue engineering, and other biomedical applications. Pluronic copolymers can form thermo-sensitive physical gel state, thus applicable for injectable hydrogels. However, they are not stable *in vivo*, showing a very fast dissolution, which limits their applications. We propose a novel Pluronic-based physical hydrogel with enhanced stability by simply adding a small quantity of graphene oxide (GO) which has a large surface area and can make strong interactions with Pluronic. Further carboxylated GO could act as a more efficient additive. The addition of GO increased the moduli of hydrogels, but more importantly, it enhanced the stability of Pluronic gel dramatically. The *in vitro* dissolution rate of Pluronic hydrogel could be systematically modulated by increasing GO content. Upon subcutaneous injection at a sol state, GO-containing hydrogel induced a stable gel state, and was maintained over several weeks whereas very fast degradation was observed without the addition of GO. Furthermore, histological analyses demonstrated that the GO-containing Pluronic hydrogel was biocompatible and showed no severe inflammatory response. Similarly, GO-containing hydrogel resulting from the packing of Pluronic-based nanogel also showed the more enhanced stability by the addition of GO both *in vitro* and *in vivo*. In both systems, hydrogels with remarkably enhanced stability by the addition of GO were also effective for the sustained release of loaded protein, and the release rates were mainly determined by the degradation rates of hydrogels. Thus, these GO-containing Pluronic systems can be used as a thermo-sensitive injectable system with a sufficient stability *in vivo*.

1. Introduction

Hydrogels have many advantages such as biocompatibility, ability for absorbing water and low inflammatory responses [1], applicable as a reservoir for slow elution of drugs or proteins [2]. Particularly, injectable hydrogels are more preferred for drug/protein delivery and tissue engineering. Injectable hydrogels can be made by using responses to environmental stimuli such as temperature [3–6] or pH [7]. For example, these materials are in a flow state before administration, but once injected, they rapidly become a gel under physiological conditions. Such systems can provide several advantages including delivery of large amount in a minimally invasive manner, gelation at the desired tissue sites, and minimized scar formation, thus reducing the risk of infection [6,8]. In addition, bioactive molecules or cells can be incorporated by simple mixing before injection [9].

Pluronic, biocompatible triblock copolymers composed of hydrophobic poly(propylene oxide) and hydrophilic poly(ethylene oxide), are a good example of thermo-sensitive polymers as an injectable hydrogel systems [10]. Pluronic can self-assemble into micelles in aqueous solutions above critical micelle concentration (CMC) and critical micelle temperature (CMT), and exhibit temperature-responsive sol–gel transition behaviors [5]. Above CMT and at high concentration of Pluronic, gelation occurs due to micelle packing, resulting in a dramatic change in their rheological properties [11,12]. Using this thermo-reversible gelation, Pluronic F127 (PF127) has been studied widely, especially as an injectable system for local drug delivery [13]. However, the application of PF127 hydrogel has been limited because of a very fast dissolution, thus fast drug release as well as weak mechanical properties *in vivo*. Therefore, various reports have attempted to enhance the stability of PF127 gel *in vivo*. Chen et al. showed a thermo-responsive nanocomposite hydrogel composed of hexamethylene diisocyanate, PF127 and hyaluronic acid [6]. Our group also reported an injectable and photo-polymerizable Pluronic based hydrogel by using a

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sufficiently long induction period during photo-polymerization [14].

Recently, there has been a remarkably increased attention to biological applications of graphene and graphene oxide (GO). Graphene is a single layer of sp^2-hybridized carbon atoms in a 2-d crystalline lattice [15]. GO is an oxidized derivative of graphene to make it water dispersible. The planar structure and ultra-high surface area of graphene and GO make them suitable for loading a large number of substances including metal, drugs, biomolecules, and even cells [16–18]. Graphene is very hydrophobic, thus graphene can interact with hydrophobic molecules based on π–π stacking and van der waals interaction, whereas GO is negatively charged with carboxyl and hydroxyl groups, so hydrogen bonding and charge interaction as well as hydrophobic association can contribute to the interaction with other molecules. Therefore, GO can associate with variety of molecules including amphiphilic polymers. Based on the non-covalent interaction of GO with variety of molecules, GO composite was shown to significantly increase the mechanical and thermal properties of host materials [18–20]. For e.g., Zhang et al. added GO as a nano-filler into a polyvinyl alcohol (PVA) matrix, and showed the increase in tensile strength, breaking elongation, and compressive strength [18]. There have also been several studies reporting the formation of hydrogel by mixing GO with polymers that can provide sufficient interaction with GO [21–25], including PVA [21,22], DNA [23], chitosan [24], and Pluronic [25] in certain concentration ranges. Also, GO modified with cyclodextrin (CD) was used to form complex hydrogel with azo-functionalized polymer [26], and Pluronic-coated GO was used to make a complex hydrogel with CD [27]. In all of these reports, both GO or modified GO and polymer were necessary for hydrogel formation. Without GO, no gelation occurs, thus GO functions as a main gelator. In contrast, there has been no study reporting that the addition of a small amount of GO can increase and modulate the thermodynamic stability of pre-existing physical gel systems.

GO interacts strongly with Pluronic, mainly by the hydrophobic association between PPO part of Pluronic and GO, so the Pluronic coating of GO nanosheet (~40 nm) could increase the colloidal stability of GO in physiological media and neutralize the surface charge of carboxylated GO [28]. In addition, the presence of carboxyl group and hydroxyl group of carboxylated GO can induce the hydrogen bonding with Pluronic, evidenced by the effect of pH in the interaction between carboxylated GO and Pluronic [25], similar to the interaction between PVA and GO [22].

In this paper, we propose GO as an effective additive to increase and modulate the stability of physical hydrogel systems, here hydrogels formed by Pluronic or Pluronic-based nanogel, in addition to its contribution to the mechanical properties of the hydrogels. We investigate the physical gel system at high concentration of Pluronic (17 wt%) or Pluronic-based nanogel (11 wt%), where they can form hydrogel by themselves by the packing of micelle or nanogel. However, since these gels are achieved by lyotropic transition, the hydrogels formed by themselves are not stable but quickly disappear when excess water is added. We report here that by adding a very small amount GO to Pluronic gel systems, the stability of physical gels already formed without GO can be greatly enhanced and modulated in an excess water environment like physiological situation.

2. Materials and methods

2.1. Materials

Pluronic F127 (PEO100 PPO65 PEO100, MW 12.6 kDa) was donated from BASF Corp. (Seoul, Korea). Acryloyl chloride, triethylamine, anhydrous toluene, potassium phosphate monobasic, sodium phosphate dibasic, potassium chloride, sodium chloride, and sodium azide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous diethyl ether was purchased from Fisher Scientific Inc. (Pittsburgh, PA, USA). 4-(2-Hydroxyethoxy) phenyl-(2-hydroxyl-2-propyl) ketone (Irgaure 2959) was obtained from Ciba Specialty Chemicals Inc. (Basel, Switzerland). Single layer graphene oxide (*X*, *Y* dimension < 500 nm) was obtained from Angstrom Materials Inc. (Dayton, OH, USA) as a 0.5% (w/v) solution. Chloroacetic acid and sodium hydroxide were purchased from Aldrich (Milwaukee, WI, USA). Dialysis membrane [MWCO 50,000 & 3500] was the product of Spectrum Laboratories, Inc. (Houston, TX, USA). 0.2 μm cellulose sterilization syringe filters and 0.8 μm cellulose sterilization syringe filters were purchased from Toyo Roshi Kaisha. Ltd. (Tokyo, Japan). 0.2 μm nylon syringe filter was purchased from Whatman (Florham Park, NJ, USA). Human VEGF ELISA Development Kit (900-K10) was purchased from Peprotech (Rocky Hill, NJ, USA).

2.2. Preparation of Pluronic-based nanogel

Chemically crosslinked, Pluronic-based nanogel was prepared by simple photo-crosslinking of diacylated Pluronic [3]. First, diacrylated Pluronic F127 (DA-PF 127) was synthesized as previously reported [3,29,30]. Shortly, dried Pluronic F127 were reacted with 10 molar excess of triethyamine and acryloyl chloride in anhydrous toluene with stirring under argon over 17 h, then it was precipitated in anhydrous diethyl ether in an ice-water bath, filtered, and dried under vacuum for a few days. The degree of acrylation of Pluronic was over 98%, as determined by comparing the acryl protons (=CH_2, 5.7–6.4 ppm) and methyl protons in propylene oxide units (—CH_3, 1.1 ppm) in 40 MHz ^{1}H NMR spectroscopy (D_2O, JNM-ECX-400P, JEOL, Japan). After that, 0.77 wt% of DA-PF 127 in de-ionized water (DIW) was photo-crosslinked by adding a photoinitiator Irgacure 2959 in 70% (v/v) ethanol and DIW and UV-irradiation using an unfiltered UV lamp (VL-4.LC, 8 W, Vilber Lourmat, France) for 15 min with 1.3 mW cm^{-2} intensity. Finally, the unreacted precursors were removed by dialysis (MWCO 50,000) for 1 day.

2.3. Preparation of carboxylated graphene oxide

The suspension of carboxylated GO sheet was prepared by chemical treatment and ultrasonication, according to the reference [31]. To 10 ml of GO (0.1 w/v%) dispersion in DIW, chloroacetic acid (150 mg) and sodium hydroxide (200 mg) were added. The solution was stirred at 45 °C for overnight. Then, the solution was sonicated by an ultrasonic probe with 750 W, 30% intensity (Vibracell VCX 500, Sonics & Materials Inc., Newtown, CT, USA) for 2 h. The solution was neutralized by dialysis (dialysis bag MWCO 3500) against distilled water for a few days. After dialysis, the suspension was sonicated for 30 min and then filtered through a 0.8 μm cellulose acetated filter. The size of carboxylated GO was ~400 nm, analyzed by using an electrophoretic light-scattering spectrophotometer (632.8 nm, ELS-8000, Otsuka Electronics Co., Toyko, Japan).

2.4. Preparation of the sol state of injectable hydrogels

Two kinds of injectable state hydrogels were prepared by dissolving PF 127 itself, or Pluronic-based nanogel in GO solution at 4 °C for 48 h by using a rotary shaker after vortexing. The final concentration of PF 127 was 17 wt%, and that of nanogel was 11 wt%. Three different concentration of GO was used: 0.04, 0.08, and 0.1 wt%.