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DNA nanogels composed of chitosan and Pluronic with thermo-sensitive and photo-crosslinking properties

Jung Im Lee [a], Hye Sung Kim [a], Hyuk Sang Yoo [a,b,*]

[a] Department of Biomaterials Engineering, School of Bioscience and Bioengineering, Kangwon National University, Chuncheon 200-701, Republic of Korea
[b] Institute of Bioscience and Bioengineering, Kangwon National University, Republic of Korea

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ABSTRACT

Chitosan/Pluronic hydrogels were prepared to develop injectable depot systems for gene therapy to enhance local transgene expression at injection sites. Water-soluble chitosan and Pluronic were separately acrylated to prepare photo-crosslinkable polymers. A mixture of acrylated polymers was mixed with plasmid DNA and temperature was elevated to 37 °C to physically crosslink polymers to form hydrogels. Chitosan/Pluronic hydrogels were chemically crosslinked by photo-irradiated hydrogels at 37 °C. Mass erosion rates and release profiles of photo-crosslinked hydrogels were determined with varying photo-irradiation periods and chitosan contents of the hydrogels. The hydrogels with short photo-irradiation times degraded fast while high chitosan content in the hydrogels accelerated degradation rates. Release rates of plasmid DNA in the hydrogel were also controlled by changing chitosan content and photo-irradiation times. Released plasmid DNA was complexed with released Pluronic or chitosan and could be dissociated by adding sodium dodecyl sulfate. Scanning electron microscopy revealed that released plasmid DNA formed nanoparticles with released Pluronic or chitosan; released chitosan formed a condensed complex with plasmid DNA compared to released Pluronic. Transfection studies employing HEK293 cells showed that released fractions from chitosan/Pluronic hydrogels showed better transfection efficiency than those from Pluronic hydrogels. This result suggested that local transfection efficiencies of plasmid DNA in hydrogels were controlled by chitosan contents in chitosan/Pluronic hydrogels.

1. Introduction

Gene therapy employing non-viral carriers has received much attention because of such carriers' superior safety in comparison with viral counterparts (Park et al., 2006). While colloidal gene carriers have been widely employed for the purpose of systemic circulation, local treatments of gene carriers have been relatively less investigated due to lack of proper delivery systems. Several research projects have been done to develop efficient delivery carriers with the aim of localizing gene expression around injection sites (Ta et al., 2008; Wieland et al., 2007). Among those, hydrogels were employed for this purpose because of their superior injectability and biocompatibility. Hydrogels composed of triblock copolymers were most often employed to prepare thermo-sensitive hydrogels for controlled release of plasmid DNA (Jeong et al., 2002; Kim and Park, 2002). A triblock copolymer composed of poly (lactide-co-glycolide) [PLGA] was employed to express luciferase genes in a skin wound (Li et al., 2003). Researchers found that maximal expression of transgene was measured in the skin wound at 24 h and the expression level gradually dropped after 72 h. Cationized gelatin was employed to efficiently control release of negatively charged DNA from hydrogels (Fukunaka et al., 2002). In that study, release rates of DNA were dependent on cationized gelatin contents in the hydrogel because of ionic interactions between DNA and cationized polymers. In another study, Pluronic was employed to prepare photo-crosslinked Pluronic hydrogels for controlled release of plasmid DNA (Chun et al., 2005). After photo-irradiation, transfection efficiencies of plasmid DNA slightly decreased compared to unexposed DNA. Degradation of the hydrogel and release of plasmid DNA were dependent on photo-irradiation time.

Triblock copolymers exhibiting temperature-sensitive phase transitions, however, had several disadvantages including low mechanical strength and slow sol–gel transition time. In order to overcome the low mechanical strength of hydrogels composed of triblock copolymers, many researchers chemically associated physically crosslinked hydrogels (Chun et al., 2005; Quick and Anseth, 2004; Lee and Tae, 2007). One of the most often employed methods was to photo-irradiate polymeric hydrogels with photo-reactive groups such acrylate or azo-type moieties (Chun et al., 2005; Quick and Anseth, 2004; Lee and Tae, 2007; Fukuda et al., 2006).

* Corresponding author at: Kangwon National University, Department of Biomaterials Engineering, Chuncheon, Kangwon 200-701, Republic of Korea.
Tel.: +82 33 250 6563; fax: +82 33 253 6560.
E-mail address: hsyoo@kangwon.ac.kr (H.S. Yoo).

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Upon photo-irradiation, photo-reactive moieties in the polymer formed inter-molecular crosslinking with other proximal groups and the degree of crosslinking explosively increased. These types of photo-crosslinking were widely employed to strengthen mechanical properties of polymeric hydrogels (Wang et al., 2003). For example, azo moieties were added to prepared chitosan hydrogels for photo-irradiated crosslinking (Fukuda et al., 2006). The photo-crosslinkable chitosan hydrogel was employed for fabricating microarrays with biomimetic cellular microenvironments. Other studies employing acrylated Pluronic prepared hydrogel gel formulations and in vitro characterization of in situ gelable and photo-polymerizable Pluronic hydrogels suitable for injection (Lee and Tae, 2007; Yoon et al., 2007). In those studies, the method of controlling release rates of bioactive molecules from these hydrogels was to control photo-irradiation time with the aim of changing degradation rates and mechanical strength of photo-crosslinkable hydrogels. However, adjusting photo-reaction time was not sufficient to precisely control crosslinking reactions between acrylated polymers because the radical polymerization reaction by photo-irradiation is considered to be difficult to control (Chun et al., 2005; Stevens, 1999).

In this study, we prepared photo-crosslinkable chitosan/Pluronic hydrogels containing plasmid DNA. Various amounts of chitosan were added to Pluronic hydrogels to determine the effects of chitosan on mass erosion rates and release rates of encapsulated plasmid DNA. Released fractions were also analyzed by gel electrophoresis and electron microscopy to confirm complex formation of plasmid DNA with released chitosan. Finally, in vitro transfection efficiencies were measured to determine effect of chitosan on transfection of released DNA.

2. Materials and methods

2.1. Materials

Pluronic® F127 [$(PEO)_{100}$–$(PPO)_{65}$–$(PEO)_{100}$] was a gift from the BASF Corporation (Germany). Chitooligosaccharide (molecular weight range: 1–3 kDa) was purchased from Kitto Life (South Korea). Triethylamine was purchased from Sigma (St. Louis, MO). Acryloyl chloride and glycidyl methacrylate were purchased from Junsei Chemical (Japan). Irgacure® 2959 was a gift from Ciba Specialty Chemicals Corp. (Basel, Switzerland). Plasmid DNA (pEGFP-N1) encoding a red shifted variant of wild-type Green Fluorescent protein (GFP) was purchased from Clontech Laboratories Inc. (Palo Alto, CA). Human Embryonic Kidney cell (HEK293) was purchased from the Korea Cell Line Bank (Seoul, Korea). All other chemicals were of analytical grades.

2.2. Synthesis of di-acrylated Pluronic F127

Di-acrylated Pluronic was prepared by conjugating acryloyl chloride to terminal hydroxyl groups of Pluronic as described previously (Yoo, 2007). Briefly, dried Pluronic F127 (40 g) and triethylamine (4.43 ml) dissolved in 60 ml of dichloromethane were charged in a round-bottomed flask. Acryloyl chloride (2.57 ml) was added in a drop-wise method. The flask was sealed and gaseous nitrogen purging was flowed through the system. The reaction mixture was stirred at 4 °C for 12 h and then incubated at room temperature for another 12 h with gentle stirring. The reaction product was purified by precipitating the reaction mixture in an excess amount of ice-cold diethylether and dried under vacuum for 1 day after filtration. The extent of acrylation was 85.8%, which was determined by 400 MHz ^{1}H NMR in $CDCl_3$ at the Core Laboratory of Kangwon National University (DPX 400, Bruker).

2.3. Synthesis of glycidyl methacrylated chitooligosaccharide

Glycidyl methacrylated chitooligosaccharide (COS) was synthesized by conjugating glycidyl methacrylate to hydroxyl groups of COS. COS (5 g) dissolved in deionized water (30 ml) was poured into a round-bottom flask and glycidyl methacrylate (12 ml) was slowly added. The reaction mixture was stirred at 40 °C in a nitrogen atmosphere for 12 h. The final product was cooled in an ice bath for 15 min to terminate further reactions and subsequently precipitated in ice-cold acetone. After completely drying in vacuum at room temperature, the degree of acrylation was 57.2%, which was determined by 400 MHz ^{1}H NMR in D_2O.

2.4. Preparation of DNA-encapsulated chitosan/Pluronic hydrogel

Chitosan/Pluronic hydrogels loaded with plasmid DNA were fabricated by dissolving di-acrylated Pluronic F127 and glycidyl methacrylated COS at different weight ratios at polymer concentration of 25% (w/v), in 0.5 ml of deionized water containing 0, 1, 5 and 10% (w/w) methacrylated COS, respectively. A water-soluble photo-initiator, Irgacure 2959 (0.1%, w/w) was added to the reaction mixture. The sol-state solutions were homogeneously mixed at 4 °C for 12 h and placed in a silicon-coated 24-well plate with plasmid DNA. The solution in the silicon-coated 24-well plate was incubated at 37 °C for 3 h to prepare physically crosslinked gels. The physical gel was subsequently photo-crosslinked by exposure to long-wavelength UV light (365 nm) for 3, 5 and 8 min under a nitrogen atmosphere (Omnicure® 1000, EXFO, Canada). The distance between the hydrogel and the light source was 2.5 cm.

2.5. Mass erosion and in vitro release of plasmid DNA of chitosan/Pluronic hydrogels

Hydrogels containing plasmid DNA (25 μg/device) were incubated in a 50-ml conical tube with 5 ml of phosphate buffered saline (PBS, pH 7.4) at 37 °C. At pre-determined times of 1, 3, 5, 7, 14 and 21 days, degraded hydrogels were freeze-dried and weighed. Mass loss percentage = (degraded polymer weight)/(original polymer weight). Released DNA in the release medium and the working reagent of PicoGreen® assay kit (1:1, v/v) were thoroughly mixed and incubated at room temperature for 5 min. Fluorescence of the sample was measured at an excitation wavelength of 488 nm and an emission wavelength of 530 nm (Molecular Probes, Eugene, OR).

2.6. Agarose gel electrophoresis

Released fractions at 21 days were analyzed by agarose gel electrophoresis (0.8%, w/v). Electrophoresis was carried out in a TBE buffer solution (89 mM Tris base, 89 mM Boric acid, and 2 mM EDTA) with a current of 100 V for 20 min. In order to dissociate ionic complexes composed of released chitosan and released DNA in the release buffer, the release medium was treated with 2% (w/v) sodium dodecyl sulfate (SDS) and further incubated at 37 °C for 2 h before agarose gel electrophoresis.

2.7. Field emission scanning electronic microscopy (FE-SEM)

Chitosan/Pluronic hydrogels with a chitosan content of 0 and 10% (w/w) were dried at 37 °C for 2 h under vacuum. After drying, the samples were mounted on metal stubs using a carbon adhesive tab and vacuum-coated with a gold layer prior to FE-SEM examination (Hitachi, S-4300, Japan).