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

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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).

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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).

2.8. In vitro transfection

HEK293 cells at a density of 2×10^5 cells/well were plated in six-well plates in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and streptomycin/penicillin. After 24 h, cell culture medium was exchanged for DMEM supplemented with 1% FBS prior to addition of released fractions at 21 days and further incubated for 3 h with released fractions. We employed 1% FBS in the transfection medium to minimize serum effects of destabilizing chitosan–DNA complex (Kiang et al., 2004; Bowman and Leong, 2006). The cultivated cells were fed again with fresh culture medium containing 10% (v/v) FBS and incubated for another 45 h. Expression levels of GFP were spectrofluorometrically measured at an excitation wavelength of 488 nm and an emission wavelength of 507 nm (PerkinElmer, LS-55B, Boston, MA). Total protein concentrations were determined by a micro-BCA protein assay kit (Pierce, Iselin, NJ). Transfection efficiencies were calculated by dividing fluorescence intensities both by the amount of total proteins and the amount of plasmid DNA in each well. All transfection experiments were performed three times.

2.9. Statistical analysis

All results were expressed in means ± standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by a Shaffer's post hoc test for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results and discussion

Fig. 1 shows a schematic diagram of the preparation of chitosan/Pluronic hydrogels containing plasmid DNA. A mixture of acrylated chitooligosaccharide, di-acrylated Pluronic, and plasmid DNA formed a physical hydrogel above LCST. In order to prepare an interconnected network of Pluronic and chitosan, the hydrogels in gel state were chemically crosslinked by photo-irradiation (Yoo, 2007). We previously showed that release rates of human growth hormone (hGH) with an isoelectric point of 5 were controllable by modulating photo-irradiation time (Yoo, 2007). This was attributed to the fact that negatively charged hGH at pH 7.0 formed an ionic complex with chitosan/Pluronic hydrogel because chitosan is positively charged at neutral pH (Yoo, 2007; Chung et al., 2005). Furthermore, preparation of acidic solutions for dissolving chitosan could be avoided because a water-soluble chitosan, chitooligosaccharide, was employed to prepare hydrogels. Therefore, we speculated that negatively charged DNA could be released in a controlled release manner because chitosan was chemically attached to the hydrogel. However, it should be mentioned that 100% Pluronic hydrogel cannot be able to control release of negatively charged DNA.

Degradation rates of hydrogels were measured with changing chitosan content and photo-irradiation times as shown in Fig. 2. All samples showed decreased mass erosion rates with increasing photo-irradiation times from 3 min to 8 min. This could be attributed to a high degree of intermolecular crosslinking between acrylated chitosan and acrylated Pluronic. Increased intermolecular networks significantly increased molecular weights of whole polymers composing hydrogels and thus decreased in vivo degradation rates of hydrogels. It should be noticed that increasing chitosan content in hydrogel increased degradation rates at 3 min and 5 min. Chitosan molecules in Pluronic solutions interfered with hydrophobic interaction among Pluronic chains, thus keeping the hydrogel from forming hydrogel above LCST. Hydrogels containing chitosan consequently showed inferior mechanical strength compared to those without chitosan. However, because these polymers were photo-crosslinkable, increasing photo-irradiation increased intermolecular networks and reversed the weak mechanical strength shown in chitosan/Pluronic hydrogels with low photo-irradiation time. Therefore, at photo-irradiation times of 5 min and 8 min, a hydrogel with 1% chitosan (1/99) showed a slower degradation rate compared to a 100% Pluronic hydrogel (0/100). However, it is of interest that degradation rates of a hydrogel with 5%

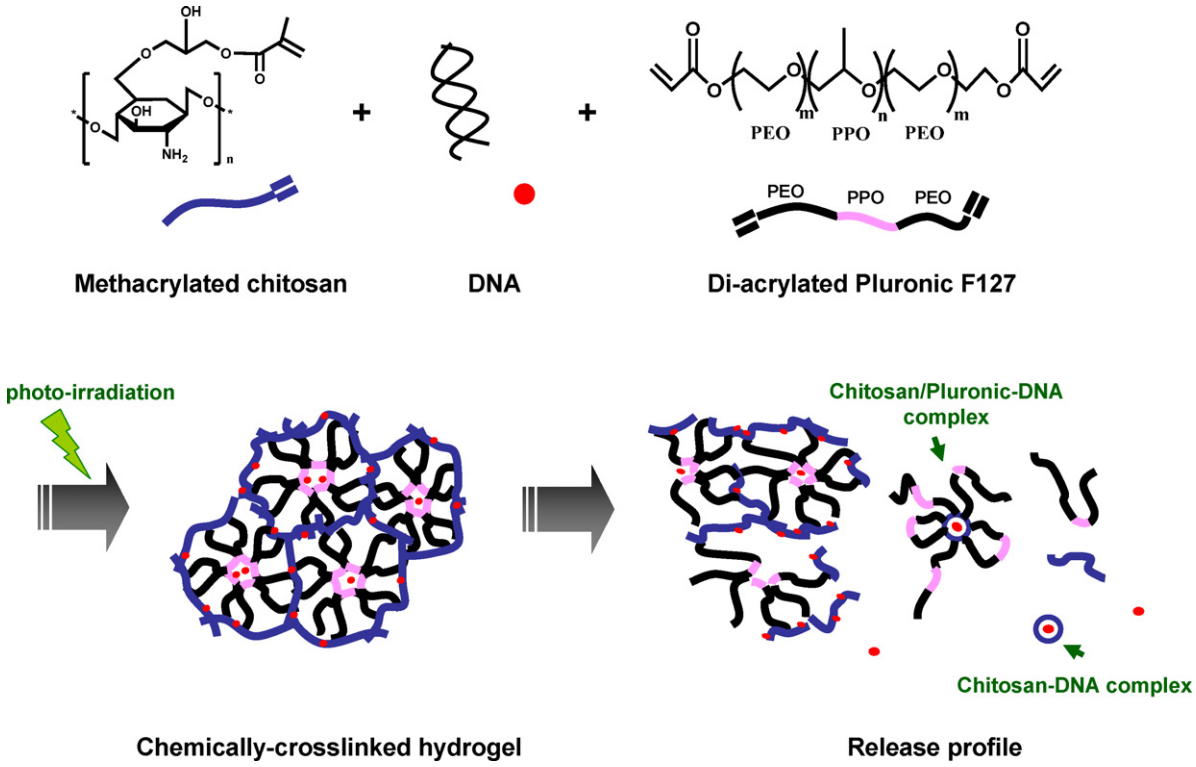

Fig. 1. Schematic presentation of preparing photo-crosslinkable chitosan/Pluronic hydrogels for controlled release of plasmid DNA.

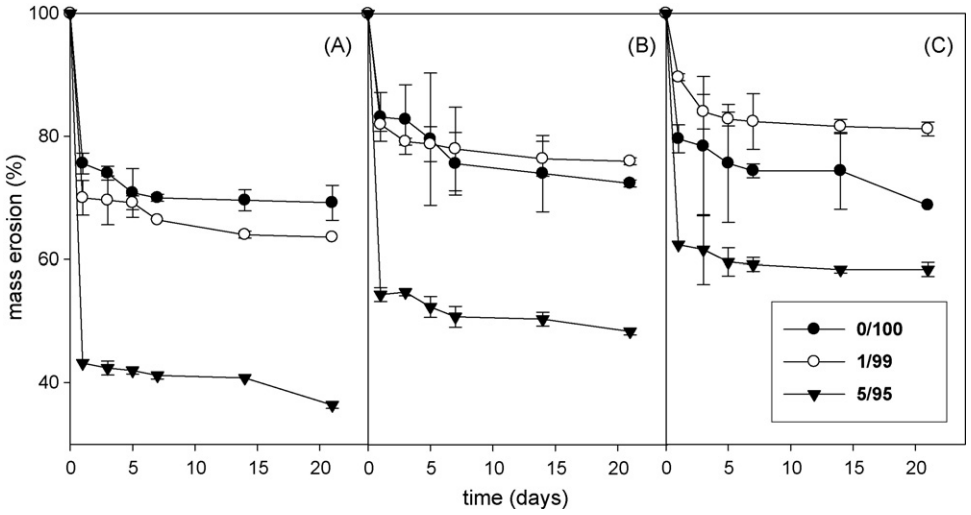

Fig. 2. Mass erosion rates of photo-crosslinked chitosan/Pluronic hydrogels with photo-crosslinking time of 3 min (A), 5 min (B), and 8 min (C) at 37 °C. Blend ratios of chitosan in the chitosan/Pluronic hydrogels were 0%, 1%, and 5% (0/100, 1/99, and 5/99).

chitosan (5/95) rapidly decreased compared to those of other polymers when photo-irradiation time increased while other samples showed a slow decrease of degradation rates. This result clearly showed that acrylated chitosan acted as a crosslinking center to tether acrylated Pluronic chains in hydrogels because one chitosan had multiple acrylate groups in one molecule. This significantly increased the degree of intermolecular networking in chitosan/Pluronic hydrogels. Therefore, degradation rates could be more precisely controlled by modulating chitosan contents and photo-irradiation time.

Fig. 3 shows release profiles of plasmid DNA from chitosan/Pluronic hydrogels with different photo-irradiation times and chitosan content. Long exposure to a light source significantly attenuated DNA release from hydrogels while chitosan in hydrogels reversed this effect. This could be explained by the degradation rates of chitosan/Pluronic hydrogels as shown in Fig. 2. High degrees of crosslinking prohibited plasmid DNA from diffusing out from chitosan/Pluronic because plasmid DNA should be released out by a simple diffusion. At 5 min of photo-irradiation time, a hydrogel with 1% chitosan showed an attenuated release profile of plasmid DNA compared to that with 100% Pluronic. It was of interest that the released amount of plasmid from 100% Pluronic hydrogels and chitosan/Pluronic hydrogels were switched after 5 min of photo-irradiation time. In fact, the released amounts from 100% Pluronic at 14 days and 21 days were approximately twice as much as those from 1% chitosan/Pluronic hydrogels. This result coincides with degradation profiles of chitosan/Pluronic hydrogels as shown in Fig. 2B, where degradability of 1% chitosan hydrogel at 5 min decreased compared to that at 3 min. Thus, release of plasmid DNA from chitosan/Pluronic was controlled by degradability of the hydrogels. However, it should also be noticed that amounts of released DNA were relatively small in all cases (<15%). This could be attributed to the fact that the hydrogel composed of 100% Pluronic showed the lowest degradation rate. In the absence of chitosan, hydrophobic interactions between PPO blocks become stronger and thus more strong association between Pluronic chains occurs. Therefore, irrespective of photo-crosslinking intensities, degradation rates of 100% Pluronic were almost constant (Fig. 2). This strong physical association at the 37 °C subsequently increased mechanical strength of 100% Pluronic hydrogels, compared to chitosan/Pluronic hydrogels. Thus, the encapsulated DNA within the hydrogel could not be easily released out although moderate degradation of Pluronic chains occurred.

Furthermore, chitosan moieties in chitosan/Pluronic hydrogels were strongly associated with negatively charged DNA molecules

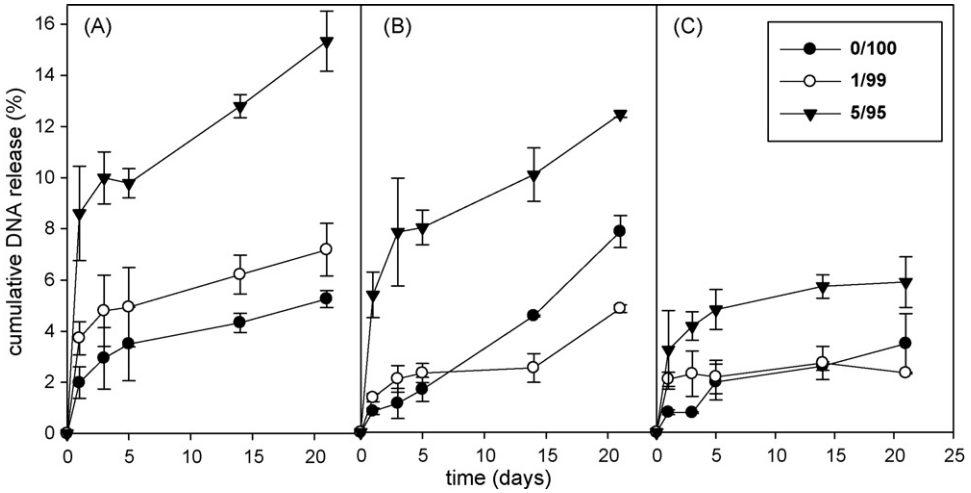

Fig. 3. Release profiles of plasmid DNA from photo-crosslinked chitosan/Pluronic hydrogels prepared with photo-crosslinking time of 3 min (A), 5 min (B) and 8 min (C) at 37 °C. Blend ratios of chitosan in the chitosan/Pluronic hydrogels were 0%, 1%, and 5% (0/100, 1/99, and 5/99).

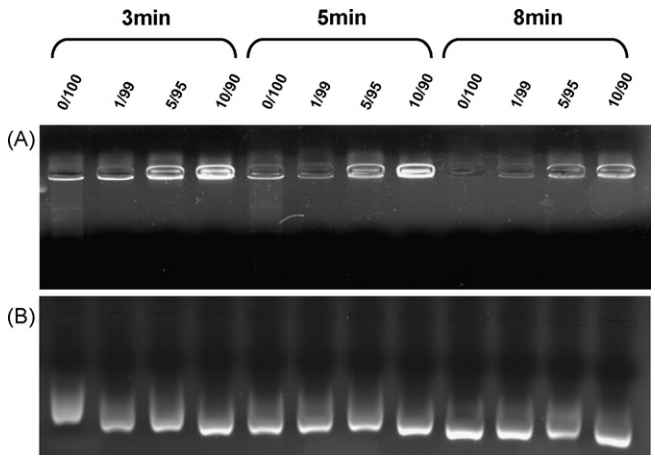

Fig. 4. Gel electrophoresis of released fractions from the photo-crosslinked chitosan/Pluronic hydrogels at 21 day (A) and released fractions at 21 days treated with 2% SDS (B). Chitosan contents in the hydrogels were 0, 1, 5, and 10% (0/100, 1/99, 5/95, and 10/90).

and release of the plasmid DNA was further attenuated compared to hydrogels composed of 100% Pluronic. However, a chitosan/Pluronic hydrogel with 10% chitosan showed a fast release of plasmid DNA because the degradation rate of the hydrogel was much faster than that of other hydrogels because of the decreased mechanical strength as shown in Fig. 2C.

Released fractions from chitosan/Pluronic hydrogels were analyzed by agarose gel electrophoresis to confirm complex formation between released polymers (chitosan and Pluronic oligomers) and DNA as shown in Fig. 4. In Fig. 4A, no prominent bands of plasmid DNA were observed while most DNA was found in loading wells. Negatively charged DNA normally moves downwards and is separated according to molecular weight. However, DNA lost its mobility because of the complex formation with released chitosan/Pluronic. In 100% Pluronic hydrogel, released Pluronic encapsulated plasmid DNA and formed micellar structures. Therefore, negative charges were shielded by micelles and not exposed to surfaces, suggesting that plasmid DNA lost its mobility. In the cases of chitosan/Pluronic hydrogels, complex formation became stronger because chitosan electrostatically interacted with plasmid DNA. Upon increasing chitosan content from 1% to 10%, the amount of plasmid DNA measured in each well also increased. However, as shown in Fig. 4B, the complex was disrupted by SDS, a strong ionic surfactant. Massive amounts of SDS replaced chitosan/Pluronic oligomers of the complex and strongly bound to chitosan/Pluronic oligomers because SDS competed with negatively charged DNA. SDS-bound DNA showed a strong negative charge and recovered electric mobility because of the negatively charged SDS. Therefore, strong bands of plasmid DNA re-appeared,

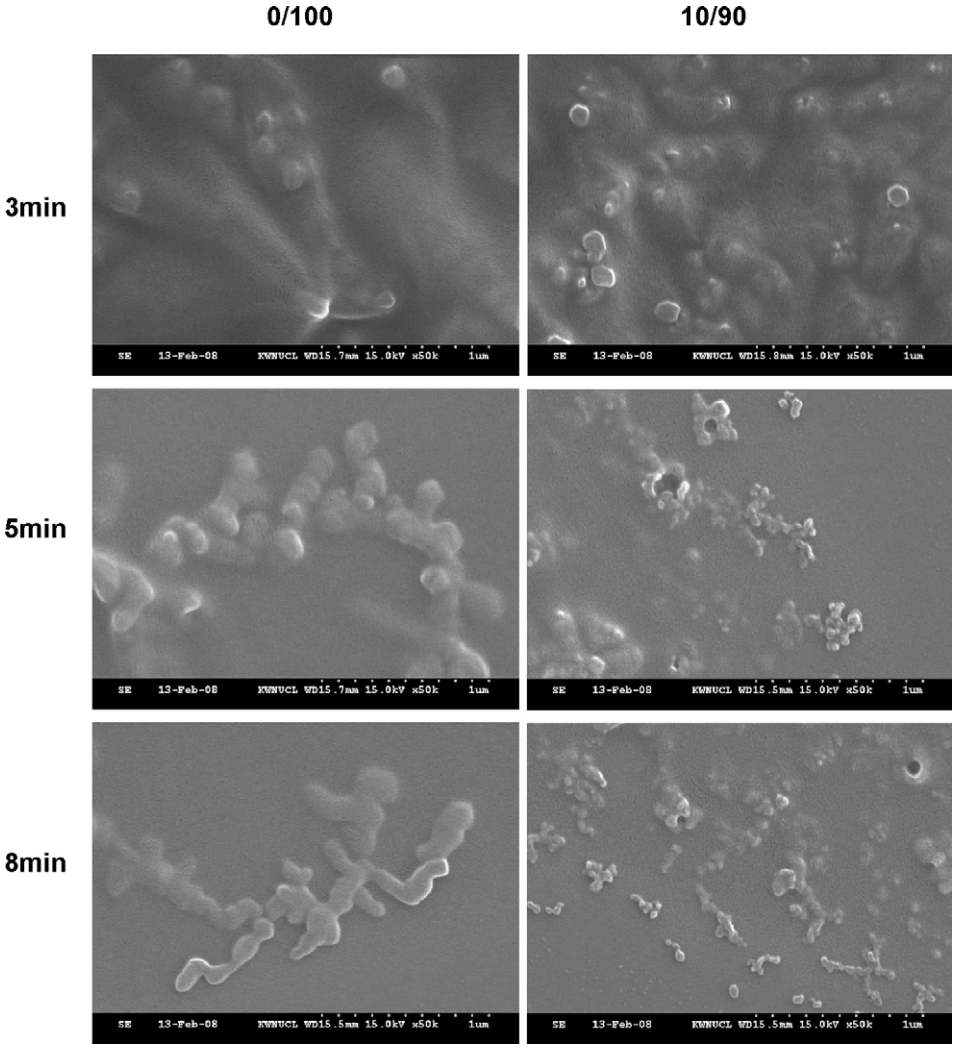

Fig. 5. Scanning electron microscopy of released fractions at 21 days from photo-crosslinked chitosan/Pluronic hydrogels with different photo-irradiation time (3, 5, and 8 min). Chitosan contents in the chitosan/Pluronic hydrogel were 0% and 10% (w/w).

as shown in Fig. 4B. In many studies, chitosan was widely employed as a gene delivery vehicle because positively charged chitosan efficiently condensed plasmid DNA to form nanoparticles. These particles were easily endocytosed by cells and transfection efficiencies were further enhanced. In this study, released fractions containing chitosan/Pluronic oligomers efficiently condensed plasmid DNA and were expected to increase transfection efficiency when hydrogels was incubated to release encapsulated DNA. It should be noticed that amount of plasmid DNA in the loading well increased when photo-irradiation time increased from 3 min to 8 min. This could be attributed to the fact that the released amount of DNA from each hydrogel was dependent on photo-irradiation time as shown in Fig. 3. In addition, the relatively small amounts of released DNA in Fig. 3 could be attributed to complex formation between chitosan/Pluronic oligomers and DNA. Because PicoGreen® dye was employed to measure released amounts of DNA, it should be intercalated into nucleotides to be capable of fluorescence. However, complexed DNA could not be easily detected because intercalation process was hindered by complex chitosan/Pluronic oligomers. Because charge ratios of DNA to chitosan/Pluronic oligomers were very high (>20), strong ionic interactions between two molecules prevent PicoGreen® dye from being incorporating into DNA. Therefore, actual amount of released DNA would be much higher than apparent values detected by a spectrophotometer. Alternatively, radio-labeling of nucleic acid will more accurately measure released amounts of DNA from the hydrogel because radioactivity is not affected by structural changes.

In order to confirm the formation of a nano-particulate complex between released DNA and released chitosan/Pluronic polymers, morphology of released fractions was examined by scanning electron microscopy (Fig. 5). Regardless of chitosan content in hydrogels, a particulate form of DNA was found in all samples; it was not a linear form, suggesting that released Pluronic and chitosan formed a complex with released DNA. In the case of 100% Pluronic hydrogel (0/100), Pluronic micelle formation did not cause efficient encapsulation and condensation of released DNA within the micelle. However, condensation of released DNA became stronger at a chitosan content of 10% (10/90). Compared to hydrogel 100% Pluronic, a hydrogel with 10% chitosan showed a smaller size and a condensed structure of particles. In fact, in the case of a hydrogel with 100% Pluronic (0/100), particle diameters ranged from *ca.* 160 nm to *ca.* 250 nm while those of 10% chitosan hydrogel (10/90) ranged from *ca.* 80 nm to *ca.* 170 nm. This result could be attributed to the condensation of released DNA by released chitosan/Pluronic oligomers. Released DNA with the released oligomers lost negative-charge on its phosphate backbone and collapsed into particulate form with the oligomers because of reduced electrostatic repulsion. Therefore, more condensed particles were formed in released fractions from 10% chitosan hydrogel (10/90) compared to 100% Pluronic hydrogel (0/100).

Transfection efficiencies of released fraction from chitosan/Pluronic hydrogels were measured as shown in Fig. 6. As photo-irradiation time increased from 3 min to 8 min, transfection efficiencies were slowly decreased twofold in the case of released fractions from 100% Pluronic hydrogels. Long exposure to UV light has been thought to damage nucleic acid by deletion or pyrimidine dimerization as shown in other studies (Lang, 1975; Lyamichev et al., 1990). Thus, photo-irradiated DNA unavoidably suffered from inactivation of DNA. Because deleted or dimerized DNA was not normally translated into active proteins, diminished fluorescent intensity of GFP was observed in released fractions with longer photo-irradiation time. However, at the same photo-irradiation time, transfection efficiencies increased as chitosan content increased. Increased transfection efficiencies

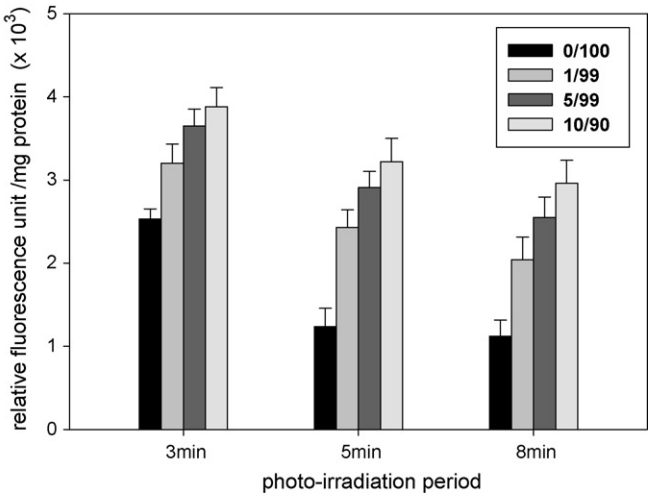

Fig. 6. Transfection efficiencies of released fractions (at 21 days) from chitosan/Pluronic hydrogels with different photo-irradiation time (3, 5, and 8 min) and chitosan contents (0, 1, 5, and 10%). Relative fluorescence intensities were normalized with respective to 1 μg of plasmid DNA based in Fig. 3.

could be attributed to the high charge ratio of released chitosan oligomers to released DNA. In other studies employing chitosan as a gene delivery carrier, charge ratios of 8 or above showed the best transfection efficiencies when unmodified chitosan was employed (Lavertu et al., 2006). In the current study, charge ratios of chitosan to DNA in 1/99, 5/95, and 10/90 chitosan/Pluronic hydrogels were 82, 411, and 822, respectively. These values seem to be very high compared to those of other studies employing unmodified chitosan. However, it should be taken into consideration that the chitosan in chitosan/Pluronic hydrogels was crosslinked to Pluronic molecules. Pluronic-attached chitosan was inferior to unmodified chitosan in terms of binding to DNA because of steric hindrance. Thus, at the same charge ratio, released chitosan was less accessible to released DNA compared to native chitosan and much higher charge ratios were required to efficiently condense DNA. Furthermore, it should also be noticed that only a small fraction of chitosan from chitosan/Pluronic hydrogels was released into the release medium and subsequently formed a complex with released DNA. Therefore, a large amount of chitosan should be added to chitosan/Pluronic hydrogels to efficiently condense released DNA for high transfection efficiencies. In addition, it should be mentioned that transfection efficiencies were normalized with respect to one microgram of released DNA, which was measured in Fig. 3. Thus, all transfection efficiencies were expressed in the same amount of released DNA. It should be of noticeable that DNA encapsulated within the chitosan/DNA complex did not show a decrease of transfection efficiency because the complexed DNA was less exposed to UV damage compared to naked DNA. Further in vivo study should be performed to confirm transfection efficiency of the hydrogel because in vitro and in vivo transfection results may significantly differ.

4. Conclusion

Photo-crosslinkable hydrogels composed of chitosan and Pluronic were fabricated for enhanced local delivery of transgenes. Complex formation of released DNA and released medium was observed by agarose gel electrophoresis and scanning electron microscopy. Enhanced transfection efficiencies were observed when released fractions from chitosan/Pluronic hydrogels were employed for in vitro studies.

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