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Efficient gene delivery by oligochitosan conjugated serum albumin: Facile synthesis, polyplex stability, and transfection

Monika Kumari[a], Chi-Hsien Liu[b,c,d,e,*], Wei-Chi Wu[e,f]

[a] Department and Graduate Institute of Chemical and Materials Engineering, Chang Gung University, 259, Wen-Hwa First Road, Kwei-Shan, Tao-Yuan 333, Taiwan
[b] Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University, 259, Wen-Hwa First Road, Kwei-Shan, Tao-Yuan 333, Taiwan
[c] Research Center for Food and Cosmetic Safety, College of Human Ecology, Chang Gung University of Science and Technology, 261,Wen-Hwa First Road, Taoyuan, Taiwan
[d] Department of Chemical Engineering, Ming Chi University of Technology, 84, Gung-Juan Road, New Taipei City, Taiwan
[e] Department of Ophthalmology, Chang Gung Memorial Hospital, Linkou, 5, Fu-Hsing Street, Taoyuan, Taiwan
[f] College of Medicine, Chang Gung University, 259,Wen-Hwa First Road, Taoyuan, Taiwan

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ABSTRACT

Chitosan and its derivatives have shown to be potential gene carriers with biocompatiblility and safety. However, their practical delivery is far from being ideal because of the low transfection efficiency. The present work describes the potential of a natural protein, bovine serum albumin (BSA), conjugated with a natural oligosaccharide, oligochitosan (OC), as a considerable promising approach for a safe and efficient non-viral gene delivery vector. The FTIR spectra proved the effective conjugation of BSA with OC through covalent bond. The condensation ability of plasmid DNA (pDNA) with a BSA-OC biopolymer was analyzed by gel retardation assay, competition binding assay, and dynamic light scattering used to measure the nanoparticle size. In addition, the BSA-OC biopolymer showed the protection of pDNA from enzymatic degradation by DNase I and showed good stability when exposed to 50% fetal bovine serum. The transfection efficiency was evaluated in the presence of 10% serum-supplemented media or serum-free media on three kinds of mammalian cells. Our results showed that the BSA-OC biopolymer is a good non-viral vehicle for gene delivery. We investigated the parameters such as the pDNA payload, temperature, incubating duration, and biopolymer/pDNA ratio on the transfection efficiency. This hybrid vehicle had the ability to transfect 90% of cells and to maintain 80% of cell viability. The aforementioned results suggest that the facile synthesis of the BSA-OC biopolymer could overcome the cytotoxicity problem and transfection barriers during *in vitro* gene delivery.

1. Introduction

Gene delivery implies the transfer of the exogenous gene into host cells involving the production of desired protein (Corsi, Chellat, Yahia, & Fernandes, 2003). However, the delivery of naked DNA is not suitable for *in vivo* application because there is a chance of DNA degradation by serum nucleases. The negative charge of DNA leads to electrostatic repulsion with the cell membrane. This phenomenon could not allow naked DNA to enter the host cells. To remediate this problem, a delivery vehicle is necessary that can modulate the DNA complex charge, protect DNA from degradation by serum nucleases, and maintain high stability in the blood stream (Dang et al., 2011). In recent years, the focus on non-viral gene delivery vectors has emerged with several advantages over viral vectors, like being easy to conjugate, as well as their economical production, low toxicity, and insignificant immunogenicity (Taranejoo, Chandrasekaran, Cheng, & Hourigan, 2016; Yan, Du, Chen, You, Yuan, & Hu, 2013). The development of a non-viral vector having low cytotoxicity at a high concentration and high gene transfer ability remains a major challenge. Among non-viral vector systems, cationic polymers offer several advantages, such as being easy to control and their versatile modification procedure. Polyethylenimine (PEI) and poly-L-lysine (PLL) is an effective cationic polymer used for gene delivery systems because of its high buffering capacity (Huang, Li, Chen, & Li, 2017; Kim, Ihm, Choi, Nah, & Cho, 2003). However, its major disadvantage is that the large molecular weight and high concentration of PEI showed more toxicity toward cells. The key factor of high cytotoxicity is due to the high positively charged density of polymer interacting with negative cell membranes, which damages the cell membranes (Thomas & Klibanov, 2003).

Chitosan is a natural biodegradable cationic polysaccharide

* Corresponding author at: Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University, 259, Wen-Hwa First Road, Kwei-Shan, Tao-Yuan 333, Taiwan.
E-mail address: CHL@mail.cgu.edu.tw (C.-H. Liu).

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composed of two subunits, D-glucosamine and N-acetyl-D-glucosamine units linked together through β (1,4) glycosidic bonds (Bae, Lee, Lee, Han, Ko, & Choi, 2016; MacLaughlin et al., 1998). The glucosamine group of chitosan shows a strong electrostatic interaction with DNA and thus reduced transfection efficiency because of its slow endosomal escape ability (Kim, Ryu, & Kim, 2014). However, the major limitation of chitosan is its low water solubility and fair transfection efficiency compared to highly charged density cationic polymer (Jiang et al., 2007; Sajomsang et al., 2014). Chitosan has been modified to obtain various cationic polymers that can be used as a gene delivery vector. While there are numerous gene delivery publications using water-soluble chitosan (molecular weight (mw) < 10 k) (Duceppe & Tabrizian, 2009; Huang, Fong, Khor, & Lim, 2005), the ultra-low mw oligochitosan (OC, MW 0.3 ~ 1.3k) has not yet been studied as a partner of delivery vectors. In fact, the chitosan with the 3 ~ 7 k molecular weight only has fair transfection efficiency since the weak interaction and the quick release of DNA from the complexes (Huang, Fong, Khor, & Lim, 2005; Koping-Hoggard et al., 2004). Moreover, the transfection efficiency of the chitosan-based polyplexes are influenced by the following parameters such as the degree of deacetylation, pH, types of transfected cells, and modification processes(Andrey, Stanislav, & Yury, 2017). Many researchers have enhanced the transfection efficiency by modifying chitosan-based materials (Lin & Hsu, 2015). The applications of modified chitosans regarding specificity to target cell and efficient gene expression have been recently reviewed (Saranya, Moorthi, Saravanan, Devi, & Selvamurugan, 2011). Chae et al. (2005) have shown that the modification of chitosan oligosaccharide with deoxycholic acid could improve the DNA condensation ability and enhance the transfection efficiency. However, the transfection efficiency was not very high for this system in HEK 293 cells (Chae, Son, Lee, Jang, & Nah, 2005). The addition of anionic polymers, such as hyaluronic acid with chitosan, enhances the transfection efficiency of chitosan compared with only the chitosan/DNA complex (Duceppe & Tabrizian, 2009). Others have shown that the conjugation of amino acids, such as histidine, with chitosan influence the transfection efficiency by enhancing its buffering capacity, which improves the endosomal escape ability of chitosan (Chang, Higuchi, Kawakami, Yamashita, & Hashida, 2010).

To obtained high transfection efficiency and good cell viability, some authors have suggested bioconjugation since it is a simple, less time-consuming process and offers an interesting way to design a safe and effective gene delivery vector (Ewe, Przybylski, Burkhardt, Janke, Appelhans, & Aigner, 2016). Natural protein conjugated with a cationic polymer could obtain high transfection efficiency and a low cell toxicity profile. The major advantages of using biomaterials in gene delivery systems are their low cytotoxicity, biocompatible, and biodegradable properties. On the other hand, it offers higher stability *in vivo* condition (Syga, Nicolì, Kohler, & Shastri, 2016). Considering the above mentioned findings, we designed a protein-based gene delivery system. In this study, we chose bovine serum albumin (BSA) as a serum protein to conjugate with oligochitosan (OC) to achieve high transfection efficiency by enhancing the endosome escape ability of OC and a low cytotoxicity gene delivery vector. Albumin is a natural abundant protein that is, widely used for gene or drug delivery systems. In fact, albumins are easily soluble in water, stable over a wide range of pH and temperature conditions and easy to control in chemical reactions. Gene delivery vectors incorporating serum albumin protect DNA from serum nuclease and avoid unfavorable reactions with serum in *in vivo* microenvironment conditions (Simões et al., 2000; Zhang et al., 2015). BSA is a versatile delivery carrier, has beneficial properties for modification such as water-solubility and amine group bearing (Karimi, Avci, Mobasseri, Hamblin, & Naderi-Manesh, 2013). However, BSA itself has very limited transfection ability. To address these issues, we hypothesize that the conjugation of BSA with OC could produce a novel transfection agent with slow DNA release and high transfection capability. The reasons for the expected high transfection efficiency could be summarized as follows. OC provides the cationic charge density for the plasmid interaction, while the presence of BSA will provide stability to the hybrid carrier and act as the shuttle through the cytoplasmic membrane. We exploit the unique modification of BSA, namely the tailor of BSA using cationic oligochitosan (average 4-mer) to achieve the delivering ability of plasmid DNA.

To the best of our knowledge, we report for the first time a BSA-OC biopolymer developed through a chemical reaction using glutaraldehyde as a crosslinking agent to achieve high transfection efficiency and low cytotoxicity as a gene delivery system. BSA possesses several amino groups on its surface for chemical modifications. This functional group can allow the conjugation of the amino group of BSA with the amino group of OC using a dialdehyde crosslinker. In addition, to understand the interaction of BSA with OC, the secondary structure has been studied by using FTIR. In this study, a BSA-OC biopolymer was used as a gene delivery vector and showed high buffering capacity, which may improve the transfection efficiency. Furthermore, to study the BSA-OC biopolymer's potential, the transfection efficiency was evaluated in three different cell lines: CHO-K1, HaCat, and HEK293T cells.

2. Materials and methods

2.1. Materials

The oligochitosan (Cat. No. COS-YS, Lot No. 0404011) obtained from Yaizu Suisan Kagaku Industry is directly used without further purification. The oligochitosan is derived from crab shells by the enzymatic method and is composed by 9.8% (2-mer), 23.8% (3-mer), 27.9% (4-mer), 23.9% (5-mer), 9.9% (6-mer), and 4.7% (7-mer). This water soluble oligochitosan has an average molecular weight of 662 g/mol, 100% yield of glucosamine and 99% degree of deacetylation. Bovine serum albumin (BSA), Hoechst 33342, ninhydrin, trypsin-EDTA, glutaraldehyde, ethidium bromide (EtBr) and potassium bromide (KBr) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Plasmid EGFP-C3 (size 4.7 kb) is obtained from Takara Bio (Shiga, Japan). The plasmid was amplified in *Escherichia coli* DH-5 alpha and purified using a purification kit (GeneMark, Taipei, Taiwan). DNase I was obtained from the Worthington Co. (Lakewood, NJ, USA). Fetal bovine serum was purchased from Biological Industries (Haemek, Israel). The BCA Protein Assay Kit was purchased from Thermo Scientific (Rockford, IL, USA). All reagents were used without further purification.

2.2. Synthesis and characterization of BSA-OC biopolymer

BSA and OC were conjugated through one step process using glutaraldehyde as a crosslinker. For an effective conjugation reaction, we optimized the molar ratio of BSA and OC to control the extent of crosslinking. At the BSA/OC molar ratio of 1:10 (mass ratio of BSA/OC = 2 mg/0.2 mg,), OC molecules had more chances to conjugate with BSA molecules by inducing the excess amount of OC. BSA can avoid the self-conjugation at this ratio. Briefly, 2 mg BSA was dissolved in 500 μL distilled water, separately 0.2 mg OC was added to 450 μL distilled water. Finally, both solutions were mixed together while adding 50 μL of 25% glutaraldehyde (final volume was 1 mL). The conjugation reaction was allowed to take place at room temperature for 2 h with constant vortexing (Genie2, Scientific Industries). To quench the free aldehyde, 0.1 mg/mL glycine was added to the resultant product (BSA-OC) and vortex for 30 min. The final product was washed two times using 50 kDa cutoff Vivaspin tube by centrifugation with deionized water to remove free OC and glutaraldehyde. However, there is also the possibility of BSA–BSA and OC-OC crosslinking through glutaraldehyde crosslinker. To confirm this hypothesis, we separately conjugated BSA–BSA and OC-OC using glutaraldehyde crosslinker. The BSA containing fractions were determined by a bicinchoninic acid (BCA) test (BCA assay kit, Thermo scientific) and OC containing fractions were analyzed by ninhydrin test. The UV–vis absorbance of pure