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Nanomicelles of Fatty Acid Grafted Chitosan Polymer for Drug and Gene Delivery

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Abbreviations

CS: Chitosan (50 kDa); pDNA: Plasmid Deoxyribonucleic Acid; mRNA: Messenger Ribonucleic Acid; siRNA: Small Interfering Ribonucleic Acid; miRNA: Micro Ribonucleic Acid; EPR: Enhanced Permeability and Retention; CMC: Critical Micelle Concentration.

Short Communication

Chitosan is a naturally occurring cationic copolymer of randomly distributed D-glucosamine and N-acetyl-D-glucosamine units. It is derived from partial deacetylation of a linear chain polysaccharide chitin, a characteristic component found in the exoskeleton of crustaceans (shrimps, crabs, lobsters, etc.) and insects. Chitosan based delivery systems are biocompatible, biodegradable and can be chemically modified for targeting as well as for enhanced permeability through membranes. Due to its innate characteristics chitosan based systems show low immunogenicity and a good safety profile which translate into a widely applicable drug carrier [1]. Additionally, chitosan can form dissociable complexes with exogenous nucleic acids (pDNA, mRNA, siRNA, miRNA, antisense oligonucleotides, etc.), proteins, and several drugs owing to hydrophobic, hydrogen-bonding, and electrostatic interactions [2]. Such interactions serve to protect the therapeutic agent from enzymatic degradation as well as other physiological factors owing to enhanced stability in the complex form [3-5].

Polymeric micelles form self-assembled structures in aqueous environment containing a hydrophobic core with the ability to encapsulate poorly water soluble therapeutics and a hydrophilic shell imparting stability to the micellar structure [6]. Fatty acid substitution onto chitosan aids in the formation of amphiphilic chitosan based polymers capable of forming nanomicelles in aqueous environment. It has been well established that such nanomicelles show enhanced permeability and retention effect (EPR effect) which is a desired property for most drug delivery strategies [2,7-9]. Fatty acid modification of chitosan enables formation of nanomicelles at relatively low concentration which demonstrate enhanced permeability through the phospholipid cell membrane, allow efficient release of nucleic acid, and result in increased transfection efficiency [10]. According to multiple investigations it has been found that 18 carbon chain fatty acid derivatives result in higher transfection efficiency compared to shorter and longer carbon chain fatty acids. Additionally, with an increase in the degree of unsaturation in the fatty acids it has been shown that channel formation occurs in the phospholipid bilayer membranes increasing the transport of macromolecules across the membrane barrier [11,12].

Fatty acid grafting onto chitosan backbone allows for the synthesis of amphiphilic cationic polymers. Chitosan modified with fatty acids can be synthesized by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC. HCl) and N-hydroxysuccinimide (NHS) mediated coupling reaction where EDC. HCl and NHS sequentially react with the carboxylic group of fatty acid to produce a semi-stable NHS-ester, which in turn reacts with the amine group of chitosan via the formation of an amide bond to form fatty acid grafted chitosan polymer. The water soluble by products are removed by dialyzing against water. The unreacted fatty acid molecules can be removed by purifying the dialyzed and lyophilized product using ethanol. The precipitate obtained by filtering the suspension in ethanol through a 0.2 μm nylon filter paper can be vacuum dried to get the purified fatty acid grafted chitosan polymer ready for further characterization [13]. Linoleic and oleic acid grafted chitosan polymers were prepared in this manner. The substitution of fatty acid on chitosan was confirmed using proton nuclear magnetic resonance (^{1}H NMR) (Figure 1) and Fourier transform infrared spectroscopy (FT-IR) (Figure 2). The new peaks in the ^{1}H NMR spectra at 1.1 and 2.4 - 2.7 ppm can be attributed to the resonances of –$CH_2$and -(CO)-CH_2- (amide) groups, respectively, as a result of fatty acyl substitution on the chitosan backbone. The degree

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of substitution of fatty acid onto chitosan can be calculated using FT-IR absorption peaks at 1655 and 2870 cm^{-1} [14]. The degree of substitution of linoleic acid and oleic acid on chitosan was found to be 35% and 29%, respectively.

As mentioned earlier, the amphiphilic fatty acid grafted chitosan polymers self-assemble in aqueous solution to form polymeric micelles. The hydrophobic fatty acyl domain of these polymers induces this assembly above a specific concentration known as the critical micelle concentration (CMC). The CMC can be determined by pyrene fluorescence probe method by adding a constant concentration of pyrene (~0.6 μM) to increasing concentrations of the sample polymer and measuring the pyrenefluorescence spectra using a spectrofluorometer set at excitation wavelength 336 nm and emission wavelength 360 - 450 nm. Graphically plotting emission intensity ratio of the first peak (I_1, ~373 nm) to the third (I_3, ~393 nm) (I_1/I_3) against the logarithmic polymer concentration results in a sharp decrease in the intensity ratio with increasing polymer concentration. This can be explained by inclusion of pyrene into the hydrophobic core of in situ forming micelles, indicating the CMC of the polymer [13]. The CMC of linoleic and oleic acid grafted chitosan polymers was found to be 50 and 65 μg/mL, respectively (Figure 3). As expected, the CMC increases with an increase in the degree of unsaturation due to the resultant increase in hydrophilicity and hence solubility in water [15].

Fatty acid grafted polymeric nanomicelles can thus be prepared by dissolving the polymer in slightly acidic buffer above their CMC. The size of these nanomicelles ranges from 200 – 250 nm and zeta potential is slightly positive. Hydrophobic drugs can be entrapped in the core of these micelles while negatively charged substances (such as nucleic acids) can interact with the positively charged free amino groups on the polymer to form polyp lexes. Furthermore, the most important feature of so formed nanomicelles as drug and gene carriers is their biocompatibility. These nanomicelles have been shown to have negligible hemo- and cytotoxicity in various studies from our research group [16-20]. Genetic modification has emerged as a powerful tool over the years to treat countless number of diseases but the application of gene therapy is predominantly affected by low transfection efficacy of gene delivery vectors alongside their toxicity and immunogenicity. Rigorous studies have given promising data, for transfection as well as biocompatibility, asserting the potential application of nanomicelles of fatty acid grafted chitosan polymer based delivery system for drug and gene therapy [2,7,21-26]. The challenges associated with viral and non-viral cationic vectors can successfully be resolved by the use of biodegradable, biocompatible, non-immunogenic, and non-toxic fatty acid grafted chitosan polymers making them a potentially high caliber drug and gene delivery system.

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References

1. Layek B, Singh J, N-hexanoyl, N-octanoyl, N-decanoyl chitosans. Binding affinity, cell uptake, and transfection. Carbohydr Polym [Internet]. 2012;89(2):403-10.
2. Hu FQ, Wu X ling, Du YZ, You J, Yuan H. Cellular uptake and cytotoxicity of shell crosslinked stearic acid-grafted chitosan oligosaccharide micelles encapsulating doxorubicin. Eur J Pharm Biopharm. 2008;69(1):117-25.
3. Oak M, Singh J. Controlled delivery of basal level of insulin from chitosan-zinc-insulin-complex-loaded thermosensitive copolymer. J Pharm Sci [Internet]. 2012;101(3):1079-96.
4. Lee E, Lee J, Jon S. A novel approach to oral delivery of insulin by conjugating with low molecular weight chitosan. Bioconjug Chem. 2010;21(10):1720-3.
5. Mao S, Bakowsky U, Jintapattanakit A, Kissel T. Self-Assembled Polyelectrolyte Nanocomplexes between Chitosan Derivatives and Insulin. J Pharm Sci.;95(5):1035-48.
6. Du YZ, Wang L, Yuan H, Hu FQ. Linoleic acid-grafted chitosan oligosaccharide micelles for intracellular drug delivery and reverse drug resistance of tumor cells. Int J Biol Macromol [Internet]. 2011;48(1):215-22.
7. Kim JH, Kim YS, Park K, Lee S, Nam HY, Min KH, et al. Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. J Control Release. 2008;127(1):41-9.
8. Hu FQ, Ren GF, Yuan H, Du YZ, Zeng S. Shell cross-linked stearic acid grafted chitosan oligosaccharide self-aggregated micelles for controlled release of paclitaxel. Colloids Surfaces B Biointerfaces. 2006;50(2):97-103.
9. Kim JH, Kim YS, Kim S, Park JH, Kim K, Choi K, et al. Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel. J Control Release. 2006;111(1-2):228-34.
10. Mandke R, Singh J. Synthesis and evaluation of cationic nanomicelles for *in vitro* and *in vivo* gene delivery. 2012.
11. Bhatia KS, Singh J. Synergistic effect of iontophoresis and a series of fatty acids on LHRH permeability through porcine skin. J Pharm Sci. 1998;87(4):462-9.
12. Rastogi SK, Singh J. Iontophoretic Enhancement of Leuprolide Acetate by Fatty Acids , Limonene , and Depilatory Lotions Through Porcine Epidermis. Pharm Dev Technol. 2004;9(4):341-8.
13. Layek B, Singh J. Cell penetrating peptide conjugated polymeric micelles as a high performance versatile nonviral gene carrier. Biomacromolecules. 2013;14(11):4071-81.
14. Kasaai MR. Determination of the degree of N-acetylation for chitin and chitosan by various NMR spectroscopy techniques: A review. Carbohydrate Polymers. 2010.
15. Sun YE, Xia W, Tang X, He Z, Chen J. Effects of fatty acid chain length and degree of unsaturation on the surface activities of monoacyl trehaloses. Front Chem Eng China. 2009;3(4):407-12.
16. Layek B, Lipp L, Singh J. APC targeted micelle for enhanced intradermal delivery of hepatitis B DNA vaccine. J Control Release [Internet]. 2015;207:143-53.
17. Mandke R, Singh J. Cationic nanomicelles for delivery of plasmids encoding interleukin-4 and interleukin-10 for prevention of autoimmune diabetes in mice. Pharm Res. Pharm Res. 2012;29(3):883-97.
18. Layek B, Singh J. Caproic acid grafted chitosan cationic nanocomplexes for enhanced gene delivery: Effect of degree of substitution. Int J Pharm [Internet]. 2013;447(1-2):182-91.
19. Mandke R, Singh J. Effect of acyl chain length and unsaturation on physicochemical properties and transfection efficiency of N-acyl-substituted low-molecular-weight chitosan. J Pharm Sci. 2012;101(1):268-82.
20. Layek B, Haldar MK, Sharma G, Lipp L, Mallik S, Singh J. Hexanoic acid and polyethylene glycol double grafted amphiphilic chitosan for enhanced gene delivery: Influence of hydrophobic and hydrophilic substitution degree. Mol Pharm. 2014;11(3):982-94.
21. Chen Y, Feng S, Liu W, Yuan Z, Yin P, Gao F. Vitamin E Succinate-Grafted-Chitosan Oligosaccharide/RGD-Conjugated TPGS Mixed

Micelles Loaded with Paclitaxel for U87MG Tumor Therapy. Mol Pharm [Internet]. 2017;14(4):1190–203.

22. Du YZ, Lu P, Zhou JP, Yuan H, Hu FQ. Stearic acid grafted chitosan oligosaccharide micelle as a promising vector for gene delivery system: Factors affecting the complexation. Int J Pharm [Internet]. 2010;391(1-2):260-6.

23. Hu FQ, Liu LN, Du YZ, Yuan H. Synthesis and antitumor activity of doxorubicin conjugated stearic acid-g-chitosan oligosaccharide polymeric micelles. Biomaterials [Internet]. 2009;30(36):6955-63.

24. Du YZ, Wang L, Yuan H, Wei XH, Hu FQ. Preparation and characteristics of linoleic acid-grafted chitosan oligosaccharide micelles as a carrier for doxorubicin. Colloids Surf B Biointerfaces. 2009;69(2):257-63.

25. Lee KY, Kwon IC, Jo WH, Jeong SY. Complex formation between plasmid DNA and self-aggregates of deoxycholic acid-modified chitosan. Polymer (Guildf). 2005;46:8107-12.

26. Hu FQ, Zhao MD, Yuan H, You J, Du YZ, Zeng S. A novel chitosan oligosaccharide-stearic acid micelles for gene delivery: properties and *in vitro* transfection studies. Int J Pharm. 2006;315(1-2):158-66.