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Preparation and characterization of oligochitosan–tragacanth nanoparticles as a novel gene carrier

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ABSTRACT

The nanoparticles of oligochitosan–water soluble tragacanth (OCH–WST) as novel gene carriers have been prepared and their transfection efficiency has been investigated on Hela and HepG2 cell lines. Different OCH:WST weight ratios were prepared to obtain particles with low size distribution and high surface charge, and also in range of below 200 nm. Nanoparticles with 132.5 ± 6.77 nm size, polydispersity index 1.92 ± 0.061, surface charge 30.45 ± 1.84 and spherical morphology, have been chosen as gene carrier. Nanoparticle–DNA complexes (nanoplexes) showed better transfection efficiency in both Hela and HepG2 cells than chitosan polyplexes, with 1.26×10^6 versus 9.05×10^5 and 7.76×10^5 versus 2.16×10^5, respectively. Higher transfection efficiency of nanoplexes could be attributed to their weaker complexation. Decreasing of transfection in presence of galactose in HepG2 cells, indicated receptor mediated endocytosis of nanoplexes. These properties all together, make OCH–WST nanoparticles as potential gene carrier for active gene delivery into cells containing sugar receptors.

1. Introduction

Gene therapy as promising therapy for genetic malformations, cancers, and autoimmune system diseases like AIDS and rheumatoid arthritis, has attracted much attention during three past decades (Brunett-Pierri, & Ng, 2008; Kay, 2011; Leung, Dhirapong, Wu, & Tao, 2010; Park et al., 2012; Qasim, Gaspar, & Thrasher, 2009). Despite all developments in molecular biology and genetic sciences, delivery of the genes to the targeted cells is the main problem of gene therapy. There are various methods to deliver gene of interest, which can be categorized as viral and non-viral methods. While viral vectors have a high transduction, their application has been restricted because of acute immune response, infections, probability of carcinogenicity, and lack of control release and low capacity of vector for transformed genes. On the other hand, non-viral vectors are more safe and biocompatible but are restricted by low transfection (Pack, Hoffman, Pun, & Stayton, 2005; Pathak, Patnaik, & Gupta, 2013). Chitosan, structure component of shellfish, insect and cell wall of bacteria and mushrooms, and the most ample natural polymer after cellulose, has been used more than 15 years as a non-viral vector because of its positive charge, biocompatibility and biodegradability. Structurally, it is partially deacetylated chitin derivative, containing D-glucosamine and N-acetyl-D-glucosamine (Mao, Sun, & Kissel, 2010). An ideal non-viral gene delivery vector must have the balance between extracellular stability and interacellular dissociation of polyplex which consequences gene release and gene expression (Pack et al., 2005). The strength of interaction between chitosan and DNA which is attributed to primary amine of chitosan, results in highly stable particles, thereby prevents dissociation within the cell and results in low transfection (Douglas, Piccirillo, & Tabrizian, 2006). Molecular weight and degree of deacetylation of chitosan are the most important factors which influence this balance for chitosan mediated gene delivery (Kiang, Wen, Lim, & Leong, 2004; Sato, Ishii, & Okahata, 2001). In vitro studies have shown that chitosan oilgomers (5–10 kDa) with the high degree of deacetylation (upper 80%) have better transfection than other types of chitosan (Köping-Höggård et al., 2004; Strand, Lelu, Reitan, de Lange Davies, Artursson, & Vårum, 2010). An approach for decreasing stability of DNA/chitosan and improving transfection efficiency involves the association of chitosan oligomers with an anionic biopolymer, such as alginate and hyaluronic acid, prior to the

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addition of DNA (Douglas et al., 2006; Douglas & Tabrizian, 2005, 2009).

Water soluble tragacanth (WST) is a de-esterified derivative of tragacanth gum (TG) (Fattahi et al., 2013). Tragacanth is a heterogeneous branched anionic polysaccharide contains tragacanthic acid which consists of repeated galacturonic acid units in the backbone, and galactose, fucose and xylose in the side chains (Aspinall & Baillie, 1963). The presence of these sugars on the side chain of carbohydrate polymers makes them a good candidate for targeted delivery by carbohydrate recognizing receptor found on the cell surface (Zhang, Ma, & Sun, 2010).

In this study, for the first time, nanocomposites of oilgochitosan–WST (OCH–WST) have been synthesized and characterized, and their transfection efficiency has been investigated.

2. Materials and methods

2.1. Materials

The OCH was supplied by Yuhuan Marine Biochemistry Co., Ltd., Zhejiang, China. Ribbon type tragacanth from Astragalus-gossypinus was purchased from local market of Isfahan, Iran. pGl3 luciferase plasmid and steady-Glo luciferase assay kit were supplied by Promega. Other chemical compounds were purchased from Sigma–Aldrich and were used without any purification.

2.2. Preparation of OCH–WST particles

WST has been prepared by de-esterification of TG base of Fattahi et al. (2013) with minor modification. Briefly, 2 g TG was dispersed in 1 L (0.1 M) sodium hydroxide and stirred for 4 h at 4 °C, followed by precipitation of WST in 60% ethanol. Then precipitated WST was dissolved in deionized water. In order to remove sodium traces, acetic acid was added to the WST solution and washed several times with increasing concentrations of ethanol (60, 70 and 94%). Finally, the stock solution was prepared by dissolving WST in deionized water or desired buffer at 0.05% (w/v). OCH (90% deacetylated; verified by 1HNM0052, Mw = 8.6 kDa; verified by CTO-20 AC-Shimadzu high performance liquid chromatography using refractive index detector and Ultrahydrogel 250 column, and PEG as standard) dissolved in deionized water or desired buffer at a concentration of 0.01% (w/v). Before preparation of nanoparticles, both solutions were filtered through a 0.4 μm filter. To synthesize particles, 0.5 ml of WST solution in concentration of 0.4, 0.2, 0.1, 0.066, 0.05 or 0.04 mg ml^{-1} was added to 1 ml of Chitosan solution with a constant concentration of 0.1 mg ml^{-1} which was under agitation to achieve particles with OCH:WST weight ratio of 0.5, 1, 2, 3, 4 and 5, respectively. Final pH of dispersant was 6 except where noted otherwise.

2.3. Particle size and zeta potential measurements

The particle size and zeta potential of the OCH/WST particles and nanoparticle–pDNA complexes (nanoplexes) were measured by a zetasizer (Zetasizer-ZEN3600 Malvern Instrument Ltd., Worchestershire, UK). All particle-size measurements were performed in a He–Ne laser beam at 658 nm. For measurement in different pHs, samples were diluted either in water (pH 4, 5, 6 and 7; adjusted by NaOH and HCl) for zeta measurements or acetate buffer (pH 4 and 5) and PBS (pH 6, 6.3, 6.6 and 7) for size measurements. Zeta potential of particle–pDNA complexes were observed at pH 6 in water after 15 min incubation of the complexes in the room temperature (r.t.). The device calculated the zeta potential of the nanoparticles from their electrophoretic mobility using Smoluchowski's equation. All the experiments were performed in triplicate.

2.4. FTIR analysis

FTIR spectra were acquired in transmission mode from OCH and WST powders, dried OCH/WST particles and nanoplexes (Calf thymus DNA as model nucleic acid and nanoparticles in w/w ratio 3 were used to prepare nanoplex at CR20). The spectra were recorded with an FTIR spectrometer (IR prestige-21, Shimatzou), in the spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

2.5. Morphological studies

Morphology of the particles was examined, using a scanning electron microscope (SEM, SERON technology, AIS2100). Prior to examination, the samples were lyophilised, fixed on a brass stub and coated with a gold–palladium layer under argon atmosphere, using a gold sputter module in a high vacuum evaporator.

2.6. Preparation of nanoplexes

The incorporation of DNA within the nanoparticle was performed through electrostatic interactions between DNA and OCH–WST nanoparticle (w/w ratio of 3). This simply involved adding the aqueous solution of the DNA at the different concentration to the nanoparticles with the constant concentration in PBS and at pH of 6, followed by 15 min incubation in r.t., prior to use.

2.7. Calculation of charge ratio of nanoparticles/DNA

Titration method was used to measure weight percent of galacturonic acid in WST. Briefly, 500 mg WST (DM of 74.28 and MW of 300 kDa) was titrated by 0.5 M sodium hydroxide according to USP method with a little modification (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007). Weight of WST equal to 1 M galacturonic acid (GA) was calculated by following formula:

$$W_{WST} = \frac{(V \times 97.04 \times DM)/194.139}{500} \quad (1)$$

where W_{WST} is weight of WST equal to 1 M GA, V is volume of 0.5 M sodium hydroxide used in titration and DM is degree of methylation of GA.

Total charge ratio consists of nitrogen/phosphate (N/P) ratio and nitrogen/carboxyl (N/C) ratio was calculated by Eq. (2).

$$\text{Total charge ratio} = \frac{(A/161.2)}{[(B/330) + (C/W_{WST})]} \quad (2)$$

where A is weight of Chitosan, B is weight of pDNA and C is weight of WST in solution.

2.8. Gel retardation assay

Samples containing 0.165 μg DNA either alone or complex with OCH or OCH–WST nanoparticles at desired charge ratio (CR), were mixed with loading buffer and loaded onto 0.8% agarose gel containing ethidium bromide (0.2 μg ml^{-1}). Samples were run in TAE running buffer, using a horizontal gel electrophoresis apparatus. The gel was exposed to an electric field (80 V) for an hour and visualized by UV illumination.

2.9. Cell culture

HepG2 (human hepatocellular carcinoma cell line) as an acyaloglycoprotein receptors (ASGPR) posetive cell line and Hela (human epithelial cervical cancer cell line) as ASGPR negative cell line were

obtained from Pasteur Institute, Iran. Cells were maintained in RPMI-1640 supplemented with 10% (v/v) FBS (fetal bovine serum) and penicillin/streptomycin (50 IU ml^{-1}, 500 μg ml^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were subcultured regularly using trypsin/EDTA.

2.10. In vitro cytotoxicity

The MTT assay was performed to evaluate in vitro cytotoxicity of OCH–WST nanoparticles. The cytotoxicity of nanoparticles was performed against HepG2 and Hela cell lines. Briefly, 5×10^4 HepG2 or Hela cells were plated in 96-well plates, and grown for 24 h. The cells were exposed to a series of concentrations of OCH–WST (1–100 μg ml^{-1}), at 37 °C for another 72 h. At the end of incubation, 20 μl of MTT solution with the concentration of 5 mg ml^{-1} was added and incubated for a further 3 h at 37 °C. The medium containing unreacted MTT was removed, and 150 μl of DMSO was added to each well to dissolve the formazan crystals. Finally, the absorbance of dissolved formazan was measured at 540 nm in an ELISA reader (Bio-Rad, Model 680, USA) (Fattahi et al., 2013). All the experiments were performed in triplicate.

2.11. In vitro transfection

The HepG2 and Hela cells were plated at 10,000 cells per well and 300 μl of media in 96 well tissue plates (Corning, USA). After incubation for 24 h, the medium was discarded, and the cells were washed once with PBS with the pH = 6. Nanoplexes were added to the serum free medium (pH = 6), vortexed vigorously for 30 s then prepared medium was added to cells and incubated for a period of 4 h. The final concentration of pDNA was 1.5 μg cm^{-1}. After this process, the nanoplexmedium was discarded and fresh culture medium (pH = 7.4), supplemented with serum and antibiotics, was added to the cells. The luciferase assay was carried out according to the manufacture's instruction (Promega, USA). Relative luciferase units (RLUs) due to luciferase activity were measured with plate reader chemiluminometer (Synergy H1, Biotech USA). RLUs normalized to protein concentration measured by bicinconinic acid (BCA) method. The effect of galactose and galactoronic acid of WST as the ligand of ASGP on transfection was investigated by adding free galactose (100 mM) to the transfection medium prior to transfection study (Jiang et al., 2008).

2.12. Statistics

Statistical analysis was achieved using the SPSS software package (v.17). One-way ANOVA tests were used with a confidence level of $p \leq 0.05$ to assess statistically significant homogeneous subsets.

3. Results and discussion

3.1. Synthesis and characterization of OCH–WST nanoparticles

OCH–WST nanoparticles were successfully prepared by the simple ionic interaction of WST and OCH. The effect of weight ratio on particle size diameter, PDI and zeta potential are summarized in Fig. 1. While there is slight increase in particle size, it is not too much decreasing in zeta potential with increasing percent of WST as negative charge polymer in nanoparticles (Fig. 1A and C), and only surface charge of particles in ratio one shows significant difference. It can be attributed to the branched structure of WST which cause low charge density of WST in unit of weight, and difference between molecular weight of chitosan (10 kDa) and WST (300 kDa) which caused small size and high surface charge even in presence of high weight percent of WST in particles. In a similar study, Duccepe and Tabrizian (Douglas et al., 2006) have reported small particle with high surface charge and low PDI prepared by chitosan and hyaluronic acid as low charge density polymer.

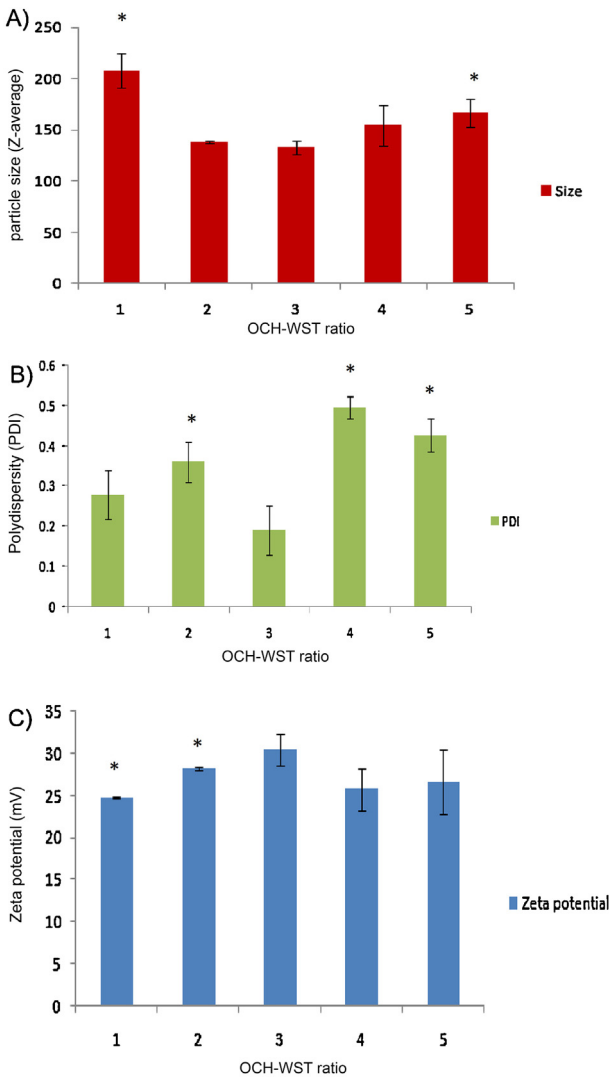

Fig. 1. (A) Particle size, (B) PDI and (C) zeta potential of OCH–WST nanoparticles with different OCH/WST weight ratio.

According to Delair (2011), when oligomeric positively charged polymer interact with high MW negative charged polymer, in the case that molar ratio of positively charged oligomer is higher than negatively charged polymer, a small portion of oligomer interacts with negatively charged polymer and makes a core. The rest of the oligomer will cover the core and make a positive shell. Accordingly, with increasing of chitosan percent in our study, the chitosan shell was saturated and excess amount of chitosan did not have a significant effect on zeta potential but it could increase aggregation rate and consequently increase particle size. Other studies have shown that the higher molecular weight of anionic polymer, the smaller particle size forms with positive oligomer (Duceppe & Tabrizian, 2009); so, particles in weight ratio of 3 with *Z*-average diameter of 132.5 ± 6.77 nm (mean ± S.D.) and PDI of 0.193 ± 0.061 (mean ± S.D.) had the smallest size and the narrowest size distribution (Fig. 1A and B). Zeta potential measurements indicated a positive charge of 30.44 ± 1.84 mV (mean ± S.D.) for weight ratio of 3 (Fig. 1C), suitable for complex formation with anionic DNA. Therefore, particles with weight ratio of 3 were chosen for further studies. The effect of pH on size and zeta potential of OCH–WST particles

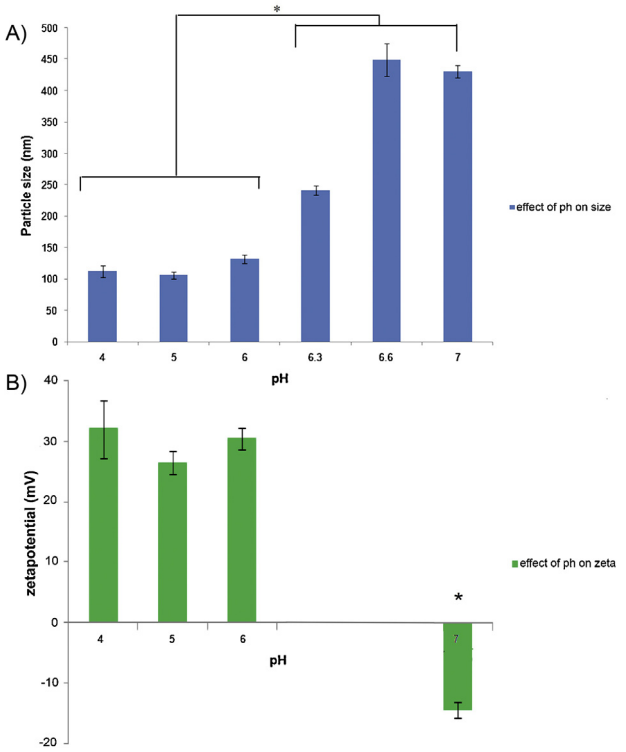

Fig. 2. Effect of pH on size (A) and surface charge (B) of OCH–WST particles. Significance was calculated by ANOVA ($^*p \leq 0.05$).

(CR of 3) is shown in Fig. 2A and B. With increasing pH from 4.5 to 7, zeta potential of particles was reduced, and the size of particles was enhanced as a consequence. From pH 4.5 to 6, size was slightly increased while in pH 6.3 which is near the p*Ka* of chitosan oligomer (p*Ka* = 6.5) particle size was significantly enhanced above than 240 nm. In pH 6.6 and 7, which are higher than p*Ka* point, more than 50% of amines of chitosan are neutral (Liu et al., 2005). Therefore, zeta potential was reversed and decreased to −14.2 mV for pH 7, and particle size was increased to 448.9 for pH 6.6 and 430.33 for pH 7 which are not suitable for biological application.

SEM imaging (Fig. 3) showed spherical particles (weight ratio of 3 and pH of 6) which are aggregated. The aggregation is the main problem of particle's freeze-drying, and can be solved by adding mono and disaccharides like dextrose and trehalose into the colloidal system prior to freeze-drying (Troiano, 2011).

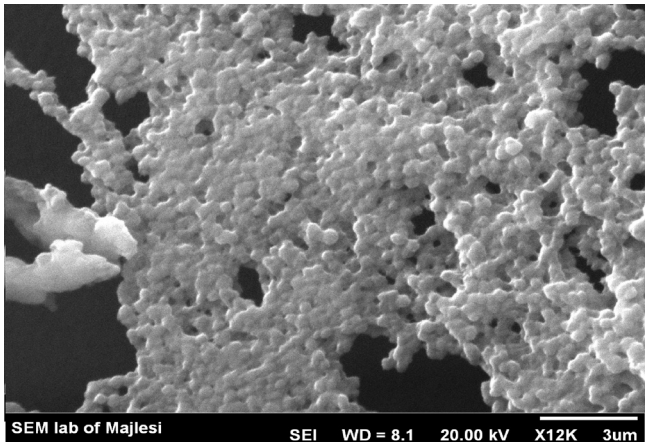

Fig. 3. SEM of nanoparticles in weight ratio 3.

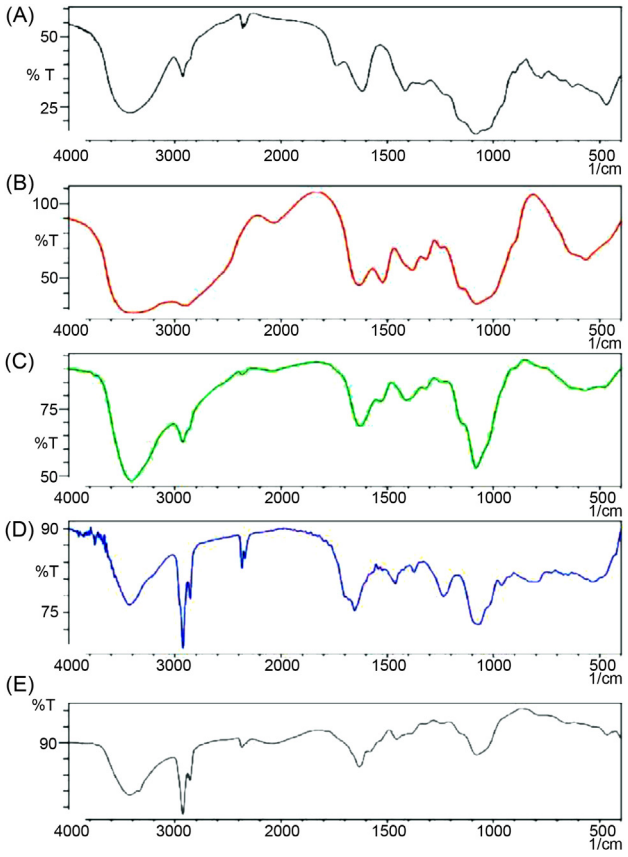

Fig. 4. FTIR spectra of (A) WST, (B) OCH, (C) OCH–WST nanoparticles in weight ratio 3, (D) ctDNA and (E) nanoplexes.

Fig. 4 describes FTIR spectra of WST, OCH, OCH–WST particles in weight ratio 3, calf thymus DNA (ctDNA) and nanoplex at CR20, respectively. The characteristic peaks of WST consist of asymmetric stretching of C=O at 1735.9 cm^{-1}, asymmetric stretching of COO− (attributed to sodium salt of carboxylic acid) at 1616.35 and symmetric stretching of COO− at 1415.75 (Fig. 4A). Characteristic infrared absorption bands of water soluble chitosan (Fig. 4B) appeared at 1631.78 cm^{-1} (amide I), 1516 cm^{-1} (NH bend) and 1381.1 cm^{-1} (CH_2 bending). In OCH–WST nanoparticle spectrum (Fig. 4C), intensity of NH bend of chitosan is decreased and shifted to a higher wavenumber while the amide band intensity is increased and shifted to a lower wavenumber. Exciting the peak at 1527 indicates that a part of amines stay in sodium salt form and in no interaction with carboxylic acids of WST. Probably ionic interaction of carboxylic acid of WST with amine of OCH was strong and shows amide properties which cause increase in the amide band. The shifts in amine and amide bands in the nanoparticle spectrum can be attributed to the interaction of amine group of chitosan and carboxylate of WST and the replacing of sodium salt with amine. Appearance of broad and intense peak at 1411.8, attributed to symmetric stretching of COO− also indicates interaction of OCH and WST, and producing new salt.

The characteristics bands at the range of 2000–1000 cm^{-1} in DNA and nanoparticles spectra have contribution (Fig. 4C and D). Therefore, they are not useful for evaluation of the nanoplex. Increase of N—H and C—H stretch bands in nanoplexes spectrum (Fig. 4E) indicates presence of DNA in the nanoplex.

Cytotoxicity of nanoparticles was investigated by MTT assay which has indicated no significant cytotoxicity, neither on HepG2 nor Hela cells even in 100 $\mu g\,ml^{-1}$ which makes nanoparticles a safe vector for gene delivery (Fig. 5).

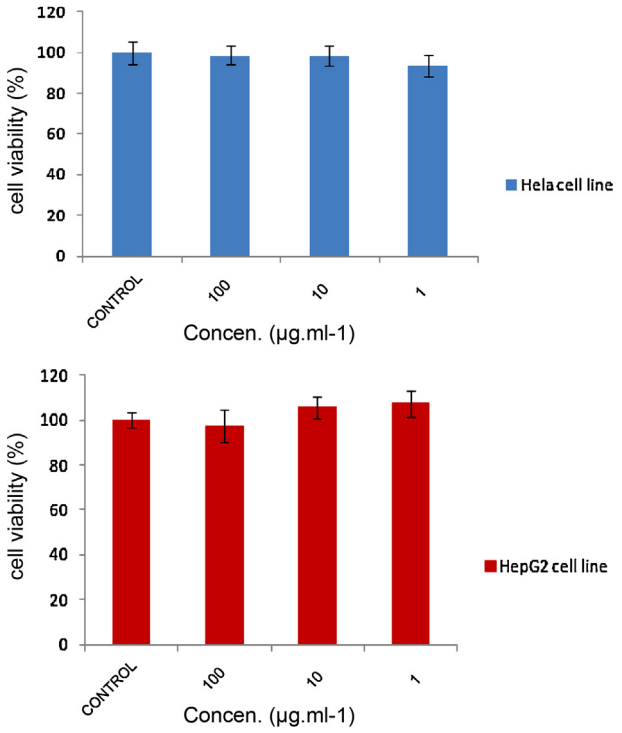

Fig. 5. Relative cell viability of OCH–WST particles ratio 3 on (A) Hela cells and (B) HepG2 cells. The relative cell viability read for the control (TCPS, tissue culture polystyrene from culture plates) after 72 h of incubation was taken as the reference (100%). Significance was calculated by ANOVA ($^*p \leq 0.05$).

3.2. Nanoplexes formation

The ability of OCH–WST nanoparticles to make complex with DNA was assessed by agarose gel retardation assay. The electrostatic interaction between OCH–WST nanoparticles and plasmid DNA (pDNA), neutralizes the negative charge of phosphate groups on DNA backbone, thus retarding the DNA mobility under the influence of electric field. The OCH–WST nanoparticles were incubated with pDNA at different weight ratios, keeping the amount of DNA constant. The samples were analyzed on 0.8% agarose gel and charge ratio required for the formation of an electroneutral complex was investigated. As shown in Fig. 6A, increasing amounts of nanoparticle in nanoplexes leads to decreased electrophoretic mobility. The chitosan polyplex completely inhibited the migration of pDNA at CR of 3 while nanoplexes showed complete retardation at charge ratio of 10 which indicates neutralization of some amine groups of OCH by WST. As shown in Table 1, the total CR of nanoplex was lower than the theoretical CR. However, complex of nanoparticle even base of total CR was weaker than chitosan complexation. Nanoplex could condense pDNA completely in total CR of 6.11 or higher while complete condensation for polyplex happened in CR of 2 or higher. This difference can be explained by the load of pDNA on the surface of nanoparticle. Our results are in agreement with those of other similar studies (Duceppe & Tabrizian, 2009).

To approve the results of gel retardation assay, zeta potential of nanoplexes in different CRs was investigated (Fig. 6B). CR1 had a negative zeta potential (-3.63 ± 0.4 mV) while CR5 and CR10 showed neutralized surface charge with zeta potential of $+1.35 \pm 0.45$ mV and $+1.99 \pm 0.71$ mV, respectively. Based on zeta potential value, at CR5 or higher CRs, nanoparticles can completely condense pDNA while the gel retardation results had indicated that the complete condensation was happened in CRs higher than CR5. This difference can be explained by the effect of the pH used in these experiments. All zeta potentials were measured at pH = 6, while for the retardation assay, nanoplexes prepared at pH = 6 were run in TAE running buffer at pH = 8. The zeta potential study showed that surface charge of nanoplexes at CR5 and pH = 6 is almost neutralized which means at this pH, almost all of amines on the surface of nanoparticles had interaction with phosphate group of pDNA. The pH of running buffer in retardation assay was much higher than p*K*a of chitosan, and at this pH a part of the amines which interacted with phosphates was de-protonated caused release of pDNA from the nanoplexes.

The zeta potential of nanoplex at CR20 and pH = 6 was $+20.7 \pm 3.75$ mV which means a portion of amines was free at this CR. Probably, in running buffer of retardation assay, the amines of chitosan which interacted with phosphate were not affected at pH = 8, and only free amines were de-protonated. Therefore, complex of pDNA and nanoparticle was stable even at pH = 8, and no pDNA was released at this pH. However, nanoplexes at CR5 and 10 are not good choices for transfection, and their uptake is potentially lower than nanoplexes at CR20 because of their neutralized surface charge.

Table 1
Theoretical charge ratio (CR) of OCH–WST nanoplexes which considered equal to CR of used chitosan, and total CR of nanoplexes calculated by Eq. (2).

Total CR of polyplex and theoritical CR of nanoplex	1	5	10	20
Total CR of nanoplex	0.94	3.25	6.11	8.79

3.3. In vitro cell transfection studies

The various properties of OCH–WST nanoparticles indicated that these systems have most of the appropriate properties required to be a good transfecting agent. The transfection efficiency of the nanoplexes was assessed on Hela and HepG2 cell lines, using a plasmid DNA containing reporter gene encoding luciferase. CRs greater than CR at which complete DNA retardation was observed, were used for transfection. The cells were exposed to either polyplexes or nanoplexes in serum free medium for 4 h. All transfection studies were carried out at pH = 6, as optimum pH. On the one hand, the higher pH caused decrease of surface charge and increase of size, which is not acceptable for biological applications, and particles could not condense pDNA completely, as well. On the other hand, pH values lower than 6 could enhance complex stability and decrease DNA release in site of action (nucleus) which consequence lower transfection. Furthermore, pH = 6 mimics some tumors' environment, and can be used for such conditions in vivo (Lee, Gao, & Bae, 2008).

Transfection efficiency of nanoplexes at different CRs was assessed (Fig. 7). Chitosan polyplexat CR10 (the optimum Chitosan polyplexe) was used as positive control and naked DNA was considered as negative control. For both HepG2 and Hela cells, CR30 was shown higher transfection with 7.76×10^5 and 1.06×10^6, respectively (Fig. 7). With increasing CR from 5 to 30, transfection efficiency was increased, which can be attributed to complete condensation and increasing of positive charge of particles. Thereafter, transfection was dropped because of strong interaction between pDNA and nanoparticle, and probably slow release of DNA (Fig. 7).

Transfection of both nanoplex and polyplex in HepG2 was significantly lower than Hela cells in the same condition (Fig. 7), which is not a surprising result. Other research groups also have indicated low transfection of chitosan in HepG2 (Erbacher, Zou, Bettinger, Steffan, & Remy, 1998; Kim, Kim, Akaike, & Och, 2005). Uptake in HepG2 is mediated by clathrine-mediated endocytosis and fluid-phase pinocytosis, and caveolar endocytosis does not have any role in uptake by this cell. Caveolar vesicles are independent of lysosomes and avoid degradation of pDNA (Adler &

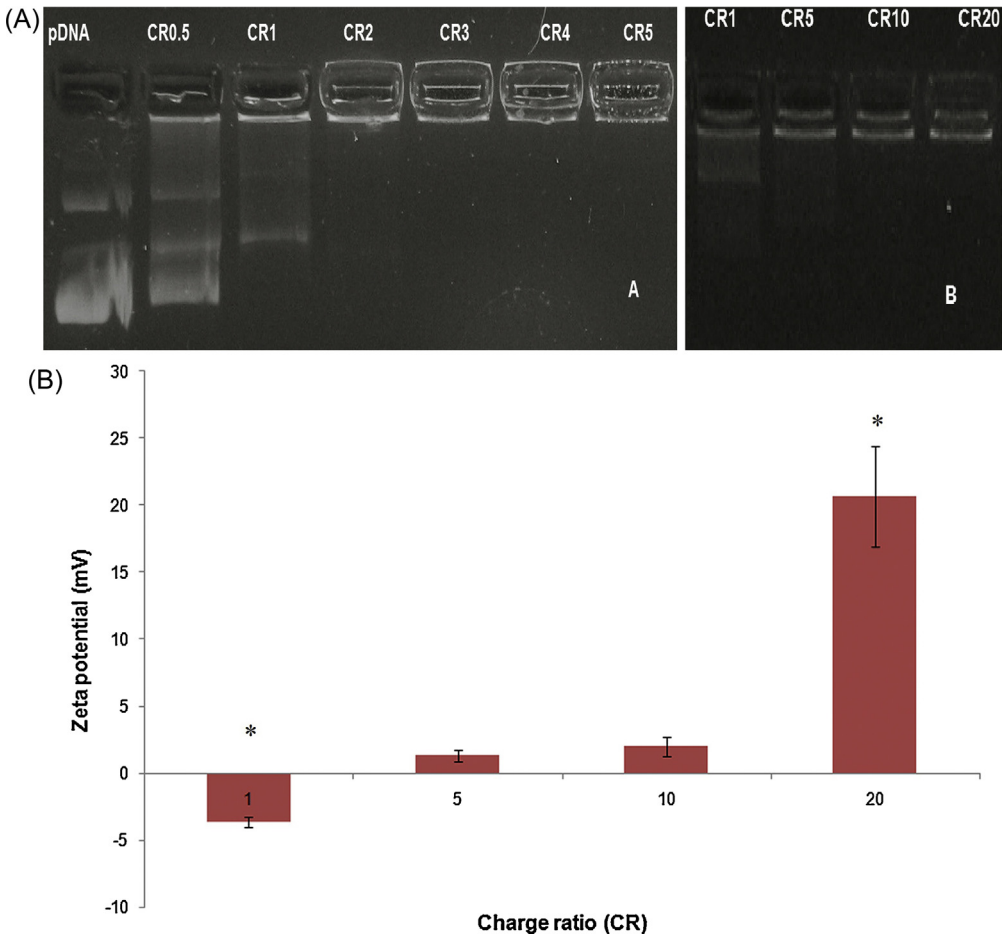

Fig. 6. Agarose gel electrophoresis of chitosan polyplexat different CRs (A) and OCH–WST nanoplex (B) in different theoretical CR. (C) Surface charge of nanoplexes at different CRs.

Leong, 2010). Therefore, transfection of polymeric vectors with low proton sponge effect (e.g. chitosan) is mainly dependent on this type of endocytosis. Receptor mediated endocytosis cannot be a main way for uptake of chitosan because there is no known receptor on the cell membrane of HepG2 for chitosan. So, the uptake happens mainly by pinocytosis.

In the case of HepG2, transfection of nanoplexes in charge ratios higher than 10 is significantly higher than chitosan polyplexes (Fig. 7B). Two special properties of OCH–WST can explain this phenomenon: (1) easier dissociation of nanoplexes and consequently, faster release of pDNA in the cell, (2) presence of galactose in the side branch and galacturonic acid in the backbone

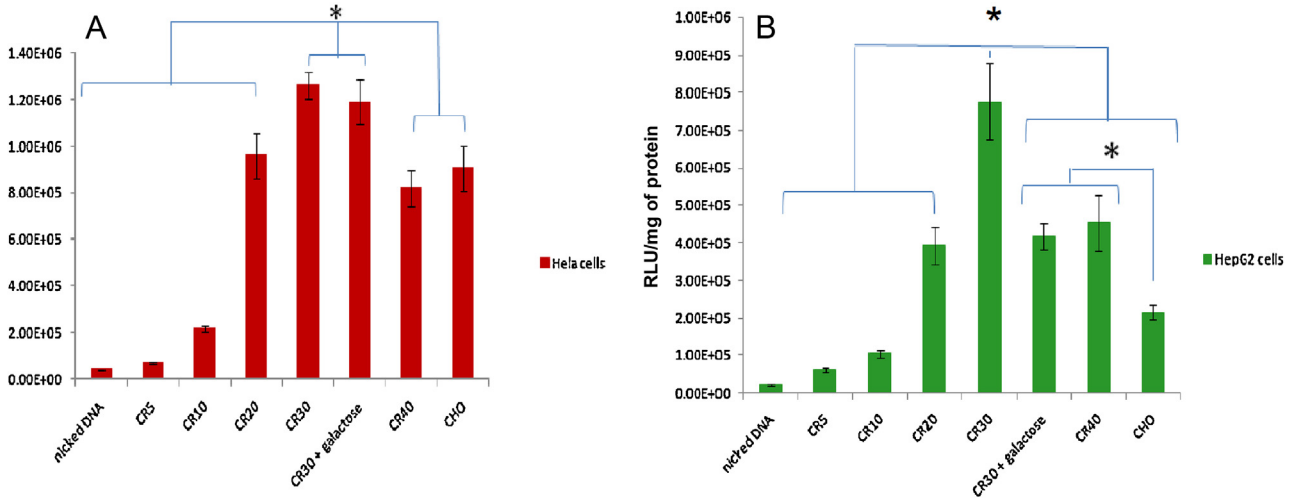

Fig. 7. Evaluation of transfection efficiency of luciferase in Hela (A) and HepG2 (B) cells. Transfection was induced for 4 h with 1.5 μg cm^{-1} in antibiotic and serum free medium. Gene transfection was assayed after 48 h and reported as relative luciferase unit per mg of protein (RLU/mg of protein). Chitosan polyplex at CR ratio of 10 was used as positive control and naked DNA as negative control. Significance was calculated by ANOVA ($^{*}p \leq 0.05$).

of WST which can interact with ASGPR and improve uptake of particles by receptor mediated endocytosis (Kim, Park, Nah, Ochi, & Och, 2004). Ciechanover, Schwartz, and Lodish (1983) showed that ASGPR's ligands trigger a rapid and specific endocytosis of receptor–ligand complexes. However, transfection of nanoplexes at CR30 was significantly higher than chitosan polyplex; its transfection enhancement in Hela cells was lower than its transfection in HepG2 where it increased for three times. Lack of ASGPR on the cell membrane of Hela cells may cause this difference (Jiang et al., 2008; Zhang et al., 2005).

Significant decrease of transfection efficiency in HepG2 cells was obtained by adding free galactose into the medium which confirmed our hypothesis regarding the possibility of interaction of nanoplexes and ASGPRs. Free galactose competed with nanoplexes for ASGPRs and inhibited the interaction of particles and receptors in HepG2 cells while lack of ASGPRs on Hela cells caused no significant decrease in transfection (Fig. 7).

4. Conclusions

Here we successfully prepared and evaluated novel OCH–WST nanoparticles as a non-viral vector. Small size, low polydispersity, high surface charge and spherical nanoparticles obtained by a simple complexation of OCH and WST. Nanoparticles condensed pDNA at CR slightly higher than CR of chitosan polyplexes. Nanoparticles had low toxicity and exhibited higher transfection efficiency when compared with chitosan in Hela and HepG2 cells. Comparing to chitosan polyplexes, transfection of nanoplexes in HepG2 cells was much more increased than that in Hela cells, and free galactose could only inhibit transfection in HepG2 cells which indicated specific cell targeting due to the presence of galactose in the side chain of WST as ligand of ASGP receptor. These properties, all together make OCH–WST nanoparticles a safe and effective vector for in vitro and potentially in vivo gene delivery.

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