Effect of cross-linking agent on the characteristics of chitosan nanoparticles designed for the delivery of siRNA to ovarian cancer tissues

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INTRODUCTION

Ovarian cancer is a leading gynecologic cause of death in women around the world. Bionanotechnology based on siRNA nanocarriers offer many promising solutions for the problems associated with the current ovarian cancer therapy. In vivo systemic delivery of siRNA remains a challenge. The major limitations against the use of siRNA are its degradation by serum nucleases, poor cellular uptake and rapid renal clearance following systemic administration (Chen et al., 2009). Further development of siRNAs for anti-cancer therapy depends on the development of safe and effective carriers for systemic administration. Chitosan nanoparticles have received considerable attention in gene therapy and targeting (Ozpolat et al., 2009). Most of chitosan nanocarriers have been performed with Tri polyphosphate (TPP) as a cross-linking agent due to its quick gelling capability and non-toxic property, however materials of natural origin are often preferred in development of dosage forms for human use. The aim of this study was to design a carrier for the delivery of siRNA to ovarian cancer tissues based on chitosan and to investigate alginate and acacia as potential natural cross linking agent to chitosan.

MATERIALS AND METHODS

Synthesis of chitosan nanoparticles

Chitosan nanoparticles were prepared by ionic gelation method. Different concentrations of polymer were dissolved in 1.5 % v/v acetic acid solution. Three different types of cross-linking agents namely Sodium tripolyphosphate (TPP), acacia and alginate solutions were dissolved in distilled water. Cross-linking agent solution was added dropwise with a syringe to chitosan solution while stirring. The nanosuspentions were analyzed immediately for their physical properties after preparation.

For the association of siRNA with the chitosan nanoparticles, a solution of siRNA in double distilled water was added to the cross linking agent solution before adding this drop-wise to the chitosan solution under constant magnetic stirring at room temperature.

Particle size and Zeta potential

The average particle size and zeta potential of nanoparticles were measured by light scattering technique (Malvern zetasizer, ZEN 3600, Malvern instruments, UK).

Determination of siRNA Entrapment Efficiency

The Entrapment Efficiency of siRNA in chitosan nanoparticles was obtained by determination of unbound siRNA concentration in the supernatant after particle centrifugation using a Nanodrop. The siRNA Entrapment Efficiency (%) is expressed as the percentage of bound siRNA (difference between the total amount of siRNA initially added for particle preparation and the amount of unbound siRNA remaining in the supernatant after centrifugation) to the total amount of siRNA initially added.

RESULTS AND DISCUSSION

Chitosan nanoparticles were successfully formulated (Figure 1). The nanoparticles possessed a spherical morphology and compact structure.

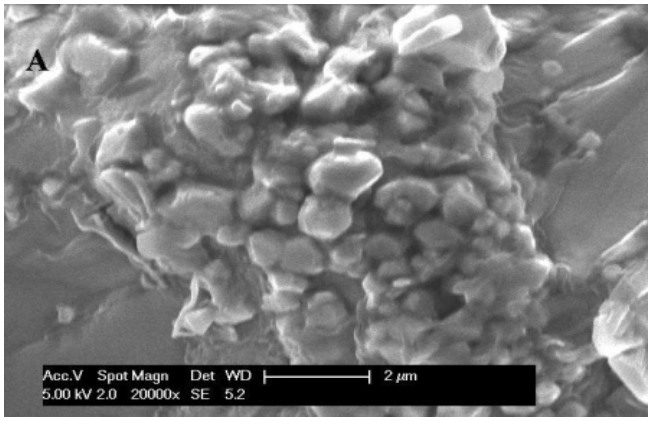

Figure 1: SEM images of chitosan nanoparticles.

The effect of types of cross-linking agent on the average diameter of particles prepared using medium molecular weight of chitosan was studied (table 1). Particles with sizes less than 150 nm were produced using acacia and TPP indicating the effectiveness of the cross-linking property of the two cross-linkers (Table 1). On the other hand, chitosan particles prepared using alginate as cross linking agent were in micrometer size range.

Table 1: Effect of type of cross-linking agent on the properties of chitosn loaded siRNA nanoparticles

Cross-linking agent	Size (nm)	Zeta (mV)	PDI	EE (%)
TPP	124	46	0.48	66.7
Acacia	132	3.5	0.7	83.3
Alginate	1650	70	0.5	50

These results could be explained by that the interaction of the positive charges of chitosan was at much higher level with the negative charge of acacia or TPP than that with alginate which resulted in a decrease of particle size of the nanoparticles produced.

Evaluation of the effect of acacia and TPP on particle size of the systems was investigated further using different molecular weights of chitosan (Figure 2). The results reveal that the mean diameters of particles produced by the two cross-linkers were in submicron size range and were comparable at the three molecular weights investigated.

Figure 2: Effect of type crossing linking agent on chitosan nanoparticles prepared using Low (LMW) medium (MMW) and high (HMW) molecular wight chitosan.

The other parameters for characterization are the surface charge of the nanoparticles as indicated by zeta potential and the polydispersity (PDI) (table 1). Chitosan nanoparticles were all positively charged as shown in the table. PDI results shows that the nanoparticles were homogeneously dispersed. Of all cross linking agents studied, acacia formulations possessed highest entrapment efficiency (EE%).

When siRNA is dissolved in chitosan solution and when the acacia with negative charge is added to the positively charged chitosan solution, the nanoparticles are formed rapidly and the siRNA is surrounded by a polymeric network due to interaction between two polymers. The higher degree of interaction between the two polymers resulted in more entrapment of siRNA in nanoparticles in comparison with the other formulations prepared by TPP and alginate.

Table 2: Effect of acacia on the properties of chitosan nanoparticles prepared using different molecular weight chitosan.

Molecular weight of chitosan	Size (nm)	Zeta (mV)	PDI	EE (%)
Low	300	27.5	0.5	50
Medium	450	55	0.9	83
High	640	75	1	25

Table 2 shows effect of acacia on the on the properties of particle prepared using different molecular weights of chitosan. Analysis of particle size revealed that, at the same chitosan concentration, as the molecular weight increases the particle size increases. The surface charges also increased with increasing the molecular weight of the polymer. However, low molecular weight chitosan nanopartilces exhibited the narrowest particle size distribution, as indicated by low PDI value. The lowest EE% was observed for particles prepared using high molecular weight chitosan which could be attributed to the larger particle size of the formulation and the less surface area available for entrapment of siRNA.

CONCLUSION

The results revealed that acacia is an effective natural cross-linking agent for production of chitosan nanoparticles with desirable properties for the delivery siRNA to ovarian cancer tissues.

REFERENCES

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