CHAPTER I

INTRODUCTION

Gene therapy enables physicians to treats the cause of a disease rather than the symptom. Not only genetic diseases, but gene therapy can also treat acquired diseases. Although gene therapy has many advantages, the process of gene therapy involves many obstacles (Patil et al., 2005). Poor cellular uptake and rapid degradation of naked DNA necessitate the use of delivery system. The therapeutic success of gene therapy is dependent on the development of efficient and safe gene delivery system. To deliver genes to targeted cells, the delivery system must use the carrier molecule or particle called the vector.

Nonpathogenic attenuated viruses are the initial vectors for gene delivery system. The examples of viral vectors are retroviruses (Crcareva et al., 2005), adenoviruses (Bouquet et al., 2006) and adeno-associated viruses (Wu et al., 2006). Although, gene expression using viral vectors is achieved with extremely high transfection efficiency (Shi et al., 2004), the major drawbacks are the toxicity (Favre et al., 2001), generation of immune response (Raper et al., 2003) and stimulation of carcinogenesis (Simon et al., 1993).

Non-viral delivery is emerging as favorable alternatives to viral vectors owing to their biosafety (Pouton and Seymour, 1998) and lack of immune response. Moreover, other advantages of non-viral gene vectors are ease of formulation, stability and ability to be produced in large quantities, flexibility in the size and sequence of DNA that can be delivered (de Jong et al., 2001; Kreiss et al., 1999), low cost and consistent of production. Commonly used non-viral vectors for gene delivery can be classified into 2 major types, cationic phospholipids and cationic polymers. Cationic phospholipids offer several advantages over viral vector, such as low immunogenicity and ease of preparation. However, the transfection efficiency of cationic phospholipids is lower than those of viral vectors, attributed to the instability of complexes (Lee et al., 2001).

Polymer-DNA complexes, on the other hand, are more stable than phospholipid-DNA complexes. Versatility of physicochemical properties and easy manipulation are some of the most important advantages of cationic polymers. However, the efficiency of gene delivery by cationic polymers is still relatively low compared to viral vectors. Commonly used polymers include polyethyleneimine (Weiss et al., 2006), polylysine (Yamagata et al., 2007), polyamidoamine dendrimer (Choi et al., 2006) and chitosan (Borchard, 2001).

Chitosan is obtained by deacetylation of chitin, a polysaccharide widely distributed in nature. Therefore, chitosan has been shown to be both biocompatible (Illum, 1998) and biodegradable (Onishi and Machida, 1999). Chitosan has been shown to be non-toxic (Dodane and Vilivalam, 1998). It is used as wound healing materials (Ueno et al., 1999) and scaffold materials (Shi et al., 2006). Because of its mucoadhesive ability (Freier et al., 2005), it is mostly used in the development of mucoadhesive dosage forms.

Chitosan has been widely used as a vector for gene delivery for a decade (Mao et al., 2001). Most studies concentrated on the generating of new chitosan derivatives such as, galactosylated chitosan (Kim et al., 2004), to achieve high transfection efficiency. Although some studies used different formulation variables, the results could not be compared because of the difference of other factors among each study. Therefore in this study, the formulation variables were collected and the effect of these formulation variables on their physical properties and transfection efficiency of chitosan-pDNA nanoparticles were investigated. The purposes of this study were:

1. To determine the effect of formulation variables on the physical properties of chitosan-pDNA nanoparticles.
2. To determine the effect of formulation variables on the nanoparticles formation of chitosan-pDNA nanoparticles.
3. To determine the protection effect of chitosan-pDNA nanoparticles.
4. To determine the effect of formulation variables on *in vitro* transfection efficiency of chitosan-pDNA nanoparticles in HeLa cells.
5. To determine the cytotoxicity of chitosan-pDNA nanoparticles in HeLa cells.