P-046	**Development of chitosan nanocapsules for cancer gene therapy applications**	

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INTRODUCTION AND OBJECTIVES

In the last decade the use of nanoparticulated systems for the delivery of genetic material has become a promising approach to treat many incurable diseases including cancer (Veiseh et al., 2009). Nanoparticulated systems can be defined as solid colloids that include nanospheres and nanocapsules. The latter are vesicle like carriers composed of an aqueous core revolved around a polymeric envelope forming a core-shell like structure (Parveen and Sahoo, 2008). These specific characteristics play an important role in gene delivery since the plasmid DNA (pDNA) is liable to a hostile environment composed by nucleases, the ones responsible for its degradation (Gill et al., 2009). Therefore, it becomes clear that a nanocarrier system needs not only to transport the genetic material towards the cell but also assure the maintenance of its structural integrity. In fact the structural stability of the exogenous DNA is crucial for the success of the transgene expression (Oliveira et al., 2009). Hence, the encapsulation of genetic material inside the nanoparticle core is advantageous since it enhances the transfection efficiency, a prerequisite for the outcome of a clinical application.

Chitosan, a cationic biopolymer, has been extensively used for gene delivery applications and nanoparticle synthesis due to its unique properties such as low immunogenicity, low toxicity, biocompatibility and a high positive charge density determined by the amount of deacetylated amino groups - deacetylation degree (DD) (Mao et al., 2009). The development of milder encapsulation techniques like the ionotropic gelation (Csaba et al., 2009), a method based on the electrostactic interaction between the negatively charged DNA backbone and a cationic polymer in the presence of a polyanionic crosslinker as provided the basis for the maintenance of the genetic material intrinsic properties.
This work is based on the optimization of the nanocapsule manufacturing process through the modulation of the polymer deacetilation degree and concentration that in turn influences the genetic material encapsulation efficiency and nanocapsule size.

MATERIALS AND METHODS

A 6.07-kbp plasmid pcDNA3-FLAG-p53 (Addgene plasmid 10838, Cambridge, MA, USA) encoding for the tumor suppressor p53 was amplified in a cell culture of *E.coli* DH5α and purified using the Quiagen Plasmid Maxi Kit (Quiagen, CA, USA) according to the supplier's protocol. Subsequently the commercially available cationic polymer, chitosan (Sigma Aldrich, MO, USA), was subjected to an acetylation and deacetilation process before the nanocapsule synthesis as previously described by Huang *et al.,* 2005, and the polymer deacetylation degree measured by first derivative of UV-spectroscopy.

Chitosan nanocapsules were synthesized using the ionotropic gelation technique. Briefly the pDNA (13 µg/mL) was added to a 1mL tripolyphosphate solution (0.5mg, pH 5.5), the mixture was then added dropwise to 4mL chitosan solutions (0.75mg, pH 4.9), with variable deacetilation degrees, under magnetic stirring (300 ± 50 rpm). The nanocapsules formed were then recovered by centrifugation at 17000g and the particle morphology was characterized through scanning electron microscopy (SEM) at variable magnifications. The pDNA encapsulation efficiency (EE) was determined by UV- vis analysis, after centrifugation, by measuring the amount of unbounded DNA in the supernatant.

RESULTS AND DISCUSSION

The nanocapsules formed by the ionotropic gelation technique demonstrated well defined spherical shapes both for the commercial and deacetylated polymer, however less defined morphological characteristics were obtained for the acetylated chitosan (Figure 1).

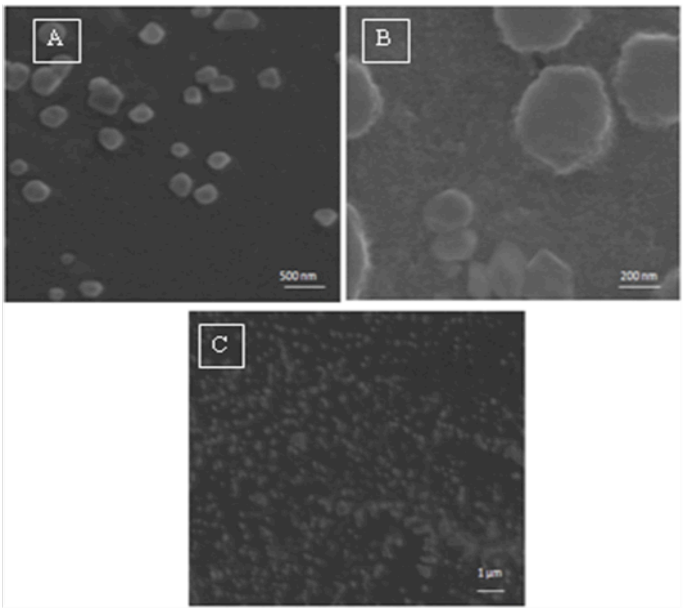

Figure 1 : Morphology of the nanocapsules obtained visualized by SEM. A.) Commercial; B.) Deacetylated; C.) Acetylated.

Analyzing the size differences between commercial (85% DD), deacetylated (98% DD) and acetylated (76% DD), chitosan nanocapsules, it can be observed that the mean particle size is smaller for the deaceylated polymer comparing with the acetylated sample (Table 1).

Table 1 : Nanocapsule particle sizes for the different chitosan formulations.

Sample	Ratio (v/v)	Particle Size (nm)
Acetylated	4:1	154 - 226
Commercial		191 - 303
Deacetylated		113 - 197

These findings are in agreement with those obtained by Huang et al, 2005, in which the decreasing of chitosan deacetylation degree from 88% to 46% resulted in the increase of the mean particle size from 181 to 239 nm (Huang et al., 2005). The encapsulation of the pDNA inside the core-shell structure of the nanocapsules is essential for the success of gene delivery and as the results in figure 2, demonstrate that the polymer deacetylation degree also influences the amount of pDNA that is included in the particle core.

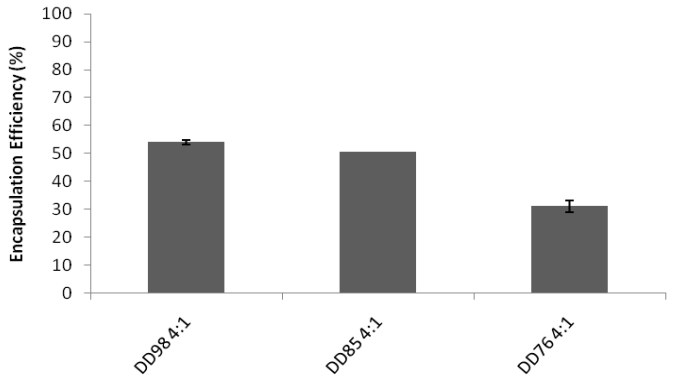

Figure 2 : Encapsulation efficiency of the nanocapsules obtained from different chitosan materials (mean ± SD, *n*=3). DD98 – Deacetylated chitosan sample; DD85 – Commercial chitosan sample; DD76 – Acetylated chitosan sample.

It is important to denote that the encapsulation efficiency of deacetylated nanocapsules is higher than that of the nanocapsules synthesized from commercial or the acetylated polymer. Our results are in agreement with those previously published by Kiang et al, 2004. They reported that the degree of deacetylation affects the DNA binding (Kiang et al., 2004).

CONCLUSIONS

This work describes the optimization of the ionotropic gelation technique that was used to synthesize biodegradable nanocapsules containing pDNA that encodes for a tumor suppressor protein, p53. The results obtained showed that the chitosan degree of deacetylatio as is the most important parameter to be controlled during the encapsulation process since in not only influences the nanocapsule size but most importantly it is also responsible for the amount of genetic material that can be included in the nanocapsule. Currently *in vitro* transfection experiments are being carried out to test the effectivness of the transgene expression in malignant cells. Overall these findings provide the basis for the future manufacture of specifically tailored nanocapsules intended for cancer gene therapy.

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