Development and characterization of chitosan- sodium deoxycholate nanoparticles

A. Cadete[1], C.C.R. Calado[2], A. J. Almeida[1] and L.M.D. Gonçalves[1]
[1]iMed.UL – Res Inst Med & Pharm Sc, Fac Farm, Univ Lisboa, Portugal; [2]Fac Eng Univ Católica Portuguesa, Rio de Mouro, Portugal.
accpires@ff.ul.pt

INTRODUCTION AND OBJECTIVES

Although the oral route of administration is the most commonly used for delivery of drugs, oral vaccine delivery still represents a major challenge. In order to improve the efficiency of mucosal vaccines, polymeric based nano and microparticles have been widely used as carrier systems for protein or DNA vaccines. (Chadwick 2010) Chitosan is one of the most promising polymers for drug delivery through the mucosal routes because of its non-toxic, polycationic, biocompatible, and biodegradable nature, and particularly due to its mucoadhesive and permeation-enhancing properties. (Kang 2009)

The use of chitosan delivery systems containing a bile salt, such as sodium deoxycholate, could be an effective strategy for improving the efficacy of an oral vaccine. Bile salts are known to interact with lipid membranes, increasing their permeability. So, the addition of bile salts to chitosan matrices may improve the delivery characteristics of the system, making it suitable for mucosal administration of bioactive substances. (Chae 2005) A few works have described chitosan and sodium deoxycholate microparticles and nanoparticles prepared by covalent attachment of deoxycholic acid to chitosan. (Kim 2005)

The aim of this study is the development of chitosan nanoparticles produced by ionotropic gelation with sodium deoxycholate and evaluate their potential as gene delivery carriers. The physicochemical properties of the particles were evaluated. Transfection studies were evaluated in cell cultures, which may be employed in future *in vivo* studies.

MATERIALS AND METHODS

Materials Chitosan low molecular weight (LMW), sodium deoxycholate, ciabacron brilliant red 3B-A, glycine, sodium chloride and HCl were obtained from Sigma-Aldrich, UK. All cell culture reagents were from Invitrogen, UK.

Preparation and physicochemical characterization of CS/DS nanoparticles CS/DS nanoparticles were prepared by ionotropic gelation technique. Different amounts of 1mg/ml DS were added to a solution of 1 mg/ml CS and stirred during 15 minutes at room temperature. Mean particle size and polydispersion index were determined by photon correlation spectroscopy (Zetasizer Nano-S; Malvern Instruments, UK). The zeta potential was measured by using electrophoretic mobility with a Zetasizer 2000 (Malvern Instruments).

The production yield of the nanoparticles was measured indirectly by a colorimetric method. (Muzzarelli 1988)

Encapsulation of plasmid DNA (pDNA) For the encapsulation, pDNA was added to the SD solution before the addition of CS solution. The theoretical DNA loadings were 10 and 20% with respect to the total amount of CS used for particle preparation. Encapsulation was assessed using agarose gel electrophoresis.

In vitro cell lines transfection The AGS and N87 (ATCC, USA), gastric carcinoma cell lines (moderately and well differentiated adenocarcinoma, respectively) were used in transfection assays. Cell cultures were performed according ATCC and HSRRB for the different cell lines at 37ºC/5.0% CO_2, humidified atmosphere. Cell transfection was performed in 24 well plates with nanoparticles containing pDNA expressing a "humanized" secreted Gaussia Luciferase as reporter gene (pCMV-GLuc, 5,7Kbp (pGLuc), New England Biolabs, USA). The activity of expression luciferase was quantified with a commercial kit (Gaussia Luciferase Assay kit, New England Biolabs, USA).

In vitro cell viability studies The end-point used on the cytotoxicity assays was the MTT reduction.

RESULTS AND DISCUSSION

Physicochemical characterization of CS/DS nanoparticles It was found that nanoparticle (NP) size and zeta potential are influenced by the amount of DS (Table 1).
Formulation number 3 was chosen for further studies (pDNA incorporations and cell culture experiments) regarding to small size of the particles, positive surface charge and formulation stability (results not shown).

Entrapment of plasmid DNA The incorporation of different amounts of pDNA (corresponding to 5, 10 and 20% loadings) into the NP increase the particle size but do not influence the transfection capacity of particles.
Independently of the amount, all the pDNA was incorporated into the NP. (Figure 1)

Table 1. Size and zeta potential of CS/DS nanoparticles (n=3); P.D.I. = polydispersion index

Sample	*CS:DS (W/W)*	*Average particle size (nm)*	*P.D.I.*	*Zeta Potential (mV)*	*Yield CS(%)*
1	1 : 1.67	403 ± 39	0.266	+56.9 ± 0.6	38.5
2	1 : 1.25	385 ± 45	0.219	+52.3 ± 0.8	41.5
3	1: 1	350 ± 27	0.275	+53.8 ± 0.6	42.4
4	1: 0.5	210 ± 10	0.302	+46.2 ± 0.5	36.9
5	1 : 0.25	153 ± 5	0.345	+33.0 ± 1.6	50.1

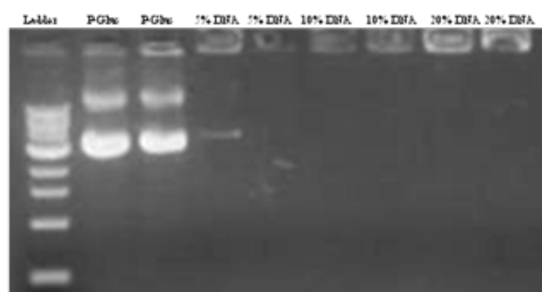

Figure 1. Agarose gel electrophoresis of chitosan- sodium deoxycholate nanoparticles with different amounts of pDNA (5, 10 and 20%). All the pDNA was loaded into de NP.

In vitro transfection efficiency of CS/DS nanoparticles

With this assay it can be evaluate three different parameters: (1) the efficiency of CS/DS nanoparticles to transfect gastric epithelial cells (GEC); (2) the influence of transfection time (1h and 24h) and (3) the effect of pDNA loading (10% and 20%).

As shown in Table 2, CS/DS nanoparticles containing encapsulated pDNA were able to transfect both AGS and N87 cell lines, being more effective with AGS cells, the less differentiated cell line. The highest enzymatic activity was achieved with 20% pDNA encapsulated and after 24h of transfection time. Furthermore, irrespectively of pDNA loading, enzymatic activity increases with time.

Table 2. Luciferase expression by different cell lines transfected with CS:DS (1:1) nanoparticles loading 10% and 20% (w/w) pDNA after one and 24 hours of transfection.

Post transfection time		2 days		3 days		9 days	
DNA loading	T.T.	AGS*	N87*	AGS*	N87*	AGS*	N87*
10%	1h	9189	72	121613	274	129924	563
10%	24h	13639	115	103291	311	172275	931
20%	1h	50196	434	208589	862	104559	222
20%	24h	333925	539	1406831	1241	449709	890

* Relative Luminescent Units (RLU)/ 10^5 cells in 24h; T.T. means transfection time

In vitro cell viability studies

No evidence of cytotoxicity was observed for the CS/DS nanoparticles (with and without pDNA) and a cell viability of 80% and 90% was observed in AGS and N87 cell line, respectively, when compared with a control.

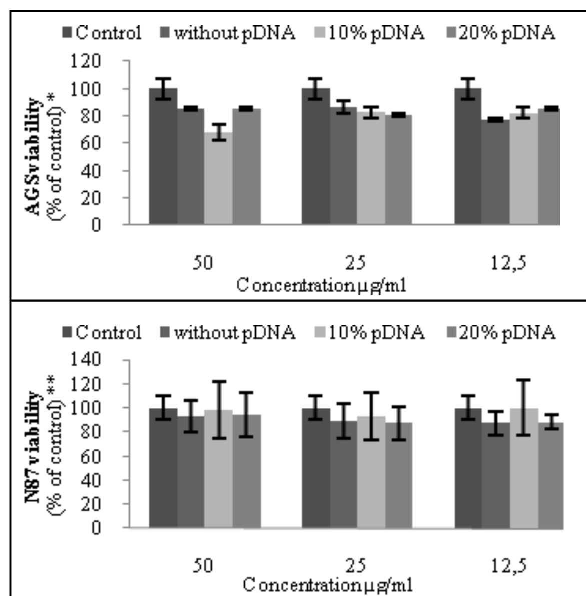

Figure 2. Viability of AGS and N87 cells measured by the reduction of MTT. Results represent the mean ± SD (n=5). Statistical differences between the control group and formulations are reported as: * p<0,001, ** p>0,05. Cell viability (% of control) = [A] test/ [A] control x 100

It can be concluded that it is possible to formulate chitosan-sodium deoxycholate nanoparticles using a suitable method and they are a new potential vehicle for oral delivery of pDNA.

REFERENCES

- Chadwick S et al (2010) *Nanotechnology solutions for mucosal immunization.* Adv Drug Del Rev (62) 394–407
- Kang ML et al (2009) *Application of chitosan microspheres for nasal delivery of vaccines*. Biotechnol Adv (27) 857-865
- Chae SY et al (2005*) Deoxycholic acid-conjugated chitosan oligosaccharide nanoparticles for efficient gene carrier* J. Cont Rel (109) 330-334
- Kim K et al (2005) *Physicochemical characterization of self-assembled nanoparticles of glycol-chitosan-deoxycholic acid conjugates* Biomacromolecules (6) 1154-1158
- Muzzarelli RAA (1988) *Colorimetric determination of chitosan*. Anal Biochem (260) 255-257

ACKNOWLEDGEMENTS

This work was supported by Fundação para a Ciência e Tecnologia (Portugal) (PTDC/BIO/69