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Preparation, characterisation and cell transfection of cationic liposomes in gene therapy

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SUMMARY

Cationic lipid-mediated gene transfer is one of the most commonly used non-viral vectors. It has been shown to be a safe and effective carrier. However, its use in gene delivery was hampered by its low transfection efficiency and stability. DOTAP, DOPE, cholesterol (CHO) and carboxymethyl-β-cyclodextrin (CD) were used to prepare cationic liposomes. Cationic liposomes were prepared using both, thin film hydration and a microfluidic method. Formulation stability was evaluated using liposome size, zeta potential and polydispersity index (PDI). Promega QuantiFluor® ONE dsDNA System was used to investigate the encapsulation efficiency. COS7 and SH-SY5Y cell lines were used to determine transfection efficiency. Results show that carboxymethyl-β-cyclodextrin increased encapsulation efficiency by 15.5% and 8% using NanoAssemblr™ and rotary evaporator, respectively compared to liposomes without CD. The addition of carboxymethyl-β-cyclodextrin to cationic liposomes resulted in an increase in transfection efficiency in both cell lines.

INTRODUCTION

Liposomes are a form of spherical vesicles that consist either of one, few or many phospholipid bilayers interacting with one another in an energetically favourable manner. Generally, liposomes form by self-assembly of dissolved phospholipid molecules. Each of which consists of a hydrophilic head group attached to hydrophobic tail by a linker (Akbarzadeh et al., 2013). The amphiphilic nature of these lipid molecules causes them to form bilayers spontaneously in aqueous environments. This results in a small spherical structure in which the surface polar heads shield the non-polar interior against water. The presence of a positively charged amine group facilitates binding with anions such as those found in DNA. In addition, liposome formulations are approved to be a safe carrier and have already been used worldwide in different therapeutic and vaccine products.

MATERIALS AND METHODS

DOTAP, DOPE and cholesterol (Lipoid Germany) with a molar ratio of 8:8:2 were dissolved, with 2mL ethanol. carboxymethyl-β-cyclodextrin were dissolved in distilled water at a concentration of 4mg/mL. using film hydration or a microfluidic method to prepare liposomes at a concentration of 10mg/mL (Table 1 shows formulations). Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcs., UK) was used to measure liposome size, zeta

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potential and PDI. Promega QuantiFluor® ONE dsDNA System was purchased from Promega (UK), and used to determine encapsulation efficiency. Gel electrophoresis at a concentration of 0.9% was used to measure binding efficiency and protection against DNase. Transfection efficiency was measured by transfecting pc DNA3.1-GFP to COS7 and SH-SY5Y cell lines using flow cytometer (FACS) BD Accuri C6 plus (Becton Dickinson Bioscience, USA) and fluorescence microscopy.

RESULTS AND DISCUSSION

Cationic liposomes transfection efficiency can be affected by the composition of the transfection reagent, liposome size and zeta potential, as well as liposome to DNA ratio. As documented in the literature a decrease in liposome size reduces complement recognition with liposome size between 70 and 200 nm.

Results in Table 1 revealed the difference in size for liposomes prepared by NanoAssemblr™ and rotary evaporator. Liposomes prepared by NanoAssemblr™ were between 79nm and 161nm, for the rotary evaporator liposome size was between 109nm and 294nm. Zeta potential (ζ) results (Table 1) revealed that all formulations were positively charged, before and after the addition of pDNA. Results of lipoplexes were close to the value obtained from liposomes alone; however there was a noticeable reduction in the zeta potential following the addition of pDNA. This could be a result of the electrostatic interaction between the cationic lipid and the negatively charged backbone of the pDNA.

Table 1. *Particle size and zeta-potential for formulations prepared by NanoAssemblr™ and rotary evaporator.*

No.	Composition	NanoAssemblr™		Rotary Evaporator	
		Size, nm	Zeta-potential, mV	Size, nm	Zeta-potential, mV
F1	DOPE+ DOTAP+CHO	79	44.1	247	56
F2	DOPE+DOTAP + CHO+ DNA	152	42.1	109	48
F3	DOPE+ DOTAP +CHO+CD	95	27.6	287	52
F4	DOPE +DOTAP +CHO+ CD+ DNA	161	24.5	186	45

In order to assess DNA protection and its degradation in the presence of DNase I, agarose gel electrophoresis of pDNA was used as a qualitative measure of DNA stability. As shown in Figure 1 free plasmid DNA was completely hydrolysed, lane (2) however all lipoplex formulations have shown protection from DNase I, lanes 4-6. After breaking liposomes shell and adding DNase, lanes 8-10, DNase was able to hydrolyse the pDNA, (lane 3 is empty liposomes). The expression of GFP following the transfection of COS7 and SH-SY5Y cell lines using different lipoplex formulations was assessed with fluorescence microscopy (Figure 2) and quantified by flow cytometry. The highest level of GFP expression was noticed after the addition of carboxymethyl-beta-cyclodextrin to cationic lipid DOTAP, DOPE and cholesterol.

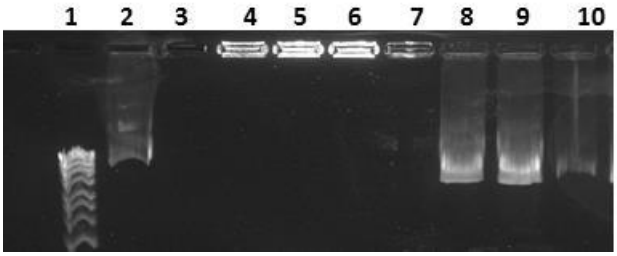

Figure 1. *Gel electrophoresis images after the addition DNase for 30min before and after breaking the liposomes using chloroform/methanol 2:1.*

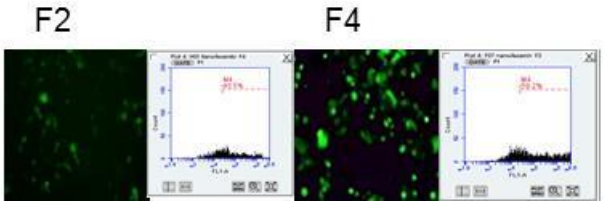

Figure 2. *Transfection efficiency for different liposomes formulations determined using fluorescence microscopy images and flow cytometry.*

CONCLUSIONS

The addition of carboxymethyl-β-cyclodextrin to cationic liposomes resulted in an increase in transfection efficiency. The NanoAssemblr™ method was shown to produce smaller, homogeneous liposomes with a low PDI compared to the rotary evaporator.

REFERENCE

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