Multilayered ABCD Nanoparticles for siRNA delivery

M Thanou [1] and A Miller [1,2]

[1]Imperial College Genetic Therapies Centre, Chemistry Department, Imperial College London, UK and
[2]Imuthes 21 Wilson Street, London EC2M 2TD, UK

ABSTRACT

Therapeutic small interference RNA is a method to treat human diseases by addressing targets that are not possible to be addressed with conventional medicines. In our Centre we design nanosize systems for siRNA delivery that consist of various components organised in concentric layers (ABCD nanoparticles). The core (A) consists of siRNA surrounded by a layer of de-novo prepared and commercially available lipids (B layer). The AB particle is surrounded by a polymer, C-layer (stealth) which is inserted in the lipid either as an amphiphile or post- coupled using aminoxy coupling techniques. The nanoparticles may be decorated (D-layer) with ligands to target receptors usually found in solid tumours or in the liver. Ligands are introduced on the surface of the Nanoparticles using similar coupling methods. We have found that the molar composition of the ABCD components can have an effect on the efficiency of the nanoparticles to transfer siRNA *in vitro* or *in vivo*. We prepared nanoparticles that were able to substantially knock down genes on leukemia cells (Jurkat) a cell line commonly characterized hard to transfect

Keywords: siRNA, ABCD nanoparticle, siRNA delivery

1 INTRODUCTION

Small interference RNA or siRNA is widely accepted as a potential strategy for therapy of a number of diseases (1). However siRNA like any genetic drug, encounters the problem of efficient delivery and cell uptake. Due to its large molecular weight (~13 kDa) and polyanionic nature (~40 negative phosphate charges), naked siRNA does not freely cross the cell membrane, and thus delivery systems are required to facilitate its access to its intracellular sites of action (2). It is widely accepted that cellular uptake of siRNA occurs via nonreceptor-mediated endocytosis. Therefore delivery systems are typically used to increase cellular accumulation of siRNA molecules and to facilitate release from endosomes to the cytosol. In our group we use assemblies of lipid and lipid amphiphiles structured in concentric layers (Figure 1) (3). Special emphasis is given at the choice of components and assembly techniques of the various layers. Using a bottom up approach, a tool kit of chemical components and coupling reactions, ABCD nanoparticles are designed and prepared for various biological targets. We present here an example of particle design for a specific cellular target. Jurkat (leukemia) cells are considered hard to transfect. Electroporation can be used however with negative effects on cell viability.

In this study we design ABC Nanoparticles to transfect Jurkat suspension cells. Emphasis is given to the lipid eB layer and the presence of stealth C layer.

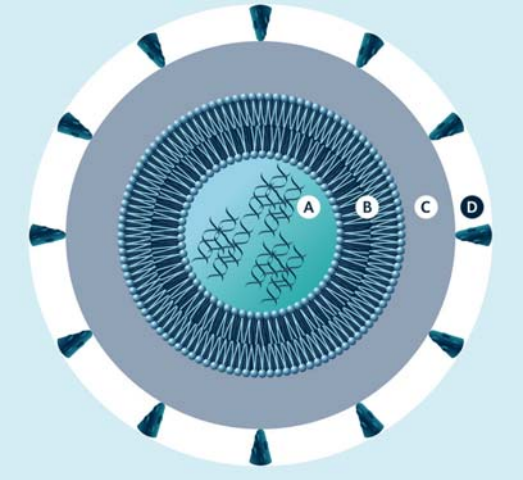

Figure 1. ABCD Nanoparticles; A is the core containing siRNA, B is the lipid envelope, C is the stealth layer and D is the targeting ligand.

2 FUNCTIONAL DELIVERY OF SIRNA IN VITRO

2.1 Materials

Jurkat Cells (clone E6-1, ATCC) were seeded at 100.000 cells/well seeding density. Jurkat cells were cultured in suspension in RPMI containing fetal bovine serum and antibiotics. siRNA-**ABC** nanoparticles were prepared from DODAG **(***N',N'*-dioctadecyl-*N*-4,8-diaza-10-aminodecanoylglycine amide; Imuthes), DOPC (dioleoyl phosphatidylcholine; Avanti polar lipids) and Cholesterol (Sigma) with DSPE-PEG^{2000} (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-polyethylene glycol2000; Avanti polar lipids). GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) siRNA and fluorimetric analysis kit were from Ambion.

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2.2 Experimental Methods

Jurkat Cells (clone E6-1, ATCC) were seeded at 100.000 cells/well seeding density. Jurkat cells were cultured in suspension in RPMI supplemented by fetal bovine serum and antibiotics. Two different siRNA-ABC nanoparticles were prepared using DODAG (as cationic lipid), DOPC, Cholesterol (both as helper lipids in the B layer) and DSPE-PEG2000 (to form the C-layer). The following BC nanoparticles were prepared at the mentioned % molar ratio at approx. 3mg/ml:

1) DODAG/DOPC/Chol/DSPE-PEG^{2000}, 20:60:19:1 mol%
2) DODAG/DOPC/Chol/ DSPE-PEG^{2000}, 50:30:19:1 mol%

Both cationic BC nanoparticles were prepared by dehydration and rehydration of lipid films in deionized water or low ionic strength buffer followed by incubation at 37°C for 30 min with mild to strong sonication. Cationic BC nanoparticles 1 and 2 were combined with siRNA (lipid:siRNA, ratio 13:1, w/w) to form siRNA ABC nanoparticles for delivery of siRNA to suspension cells at different concentrations (1, 6 and 12nM/well; 1, 6 and 12 pmol/well respectively). The siRNAs used were either anti-human Glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH, RNA+) or the corresponding RSC control (RNA). Before siRNA delivery, Jurkat cells were transferred to Optimem and then cells were incubated for 4 h in the presence of either of the siRNA ABC nanoparticles described above, prior to the addition of full media. Cell lysis was performed 24h after siRNA delivery and lysate was analysed for GAPDH content using an Ambion assay (KD-alert) and a Varioskan fluorescence plate reader. Gene knockdown was calculated as:

$$\%knockdown = 100 - \left(100x \frac{\Delta fluorescence_{GAPDH}}{\Delta fluorescence_{Neg}} \right)$$

2.3 Results

In this experiment Jurkat cells were incubated with various DODAG-containing siRNA-**ABC** nanoparticles. For housekeeping genes such as GAPDH, the rate of cell division and concomitant synthesis of the housekeeping protein during the transfection experiment also have an impact on knockdown levels. siRNA was incubated with Jurkat at different concentrations using two different Nanoparticles. Not all conditions gave substantial siRNA transfer and gene knockdown either due to inefficient cell uptake or due to cytotoxicity caused by the high levels of siRNA and nanoparticles. The conditions that gave substantial knockdown are shown in Figure 2.

Levels of GAPDH in cells post siRNA-**ABC** nanoparticle-mediated siRNA delivery are shown (Table **1**). The most effective knock down was achieved with the siRNA-**ABC** nanoparticle system prepared with 50mol% of DODAG at the lowest delivered dose of siRNA (1 pmol/well) (see Figure **2**). By way of benchmark, lipofectAMINE2000 (Invitrogen) failed to mediate functional siRNA delivery to Jurkat cells under these circumstances. This may be due to the relatively high cytotoxicity of lipofectamine formulations.

GAPDH	DODAG 20% +12pmol	DODAG 50% +1 pmol	DODAG 50% +6pmol	lipofect amine +1pmol
siRNA+	0.042	0.020	0.030	0.037
siRNA -	0.053	0.051	0.042	0.042

Table 1. Levels of GAPDH after application of DODAG nanoparticles in Jurkat cell suspensions

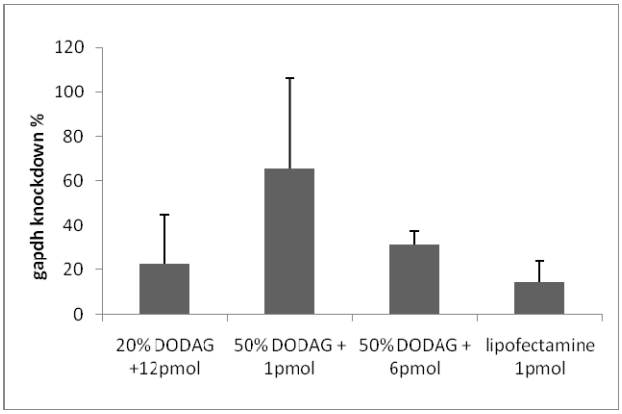

Figure 2. GAPDH knockdown in Jurkat human T-lymphocytes. Cells were seeded at 10^5 cells /well and transfection was allowed for 4 h in Optimem.

The nanoparticles tested in this study were below 100nm diameter with 100% siRNA encapsulation efficiency and zeta potential 10 and 45 mV for 20 and 50% DODAG, respectively.

2.4 Discussion

Jurkat cells are derived from a human acute T-cell leukemia line and are used extensively in the study of T cell signaling and cancer drug development. Jurkat cells are hard to transfect cells for both DNA and siRNA. In this study we present designed ABC Nanoparticles that at

certain concentrations were able to transfer siRNA into the cells.

Previous experiments in our group had focused in screening a number of different composition ABC nanoparticles. One important observation in these experiments was that suspension cells (e.g. Jurkat) are transfected by ABC nanoparticles only. This is in contrast with the general findings that polyethylene glycol corona is decreasing gene transfer in cells. It is likely that in the current situation of suspension cell lines PEG layer served colloidal stability and kept particles below 100nm throughout the experiment. This colloidal stability was observed on ABC nanoparticles that contained DODAG in combination with DOPC and Cholesterol in the B layer.

In this study we assess the levels of GAPDH protein in the cells. In general, efficient knockdown will cause a more dramatic reduction in target mRNA levels than in target protein levels. This is due probably because protein knockdown is influenced by the rates of protein synthesis and turnover, in addition to the rates of target mRNA synthesis and turnover. In the present study a 60% knockdown of GAPDH protein could be observed for a positively charged low siRNA concentration formulation. It appears that for Jurkat cells, a high charge but stealth ABC nanoparticle is required for siRNA cell uptake.

3 CONCLUSIONS

ABC Nanoparticles are assembled using designed chemical components. Their modular character allows the versatility and adaptability for any biological target. ABC nanoparticles show substantial colloidal stability and are able to transfer siRNA in Jurkat cells for functional gene knockdown.

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REFERENCES

1) Interfering with disease: a progress report on siRNA-based therapeutics. de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J. Nat Rev Drug Discov. 2007, 6(6):443-53.
2) Nonviral delivery of synthetic siRNAs in vivo. Akhtar S, Benter IF. J Clin Invest. 2007 117(12):3623-32.
3) Synthetic, self-assembly ABCD nanoparticles; a structural paradigm for viable synthetic non-viral vectors. Kostarelos K, Miller AD.Chem Soc Rev. 2005, 34(11):970-94.