

POLYAMIDOAMINE (PAMAM) POLYMERS AS NANOCARRIERS FOR DNA DELIVERY

Navarro, G. and Tros de Ilarduya, C.

*Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy,
University of Navarra, Pamplona, Spain
gnavarro@alumni.unav.es*

The application of nanotechnology to biomedical research is expected to have a major impact leading to the development of new types of diagnostic and therapeutic tools. One focus in nanobiotechnology is the development of nonviral vectors for gene therapy. The goal of gene therapy is to introduce foreign genes into somatic cells to supplement defective genes or provide additional biological functions, and can be achieved using either viral or synthetic non-viral delivery systems. Compared with viral vectors, synthetic gene delivery systems offer several advantages including reduced risk of cytotoxicity and low immunogenicity, but their use has been limited by the relative low transfection efficiency [1].

Cationic polymers are one of the most widely used vectors in non-viral gene delivery [2,3]. Polyamidoamine (PAMAM) dendrimers are cationic polymers characterized by a branched spherical shape and a net positive charge at physiological pH. The spherical structure is achieved by the assembly of polymer subunits in concentric spheres around an initiator core. The size of the molecule is determined by the number of layers or generations present in the polymer. Each generation enlarges the size and increases the charge [4].

The main objective of this study is the development, optimization and characterization of a non-viral vector composed of PAMAM dendrimers (generation 4 and 5) and DNA, which can deliver genetic material into tumour cells. The DNA-dendrimer complexes were prepared by mixing a solution of different generations of PAMAM with plasmid DNA (pCMVLuc). The quantities of both components were calculated in order to prepare complexes at charge ratio(+/-) from 1/1 to 10/1 (dendrimer/ DNA).

Complex size and zeta-potential were measured by dynamic light scattering in a Zetasizer-nanoparticle analyzer. The size of complexes prepared with G4 PAMAM ranged from 150 to 170, while complexes prepared with the generation 5 showed slightly smaller values (around 100 nm). The zeta potential was positive in all cases, except for the ratio 1/1 (electroneutrality point).

In vitro transfection efficiency was determined by measuring the luciferase expression in HepG2 cells. The cells were incubated with complexes for 4 h in the presence of 60% FBS. The complexes were then removed and the medium was replaced. After 48 h of incubation, cells were lysed and luciferase expression was measured by a luminometer. The transfection levels increased by increasing the charge ratio in the complexes in both generations, with maximum level at charge ratio 10/1 (Figure 1). Cell viability was determined by Alamar blue assay and the results showed a viability higher than 80% in all the complexes prepared.

References:

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Figures:

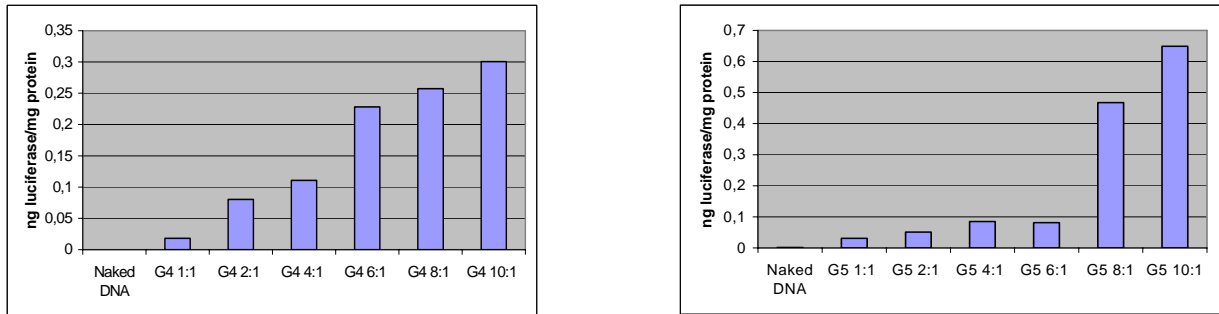


Figure 1. Transfection of HepG2 cells with PAMAM–DNA complexes at different charge ratios (+/-) in the presence of 60% FBS. Generation 4 (left) and Generation 5 (right) of polyamidoamine dendrimers were tested