Thematic day on viral vectors
17 May 2019
ICM, La Pitié Salpêtrière, PARIS

Transfection efficiency and toxicology Assessment of Poly-L-lysins Dendrimer as non-viral vectors.

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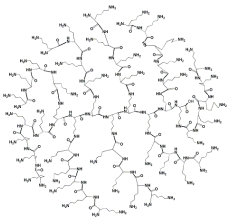
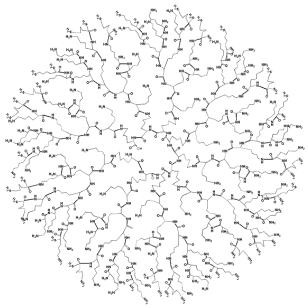
Comprehensive cancer curative treatments are based on combined chemotherapy, radiotherapy and immuno--therapy as well as recently adoptive cellular therapy. During the past three decades, enormous research in the area of gene delivery has been conducted worldwide, in particular for cancer gene therapy applications. Retroviral and lentiviral vector used for cell transfection pose certain safety concerns (1).
Non-viral PEI (Polyethyleneimine) vector used for T cell transfection induces the disruption of the cell membrane and disruption of the mitochondrial membrane after internalization can lead to delayed apoptosis (2). In this poster, we present the results of study on poly-L-lysins dendrimer vector for human cells transfection.

Methods: G2 to G5 dendrimers and PEI were respectively complexed with pEGP-C1 plasmid (473pb).
The capability of these compounds to form stable nanoparticles with plasmid has been demonstrated by gel electrophoresis, transmission electron microscopy (TEM), and dynamic light scattering (DLS) investigations.
Transfection efficiencies of the self-assembled have been evaluated in vitro on HeLa cells and human fibroblast using confocal florescence microscopy.



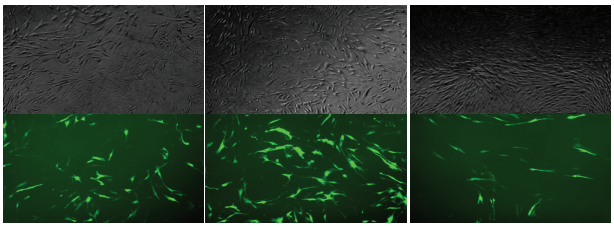
G2
50 lysines – 4,5 nm



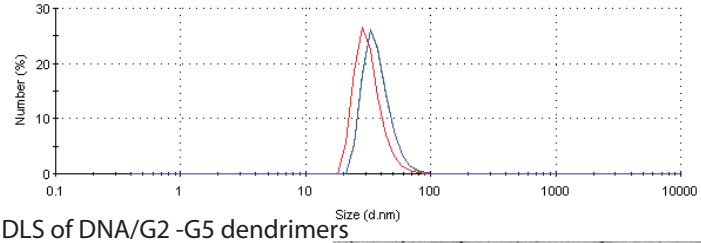
G3
120 lysines – 7 nm

Generation	G1	G2	G3	G4	G5
Structure	Linear	DGL	DGL	DGL	DGL
Molecular Mass (kDa)	1.5	8.6	22.0	65.0	170.0
Diameter (nm)	2	4.5	7	11	16
Number of groups	8	50	120	360	1000

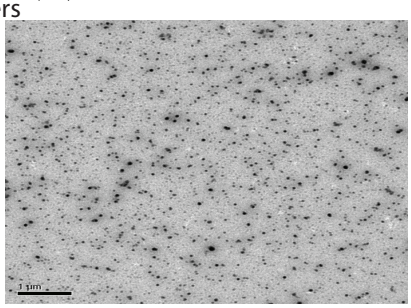
Dendrimers (DGL) Characterization



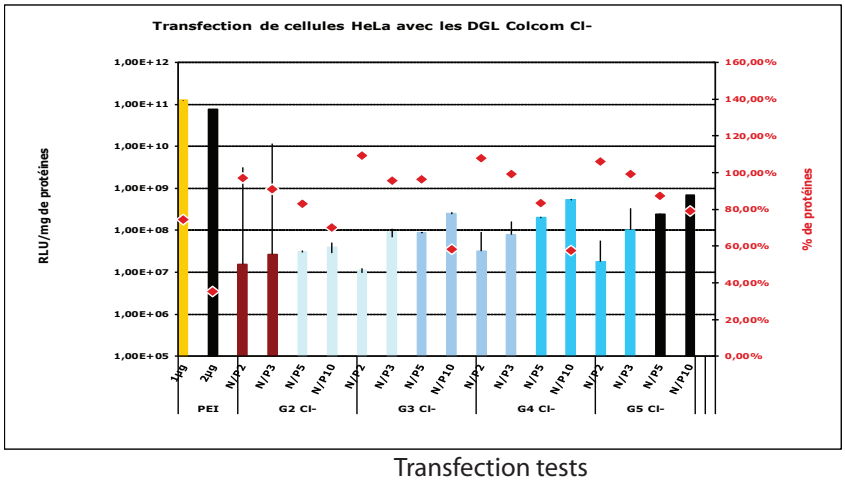
Transfection of human fibroblats by plasmid pEGFP-C1 (4731 pb) plasmid using DGL-G3



Analysis by DLS of DNA/G2 -G5 dendrimers (N/P=5) in 5% Glucose



Polyplexe DGL-G5 + DNA (N/P=5) viewed by TEM



Transfection tests

Results: Both toxicity and transfection efficiency increase with the overall cationic charge of the Dendrimer/DNA complex (N / P ratio) and the size of the dendrimer: G3 dendrimer (N/P=5) is optimum compromise. G3 Dendrimer compounds show transfection efficiency (>75%) and very low toxicity. Indeed, DGL-G3 transfected cells exhibit their functions and subcellular structures intact compared to those transfected by other synthetic vectors (as PEI).

Conclusion: Low toxic poly-L-lysins dendrimer as vector would present tremendous interest in the gene and cellular therapy field. Further works devoted to optimization of human cell transfection are in progress.

Bibliography

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2. Moghimi S. M . A two-stage poly(ethylenimine)-mediated cytotoxicity: implications for gene transfer/therapy. Mol. Ther 2005; 11: 990–995