Polyallylamine functionalized with dextran for siRNA delivery.

Sofia Zuffi[1], Aharon Steffé[2], Francesca Buco[2], Angela Roberta Cortese[2], Mario Di Pilo[1], Patrizia Andreozzi[2], Marco Marradi[2], Sergio Moya[1]

[1]CICbiomaGUNE – Soft Matter nanotechnology Group, Paseo Miramon 182, Edificio C, 20014 Donostia-San Sebastian, Spain.
[2]Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3/13, Sesto Fiorentino, Florence 50019, Italy

szuffi@cicbiomagune.es

Background

Small interfering RNA (siRNA) is a small RNA molecule able to inhibit the expression of a target protein via degradation of its messenger RNA, impairing its translation. siRNA technologies have a great potential not only in basic research but also in gene therapy [1].

Cationic polyelectrolytes can complex with nucleic acids and deliver them inside cells. Upon endocytosis and liberation of the cargo polycation with protonable amines induce an osmotic swelling in the acidic environment of the endosomes that facilitates siRNA translocation into cytoplasm where must act on mRNA. [2].

Polyallylamine (PAH) is an interesting system for intracellular delivery of siRNA. Its positive charges can efficiently interact with both nucleic acids (cargo) and cell membrane (target). Also, it responds to pH variations allowing for the liberation of the complexed siRNA in the cytoplasm upon acidification of the endosome. However, positive charges induce toxicological endpoints, limiting the application of complexes of PAH *in vivo* [3, 4]. Besides complexing nucleid acids, PAH forms nanoparticles in presence of phosphate buffer. These nanoparticles have an interesting pH response, being stable in a narrow pH range between 6 and 8 and dissolving in a reversible way at lower and higher pHs.

Aim

Given the toxicity of the positive charges of PAH, we are testing PAH functionalized with dextran. Dextran is a non charged biocompatible molecule, and we hypothesize that after complexation of dextran functionalized PAH with siRNA a protective dextran layer will form around the nanoparticle, shielding positive charges, reducing toxicity, and/or favouring other endocytic pathways. We have explored the formation of nanoparticles of dextran modified PAH in phosphate buffer and the complexation of the polymers with siRNA.

Methods

PAH was substituted with different ratios of dextran chain/polymer chain. Dextran functionalized PAH was then complexed with siRNA and complexes characterized via dynamic light scattering and transmission electron microscopy. Gel retardation assay was used to confirm nucleic acids encapsulation.

The toxicity of the system was studied in two different cell lines: Jurkat, a model for suspension cells, and A549, lung carcinoma adherent cells.

Results

DLS studies revealed that the less-substituted polymers form nanoparticles in the tested buffers. Nanoparticle dimensions are dependent on ionic strength: overall, the higher the concentration of salts, the bigger the dimensions of nanoparticles. On the contrary, highly substituted polymers do not form nanoparticles,.

DLS studies also revealed that the range of pH stability of the nanoparticles varies with the degree of substitution.

Transmission electron microscopy (TEM) allowed for the visualization of the morphology of the assemblies. Depending on the number of dextran chains attached to PAH we observe the formation of nanoparticles or capsules, with an empty core.

Next, polymers were complexed with siRNA and complexes studied via DLS. Gel retardation assay proved the encapsulation of siRNA for different N/P ratios.

Nanoparticles in absence and presence of siRNA were studied in Jurkat cell line to evaluate their cytotoxicity profile. Toxicity was observed only at high concentrations, while the systems loaded with siRNA showed an excellent profile, with cell metabolic activity always above 80% when compared to the control. Similar results were obtained in A549.

Conclusions

We show here the formation of assemblies of dextran modified PAH in phosphate buffer and in presence of siRNA. The dextran modified PAH forms different structures through association with phosphate buffer depending on the number of dextran chains present. The polymer complexes siRNA efficiently and the presence of dextran reduces toxicological endpoints in vitro.

References

[1] Whitehead, Ket et al., Nat Rev Drug Discov 8, (2009) 129–138.
[2] Vijayanathan V. et al., Biochemistry, 41(48), (2002), 14085-94.
[3] Andreozzi P. et al., ACS Appl. Mater. Interfaces 9(44), (2017), 38242-38254.
[4] Di Silvio D. et al., J. Colloid Interface Sci., 557, (2019), 757-766

Figures

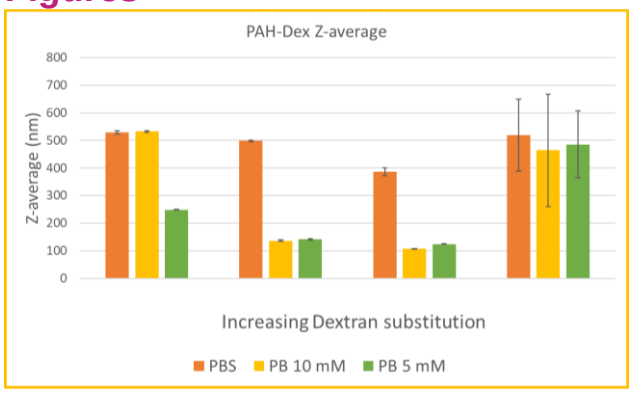

Figure 1. Dynamic light scattering of different dextran substituted polymers in different phosphate-containing buffers. Error bars represent standard deviation.