Self-Assembly of Polyamine Phosphate Nanocarriers (PANs) for Intracellular siRNA Delivery

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Silencing RNA (siRNA) technologies emerge as a promising therapeutic tool for the treatment of multiple diseases. However, the medical translation of this technology is hampered by the lack of effective delivery vehicles for siRNAs in cytosol that prevent their degradation in the bloodstream. The use of molecular complexes based on polyamines have great potential for siRNA delivery as polyamines can protect the siRNA during circulation and at the same time favor siRNA translocation in cytosol. Here, we show a novel application of polyamine phosphate NCs (PANs) based on their capacity to load negatively charged nucleic acids and their pH stability. PANs are fabricated by complexation of phosphate anions from phosphate buffer solution (PB) with the amine groups of poly(allylamine) hydrochloride as carriers for siRNAs. Once internalized in cells following endocytic pathways PANs disassembly at the low endosomal pH and release the siRNAs into the cytoplasm. Confocal Laser Scanning Microscopy (CLSM) experiments show that siRNAs are released from the PANs.[1] The stability of PANs at different pHs and in cell media is studied by fluorescence cross-correlation spectroscopy (FCCS). FCCS studies show that the nanoparticles are stable at physiological pH and in cell media but they disassemble at acidic pH.[2] Co-localization experiments with labeled endosomes and either labelled PANs or siRNAs prove the translocation of PANs and siRNAs into the cytosol, As a proof of concept, it is shown that PANs with encapsulated green fluorescence protein (GFP) siRNAs are able to silence GFP in A549 cells expressing this protein. Silencing efficacy was evaluated by flow cytometry, CLSM and western blot assay[1] (Figure 1). These results open the way for the use of poly(allylamine) phosphate nanocarriers for the intracellular delivery of genetic materials.

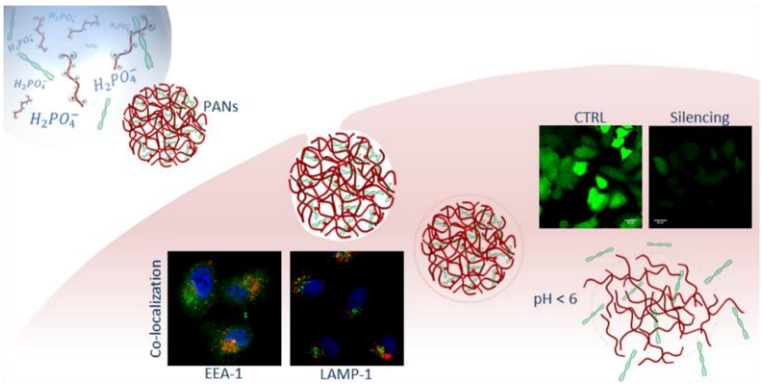

Figure 1. Scheme of PANs formation and intracellular delivery of siRNA.

References

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