Polyethyleneimine-based carbon dots for siRNA delivery

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Résumé

Carbon dots (CDs) are spherical carbon nanoparticles (NPs) that exhibit advantageous properties, including small size ($<$ 10 nm), solubility and stability in aqueous media, and photobleaching-resistant intrinsic fluorescence. These properties make them attractive for bioimaging and drug delivery applications. Small interfering RNAs (siRNA) are double-stranded non-coding RNAs (21-24 bp) that can silence any gene in a specific manner by operating within the RNA interference pathway. These nucleic acids stand as promising therapeutic tools, but their clinical use is limited by several major obstacles. Due to plasma enzymes, renal clearance or macrophage phagocytosis, their presence in the bloodstream and tissues is only transient. Furthermore, their size and negative charge prevent their spontaneous passage across the plasma membrane. As well, once internalised in cell by endocytosis, siRNAs tend to remain sequestered in endosomal compartments, where they can be degraded before reaching the cytoplasm. Consequently, the use of vectors to protect siRNAs, facilitate their delivery into cells and promote their endosomal escape is a potentially fruitful avenue for siRNA therapies. In the present work, we investigated the siRNA delivery potential of a library of 7 CDs produced from citric acid and polyethyleneimine (PEI) of varying molecular weight under different pyrolysis conditions (pH, temperature, activation mode). We characterised the size, charge and fluorescence properties of the various CDs, assessed their capacity to complex siRNA, and investigated their transfection activity and safety in two cell lines expressing the green fluorescent protein (GFP) and/or luciferase (Luc). All the CDs were able to complex siRNA. Two of them, made from PEI 600 or PEI 1200, demonstrated a potent silencing activity ($>$ 70%) with a low toxicity (viability $>$ 80%), as assessed by measurements of the luciferase activity (bioluminescence) in the U87 GFP/Luc and A549 Luc cell lines, and the cell viability (MTT assay). The silencing activity was confirmed by showing a decrease in GFP fluorescence in U87 GFP/Luc cells, by confocal microscopy. To further improve the transfection efficiency of these CD, the next step will be to functionalise them with a photosensitiser (PS), capable of producing singlet oxygen under light irradiation. Light irradiation of complexes made of PS-doped CDs will promote decomplexation of the siRNA payload and its release into the cytosol, by cleavage of a oxygen-sensitive linker, releasing the cationic charges on the NPs, and by destabilization the endo/lysosomal membrane, respectively.

Mots-Clés: Carbon Dot, Nanoparticle, Vector, siRNA delivery, Gene silencing

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