450b Cellular Interactions and Transport Mechanisms of Dendrimer-Based Nanodevices

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Dendrimers are unique and well-defined nanostructured (5-15nm) and highly branched polymers that have gained wide attention as promising intracellular drug delivery vehicles . They are characterized by a central core and multiple functionalities at the periphery. The surface functionality can be ‘tailored' to attach a variety of drugs, genes, targeting agents and imaging agents either by complexation or conjugation. Cellular interactions and cytotoxicity of dendrimers are mainly governed by the physicochemical properties including the size, generation, surface functionality and the net charge on the dendrimer. A number of literature reports deal with the applications of dendrimers for intracellular drug delivery, however, the endocytotic uptake of dendrimers have not been well characterized. The present study focuses on the dynamics of endocytotic uptake of PAMAM dendrimers in A549 lung epithelial cells as a function of surface functionality and charge.

Different PAMAM dendrimers including G4 amine terminated, G4 hydroxyl terminated and G3.5 carboxyl terminated were labeled with fluoroisothiocyanate (FITC) using either DCC or EDC as a catalyst. The cell uptake studies were carried out using A549 lung eiphtelial cells and the intracellular fluorescence was quantified using flow cytometry. Intracellular localization of dendrimers was imaged using fluorescence and confocal microscopy. Inhibition studies were carried out to identify the endocytotic pathway for the cellular uptake of different dendrimers. Temperature block and metabolic inhibition (sodium azide and 2-deoxyglucose) were used to investigate if the cellular uptake was an energy dependent mechanism. Various endocytotic blockers including sucrose, filipin, nocodazole, ammonium chloride and cytochalasin were used to identify the endocytoic pathway for the transport of different dendrimers.

Surface functionality and charge of the dendrimer has an impact on the cellular interactions and subsequent endocytotic uptake into the cell. Dendrimers enter the cell by specific electrostatic interactions or non-specific hydrophobic and hydrogen bond interactions. The endocytotic pathway followed by the dendrimers governs the cell entry kinetics of the dendrimer. Cationic dendrimers are taken up faster than anionic dendrimers. The cationic dendrimers are mainly endocytosed by fluid phase endocytosis through clathrin coated pits, while anionic dendrimers are mainly transported through caveolae mediated endocytosis. Neutral dendrimer is transported by clathrin coated endocytosis through nonspecific interactions. Therefore it is essential to consider the surface functionality and charge in the design of dendrimer based intracellular drug delivery systems. By tailoring the dendrimer surface, it is possible to target the dendrimer to a specific group of cells by exploiting the constitutive endocytotic pathway of the specific group of cells.