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Biodegradable nanoparticles for cytosolic delivery of therapeutics ☆

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Abstract

Many therapeutics require efficient cytosolic delivery either because the receptors for those drugs are located in the cytosol or their site of action is an intracellular organelle that requires transport through the cytosolic compartment. To achieve efficient cytosolic delivery of therapeutics, different nanomaterials have been developed that consider the diverse physicochemical nature of therapeutics (macromolecule to small molecule; water soluble to water insoluble) and various membrane associated and intracellular barriers that these systems need to overcome to efficiently deliver and retain therapeutics in the cytoplasmic compartment. Our interest is in investigating PLGA and PLA-based nanoparticles for intracellular delivery of drugs and genes. The present review discusses the various aspects of our studies and emphasizes the need for understanding of the molecular mechanisms of intracellular trafficking of nanoparticles in order to develop an efficient cytosolic delivery system.

Keywords: Biodegradable polymers; Nanoparticles; Sustained release; Gene delivery; Drug delivery; Cellular uptake; Endocytosis

Contents

1. Introduction

Effective intracellular drug delivery is important for therapeutic agents which have specific molecular targets inside a cell. The targets can be located in the cytoplasm (glucocorticoid receptors,

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proteins, siRNA), nucleus (DNA, antisense oligonucleotides, DNA intercalating agents such as doxorubicin), mitochondria (anti-oxidants) or other subcellular compartments of a cell. Further, cytosolic delivery is desirable for drugs which undergo extensive efflux from the cell by the efflux transporters such as multidrug resistance proteins (MRP) and P-glycoproteins (P-gp) [1]. Macromolecular drugs such as recombinant proteins and plasmid DNA usually have their site of action in the cytoplasm and nucleus, respectively. The ultimate goal of gene delivery can be fulfilled only if the plasmid DNA is able to localize and integrate with the nuclear or mitochondrial DNA. Further, these therapeutic entities are highly susceptible to enzymatic degradation and delivery systems need to be designed in order to ensure the protection of proteins/plasmid DNA from proteases and nucleases. For example, the half-life of plasmid DNA in cytosol is only 60 to 90 min. Thus protecting DNA from degradation is critical for enhancing gene expression.

2. Drug-carrier systems for cytosolic delivery of therapeutics

A number of drug-carrier systems (liposomes, cell penetrating peptides, cationic polymer conjugates, polymeric nanoparticles) have been explored for intracellular delivery of therapeutics. These are required to cross a series of membrane barriers in order to reach the site of drug action in the cells and during this process lose a significant portion of the drug molecules at each successive barrier. These barriers include the cellular association and internalization of the drug-carriers by endocytosis; intracellular trafficking and release of drug or drug-carrier into the cytoplasm; cytoplasmic translocation of drug or drug-carrier to nucleus or any other cellular organelle; and the nuclear/organellar uptake. Fig. 1 depicts a typical intracellular trafficking pathway for nanoparticles (NPs) and other colloidal drug-carrier systems. The cell contains several intracellular organelles with specific functions. Intracellular targeting of therapeutics to these specific organelles is not only expected to significantly enhance the therapeutic efficacy but also reduce non-specific effects and hence toxicity. Therefore, there is significant interest in achieving intracellular target-specific delivery of therapeutics using different carrier systems.

The efficiency of drug-carriers for cytosolic delivery of therapeutics is limited mainly by their interaction with cell membranes and the endosomal release of carriers. Most of the carriers including liposomes and polymeric NPs associate with the cell membrane and are internalized into cells by means of endocytic mechanisms. Cell recognition and association of drug-carriers with cell membrane can be enhanced by the use of targeting ligands which can bind to specific receptors on cell membranes. This promotes not only the association and binding of drug-carriers to the cell membrane but also can increase the cellular internalization by means of receptor-mediated endocytosis. Another bottleneck for cytosolic drug delivery is the sequestration of drug-carriers within the endosomal compartment, following endocytosis. This has opened numerous avenues for research into development of strategies to enhance the endosomal escape of drug-carriers, in order to improve the efficiency of cytosolic drug delivery.

2.1. Liposomes

Liposomes have been extensively investigated as a potential drug-carrier system for cytosolic delivery due to the enormous diversity of structure and compositions that can be achieved. Different formulation strategies have been developed to increase the ability of liposomes to mediate cytosolic delivery of therapeutics. These include the development of 'fusogenic' and 'pH-sensitive liposomes'. Fusogenic lipids are included in liposomes since they undergo a phase transition under acidic conditions. This facilitates an interaction and fusion or destabilization of liposomes with the endosomal membranes, resulting in release of the encapsulated therapeutic in the cytoplasm. pH-sensitive liposomes are stable at physiological pH (pH 7.4) but undergo destabilization, and acquire fusogenic properties under acidic conditions. Different hypothetical mechanisms have been proposed for the endosomal escape of pH-sensitive liposomes: (i) destabilization of pH-sensitive liposomes at acidic pH triggers destabilization of the endosomal membrane by pore formation leading to cytosolic delivery; (ii) upon destabilization of liposomes, the encapsulated molecules diffuse to the cytoplasm through the endosomal membranes; and (iii) fusion between the

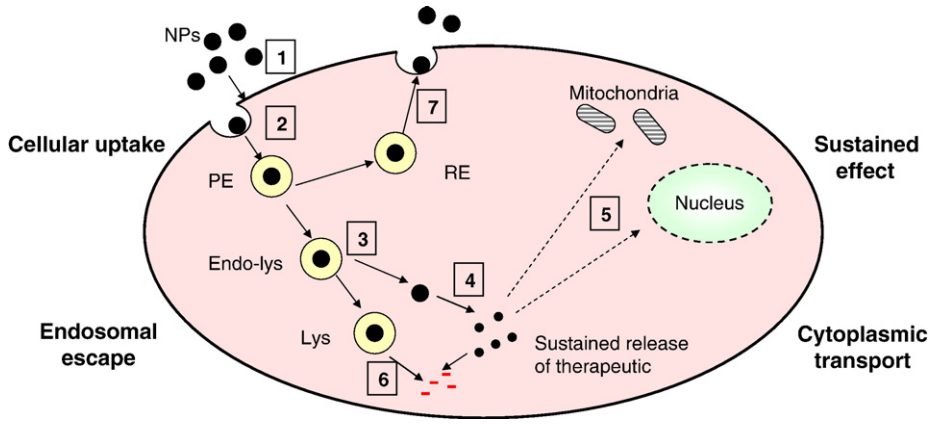

Fig. 1. Schematic drawing of steps involved in cytosolic delivery of therapeutics using polymeric nanoparticles (NPs). (1) Cellular association of NPs, (2) Internalization of NPs into the cells by endocytosis, (3) Endosomal escape of NPs, (4) Release of therapeutic in cytoplasm, (5) Cytosolic transport of therapeutic agent, (6) Degradation of drug either in lysosomes or in cytoplasm, (7) Exocytosis of NPs. Major barriers include: (A) cellular uptake of NPs, (B) endosomal escape of NPs, (C) cytoplasmic transport of therapeutic/NPs, (D) sustained therapeutic benefit. [PE: Primary endosomes, RE: Recycling endosomes, Endo-lys: Endo-lysosomes, Lys: Lysosomes, Solid circles represent polymeric NPs].