PROBING MOLECULAR INTERACTIONS OF PLGA PARTICLES WITH CELL MEMBRANES FOR THE DELIVERY OF PHARMACEUTICALS

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By

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Dedicated

to

My beloved Parents

CERTIFICATE

This is to certify that the thesis entitled "**Probing molecular interactions of PLGA particles with cell membranes for the delivery of pharmaceuticals**", being submitted by **Ms. Roohi Gupta** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her, which has been prepared under our supervision and guidance in conformity with the rules and regulations of "Indian Institute of Technology, Delhi". The research reports and the results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Cell membrane acts as an important site of interaction for many drugs and pathogenic microorganisms. The drug-surface receptor interactions generate cascade of responses leading to transport of drug inside the cell and eventually provoking or preventing some cellular activity. Thus therapeutic activity of any drug molecule will depend upon availability of the drug at the membrane surface before a drug- receptor interaction generates cascade of responses. Delivery of therapeutic agents to cells can be highly inefficient because of problems like rapid degradation, need for repeated injections, low bioavailability and poor transport of drugs through biological barrier. In order to achieve efficient drug delivery, delivery of therapeutic molecules via various nanocarrier systems such as biodegradable polymeric nanoparticles, liposomes, nanocapsules, nanospheres have been extensively investigated. Polymeric biodegradable nanoparticles are promising carriers due to their biocompatibility, biodegradability and ability to maintain therapeutic drug levels for sustained periods of time. Pharmaceutical applications of these nanoparticles depend upon delivery of the drug loaded particles to target cells followed by efficient intra-cellular uptake and subsequent biodegradation leading to the release of the entrapped molecules. An effective approach for enhancing efficacy of the encapsulated therapeutic agent should be based on better understanding of their (nanocarrier systems) cellular interactions, mechanisms of uptake and intracellular trafficking. Thus, in order to understand the interaction of nanoparticles with the cell membrane and their intracellular uptake and trafficking through cells, present work focuses on studying membrane interactions and cellular uptake of biodegradable Poly (DL-lactic-*co*-glycolic acid) (PLGA) nanoparticles loaded with model hydrophobic fluorescent marker drug (6-coumarin) with cancer cell lines. Fluorescent probe (6-

coumarin) was used so that nanoparticle uptake and distribution can be visualized by fluorescence microscopy.

PLGA (50:50) Nanoparticles (NPs) containing 6-coumarin were formulated using single emulsion-solvent evaporation technique and characterized for their size, surface morphology, surface charge, loading and encapsulation efficiencies. Uptake kinetics of NPs was studied *in vitro* for defined time intervals and at different temperature conditions with HeLa and Caco-2 cell lines. Intracellular amount of nanoparticles in terms of its fluorescence intensity was quantified by analyzing the captured fluorescent images of cells in MATLAB using self written algorithms. Both types of cells took up PLGA NPs quite readily with similar uptake profile and saturation was observed after one hour incubation period. The internalization of NPs was found to be more in Caco-2 cells when compared with HeLa cells, but the initial rate of uptake was found to be higher in HeLa cells. No statistical difference in uptake kinetics was observed between synchronized and non-synchronized cells. The internalization of NPs was confirmed by confocal laser scan microscopy. While working on intracellular uptake of coumarin loaded biodegradable polymeric nanoparticles, it was observed that coumarin enhances fluorescence of propidium iodide (nucleic acid binding fluorescent dye) by ~ 30% both inside cells and in solution. In this work, for the first time the involvement of mannose receptors (known endocytotic ligand) in uptake of poly (D, L-lactic-co-glycolic acid) (PLGA) nanoparticles by cancer cells have been reported. This was analyzed by designing a novel approach in which cell surface receptors were shaved off by trypsin after incubating cells with NPs and subjecting the tryptic peptides released into the incubation buffer to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and further identifying the 'shaved' proteins by Liquid Chromatography- Mass Spectrometry analysis. The results reported further substantiate the key

role of endocytosis in the nanoparticle entry in to the living cells and intracellular trafficking studies showed that it follows the conventional intracellular route from early to late endosomes and to lysosomes. Cytotoxicity has been studied using paclitaxel (hydrophobic anti-cancer drug) loaded PLGA NPs in order to illustrate the efficacy of an actual chemotherapeutic agent encapsulated NPs. The findings described herein is likely to enhance our basic understanding of cell surface interactions and cellular uptake of biodegradable NPs with cells which in turn could help in developing efficient nanocarrier systems for intracellular delivery of therapeutics.

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