ABSTRACT

Nanoparticles are materials with at least one dimension in the range of 1-100 nm. When the size of matter is reduced to this scale materials gain some unique size dependent optical, electronic, magnetic, mechanical and surface properties. Recently, these unusual properties of nanomaterials have attracted immense attention of researchers from almost every field of science including biology and medicine. Investigation of novel properties of nanoparticles and their application has become very active area of research. Nanoparticles have shown tremendous potential for application in biomedical research. They are being extensively investigated for both analytical and therapeutic purposes in the areas of delivery of nucleic acids and drugs inside the cell, probes for spectroscopy and microscopy, applications in diagnostics and imaging etc.

Thematically centered around nanoparticles, the work described in this thesis has been divided into following three chapters:

Chapter-1: Review of Literature

In this chapter a general introduction and an overview about Nanomaterials with special emphasis upon Gold nanoparticles, Iron nanoparticles and Hyperbranched polymers used in this study have been given. It begins with a brief history of nanoparticles and covers a brief account of important nanoparticles which are being actively investigated for biomedical research. Emphasis has been to provide an overview of synthetic methods and important application of different nanoparticles. A brief discussion of biocompatibility issues of nanoparticles, their possible impacts and required measures have also been discussed.

Chapter-2: A Transcriptomics Approach to Study the Biocompatibility and Finding out the Potential Applications of Few Metal Nanoparticles

In vivo response of the cell due to entry of nanoparticles will depend upon the amount and presentation of the biomolecules on the surface of the nanoparticles. However, due to their high surface to volume ratio and unique surface properties, nano-scale materials can interact with biomolecules differently than materials in molecular or bulk state. Therefore, nanoparticles are expected to produce unusual
effects upon living system which cannot be predicted from the effects of the same material in molecular or bulk state. These effects may be beneficial or toxic both and need to be studied separately. We rationalized an atypical non hypothesis-driven approach for studying the biological effects of nanoparticles is likely to throw light on such unique, unexpected effects. The idea was to take an unbiased account of molecular level effects caused by nanoparticles to decide therapeutic or toxic potential of nanoparticles. For this purpose, we used transcriptomics to identify effects of nanoparticles upon cultured mammalian cells. This approach would give us simultaneous clues to use nanoparticles to target genes known to be involved in diseases as well as biocompatibility of nanoparticles. As a proof of concept following two studies to understand the molecular effects of uptake of Gold Nanoparticles (GNP) and Magnetite nanoparticles (MNP) were performed.

Chapter-2A: Molecular Effects of Uptake of Gold Nanoparticles

Amongst an array of nanomaterials being synthesized, GNP have found wide acceptability because of their stability, ease in size controlled synthesis and relatively easy surface modification with amine and thiol groups, especially suited for conjugation with DNA and proteins. The naked GNP has been shown to have antiangiogenetic properties. However, fragmented and often contradictory reports of GNP having adverse effects upon cells are also there. To have a better insight of effect of interaction of GNP with biological system, GNPs of 18 nm size were synthesized by citrate reduction method. Cultured HeLa cells were exposed to these nanoparticles for different time periods, and their survival was measured by MTT assay. The GNP was found to be mildly cytotoxic. To understand the effects of exposure of GNP on cells, electron microscopy, unfolded protein response (UPR) assay, and microarray studies were performed. Electron microscopy studies showed that GNP is effectively internalized by cells. Microarray studies revealed that internalization of gold nanoparticles is not associated with any specific as well as gross changes in transcription profile of cell. The exposure to Gold nanoparticles did not induce Unfolded Protein Response (UPR) also. Therefore, it was concluded for the first time from our study that 18 nm GNP used in this study do not cause changes in gene expression or interfere with protein folding. Taken together, GNP seem to enjoy the advantages viz. small size, large surface area, cell penetrability and suitability for surface modifications of other nanomaterials, while being free of their toxic effects on cells.
Chapter-2B: Molecular Effects of Uptake of Magnetite (Fe₃O₄) Nanoparticles

The developments in methods for synthesis of superparamagnetic nanoparticles of iron and their surface modification has led to many biomedical applications like targeted drug delivery, contrast agents in magnetic resonance imaging (MRI), magnetic separation of biomolecules and cells, immunoassays, detoxification of biological fluids, hyperthermia etc. However, studies to understand detailed molecular effects of interaction MNP have not been carried out. This work, for the first time explored the molecular effect of cellular uptake of MNP and suggests that these nanoparticles can interfere with TGF-beta signaling. MNP of 40 ± 6 nm were synthesized by co-precipitation of Fe²⁺ and Fe³⁺ ions to study their molecular effects of upon cells using the transcriptomics approach. The uptake of MNP by HeLa cells was studied using electron microscopy. Genome wide expression profiles of cells exposed to MNP revealed that the uptake cause down regulation of 68 genes and up regulation of only one gene. Classification of differentially expressed genes on the basis of GO terms revealed that majority of these genes were involved in important cellular functions like transcription, growth and development. Pathway analysis and literature mining suggested that many of these genes are associated with TGF-Beta signaling. Microarray results of five of the differentially expressed genes SMAD6, SMAD7, ID1, ID2 and ID3 with well documented roles in TGF-Beta signaling were validated and their time dependent expression was studied using real time PCR. Cellular levels of CASP9 (a member of apoptosis pathway) was also found to be inhibited both at RNA and protein level. Together, these results suggest that the perturbations caused by entry of MNP in to cell are counterbalanced by modulation of transcription levels of genes associated with TGF-Beta signaling. Down regulation of CASP9 suggest that to prevent cell death, inhibition of apoptosis also seems to occur. These findings have significant implications for cancer, differentiation and development because TGF-beta signaling invokes different responses in undifferentiated cells and adult tissues in a cell-type specific manner. Many of the genes found to be affected by uptake of MNP are known drug targets for cancer. On one hand these findings can lead to therapeutic applications of MNP in diseases like cancer and angiogenesis. Whereas, MNP affecting pathways critical to cell survival and differentiation suggest that these nanoparticles can have toxic effects on normal cells.
Chapter-3: Synthesis, Characterization and Application of RNA Nanoparticles Using a PEG based Hyperbranched Cationic Polymer

It has been well established that RNA is a key molecule in regulation of gene expression and other crucial metabolic processes. It is being considered as an ideal therapeutic candidate both for metabolic and genetic disorders. However, labile nature of RNA poses practical limitations to its storage, study and manipulations. We hypothesized that RNA can be stabilized if it is bound to suitable polymers that can give nanoparticle like shape to RNA. In such scenario, it will not be accessible to nucleases which are often present in RNA preparations. Total cellular RNA isolated from yeast was complexed with a Poly Ethylene Glycol (PEG) based cationic Hyperbranched Polymer (HP). It was found that RNA spontaneously interacts with the positively charged polymer. Atomic force microscopy studies confirmed that binding of HP with RNA can give nanoparticle like shape to RNA. RNase protection assay revealed that RNA nanoparticles show considerable resistance to RNase. Recovery of atleast some amount of RNA from HP/RNA complex, by ethanol precipitation in presence of Li$^+$ ions, for subsequent use has also been demonstrated. The RNA could also be released by ion exchange with high concentration of Li$^+$ ions. However Li$^+$ concentrations required were too high and are not suitable for many molecular biology reactions. Therefore, we propose chemical modification of HP scaffold for development of a general purpose RNA stabilization agent. Such positively charged HP will be useful in RNA separation, purification, manipulation, detection, storage, long distance transportation and also delivery inside the cell.