# Toxicity Evaluation of Therapeutic Nanoparticles

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**Abstract—***Nanoparticle based drug delivery system now a day has become an extensively used aspect for its importance in medical field and is used to prevent and treat the emerging diseases. They are used to overcome the shortcomings of the conventional drugs, such as unfavorable pharmacokinetics, poor solubility, instability, drug resistance and low cellular uptake, further it has been reported that nanostructures prevents the degradation of drugs in the gastrointestinal tract (GIT) and have particularly large surface area, hence presenting diverse opportunities to place functional groups on the surface. But on the contrary these nano sized structures do have some side effects on the biological system due to their increased permeability, solubility and accumulation at the target site. This review is an attempt to analyze toxicity effect of therapeutic nanoparticles.*

**Keywords***: Polymeric nanoparticles, Therapeutic index, Intercellular responses, Nanoformulation, Cytotoxicity, Genotoxicity.*

## 1. INTRODUCTION

Nanoparticles have been extensively explored now a days in the field of therapeutics to redesign the drugs, herbal compounds, neutraceuticals, transplants and implants to increase their efficacy, bioavailability, therapeutic index, biological membrane permeability and to lower down their side effects, hepatic metabolism and degradation. Since, these therapeutic nanoparticles are in nanometric range hence, they are able to penetrate through various physiological barriers inside the body therefore; they work like an ideal carrier system for a targeted drug delivery. But, due to these site specific targeting, there are chances that excess of drug or compound along with polymers get accumulated at the same site, causing toxicity. The toxicity by nanoparticles is suspected to go beyond the intercellular changes and might also cause gene alterations. Recent studies have shown that nanoparticle toxicity can be categorized under three main forms – genotoxicity, epigenicity and cytotoxicity and hence causing a physiological menace again. Therefore, it is very important to evaluate the therapeutic utility of the developed nanoformulation and their related cellular responses given by *in vitro* models. The more explored method for drug targeting is either active or passive targeting.

Active targeting increases the delivery of drugs to a specific target with the use of specific interactions at target sites where a drug's pharmacological activities are applied whereas, in passive targeting, the physical and chemical properties of carrier systems increase the target/ non-target ratio of the quantity of drug delivered by adjusting these properties to the physiological and the histological characteristics of the target and non-target tissues, organs, and cells [2]. Recent advance tools used for the targeted deliveries are nanoscale sized nanostructures, which are able to penetrate tissues and are easily taken up by the cells. Uptake of nanostructures is 15-250 times greater than that of micro particles (1-10µm). They circumvent the drug efficacy issues like poor bioavailability, solubility, intestinal absorption and inability to cross many physiological barriers [2]. They can be made by interaction with specific antibodies and by expanding and contracting with changes in temperature or pH. More designs can be made by combining with inorganic materials and combining different classes of polymers together. With the recent advancement in chemistry, processing techniques and analytic instrumentation, whole new types of polymeric nanoparticles could be designed and are broadly classified into - metallic, ceramic, polymeric ones along with fullerenes and quantum dots. Amongst all polymeric nanoparticles are vastly used in therapeutics and makes it more important to evaluate their toxicity effects. They are prepared either from preformed polymers or by direct polymerization of monomers by micro emulsion, mini emulsion, surfactant-free emulsion and interfacial polymerization. Their size ranges between 10-1000 nm [3]. Polymeric nanoparticles have been produced for a decade now and have extensive applications, other than therapeutic fields like specialty coating, high impact resisting polymers etc. Also, either natural or synthetic polymers are being used for these types of nanoparticles.

This study is focused on evaluating the adverse effects of biodegradable polymeric nanoparticles with their probable effective mechanisms on cells.

## 2. POTENTIAL APPLICATIONS OF NANOPARTICLES

Our body is constructed from building blocks such as DNA and proteins, which are long been targeted by the pharmaceutical industry long before the emergence of nanotechnology [4,5]. Due to their extremely small size nanoparticles have site specific, large surface area to mass

ratio, and high reactivity, which are different from bulk materials (in micro scale) of the same composition. These properties can be used to overcome some of the limitations found in traditional therapeutic and diagnostic agents. Moreover, it also controls the human biological systems at molecular level. It not only covers therapeutic agents themselves, but also promises to combine the abilities to deliver those agents to specific regions or tissues in the body, to specific cells, perhaps to a specific location within a cell, and also to release therapeutic molecule responsive to a physiological condition and perform specific task[6]. Many other therapeutic techniques like – Bioscaffolding, in which tissue regeneration is performed, here it can be promoted effectively by using nanoparticles thus promoting cell growth and cell viability. Similarly other area such as drug delivery**,** advantages of using polymeric nanoparticles (PNPs) here are enormous, they generally increase the stability of any volatile pharmaceutical agents and can be easily formulated in large quantities by multitude of methods. Nanoparticles can be used in targeted drug delivery at the site of disease to improve the uptake of poorly soluble drugs along with increased bioavailability. Several anticancer drugs including paclitaxel, dexamethasone has been successfully formulated using nanoparticles.

## 3. PHYSIOLOGICAL UPTAKE AND TRANSPORTATION OF NANOPARTICLES

Upon administration of nanoparticles in body, various interactions at the interface trigger various processes such as formation of protein ring, particle twining at the cell membrane, endocytosis and intracellular biocatalysis which can lead to some potential detrimental effects. For instance, nanoparticles entering through endocytic pathway may trigger the immune reactions. The nature of interaction is influenced by nanoparticle characteristics namely size, shape, surface area, surface charge etc. which are also the factors responsible for cytotoxicity.[7]

Exposure to nanoparticles can be linked to four common routes. Uptake of different types of nanoparticles (polymeric, metal oxides, carbon, quantum dots, etc.) were studied based on in vitro model systems involving different cell lines.[8]

**Table 1: Physiolocal uptake routes of nanoparticles**

| Ingestion | Injection | Transdermal | Inhalation |
|---|---|---|---|
| CaCo2,RKO Immortal Colon Cell Lines | Primary Human Umbilical Vein epithelial,Hela,MCF-7 cell lines | Human Derived keratinocyte (HaCaT), Dermal fibroblasts cell lines | Immortal lung cell lines,A549, BEAS-2B |
| Polymeric,Metal Oxides and Carbon based nanoparticles | Quantum dots, Polymeric nanoparticles | Mostly metal oxide nanoparticles Eg.ZnO,Titanium oxide | Titanium oxide, Carbon based nanoparticles |

Once the nanoparticles enter the body, they need to be taken up by the cells for their translocation and trafficking. Nanoparticles are able to cross cellular barriers easily. Diffusion and endocytosis serve as key processes in their crossing. Endocytosis can be divided into two main categories: phagocytosis and pinocytosis.[9]

In phagocytosis, cell engulfs the substance by invagination producing a vesicle called phagosome. Materials in the phagosome are acted upon by enzymes and are degraded. In pinocytosis the cell engulfs extra cellular media via a vesicle and then releases their contents to the surrounding tissues. [10] Pinocytosis uses proteins such as clathrin and caveolin as mediators. Followed by endocytosis, vesicles fuse with each other to form endosomes. Transportation between Golgi bodies and endosomes then provide delivery to intracellular pathways. [11][12] Efficient drug delivery is important to achieve the desired responses. For biodegradable nanoparticles some of the methods include[13][14][15]:

1. Diffusion
2. Matrix erosion
3. Combined erosion and diffusion.
4. Desorption

## 4. LIMITATIONS OF THERAPEUTIC NANOPARTICLES

The small size of nanoparticles is what that makes nanotechnology so useful in medicine and industry but at the same time it is also one of the main factors that might make them potentially dangerous to human health. Sometimes the interactions of particles with cells generate free radicals which cause oxidative stress that may result in cell death. Sometimes these nanoparticles can behave like haptens to modify protein structures due to their small size[16]. Recently, toxicity of nanoparticles was seen in mammalian germiline stem cells when an experiment was conducted on a cell line with spermatogonial stem cell characteristics. This aroused great concern over the biosafety of nanoparticles[17]. The results of experiment conducted showed that of all the tested materials (Ag, Mo , and Al), silver nanoparticles were the most toxic with manifestations like drastic reduction of mitochondrial function, increased membrane leakage, necrosis, and induction of apoptosis. The findings are of significant practical implications because silver nanoparticles are now able to access human sperms via a variety of commercialized products like contraceptive devices and maternal hygiene items. Based on this, fertility problems may occur. Another experiment was conducted with *in vitro* BRL 3A rat liver cells where liver also appeared to be a major accumulation site of circulatory silver nanoparticles[18]. Oxidative stress induced by nanoparticles is reported to enhance inflammation through up regulation of redox-sensitive transcription factors. It is

observed in later studies that polymeric nanoparticles showed less toxicity as compared to the other ones.

## 5. TOXICITY ASSESSMENT OF THERAPEUTIC NANOPARTICLES

Toxicity studies shed light on the cytotoxic, genotoxic and epigenetic effects of nanoparticles on living cells. Nanoparticle localization and interaction type with cellular components leading to cytotoxicity or genotoxicity depends on its size. Evaluation of any possible toxicity after exposure to polymeric nanoparticles is an essential factor to consider while assessment of their potential in biomedical applications. It is considered that the toxicity caused by nanoparticles is due to the chemical composition of parent material, interactions at the level of nanoparticle-biological interface, or due to added effect of both. [19] Several cytotoxicity studies have reported that toxicity of nanoparticles is inversely related to their size. Accumulation of smaller sized nanoparticles in organs such as liver and kidneys is more as compared to the larger sized ones, causes toxicity.Usage of biodegradable polymers for biomedical applications has increased recently due to their biocompatibility, biodegradability, flexibility and minimal side effects. The main advantage of biodegradable polymers is that it's degradation products are either non-toxic or easily eliminated from the body[20] It is of importance to understand the properties of nanoparticles that lead to the interactions between particles and cells, to reach the objective of developing a nano carrier of required functionality.[21] In order to avoid adverse effects of biodegradable polymeric nanoparticles, it is essential to check that the polymer itself is not toxic and the nature of responses induced by the degraded particles. The intact polymeric nanoparticle starts to degrade after a certain amount of time, after its input into the body. This, sometimes, results in hazardous polymeric sub part formation. These physiological changes can lead to certain mutations which can be hard to detect. Physiological changes at the genetic level caused by nanoparticles also lead to change in gene expression, post translational modifications governing them and gene structure. Nanoparticles cause variations in cells and the traits exhibited by them giving rise to epigenetic effects. Cellular functions that are modulated depend on the extent and kind of chromatin being affected. Polymeric residue formation and degradation products of varied sizes are exposed to biological surroundings. Biodegradable polymers can cause harm by accumulating within cells which in turn leads to intracellular changes – disruption of organelle to gene alterations [22]. The degree of toxicity is dependent upon the composition and biological conditions of surroundings. [23]. Biodegradable polymer-PLGA (poly lactic co-glycolic acid) shows minimal systemic toxicity and excellent biocompatibility, both *in-vitro* and *in-vivo*; although some inflammatory reactions are reported. PGA has low solubility and a high degradation rate, with formation of an acidic product that can also provoke an inflammatory reaction [24]. Hence, substitutes of PGA in biomedical applications like caprolactone, lactide, and tri methylene carbonate have been developed to eliminate these toxic products.[25].PGA (poly glycolic acid) on the other hand has low solubility, high degradation rate and provokes inflammatory reactions. Genotoxicity studies reveal DNA strand breakages, chromosomal fragmentation, point mutations, alterations in gene expression. Nanoparticles can cause DNA damage directly or induce a series of events resulting in DNA damage. Nanoparticles can induce large chromosomal rearrangements such as aneuploidy. Further, the products of hydrolysis of biodegradable polymers (carboxylic acid and/or hydroxyl chain end) may be oxidized, resulting in production of species such as short chain carboxylic acid, that may lead to local variations in pH that trigger an inflammatory response[26]. Studies conducted for premier therapeutically important biodegradable polymers found positively charged PEG (Poly ethylene glycol) and chitosan nanoparticles to be cytotoxic on conduction of MTT-assay. It was observed that positively charged PEG of smaller size were more toxic than larger ones whereas, negatively charged.[27].PLGA (poly lactic co-glycolic acid) nanoparticles have shown minimal toxicity in endothelial cells.[28].PVA (polyvinyl alcohol), PGA and PVC (polyvinyl chloride) did not show any deformations, malformations, morphological changes, physiological changes, apoptosis, necrosis or any other forms of significant cytotoxicity.[29].In chitosan nanoparticles, a study in zebra fish embryo model system indicated a significant decrease in hatching rate and increase in mortality rate for higher concentration of smaller nanoparticles. However, even at very low concentration of smaller sized nanoparticles certain malformations were observed. Interestingly, fucoidan-chitosan nanoparticles in particle size outside nanoparticle range decrease in cell viability in Caco-2 cells, but concentrations in the range of micrograms/ milliliters were non-cytotoxic. [30]

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