

## Application and Hazards of Nanotechnology in Drug Delivery - A Review

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### ABSTRACT

**General concept:** Nanotechnology is a revolutionary field of micro manufacturing involving physical and chemical changes to produce nano-sized materials. The word “nano” is a Latin word meaning “dwarf”. Mathematically a nanometer is equal to one thousand millionth of a meter. Although, the initial properties of nanomaterials studied were for its physical, mechanical, electrical, magnetic, chemical and biological applications, recently, attention has been geared towards its pharmaceutical application, especially in the area of drug delivery. Over the last decades, different types of nanoparticles have been developed based on various components, including carbon, silica oxides, metal oxides, nanocrystals, lipids, polymers, dendrimers, and quantum dots, together with increasing variety of newly developed materials. These nanomaterials are capable to provide a high degree of biocompatibility before and after conjugation to biomolecules for specific function so as to translate into nanomedicines and clinical practice. Nanomaterials provide for a favorable blood half-life and physiologic behavior with minimal off-target effects, high specificity towards the target site, effective clearance from the human organism, and minimal or no toxicity to healthy tissues in living organisms. In addition, the nanosize also allows for access into the cell and various cellular compartments including the nucleus. Nanotechnology definitely promises to serve as drug delivery carrier of choice for the more challenging conventional drugs used for the treatment and management of chronic diseases such as cancer, asthma, hypertension, HIV and diabetes. This review provides an overview on the currently used systems of nanotechnology in drug delivery, applications and hazards of nanotechnology in pharmaceuticals release. **Conclusion:** Although for pharmaceutical use the current requirements seem to be adequate to detect most of the adverse effects of nanoparticle formulations, it can not be expected that all aspects of nanoparticle toxicology will be detected. So, probably additional more specific testing would be required.

**Keywords:** Drug Delivery, Nanoparticles, Toxicology, Pharmaceuticals.

### INTRODUCTION

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience and nanotechnology. There is increasing optimism that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease. Anticipated applications in medicine include drug delivery, both in vitro and in vivo diagnostics, nutraceuticals and production of improved biocompatible materials [1,2].

The current review was made to document and discuss the progressive status of nanotechnology, its implementation in drug delivery. Also, an overview of the risk aspects of this scientific knowledge will be provided.

#### 1. DRUG DELIVERY

Drug Delivery System (DDS) is defined by national institute of health in USA as, “Formulation of a device that enables the introduction of therapeutic substances in to the body and improves efficiency and safety by the control the rate, time and place of release of drug in the

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body.” The process of drug delivery can be mainly divided into, 1) The administration of the drug or therapeutic product can be divided as non-invasive and invasive administration. Non-invasive administration such as oral, topical (skin), nasal, and inhalation routes. Invasive administration is injection or nanoneedle array. 2) The release of the active part of the drug by the product. 3) Transport active ingredients across the biological membrane to the target site to perform action [3].

## 2. NANOTECHNOLOGY USES IN MEDICINE

National Institute of Health in USA, defined nanomedicine as “highly specific medical intervention at the molecular scale for diagnosis, prevention and treatment of disease”. This nanotechnology application in medicine also immature field and few methods already in action but some techniques only imagined while most of the techniques are under the research conditions [4]. Nanotechnology is used in field of medicine for drug delivery, treatment, diagnostic & monitoring techniques, bio sensors, antimicrobial techniques, cell repair and control the biological system are some of applications [5]. Fiber optic technology uses to monitoring diseases. Optical biosensors used to measurement physical parameters such as pH, blood flow rate, blood oxygen levels, radiation dosage. Endoscopy in next generation will extend its capability from imaging to diagnostics and therapy using nanofiber technology. Fiber optic sensors, endoscopes nano-scale bioprobes with the rapid advance of nanotechnology [6]. DDS interface, between the patient and the drug and it may be formulation of the drug or device used to deliver the drugs to the particular site [7]. The usual drug delivery systems are not up to the satisfactory level. There were many drawbacks include poor bioavailability, generate side effects, low drug loading capacity, poor ability to control the size range, plasma fluctuation of the drug levels, low therapeutic effectiveness, low in- vivo stability, low solubility, no control over the time, location and lack of target delivery to the site of action as well as some drugs are only active in a narrow range. If concentration is above the threshold level it becomes toxic, if it is low lack of therapeutic effect. These drawbacks put pressure on scientists to investigate more about new DDS and its control and determine the rate and location of drug

release [8]. Scientists developed NPs of the size of macromolecules such as DNA and proteins. The some developed nano-structures were smaller than diameter of a double stranded DNA (2nm). The smallest cellular form in the world is a bacteria named mycoplasma. Which has the size of 200nm but in comparison the largest NP is only 100nm in size. New DDS has the ability to deliver drugs to specific target cells in various areas of the body without degradation in the gastrointestinal track. It includes delivery and targeting of pharmaceutical, therapeutical and diagnostic agents by the help of NPs to the cells such as cancer cells. The ultimate goal of NP drug delivery is to improve the proper treatment diagnostics and prevention of disease [9,10]. Although solid NPs may be used for drug targeting, when reaching the intended diseased site in the body the drug carried needs to be released. So, for drug delivery biodegradable nanoparticle formulations are needed as it is the intention to transport and release the drug in order to be effective. However, model studies to the behavior of nanoparticles have largely been conducted with non-degradable particles. Most data concerning the biological behavior and toxicity of particles comes from studies on inhaled nanoparticles as part of the unintended release of ultrafine or nanoparticles by combustion derived processes such as diesel exhaust particles [11,12].

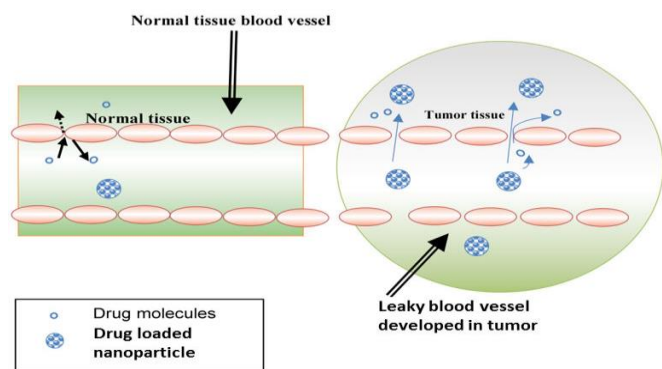
## 3. CANCER TREATMENT

Cancer is one of the leading causes of death worldwide, occupying the second place in developing countries, and showing a growing incidence over time [13]. Current cancer therapy strategies are based in surgery, radiotherapy and chemotherapy, being the chemotherapy the one that shows the greater efficiency for cancer treatment, mainly in more advanced stages [14,15]. Despite of this great response, anticancer agents are administrated at higher amounts in order to provide a final suitable concentration to the target tissues or organs, and this procedure is repeated in each cycle of chemotherapy [16]. Introduction of new agents to cancer therapy has greatly improved patient survival but still there are several biological barriers that antagonize drug delivery to target cells and tissues, namely unfavorable blood half-life and physiologic behavior with high off-target effects and effective clearance from the human organism [14,17,18]. Moreover, in cancer, there is a small

subset of cancer cells-cancer stem cells (CSC)-that, like normal stem cells, can self-renew, give rise to heterogeneous populations of daughter cells, and proliferate extensively [19,20]. Standard chemotherapy is directed against rapidly dividing cells, the bulk of non-stem cells of a tumor, and thus CSC often appear relatively refractory to those agents [19-21]. The development of side effects in normal tissues (e.g. nephrotoxicity, neurotoxicity, cardiotoxicity, etc) and multidrug resistance (MDR) mechanisms by cancer cells leads to a reduction in drug concentration at target location, a poor accumulation in the tumor with consequent reduction of efficacy that may associate to patient relapse [21-25]. To overcome these issues and still improve the efficiency of chemotherapeutic agents there is a demand for less toxic and more target specific therapies towards cancer cells, i.e. novel drugs, drug delivery systems (DDSs) and also gene delivery systems [26-29]. Nanotechnology is the manipulation of matter on an atomic, molecular, and supramolecular scale involving the design, production, characterization and application of different nanoscale materials in several key areas providing novel technological advances mainly in the field of medicine, so called Nanomedicine [30-32].

The largest database on the toxicity of nanoparticles has originated from inhalation toxicology including the PM10 literature (particulate matter with a size below 10 μm), where the 'NP hypothesis' has proved to be a powerful drive for research [33,34].

Nanoparticulate delivery systems utilize specific targeting agents for cancer cells minimizing the uptake of the anticancer agent by normal cells and enhance the entry and retention of the agent in tumor cells (Figure 1).



**Figure 1.** Schematic diagram of nanoparticle permeation and retention effect in normal and tumour tissues. Normal tissue vasculatures are lined by tight endothelial cells, hereby

preventing nanoparticulate drug delivery system from escaping, whereas tumor tissue vasculatures are leaky and hyperpermeable allowing preferential accumulation of nanoparticles or nanoliposomes in the tumor interstitial space by passive targeting

The adverse health effects of particulate matter (PM) are measurable as exacerbations of respiratory disease and deaths as well as hospitalizations and deaths from respiratory and cardiovascular disease [35]. Inflammation is the common factor that binds together these adverse effects and the ability of NPs to cause inflammation can be seen as an important property. It is not clear what effects of NPs have pulmonary inflammation as a prerequisite and what effects could potentially be driven by exposures below those causing inflammation. There is also the potential for pulmonary inflammation to result in changes in membrane permeability that in turn may impact the potential for particles to distribute beyond the lung. Some NPs may have the extra potential of affecting cardiovascular disease directly. Vascular function was impaired after inhalation of diesel exhaust particles [36]. However, data to date are limited and not all studies of nanoparticles have shown significant translocation from lung to the blood. In some studies translocation has been rather minimal [37,38]. Understanding clearance kinetics of inhaled ambient air nanoparticles will also be important in understanding their potential for adverse effects.

The current paradigm in particle toxicology is that ultrafine ambient air particles have the potential of affecting cardiovascular disease both indirectly via pulmonary inflammation and directly through particle distribution. Although important, this property of redistribution has yet to be demonstrated for NPs present in real PM10. It should be noted that there are several mechanisms whereby NPs could lead to inflammatory effects, as is the case for larger particles. These mechanisms are either based on the large surface area of particle core or on soluble components released by the NPs. In addition various chemicals including those of biological origin like endotoxin may be adsorbed onto the NP and released [12]. Several toxicological studies support the contention that NPs in PM10 could drive inflammatory effects. There are a number of components of PM10 that contribute to the mass but have little toxicity - these include salts such as sulfates, chlorides and ammonium salts and nitrates, but also

wind-blown or crustal dust. In fact within PM10 there are only few components that toxicologists would identify as likely mediators of adverse effects, i.e., particle surfaces, organics, metals and endotoxin (in some PM10 samples). In fact, a large surface area, organics and metals are all characteristic of combustion-derived particles and so these have attracted considerable toxicological attention [39]. However, it is difficult to untangle, in a combustion particle sample, the relative roles of surface, organics and metals, although this has been most attempted in vitro. The aggregation of multiple chemical species including biological compounds like endotoxin limits the extrapolation of the results on the toxicological effects of such particles.

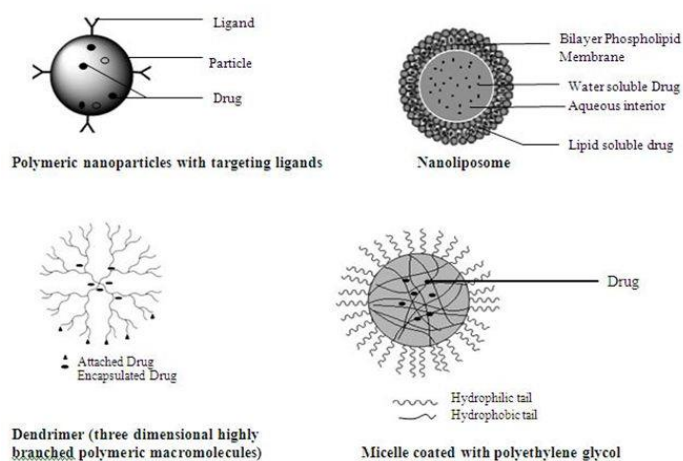
The usual drug delivery to the tumor cells develop side effects in normal tissues such as nephrotoxicity, neurotoxicity, cardiotoxicity and multiple drug resistance (MDR) reduces drug concentration at target location, poor accumulation. MDR is mostly due to the increase efflux pumps in cell membrane such as P-glycoprotein. Paclitaxel loaded NP can pass drugs without disturbing by MDR [40]. To overcome these problems NP based drug delivery system is used. The tumor sites forms new blood vessels to supply nutrients and oxygen rapidly. These newly formed vesicles are defective and have leaky vasculature allow NP to diffuse. The energy requirement increase and glycolysis occur. Ultimately acidic environment generated and the advantage of pH uses to drug releasing [41].

## 4. VARIOUS NANOSCALE DRUG DELIVERY SYSTEMS

### 4.1. Nanoparticles

Nanoparticles are submicron-sized polymeric colloidal particles with therapeutic agents of interest encapsulated or dispersed within their polymeric matrix or adsorbed or conjugated onto the surface. Commonly used synthetic polymers to prepare nanoparticles for drug delivery are generally biodegradable [42]. Nanoparticles may also be composed of or transport a variety of substances such as silica, gold or other heavy metals, medicaments, quantum dots, nanocrystals, quantum rods and various contrast agents [43]. Nanoparticle systems offer major improvements in therapeutics through site specificity, their ability to escape from multi-drug resistance and the efficient delivery of an agent. They

can be used for active drug targeting attaching ligand such as antibody on their surface (Figure 2).



**Figure 2.** Different Types of Nanocarriers for drug delivery

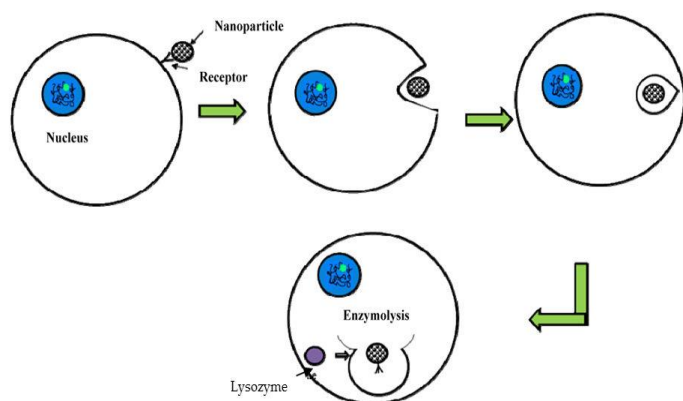
Solid lipid nanoparticles (SLNs) refer to as lipospheres or solid lipid nanospheres, or particles and are generally solid at human physiological temperature (37°C) and have a diameter less than 1000 nm [44]. They can be formed from a range of lipids, including mono-, di- and triglycerides, fatty acids, waxes and combinations thereof. SLNs must be stabilized by surfactants to form administrable emulsions. SLNs form a strongly lipophilic matrix into which drugs can be loaded for subsequent release. SLNs have been investigated for the delivery of various cancer treatments like colon cancer, breast cancer [45].

Polymer-based nanoparticles have been extensively investigated as drug nanocarriers. The most widely researched synthetic polymers include polylactide (PLA), poly (D,L-lactide-co-glycolide) (PLGA) and poly ethylene glycol (PEG). All three polymers are hydrolyzed in vivo and are biodegradable. Other polymers based on biological polysaccharides have been extensively investigated, including chitosan, Cyclodextrin and dextrans [46].

Gold nanoparticles (NPs) consist of a core of gold atoms that can be functionalized by addition of a monolayer of moieties containing a thiol (SH) group. Gold NPs can be synthesized using NaBH<sub>4</sub> to reduce AuCl<sub>4</sub><sup>-</sup> salts in the presence of thiol containing moieties that subsequently form a monolayer around the core gold atom, depending on the stoichiometric gold/ thiol ratio [47]. Drug delivery using gold NPs has been made in DNA delivery for gene therapy and imaging [48]. PEG coated micelles

containing drug are also used to deliver drug as new delivery system (Figure 1). Many other nanoparticulate synthetic, semisynthetic, natural and metals are under investigation to know their potentials as drug delivery materials.

Polymeric nanoparticles may adhere to the cell surface and release drug molecules by diffusion which may enter inside the cell to work. However the entire polymeric nanoparticles can also enter the cell by endocytosis. They bind with the cell surface receptor and formation of endosome takes place. Endosome may be lysed with the help of lysosomal enzymes and the nanoparticles release in the cytoplasm (Figure 3).



**Figure 3.** Endocytosis mediated cellular internalization of drug nanocarriers

#### 4.1.1 Nanoliposomes

Nanoliposomes are the nanosize vesicles made of bilayered phospholipid membranes generally unilamellar with an aqueous interior (Figure 1) [49]. They can be used for the delivery of low molecular weight drugs, imaging agents, peptides, proteins, and nucleic acids. Different anticancer, antiviral drugs are incorporated within the liposomes [50]. Nanoliposomes can also provide slow release of an encapsulated drug, resulting in sustained exposure to the site of action and enhanced efficacy. Usually hydrophilic drugs can be loaded in aqueous compartment and lipophilic drugs are incorporated in the phospholipid layer [51]. However unlike liposome nanoliposome does not undergo rapid degradation and clearance by liver macrophages.

As for the targeted drug delivery, nanoliposome plays an important role. It can be used for passive targeting or active targeting [52]. Due to the leaky vascular structure of the tumor tissue nanoliposomes get predominantly accumulated in the tumor and release the drug for a

prolonged period of time in passive targeting. Active targeting is achieved by incorporating antibody, ligands etc. on the nanoliposomal surface. By active targeting liposomes directly go to the targeted organs or tissues, and release drug for a prolonged period of time, so that the normal cells are not affected and only the diseased cells are affected [53]. Targeted nanoliposomal drug delivery is more efficacious than the non-targeted drug delivery systems. C6-ceramide ligand induced nanoliposome used to treat the blood cancer directly targets the over expressed leukemic cells and decreases the high expression of survivin protein in leukemic cells [54]. The concept of long-circulating or sterically stabilized nanoliposomes is derived for novelibility of delivery systems which can circulate in the blood for a long period of time. Nanoliposomal formulations containing polyethylene glycol (PEG) alter the pharmacokinetic properties of various drug molecules leading to long elimination half-life [55]. Nanoliposomes are expected to bring lots of change in drug delivery in near future.

#### 4.1.2 Dendrimers

Dendrimers are branched polymers, resembling the structure of a tree (Figure 1). Dendrimers represent three dimensional highly branched polymeric macromolecules with the diameter varying from 2.5 to 10 nm. It can be synthesized from both synthetic and natural monomers e.g. aminoacids, monosaccharides and nucleotides. Two classes of dendrimers commonly used for biomedical applications are polyamidoamines and polypropyleneimines [56]. A dendrimer is typically symmetric around the core, and when sufficiently extended it often adopts a spheroidal three-dimensional morphology in water. A central core can be recognized in their structure with at least two identical chemical functionalities. Starting from those groups, repeated units of other molecules can originate with at least one junction of branching. The repetitions of chains and branching result in a series of radially concentric layers with increased crowding [57].

The overall shapes of dendrimers range from spheres to flattened spheroids (disks) to amoebalike structures, especially in cases where surface charges exist and give the macromolecule a “starfish”-like shape. Branching of dendrimers depends on the synthesis processes. Low molecular weight drugs can be placed into the cavities

within the dendrimer molecules and are temporarily immobilized there with hydrophobic forces, hydrogen and covalent bonds <sup>[58]</sup>. The two processes for the synthesis of dendrimers are divergent and convergent methods. In the divergent method dendrimer grows outwards from a multifunctional core molecule. The core molecule reacts with monomer molecules containing one reactive and two dormant groups giving the first generation dendrimer. The convergent method is developed as a response to the weakness of the divergent synthesis. In the convergent approach, the dendrimer is constructed stepwise, starting from the end groups and progressing inwards. When the growing branched polymeric arms, called dendrons, are large enough, they are attached to a multifunctional core molecule. The convergent method is relatively easy to purify the desired product and the occurrence of defects in the final structure is minimised <sup>[59]</sup>. Due to classical polymerization dendrimers have a negligible degree of polydispersity. They are random in nature and produce molecules of various sizes. The size of dendrimers can be carefully controlled during the process of synthesis of dendrimers. Scientists are focusing on newer approaches for speeding up the synthesis process by preassembly of oligomeric branches which can be linked together to reduce the number of synthesis steps involved and also increase the dendrimer yield <sup>[60]</sup>.

Dendrimers are popularly used for transfer of genetic materials in cancer therapy or other viral diseases in different organs because of their monodispersity, high density of functional groups, well-defined shape and multivalency. In gene delivery polyamidoamines (PAMAM) dendrimer is widely used. Some other types of dendrimers are peptide dendrimers, glycodendrimers, polypropylimine dendrimers, Polyethyleneimine (PEI) dendrimers etc.

#### 4.1.3 Nanoshells

Nanoshells (100-200 nm) may be used for drug carrier of both imaging and therapy. Nanoshells consist of nanoparticles with a core of silica and a coating of thin metallic shell <sup>[61]</sup>. They can be targeted to a tissue by using immunological methods. Nanoshells can also be embedded in a hydrogel polymer <sup>[62]</sup>. Nanoshells are currently being investigated for prevention of micrometastasis of tumors and also for the treatment of diabetes. Nanoshells are useful for diagnostic purposes in whole blood immunoassays <sup>[63]</sup>.

## 4.2 Fullerenes and Nanotubes

Fullerenes composed of carbon in the form of a hollow sphere or ellipsoid tube. These are also known as 'bucky balls' because of their resemblance to the geodesic dome design of Buckminster Fuller. Fullerenes are being investigated for drug transport of antiviral drugs, antibiotics and anticancer agents <sup>[64]</sup>. Fullerenes have the potential to stimulate host immune response and productions of fullerene specific antibodies. Soluble derivatives of fullerenes such as C60 have shown great utility as pharmaceutical agents.

Nanotubes are nanometer scale tube like structure and they are of different types like carbon nanotube, inorganic nanotube, DNA nanotube, membrane nanotube etc. <sup>[65]</sup>. Carbon nanotubes can be made more soluble by incorporation of carboxylic or ammonium groups to their structures and can be used for the transport of peptides, nucleic acids and other drug molecules. The ability of nanotubes to transport DNA across cell membrane is used in studies involving gene therapy. DNA can be attached to the tips of nanotubes or can be incorporated within the tubes <sup>[66]</sup>.

## 4.3 Nanopores

Nanopores (20 nm in diameter) consist of wafers with high density of pores which allow entry of oxygen, glucose and other chemicals such as insulin to pass through. Nanopores can be used as devices to protect transplanted tissues from the host immune system, at the same time, utilizing the benefit of transplantation <sup>[67]</sup>.  $\beta$ -Cells of pancreas can be enclosed within the nanopore device and implanted in the recipient's body. Nanopores can also be employed in DNA sequencing. Nanopores are also being developed with an ability to differentiate purines from pyrimidines <sup>[68]</sup>.

## 4.4 Quantum Dots

Quantum dots (QD) are tiny semiconductor nanocrystals type of particles generally no larger than 10 nanometers that can be made to fluoresce in different colours when stimulated by light. The biomolecule conjugation of the QD can be modulated to target various biomarkers <sup>[69]</sup>. They can be tagged with biomolecules and used as highly sensitive probes. QD can also be used for imaging of sentinel node in cancer patients for tumour staging and planning of therapy. This technology also

outlines some early success in the detection and treatment of breast cancer <sup>[70]</sup>. QD may provide new insights into understanding the pathophysiology of cancer and real time imaging and screening of tumors.

## 5. APPLICATIONS OF NANOSCALE DRUG DELIVERY SYSTEMS

### 5.1 Nanotechnology for Brain Drug Delivery

The blood brain barrier (BBB) is a structure formed by a complex system of endothelial cells, astroglia, pericytes, and perivascular mast cells, preventing the passage of most circulating cells and molecules <sup>[71]</sup>. The tightness of the BBB is attributed mainly to the vascular layer of brain capillary endothelial cells which are interconnected side-by-side by tight and adherens junctions. Among the different nanodevices, nanosize drug delivery systems between 1 and 100 nm work as a whole unit in terms of transport to cross BBB <sup>[72]</sup>. Nanosize brain drug delivery systems may promote the targeting ability of drug in brain and at the same time enhance the permeability of molecules through BBB. However crossing of BBB by the nano drug carriers will depend completely on the physicochemical and biomimetic features and does not depend on the chemical structure of drug, inside the nanoparticles <sup>[73]</sup>. Nanosize drug carriers which do not cross BBB generally can be made “stealth” coated with some polymeric materials or other chemicals to avoid the reticuloendothelial system, to display long circulation time and stability in blood, and may be functionalized to successfully cross the BBB and target brain <sup>[74]</sup>.

### 5.2 Nanosize Drug Carriers in Ocular Drug Delivery

Drug loaded nanoparticles with favourable biological properties include prolonging the residence time, decreasing toxicity and high ability of drug penetration into the deeper layers of the ocular structure and minimizing precorneal drug loss by the rapid tear fluid turnover <sup>[75]</sup>. Nanoparticles could target at cornea, retina and choroid by surficial applications and intravitreal injection. Nanocarrier based drug delivery is suitable in the case of the retina, as it has no lymph system, hence retinal neovascularisation and choroidal neovascularization have similar environments to that of solid tumors, and the EPR effect as available for solid

nanoparticles in case of solid tumor may be also available for drug delivery targeted to eyes by nanoparticles <sup>[76]</sup>. Nanoparticles can deliver ocular drugs to the target sites for the treatment of various diseases such as glaucoma, corneal diseases, diabetic retinopathy etc. The uses of nanotechnology based drug delivery systems like nanosuspensions, SLNs and nanoliposomes have greater effect for ocular therapeutic efficacy <sup>[77]</sup>. Nanotechnology-based drug delivery is also very efficient in crossing membrane barriers, such as the blood retinal barrier in the eye.

### 5.3 Nanoparticle Loaded Contact Lenses

Contact lenses loaded with nanoparticles can be effective for topical administration of ophthalmic drugs. Drug loaded contact lenses can also provide continuous drug release because of slow diffusion of the drug molecules through the lens matrix. The soaked contact lenses also delivered drugs only for a period of few hours for some typical drugs <sup>[78]</sup>. The duration of drug delivery from contact lenses can be significantly increased if the drug is first entrapped in nanoformulations, such as nanoliposomes, nanoparticles, or microemulsions. Such drug nanocarriers can then be dispersed throughout the contact lens material. The entrapment of drug in nanocarriers also prevents the interaction of drug with the polymerization mixture. This provides additional resistance to drug release, as the drug must first diffuse through the nanocarriers and penetrate the drug carrier surface to reach the contact lens matrix <sup>[79]</sup>.

### 5.4 Biodistribution of Nanoparticles in The Retina

The ocular biodistribution of nanoparticles can provide insights into the bioavailability, cellular uptake, duration of drug action and toxicity. Factors such as particle size, composition, surface charge and mode of administration influence the biodistribution in the retinal structures and also their drainage from the ocular tissues <sup>[80]</sup>. Larger particles (2  $\mu\text{m}$ ) were found to remain in vitreous cavity near the trabecular meshwork from which they are discharged out from the ocular tissue within 6 days, whereas the particles 200 nm were found evenly distributed in the vitreous cavity, and the inner limiting membrane. The smaller particles 50 nm crossed the retinal barriers, and was detected in the retina even after

2 months post injection <sup>[81]</sup>. The surface chemistry can also affect nanoparticle distribution. Positively charged nanoparticles can adhere to the anionic vitreous network components and aggregate within the vitreous network. The surface chemistry can also affect nanoparticle distribution. Positively charged nanoparticles can adhere to the anionic vitreous network components and aggregate within the vitreous humor <sup>[82]</sup>. Anionic nanoparticles were found to diffuse through the vitreous humor and could even penetrate the retinal layers to be taken up by Muller Cells <sup>[83]</sup>.

Vitreous humor is regarded as the barrier for non-viral ocular gene therapy because of the strong interaction of conventional cationic nature of non-viral gene vectors with the anionic vitreous humor <sup>[53]</sup>. The cationic PEI nanoparticles aggregated within vitreous humor and were prevented from distributing to the retina by the vitreal barrier. In contrast, cationic glycol chitosan (GC) nanoparticles and GC/PEI blended nanoparticles could penetrate the vitreal barrier and even reach at the inner limiting membrane because of the existence of glycol groups on nanoparticles <sup>[84]</sup>.

## 6. TOXICOLOGICAL HAZARDS OF NANOPARTICLES

To use the potential of Nanotechnology in Nanomedicine, full attention is needed to safety and toxicological issues. For pharmaceuticals specific drug delivery formulations may be used to increase the so called therapeutic ratio or index being the margin between the dose needed for clinical efficacy and the dose inducing adverse side effects (toxicity). However, also for these specific formulations a toxicological evaluation is needed. This is particularly true for the applications of nanoparticles for drug delivery. In these applications particles are brought intentionally into the human body and environment, and some of these new applications are envisaged an important improvement of health care <sup>[2]</sup>. Opinions started to divert when toxicologists claimed that new science, methods and protocols are needed <sup>[85]</sup>. However, the need for this is now underlined by several expert reports <sup>[86,87]</sup> and more importantly by the following concepts:

1. Nanomaterials are developed for their unique (surface) properties in comparison to bulk materials. Since surface is the contact layer with the body tissue, and a crucial determinant of particle response, these

unique properties need to be investigated from a toxicological standpoint. When nanoparticles are used for their unique reactive characteristics it may be expected that these same characteristics also have an impact on the toxicity of such particles. Although current tests and procedures in drug and device evaluation may be appropriate to detect many risks associated with the use of these nanoparticles, it cannot be assumed that these assays will detect all potential risks. So, additional assays may be needed <sup>[87]</sup>. This may differ depending on the type of particles used, ie, biological versus non-biological origin.

2. Nanoparticles are attributed qualitatively different physico-chemical characteristics from micron-sized particles, which may result in changed body distribution, passage of the blood brain barrier, and triggering of blood coagulation pathways. In view of these characteristics specific emphasis should be on investigations in (pharmaco)kinetics and distribution studies of nanoparticles. What is currently lacking is a basic understanding of the biological behavior of nanoparticles in terms of distribution in vivo both at the organ and cellular level.

3. Effects of combustion derived nanoparticles in environmentally exposed populations mainly occur in diseased individuals. Typical pre-clinical screening is almost always done in healthy animals and volunteers and risks of particles may therefore be detected at a very late stage.

It may be argued that some if not all of these specific effects will be detected during routine testing and post marketing evaluation after clinical use. All would depend on the types of assays used in the preclinical evaluation, which should be considered in the light of the use of the final products. In addition, one cannot rely on the toxicological profile of the bulk material when that material is used in a nanoformulation. What is clear is that the safety evaluation and the risk benefit analysis need to be performed on a case by case basis. The use of nanoparticles as drug carrier may reduce the toxicity of the incorporated drug. In general the toxicity of the whole formulation is investigated while results of the nanoparticles itself are not described. So, discrimination between drug and nanoparticle toxicity cannot be made. So, there should be a specific emphasis on the toxicity of the “empty” non-drug loaded particles. This is especially important when slowly or non degradable particles are used for drug delivery which may show persistence and



accumulation on the site of the drug delivery, eventually resulting in chronic inflammatory reactions.

## CONCLUSION

On last few years several new technologies have been developed for the treatment of various diseases. Nanotechnologies as drug delivery systems are designed to improve the pharmacological and therapeutic properties of conventional drugs. The highly toxic and low selectivity drug are transported to the target site without accumulate in any place by using nanoparticles. The nanotechnology improves bioavailability of drugs, efficiency and selectivity as well as reduces the side-effects and toxicity<sup>[3]</sup>.

The use of Nanotechnology in medicine and more specifically drug delivery is set to spread rapidly. For decades pharmaceutical sciences have been using nanoparticles to reduce toxicity and side effects of drugs. Up to recently it was not realized that these carrier systems themselves may impose risks to the patient. The type of hazards that are introduced by using nanoparticles for drug delivery are beyond that posed by conventional hazards imposed by chemicals in delivery matrices.

The use of multiple nanoparticles that can be used together may overcome current limitations of each individual nanoformulation alone. For example, gold nanoparticles (AuNPs) have proven to be outstanding vectorisation systems for gene delivery and can be used to target molecular pathways, including those involved in drug resistance and in survival of cancer cells. These NPs may be used in combination with any other polymeric and/or metallic nanoparticles in therapeutic approaches that include drug and thermal ablation, selective delivery via out of the body triggering (light source). All of these applications of nanoparticles in therapeutics still lack enough toxicology and pharmacology studies and data that can support the effective translation into the clinics. However, nanoscale size drug delivery systems may revolutionize the entire drug therapy strategy and bring it to a new height in near future. However, toxicity concerns of the nanosize formulations should not be ignored. Full proof methods should be established to evaluate both the short-term and longterm toxicity analysis of the nanosize drug delivery systems.

## DISCLOSURE STATEMENT

The authors declare that there is no conflict of interest.

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