CHAPTER 1

INTRODUCTION

Introduction

History of Nanotechnology

Many scientists call nanotechnology the key technology of the 21st century. By some estimates, nanotechnology even promises to far exceed the impact of the Industrial Revolution (Nel *et al.,* 2006). Humans have accidentally employed nanotechnology for thousands of years in making steel, paintings and in vulcanizing rubber. 'Nano' is a prefix used to describe 'one billionth', or 10^{-9}, of something. The concept of "Nanotechnology" was introduced by physics Nobel laureate **Richard Feynman** at CalTech, in his famous lecture entitled 'There's plenty of room at the bottom' at the December 1959 meeting of the American Physical Society (Feynman, 1960). Since then many revolutionary developments in physics, chemistry and biology have demonstrated Feynman's ideas of manipulating matter at the atomic scale. In 1974, the term "nanotechnology" was coined by **Norio Taniguchi** a Professor at Tokyo Science University to describe extra-high precision and ultra-fine dimensions. He introduced the 'top-down approach' by predicting improvements and miniaturization in integrated circuits, optoelectronic devices, mechanical devices and computer memory devices. In 1979, K Eric Drexler introduced the 'bottom-up approach' and published "Molecular engineering: An approach to the development of general capabilities for molecular manipulation" where he discussed the creation of larger objects from their atomic and molecular components as the future of nanotechnology (Drexler, 1990).

Nano definitions

Nanoscience refers to the study of the fundamental principles of molecules and structures (nanostructures) with at least one dimension roughly between 1 and 100 nm (Ratner & Ratner, 2003). The application and manipulation of these nanostructures is known as nanotechnology which has a wide range of applications in drug development, water decontamination, information and communication technologies, and the production of stronger and lighter materials. Nanomaterials (NMs) are defined as a material which contains single or agglomerated/aggregated particles where a minimum of 50% of those particles have atleast one dimension in the scale of 1 – 100 nm (Commission, 2011).

Nanoparticles

Nanoparticles (NPs) are defined as particles with all three external dimensions in the nanoscale (1 – 100 nm) (Balogh, 2010). To put the size of nanoparticles (NPs) into perspective, a human hair has a width of about 80,000 nm; a red blood cell has a diameter of 7-8000 nm, while virus is similar in size to nanoparticles. Therefore size of nanoparticles is comparable to biological macromolecules such as DNA and protein (Figure 1.1). NPs have a larger surface area to volume ratio than their bulk counterparts allowing for greater reactivity and they also possess certain physicochemical properties that allow them to have very different characteristics to those particles of larger size (MacCormack & Goss, 2008). NPs are specifically engineered to exploit these electrical, thermal, mechanical and imaging properties that are lacking in their larger counterparts (Liu, 2006).

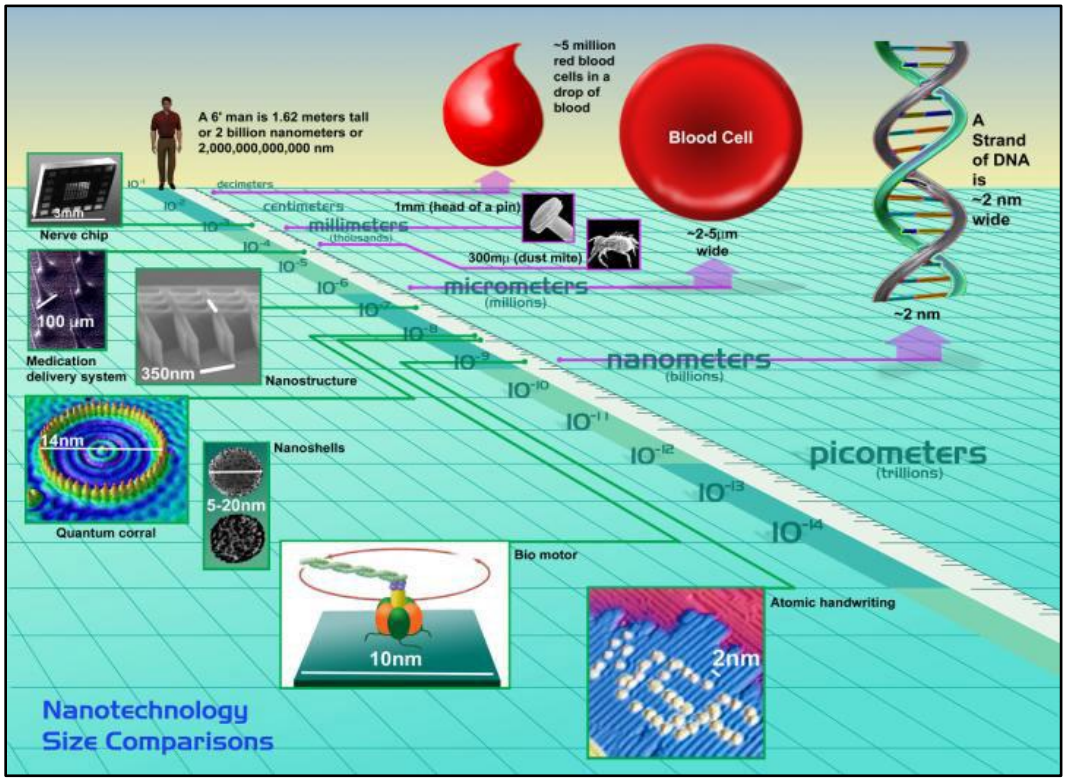

Figure 1.1: Log scale showing size of nanoparticles (NPs) compared to biological component (https://nanohub.org/resources/11965/watch?resid=12402)

Nanoparticles classification

Nanoparticles are generally classified based on their dimensionality, morphology, composition, uniformity and agglomeration.

- **Dimensionality**

Dimension	Definition	Examples
One dimension	One dimension in naometer scale	Tubes, wires, plates, fibers, rods
Two dimension	Two dimension in naometer scale	Films, coatings, multilayers
Three dimension	Three dimension in naometer scale	Particles, quantum dots, hollow spheres

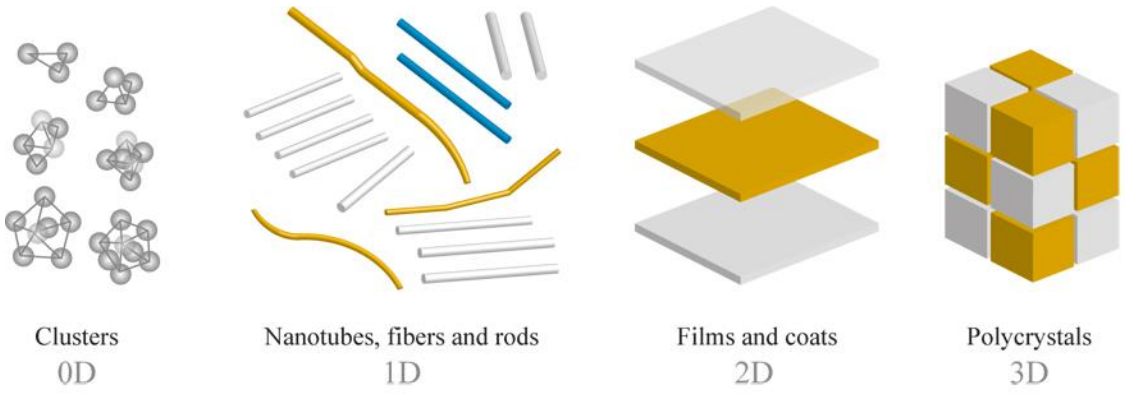

Figure 1.2: Types of nanomaterials by size of their structural elements: 0D (zero-dimensional) clusters; 1D (one-dimensional) nanotubes, fibers and rods; 2D (two-dimensional)

films and coats; 3D (three-dimensional) polycrystals. (http://eng.thesaurus.rusnano.com/wiki/article1371)

- **Morphology**

Morphology of nanoparticles is based on flatness, sphericity and aspect ratio. A general classification exists between high- and low-aspect ratio particles (Buzea *et al.*, 2007). High-aspect ratio particles include nanotubes and nanowires, with various shapes, such as helices, zigzags, belts etc. Low-aspect ratio particles include spherical, oval, cubic, prism, helical, or pillar (Figure 1.3).

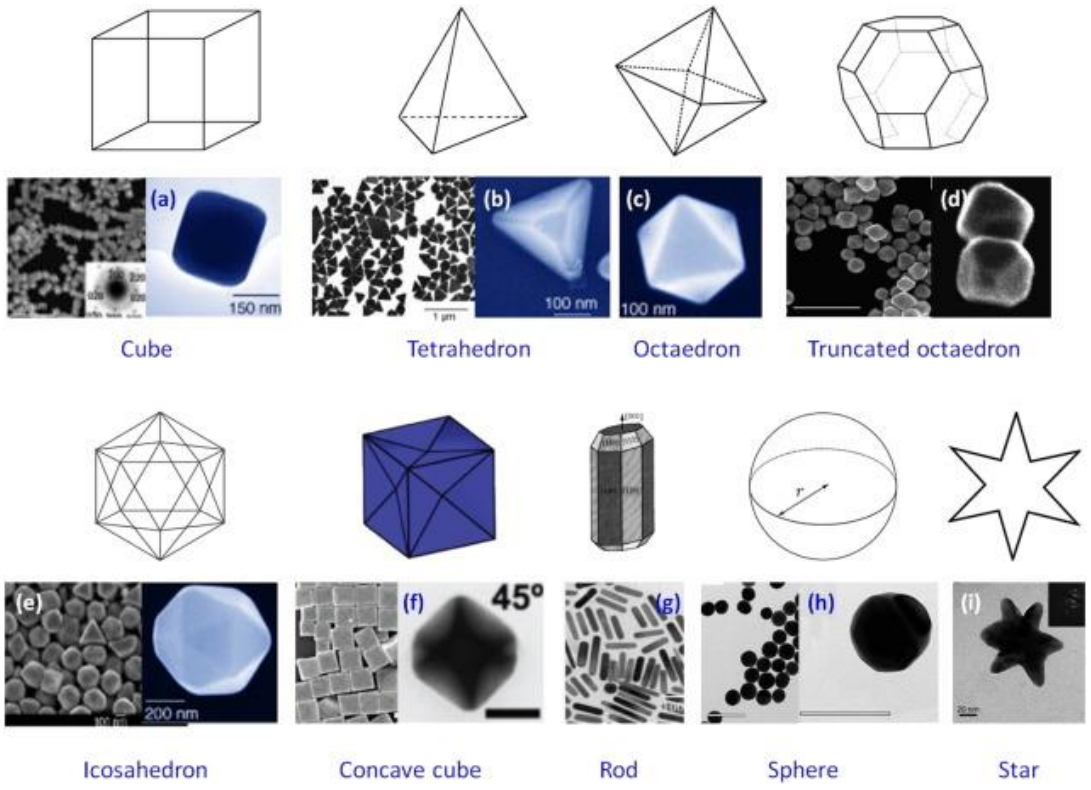

Figure 1.3: Various shapes of nanoparticles (Zaleska-Medynska *et al.*, 2016)

- **Composition**

Nanoparticles are either composed of a single constituent material or a composite of several materials. The nanoparticles found in nature are often agglomerations of materials with various compositions, whereas pure single-composition materials are the ones synthesized.

- **Nanoparticles uniformity and agglomeration**

Based on their chemistry and electro-magnetic properties, NPs can exist as dispersed aerosols, as suspensions/colloids, or in an agglomerate state. Aggregation occurs when particles are bonded strongly to each other and the total surface area of the aggregate is reduced (ISO, 2008). Agglomeration occurs when aggregates are weakly bonded together and the total surface area of the agglomerate is similar to the sum of the surface areas of the individual particles/aggregates (ISO, 2008).

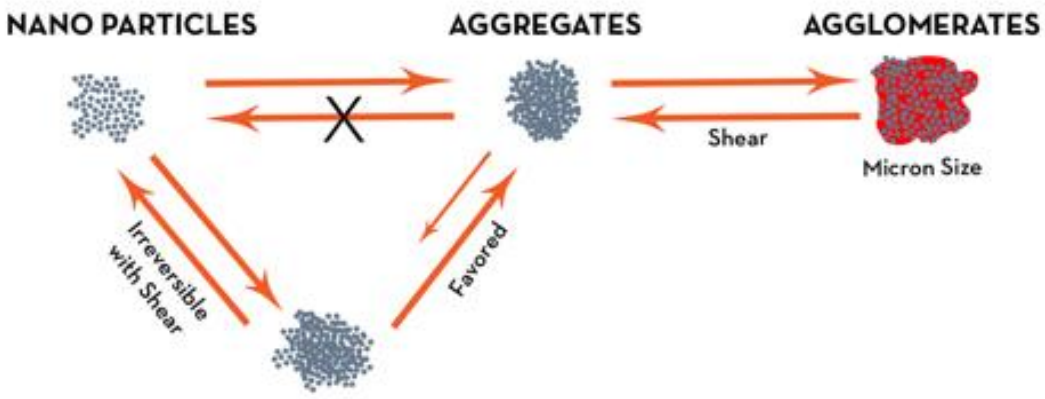

Figure 1.4: Aggregation/Agglomeration state of nanoparticles (https://knowledge.ulprospector.com/2435/pc-nano-particles-when-smaller-is-better/)

Sources of nanoparticles

Although nanotechnology is an emerging field of science, yet the exposure to nanoparticles to the human and the environment is not new. The history of NPs exposure can be traced to the human's evolutionary period. Since the evolutionary period, humans were exposed to the natural atmospheric particulates such as fires, volcanoes, sea spray, and erosion. However, currently humans are not only exposing to naturally occurring NPs but also to engineered nanoparticles (ENPs).

Natural nanoparticles

They have existed since before life began on Earth. These NPs are largely either of anthropogenic (from grinding of primary or secondary minerals, wear of metal or mineral surfaces, combustion) or pyrogenic (smoke from volcanoes or fires) origin. The most common natural NPs are soil colloids, which are constituted of silicate clay minerals, iron- or aluminium oxides/-hydroxides or humid organic matter, including black carbon. Also, airborne nanocrystals of sea salts formed from the evaporation of sea water sprays are among the most common natural NPs. Carbon black, is also formed anthropogenically, e.g. during fires, traffic and industry, are thus not new to the environment (Figure 1.5).

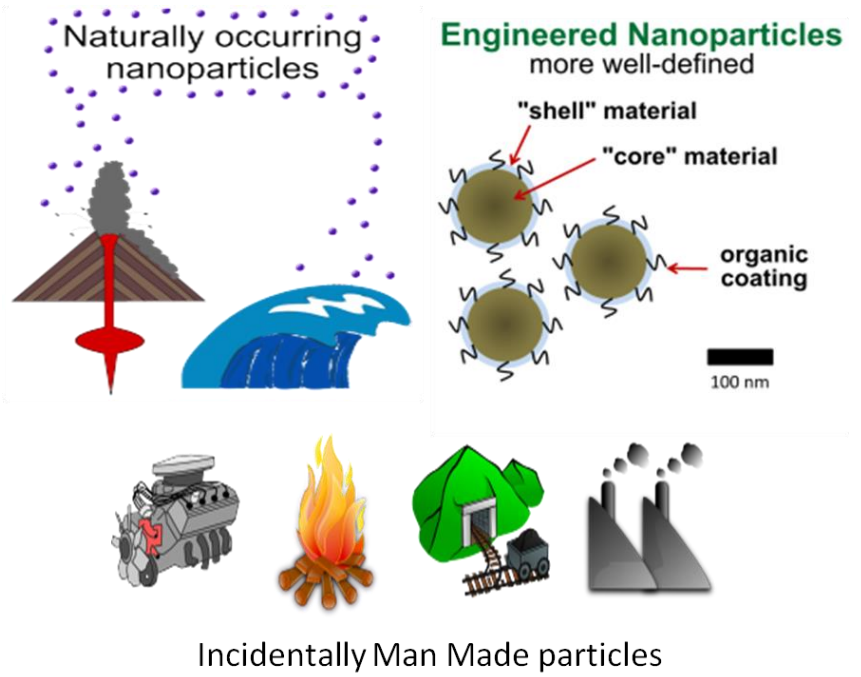

Figure 1.5: Sources of natural and engineered nanoparticles (http://sustainable-nano.com/2013/03/25/nanoparticles-are-all-around-us/)

Engineered nanoparticles (ENPs)

Engineered nanoparticles are particles intentionally created (in contrast with natural or incidentally formed) with one or more dimensions greater than 1 nm and less than 100 nm. These different kinds of engineered nanoparticles have been used in applications such as pigments, resins and cosmetics. In addition, nanotechnology will increasingly generate new materials and products that are based on NPs, devices and tools. It is the sum of existing and newly developed manufactured (intentionally made) nanoparticles that form the primary target of risk assessment to the humans and the environment (Figure 1.5).These particles are manufactured to improve the quality of products because of their unique physical, chemical or biological properties are known as engineered nanoparticles. Table 1.1 summarizes the properties of ENPs and its applications.

Table 1.1: Properties of Engineered nanoparticles and its applications

Property	Application
Optical	Anti-reflection coatings. Tailored refractive index of surfaces. Light based sensors for cancer diagnosis.
Magnetic	Increased density storage media. Nanomagnetic particles to create improved detail and contrast in MRI images.
Thermal	Enhance heat transfer from solar collectors to storage tanks. Improve efficiency of coolants in transformers.
Mechanical	Improved wear resistance. New anti-corrosion properties. New structural materials, composites, stronger and lighter.
Electronic	High performance and smaller components, e.g. capacitors for small consumer devices such as mobile phones. Displays those are cheaper, larger, brighter and more efficient. High conductivity materials.
Energy	High energy density and more durable batteries. Hydrogen storage applications using metal nanoclusters. Electrocatalysts for high efficiency fuel cells. Renewable energy, ultra high performance solar cells. Catalysts for combustion engines to improve efficiency, hence Economical
Biomedical	Antibacterial silver coatings on wound dressings. Sensors for disease detection (quantum dots). Programmed release drug delivery systems. “interactive” food and beverages that change color, flavor or nutrients depending on a diner’s taste or health
Environmental	Clean up of soil contamination and pollution, e.g. oil. Biodegradable polymers. Aids for germination. Treatment of industrial emissions. More efficient and effective water filtration.

Types of Engineered nanoparticles

Engineered nanoparticles are often classified on the basis of their chemical composition:

- Fullerenes (e.g. Buckminster fullerenes, CNTs, nanocones etc.)
- Metal ENPs (e.g. elemental Ag, Au, Fe)
- Metal Oxides (TiO_2, ZnO, etc.)
- Complex compounds (alloys, composites, nanofluids etc., consisting of two or more elements) e.g. Cobalt-zinc iron oxide
- Quantum dots (or q-dots) e.g. Cadmium-selenide
- Organic polymers (dendrimers, polystyrene, etc.)

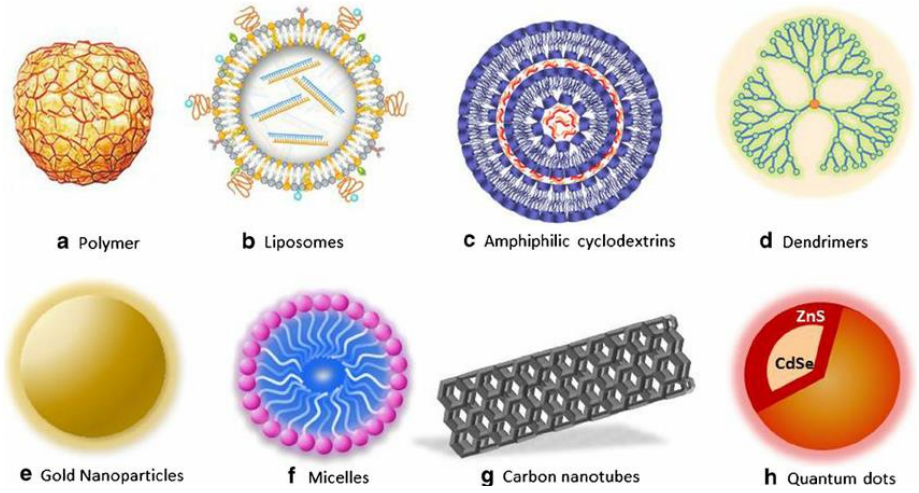

Figure 1.6: Types of engineered nanoparticles (Mc Carthy *et al.*, 2015)

Application of engineered nanoparticles

Today various products containing nanomaterials are available in the market to improve the quality of products (Figure 1.7).

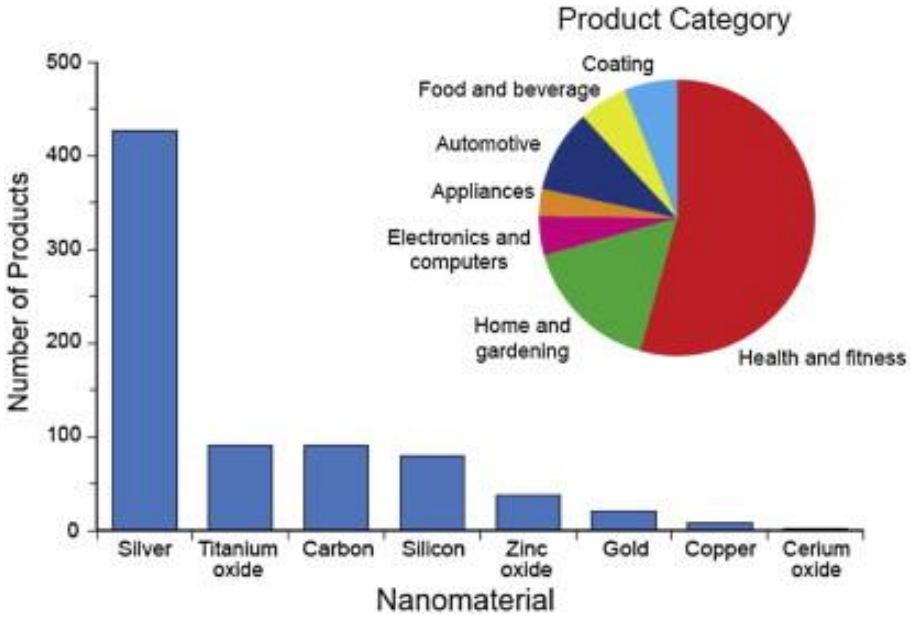

Figure 1.7: Number of products containing commercial NMs (bar chart) and NMs in products as defined by product categories (pie chart) (As listed on the Consumer Products Inventory, The Project on Emerging Nanotechnologies, http://www.nanotechproject.org/cpi/about/analysis/)

Most widely used applications of nanomaterials are microelectronics, synthetic rubber, catalytic compounds, photographic supplies, inks and pigments, coatings and adhesives, ultrafine polishing compounds, UV absorbers for sunscreens, synthetic bone, optical fiber cladding, cosmetics, fabrics and their treatments, filtration, dental materials, surface disinfectants, diesel and fuel additives, hazardous chemical neutralizers, automotive components, electronics, scientific instruments, sports equipment, flat panel displays, drug delivery systems, and pharmaceutics. Table 1.2 summarizes the major applications of some of the engineered nanoparticles.

Table 1.2: Major applications of Engineered Nanoparticles

Nanoparticle	Applications	References
Fullerene	Organic photovoltaics, antioxidants, catalysts, polymers, water purification and biohazard protective agents	Yadav and Kumar (2008)
Carbon nanotubes (SWCNT, MWCNT)	Electronics and polymer industry, batteries	Köhler *et al.* (2008)
Gold	Medical field and biological probe	(Gibson *et al.*, 2007; Horisberger & Rosset, 1977; Qian *et al.*, 2008; Tsai *et al.*, 2007)
Silver	As disinfectant in medical field, cosmetics, water purifiers, plastic wares, textiles.	(Chopra, 2007; W.-R. Li *et al.*, 2010)
Iron	Magnetic recording media, magnetic tapes, catalysts, drug delivery, remediation of contaminated sites.	(Plum *et al.*, 2005; W.-x. Zhang, 2003)
Platinum	Antioxidant	J. Kim *et al.* (2008)
Quantum dots	Biomedical imaging, targeting specific cell membrane receptors, cellular biomolecules such as peroxisomes and DNA and electronic industries	(Alivisatos, 2004; Chan *et al.*, 2002; Colton *et al.*, 2004; Dubertret *et al.*, 2002; Lidke *et al.*, 2004; Y. Wu *et al.*, 2004)

Metal oxide nanoparticles

Presently, the most commonly used nanomaterials include simple metal oxides such as titanium oxide (TiO_2), zinc oxide (ZnO), magnesium oxide (MgO), copper oxide (CuO), aluminium oxide (Al_2O_3), manganese oxide (MnO_2) and iron oxide (Fe_3O_4, Fe_2O_3) (Balasubramanyam *et al.*, 2010; Fahmy & Cormier, 2009; Oszlánczi *et al.*, 2010; Pan *et al.*, 2010; Sárközi *et al.*, 2009; N. Singh *et al.*, 2010). Metal oxide NPs are finding increasing application in a wide range of fields and represent about one-third of the consumer products in the nanotechnology market (Maynard *et al.*, 2006). Titanium dioxide NPs are among the most commonly used metal oxide NPs and can exist naturally as three mineral compounds viz. anatase, brookite, and rutile, each of them has its own unique property (Macwan *et al.*, 2011). TiO_2 Bulk is mostly used as a pigment because of its brightness, high refractive index, and resistance to discoloration. Nearly 70% of the TiO_2 produced is used as a pigment in paints and glazes, enamels, plastics, paper, fibers, foods, pharmaceuticals, cosmetics, and toothpastes (Weir *et al.*, 2012). However, more attention is being given to the applications of TiO_2 nanomaterials because of larger surface area to volume ratio and enhanced physicochemical properties. In 2005 the global production of nanoscale TiO_2 was estimated to be 2000 metric tons worth $70 million (Davis *et al.*, 2010). By 2010 the production had increased to 5000 metric tons and is expected to continue to increase till 2025 (Landsiedel *et al.*, 2010).

The two most commonly studied iron oxide nanoparticles (IONPs) are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) (Gupta & Gupta, 2005). IONPs are found naturally in the environment as particulate matter and in volcanic eruptions. Fe_3O_4 (magnetite) or γ-Fe_2O_3

(maghemite) particles can be generated as emissions from traffic, industry and power stations and also be specifically synthesized chemically for many applications (Faraji & Wipf, 2009; Hurley *et al.*, 2003; Karlsson *et al.*, 2008). Magnetic behaviour is an important parameter considered while designing and synthesizing superparamagnetic iron oxide NPs (SPIONs) so as to maximally facilitate their imaging and therapeutic efficacy (Gould, 2006). ZnO is present in the Earth's crust as a mineral zincite; however, most ZnO used commercially is produced synthetically. ZnO is nontoxic and is compatible with human skin making it a suitable additive for textiles and surfaces that come in contact with human body. The properties of ZnO, e.g. UV absorption and specific surface area, are improved at the nanoscale. ZnO NPs have been shown to be more efficient at absorbing UVA radiation than TiO_2 NPs (Pinnell *et al.*, 2000) and ZnO NPs are also transparent which is advantageous to the cosmetics and sunscreen industries (Houdy *et al.*, 2011).

Aluminium oxide (alumina) is a compound of aluminium and oxygen with chemical formula of Al_2O_3. Alumina has several phases such as gamma, delta, theta, and alpha (Piriyawong *et al.*, 2012). However, the alpha alumina phase is the most thermodynamically stable phase and commonly occurring crystalline form of aluminum oxide and its hardness make it suitable for applications as an abrasive and as a component in cutting tools. Magnesium oxide (MgO) occurs naturally as periclase and exhibits unique properties like good refractoriness, good corrosion resistance, high thermal conductivity, low electrical conductivity and transparency to infrared.

Table 1.3: Major applications of Metal oxide Nanoparticles

Nanoparticle	Applications	References
Titanium dioxide (TiO_2)	Photo catalyst, in photovoltaic devices, cosmetics, paintings, electronic devices and sensors.	(Adams *et al.*, 2006; Contado & Pagnoni, 2010; Davis *et al.*, 2010; Kägi *et al.*, 2008; Lorenz *et al.*, 2010)
Iron oxide (Fe_2O_3)	Ferrofluids, rotary shaft sealing, loudspeakers, computer hard drives and in magnetic resonance imaging.	(Bulte & Kraitchman, 2004; Schlorf *et al.*, 2010); (Kumar & Mohammad, 2011)
Zinc oxide (ZnO)	UV blocker in sunscreens, sensors, non linear optical systems.	(Nakada *et al.*, 2004; Nomura *et al.*, 2003).
Aluminum oxide (Al_2O_3)	In drug delivery and as an anti-microbial.	(Balasubramanyam *et al.*, 2010; W. Jiang *et al.*, 2009)
Magnesium oxide (MgO)	As scrubber for air pollutant gases (CO2, NOx, SOx), sensors and catalysts.	(S.-t. LI *et al.*, 2005; Mahmoud *et al.*, 2016; Yeheskel *et al.*, 2005).

Nanotoxicology

Nanotoxicology refers to the study of the potentially harmful effects of nanomaterials, in particular nanoparticles (Donaldson *et al.*, 2004). Nanotoxicology focuses upon gaining a thorough understanding of the relationship between the toxicity of NPs depending on their dose levels and physicochemical properties such as size, shape, reactivity and material composition (Paur *et al.*, 2011). The rapid growth of nanotechnology industry and its ever increasing applications will inevitably increase the concentration of nanomaterials in the environment, with potential human and environmental exposure as a consequence (Figure 1.8)(Europea, 2004). The human body is exposed to NPs through four possible routes: inhalation of airborne NPs, ingestion of drinking water or food additives, dermal penetration by skin contact, and injection of engineered nanomaterials (Oberdörster, Oberdörster, *et al.*, 2005). Regarding the environment, nanomaterials may potentially affect it in three possible ways: (i) direct effect on micro-organisms, invertebrates, fish and other organisms; (ii) interaction with contaminants that may change the bioavailability of toxic compounds and/or nutrients; and (iii) changes to non-living environmental structures (Lead & Smith, 2009). Thus, it is essential to know the toxicity of nanomaterials before using it for the wide range of applications mentioned above (Donaldson & Poland, 2013; Gao *et al.*, 2015; Haynes, 2010).

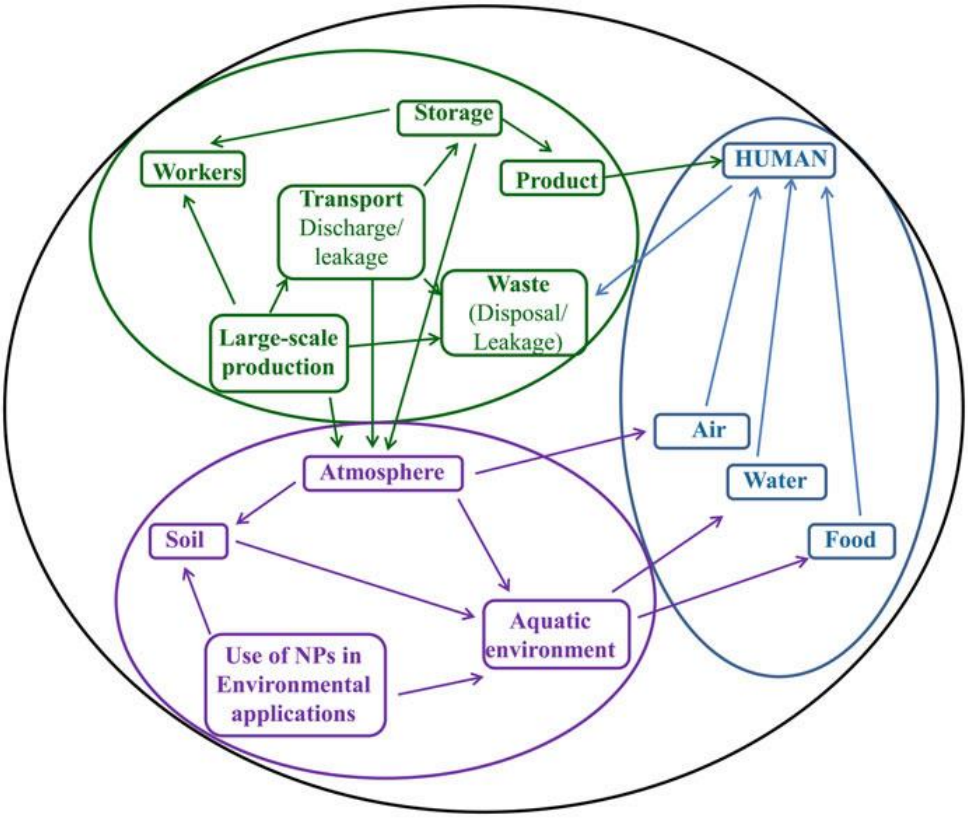

Figure 1.8: Possible ways through which nanoparticles enter into environment and humans (Viswanath & Kim, 2016)

Fate and Transport of Engineered Nanoparticles in the Environment

Nanoparticles are emitted into the environment by primary sources such as natural phenomena, combustion processes or industrial activities (e.g. welding) or are released during generation and handling of engineered NPs. After ENPs are released to the environment, they may remain as they are, or their characteristics may be altered by the action of the environment. Moreover, the physico-chemical characteristics (e.g., pH, ionic strength, presence of organic matter) of various environmental media may affect the transport of ENPs (Soni *et al.*, 2015).

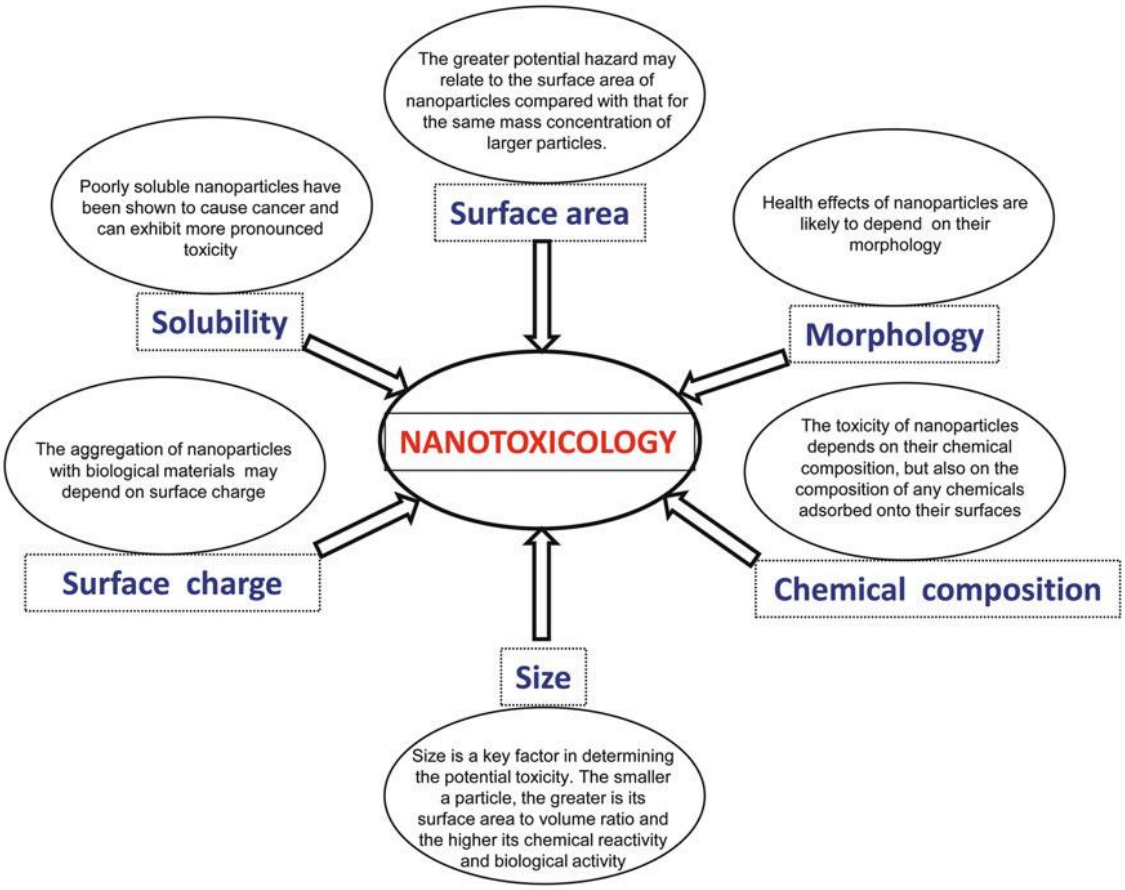

Figure 1.9: Physicochemical properties of engineered nanomaterials leading to nanotoxicology (Viswanath & Kim, 2016)

Internalization of nanoparticles in biological systems

Understanding cellular uptake of NPs is important for assessment of the NP-cell interaction. Different mechanisms have been proposed for internalization of NPs into the plasma membrane. Reports suggest endocytosis to be one of the most commonly used pathway for transport across the membrane. Table 1.4 summarizes some of the major physiological mechanisms, which are involved, in cellular uptake along with the intracellular transport.

Table 1.4: Pathways of entry of nanoparticles into cell

Type of endocytosis	Description
Phagocytosis	An actin-based mechanism occurring primarily in professional phagocytes, leading to phagosomes and phago-lysosomes.
Pinocytosis	It is fluid phase uptake of smaller particles (around 5-10 nm) and its various types are described below (Caballero-Díaz & Cases, 2016)
Clathrin mediated endocytosis	Associated with the formation of a clathrin lattice and depending on the GTPase dynamin, forming primary endosomes and late endosomes including multivesicular bodies (Zhao *et al.*, 2011)
Caveolae mediated endocytosis	Flask-shaped invaginations made of caveolin dimers, also dynamin-dependent and forming caveosomes, which fuse with the endoplasmic reticulum (S. Zhang *et al.*, 2015)
Adsorptive pinocytosis	Internalization through non-specific interactions with complementary binding sites on cell surface (Caballero-Díaz & Cases, 2016)
Macropinocytosis	An actin-based pathway, leading to macropinosomes which might be exocytosed or fuse with lysosomes (Kou *et al.*, 2013)

Apart from endocytosis, NPs have been reported to penetrate the cell also by passive diffusion where there is transport across the cell membranes without the need of energy, due to a chemical gradient (Y. Jiang *et al.*, 2015)

Toxicity of Metal oxide nanoparticles

Recently, there has been an increase in studies pertaining to the undesirable effects of nanotechnology. However, researchers have not been fully successful in comprehending

the consequences of the interaction of nanoparticles with biological systems. We summarize below, the results of the few studies that have been performed to test the toxicity of metal oxide nanoparticles on cell lines and on various animal models.

Table 1.5: *In vitro* studies on toxicity of metal oxide nanoparticles

Name of Metal oxide ENPs	**Cell line**	**Results**	**Reference**
Titanium dioxide (TiO_2)	Human bronchial epithelial cell line (BEAS-2B)	Rutile TiO_2 nanoparticles (200 nm) induced hydrogen peroxide and oxidative DNA.	Gurr *et al.* (2005)
	Human lymphoblastoid cells	TiO_2 nanoparticles treatment increased MNBC and comet tail moments.	Sanderson *et al.* (2007)
	Chinese hamster ovary cells (CHO)	No chromosomal damage was observed after TiO_2 NPs treatment.	Theogaraj *et al.* (2007)
	Peripheral blood lymphocyte	TiO_2 NPs induces ROS generation in lymphocytes, thereby activating p53-mediated DNA damage checkpoint signals.	Kang *et al.* (2008)
	Human lung	TiO_2 NPs treatment	Bhattacharya *et*

	fibroblasts IMR-90 and BEAS-2B cells	neither showed DNA breakage nor free radical generation.	*al.* (2009)
Zinc oxide (ZnO)	Human sperm and lymphocyte cells	Significant DNA damage was observed in both the cells exposed to the nanoparticles	Gopalan *et al.* (2009)
	Human carcinoma intestinal cells (Caco-2 cells)	ZnO nanoparticles caused DNA damage	Gerloff *et al.* (2009)
	Human epidermal cell line (A431)	Significant DNA damage and oxidative stress was observed after ZnO NPs treatment	Sharma *et al.* (2009)
	Primary Human keratinocyte cell (HEK)	ZnO NPs caused significant DNA damage and induced oxidative stress	Sharma, Singh, *et al.* (2011)
	Human nasal mucosa cells	ZnO NPs caused genotoxicity	Hackenberg *et al.* (2011)
	Human Hepatocellular cells (HepG2)	ZnO nanoparticles caused significant increase in DNA damage with an increase in the Olive tail moment (OTM)	Sharma, Anderson, *et al.* (2011)
	Human lung	Iron oxide nanoparticles	Karlsson *et al.*

	adenocarcinoma A549 cells	caused oxidative DNA damage	(2009)
Iron oxide (Fe_2O_3)	Human lung diploid IMR-90 fibroblasts	Fe_2O_3 NPs did not induce 8-OHdG adduct formation	Bhattacharya *et al.* (2009)
	Human carcinoma intestinal cells (Caco-2 cells)	No genotoxic effect was observed.	Gerloff *et al.* (2009)
	Rat pheochromocytoma PC12 cells	Iron oxide nanoparticles caused dose dependent cell mortality and arrested cell cycle in G2/M phase	J. Wu and Sun (2011)
	Syrian hamster embryo cells	No significant DNA damage was detected after iron oxide nanoparticles treatment	Guichard *et al.* (2012)
	Human lymphoblastoid MCL5 cells	Iron oxide nanoparticles induced chromosomal abberations	Sharma *et al.* (2012)
	Human skin epithelial A431 cells	Iron oxide nanoparticles caused DNA damage	Ahamed *et al.* (2013)
	Human lymphocytes	Fe_2O_3 NPs caused DNA damage and chromosomal abberations	Rajiv *et al.* (2016)
	Human cervix adenocarcinoma cells	MgO NPs induced oxidative stress and	Krishnamoorthy *et al.* (2012)

	(HeLa)	apoptosis	
Magnesium oxide (MgO)	Mouse lymphoma cell line (L5178Y) and human bronchial epithelial cells (BEAS-2B)	Al_2O_3 nanoparticles induced DNA damage.	H. W. Kim *et al.* (2009)
	Chinese hamster ovary cells (CHO)	Micronuclei formation was observed after Al_2O_3 NPs treatment.	Di Virgilio *et al.* (2010)
Aluminium Oxide (Al_2O_3)	Human brain microvascular endothelial cells (HBMEC)	Al_2O_3 nanoparticles reduced HBMEC viability, altered mitochondrial potential, increased cellular oxidation and decreased tight junction protein expression	Chen *et al.* (2008)
	Human mesenchymal stem cells	Al_2O_3 nanoparticles induced oxidative stress	Alshatwi *et al.* (2013)

Table 1.6: *In vivo* studies on toxicity of metal oxide nanoparticles

Name of Metal oxide ENPs	**Cell line**	**Results**	**Reference**
	Freshwater crustacean Daphnia magna	TiO_2 NPs did not induce genotoxicity	Lee *et al.* (2009)

Titanium dioxide (TiO_2)	Earthworm Eisenia fetida	DNA damage was reported at the higher doses, the nanoparticles were accumulated in the mitochondria	Hu *et al.* (2010)
	Male Sprague Dawley rats	Macrophages and neutrophils were detected in the alveolus of the lung, no genotoxicity was induced.	Naya *et al.* (2012)
	Male B6C3F1 mice	TiO_2 NPs reached the bone marrow cells and caused cytotoxicity, but were not genotoxic.	Sadiq *et al.* (2012)
	Adult male Wistar rats bone marrow cells	TiO_2 NPs were not cytotoxic but increased the frequency of micronuclei formation	Dobrzyńska *et al.* (2014)
Zinc oxide (ZnO)	Mice	ZnO NPs induced oxidative stress and were cytotoxic	C.-H. Li *et al.* (2012)
	Liver and kidney cells of mice	ZnO NPs accumulated in the liver and caused injury and oxidative stress mediated DNA damage and apoptosis	Sharma *et al.* (2012)

	Mice	Surface modified ZnO NPs did not induce genotoxicity	Kwon *et al.* (2014)
	Drosophila Melanogaster	ZnO NPs did not induce genotoxicity or toxicity	Alaraby *et al.* (2015)
Iron oxide (Fe_2O_3)	Male wistar rats	Fe_2O_3 NPs mainly caused mydriasis, exophthalmos and effects on spontaneous locomotor activity	Bourrinet *et al.* (2006)
	Female Swiss mouse bone marrow erythrocytes	Iron oxide nanoparticles did not cause micronuclei formation	Estevanato *et al.* (2011)
	Different tissues of female Wistar rats	IONs affected synaptic transmission and nerve conduction	Kumari *et al.* (2012)
	Wistar rat leucocytes and Wistar rat bone marrow cells	Fe_2O_3 NPs did not induce genotoxicity	S. P. Singh *et al.* (2013)
	Adult female Sprague-Dawley rats	Biomarkers of inflammation and apoptosis were found to be increased after iron oxide nanoparticles treatment. Endothelial and Schwann cells were	Y. Kim *et al.* (2013)

		vulnerable to cell death.	
	Wistar rats	Iron oxide nanoparticles lowered ROS production and protein expression of p53	Nora *et al.* (2016)
Magnesium oxide (MgO)	Swiss Albino Mice	MgO NPs influence the behavior and internally affect the organs of mice	Shaikh *et al.* (2015).
	Zebra fish embryo	MgO NPs decreased the hatching rate and survival of embryos	Ghobadian *et al.* (2015)
Aluminium Oxide (Al_2O_3)	Zebra fish **(*Danio rerio*)** embryo	Aluminium Oxide nanoparticles did not affect larval development or the hatching of the embryos	Zhu *et al.* (2008)
	ICR strained mice	Aluminium Oxide nanoparticles impaired neurobehavioral functions and induced cell necrosis and apoptosis	Q. Zhang *et al.* (2011)

Possible Mechanisms by Which Nanoparticles Induce Toxicity

Nanoparticles are toxic to living organisms more because of their aggregation behavior than their solubility. In addition, the shape and size of NPs also affect their interactions

with the cell surface and cytosolic components. NP-induced toxicity is mainly mediated through the generation of reactive oxygen species (ROS) in cells.

- **Generation of ROS**

ROS, key molecules during cell signaling and homeostasis, are reactive species of molecular oxygen. ROS constitute a pool of oxidative species including superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) (Manke *et al.*, 2013). Over-production of ROS caused by nanoparticle exposure can perturb the cellular balance between ROS and antioxidants, resulting in oxidative stress, which can be detrimental. High ROS levels indicate oxidative stress and can damage cells by peroxidizing lipids, altering proteins, disrupting DNA, interfering with signaling functions, and modulating gene transcription (Oberdörster, Oberdörster, *et al.*, 2005) and finally ending up in cancer, renal disease, neurodegeneration, cardiovascular or pulmonary disease. ROS can steal electrons from lipids in cell membrane resulting in decline in physiological function and cell death (Sayes *et al.*, 2005). Reduced particle size results in structural defects and altered electronic properties on the particle surface creating reactive groups on the NP surface (Donaldson & Tran, 2002; Oberdörster, Maynard, *et al.*, 2005). Within these reactive sites, the electron donor or acceptor active sites interact with molecular O_2 to form $O_2^{\bullet-}$ which in turn can generate additional ROS via Fenton-type reactions (Nel *et al.*, 2006). Fenton reactions usually involve a transition metal ion that reacts with H_2O_2 to yield $OH^{\bullet}$ and an oxidized metal ion. ROS are also induced endogenously where the mitochondrion is a major cell target for NP-induced oxidative stress. Nanoparticles enter the mitochondria and stimulate ROS via impaired electron transport chain, structural

damage, activation of NADPH-like enzyme system, and depolarization of the mitochondrial membrane (Sioutas *et al.*, 2005; Xia *et al.*, 2006).

- **DNA Damage**

There are several studies which have shown that size-dependent entry of ENPs into the nucleus may lead to disturbance in nuclear organization and lead to DNA damage (Bhattacharya *et al.*, 2009). ROS can oxidatively attack DNA and thereby cause structural alterations of the DNA. ROS (usually •OH) are known to attack guanine residues (the most easily oxidized base) in DNA to induce the formation of 8-OH-dG. 8-Hydroxydeoxyguanosine (8-OHdG), is the major oxidative DNA-damage that can produce mutations - A: T to G: C or G: C to T: A transversion mutation, because of its base pairing with adenine as well as cytosine (Shibutani *et al.*, 1991; Wood *et al.*, 1992). Thus the oxidative DNA damage is one of the factors for spontaneous mutagenesis. ENPs can cause DNA damage either by direct binding with the DNA or the free radicals produced by them. ROS are known to produce chain breaks, modification of carbohydrate parts and nitro bases by oxidation, nitration, methylation or deamination reactions leading to DNA damage. It can also activate the NADPH oxidase enzymes and reduce the disulfides, protein-protein cross linking, peptide fragmentation and modification of prosthetic group that may result in the depletion of glutathione in the cell. The enhancement of endogenous generation of free radicals results in oxidative stress and can lead to the induction of oxidative DNA damage. Figure 1.10 shows the mechanism of nanoparticles induced toxicity.

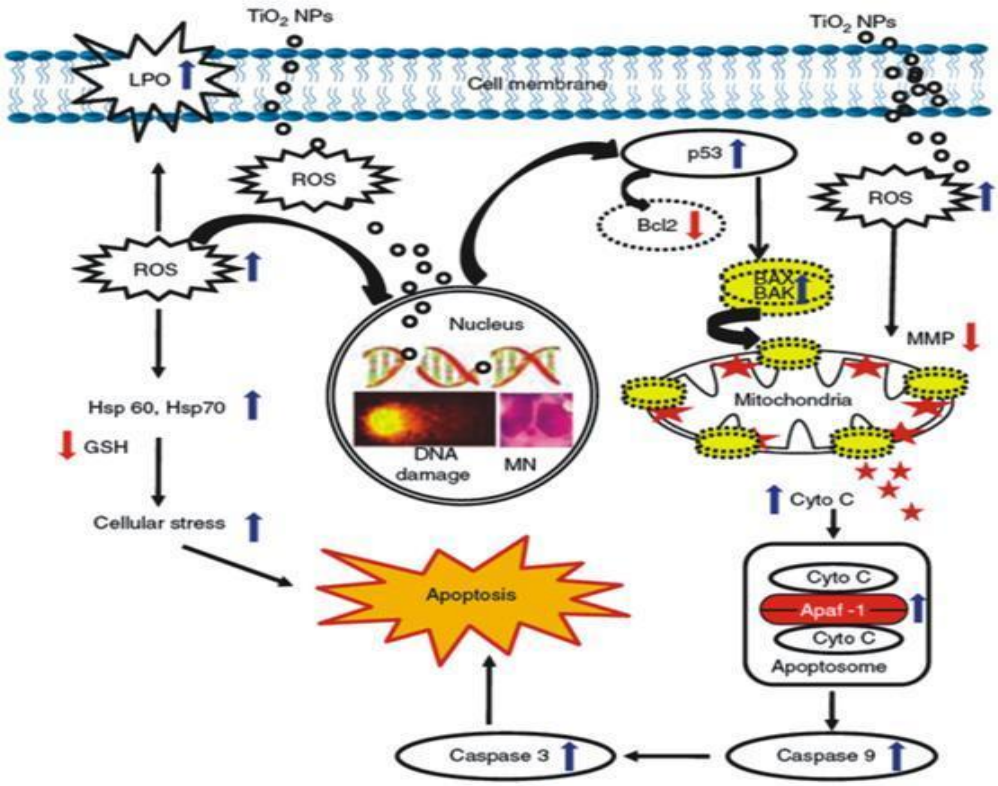

Figure 1.10: Possible mechanism of TiO_2 NPs induced cellular toxicity (Shukla *et al.*, 2013)

- **Interaction with Proteins**

It is now well-recognized that the surfaces of biomaterials (e.g., implants and medical devices) are immediately covered by biomolecules (e.g., proteins, natural organic materials and enzymes) when they come in contact with a biological medium (Engel *et al.*, 2004; Mahmoudi *et al.*, 2011; Norde & Gage, 2004). The absorption of biomolecules to such surfaces confers a new "biological identity" which determines the subsequent cellular/tissue responses. The physico-chemical properties (size, shape, composition, surface functional groups, and surface charges) of NPs influence how they interact with proteins. Lynch and Dawson (2008) suggested that the interaction of NPs with proteins could form a "protein corona", which promotes cell internalization of NPs. The

biomolecules in the corona can be recognized by receptors of the cell membrane leading to uptake of NPs (Lynch & Dawson, 2008). It has been reported that multireceptor sites on rat brain epithelial cells (RBEC) facilitate the interaction of NPs with apolipoproteins in ways that allow the NPs to enter the cells (H. R. Kim *et al.*, 2007). Also, NP-protein interactions alter the 3D confirmation of proteins, leading to impairment of protein activity (Aggarwal *et al.*, 2009). Halliwell and Gutteridge (2015) reported that metal NPs interact with sulfhydryl (-SH) groups present on proteins which could oxidize GSH and inhibit antioxidant pathways.

Proteins are chains of amino acids, where the exact sequence of the amino acids determines the protein's shape, structure, and function. Since protein function is linked with protein conformation, understanding the type of conformational changes undergone by the proteins upon interaction with NPs is important. Factors such as electrostatic interactions, hydrophobic interactions, and specific chemical interactions between the protein and the nanoparticles play important role in their interaction (Lynch & Dawson, 2008). The subsequent biological responses to the unfolded proteins are highly affected by their conformational changes. Therefore, a better understanding of the consequences of interaction with NPs on the protein conformation and activity is essential to develop functional as well as safe NPs.

Rationale of the study

Development of an avian embryo and production of a progeny is the essence of survival of an avian species in the wild. After all, lack of production of a progeny by a particular bird species can be a crucial factor for its speedier extinction. NPs are known to cross biological barriers and hence it is assumed that it can possibly cross an avian eggshell as

well as the shell membranes and the choriallantoic membrane. Therefore, the ground and marsh nesting birds are a high risk group of species susceptible to the impact of NPs. Many studies have reported impact of NPs on avian embryonic tissue such as the bursa of fabricius, brain, liver etc. In an avian egg, albumen is of crucial importance in regulating whole-body protein synthesis in chicken embryos during incubation. Nanoparticles are known to interact with proteins and form a protein corona that may transmit biological effects due to altered protein conformation, exposure of novel epitopes and perturbed function (due to structural effects or local high concentration). These facts warranted a detailed study to decipher avian-metal oxide nanoparticles interaction. Hence, the proposed study answers basic questions pertaining to avian- nanoparticle interaction in terms of its impact on avian embryonic development.

Objectives of the study

The objectives of the study to decipher the avian-metal oxide nanoparticles interaction are:

- Toxicological evaluation of NPs on chicken embryo and chicken embryonic fibroblasts.
- Physicochemical and particokinetic evaluations of NPs
- To identify toxicity targets during NP-avian embryo interaction.
- To assess the ability of chosen NPs in crossing *in ovo* biological barriers.