REVIEW

Nanoparticles toxicity and their routes of exposures

Clarence Suh Yah[1]*, Geoffrey Simate Simate[2] and Sunny Esayegbemu Iyuke[2]
[1]Biochemistry and Toxicology Section, National Institute for Occupational Health NIOH), 25 Hospital Road, Constitutional Hill, Johannesburg, Gauteng, South Africa
[2]School of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg, P/Bag 3, Wits 2050, South Africa

Abstract: The new scientific innovation of engineering nanoparticles (NPs) at the atomic scale of 100 nm or less, has led to numerous novel and useful wide applications in electronics, chemicals, environmental protection, biological medicine. Manufacturers and consumers of the nanoparticles-related industrial products however, are likely to be exposed to these engineered nanomaterials which have various physical and chemical properties. These nanosize particles are likely to increase an unnecessary infinite toxicological effect on animals and environment, although their toxicological effects associated with human exposure are still unknown. In order to understand the effects of these exposures, this review seeks to examine the various toxicological portal routes associated with NPs exposures. These NPs can enter the host systems via skin spores, debilitated tissues, injection, olfactory, respiratory and intestinal tracts. These uptake routes of NPs may be intentional or unintentional. Their entry may lead to various diversified adverse biological effects. Until a clearer picture emerges, the limited data available suggest that caution must be exercised when potential exposures to NPs are encountered. Methods used in determining NPs portal of entry into experimental animals include pharyngeal instillation, injection, inhalation, cell culture lines and gavage exposures. This review also provides a step by step systematic approach for the easy identification and addressing of occupational health hazards arising from NPs.

Keywords: Nanoparticles, exposure, routes.

INTRODUCTION

The term nanotechnology encompasses the production of new materials at atomic scale. It builds nanoparticles (NPs) whose diameter is below 100nm by manipulating matter (Mohanpuria *et al.,* 2008; Simate *et al.,* 2010; Ngoy *et al.,* 2011; Yah *et al.,* 2011a, b). According to Stern & McNeil (2008) NPs can be classified into two groups depending on the nature of the particle (i.e., engineered or incidental). NPs such as the quantum dots, carbon nanotubes, dendrimers and fullerene which have diameters < 100nm are termed as engineered. These particles can be compared to sizes of living things (fig. 1). Also NPs like diesel particles are incidentally generated while living things such as bacteria and larger viruses are natural living cells with diameter < 100nm (fig. 1).

The technology can be applied to biological systems, or derivatives thereof, to make nanomaterials for specific use. It incorporates a wider range of useful industrial and biological processes that modify the needs of humanity at the nanoscale level. Studies have also shown that

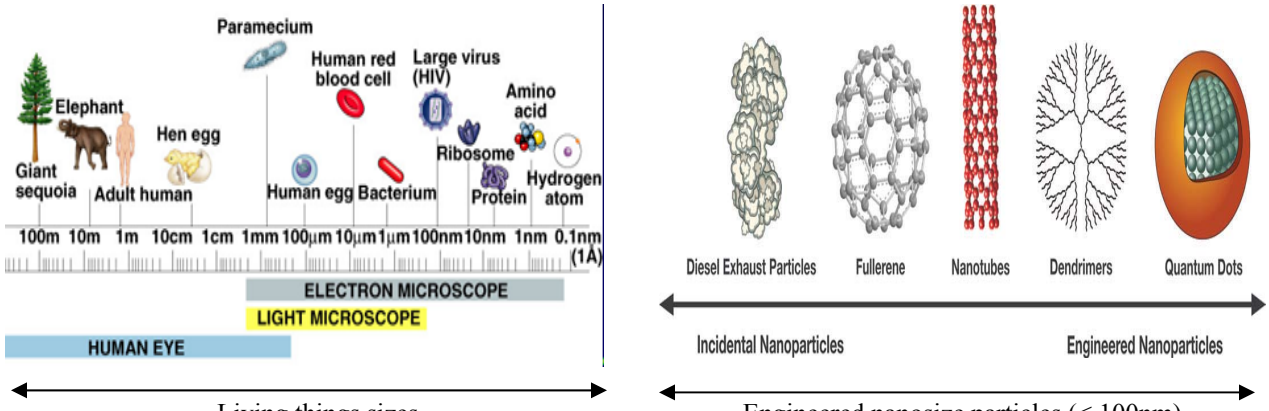


Fig. 1: Structures of some nanoparticles (Stern and McNeil, 2008; Mohanpuria *et al.,* 2008).

*Corresponding author: e-mail: yahclar@gmail.com

microorganisms can as well be used as potential developers of NPs (Roco and Bainbridge, 2005; Andrew *et al.,* 2004; Deendayal *et al.,* 2006; Jail *et al.,* 2007; Sadowski *et al.,* 2008). In view of these developments, nanotechnological companies (fig. 2) are gaining new prospects that enable them to improve the performance of products and life with uncertain health safety issues.

However, the benefits of nanotechnology have been offset by substantial discussion about the health issues arising from nanotechnologies (Giles, 2003; Maynard and Kuempel, 2005). Occupational illnesses, however, do occur as a result of respiratory dust particle. This is attributed mainly to ultrafine NPs (Donaldson *et al.,* 2006). These occupational diseases tend to be characterized by temporal or permanent physiological dysfunction with only a few visible symptoms. On the other hand, there is a possibility that they may gain access to the body and pose serious toxicological problem. These NPs might enter the host via the lungs, dermal, wound tissues, intestinal tract either intentionally or unintentionally (Peter *et al.,* 2004; Oberdorster *et al.,* 2005; Chen *et al.,* 2006; Mayank and Mansoor, 2007). NPs can enter the environment and animals system through different pathways. For instance, it could be through effluent, spillage, consumer products and disposal. The intake is usually tolerated by the organism system but when a certain range is exceeded, it would cause toxic effect and even deaths. Since NPs have environmental and animal health risks, it is, therefore, inevitable to carry out research so as to understand and anticipate such risk through risk assessment and risk management. However, in view of scarce health information arising from NPs, it is paramount to take some remedial actions so as to reduce the hazards to workers and the environment.

The ability of nanoscale materials to enter the host system, however, is amongst the numerous features that researchers need to observe in ascertaining whether such substances may pose any hazards. Ultrafine materials (e.g., agglomerates, aggregates) have the highest probability of entering the system especially when they are airborne (Ku and Maynard, 2005). Once NPs are in the body they can transverse the cells by persorption, intermingle with the tissue cells causing malfunctioning of the organs (Oberdorster *et al.,* 2005). Studies have shown that airborne nanomaterials can be deposited in the respiratory tract when inhaled. From there, the NPs can transverse the blood stream, and be relocated to other organs (Warheit *et al.,* 2004; Zhang *et al.,* 2005). In order to understand the effects of NPs exposures on health, this review seeks to examine the various toxicological portal routes associated with nanoparticles exposures. Until a clearer picture emerges, the limited data available suggest that caution must be exercised when potential exposures to NPs arise. Previous studies have shown decrements in the functioning of the lungs and adverse respiratory symptoms to workers that were exposed to NPs (Borm *et al.,* 2004; Beck-Speier *et al.,* 2005; Bottini *et al.,* 2006; Donaldson *et al.,* 2006). Therefore, the portal routes of entry of engineered NPs need to be understood.

Health exposure concerns of nanoparticles

As earlier stated, NPs have diameters between 1 and 100nm that may be in the gas, liquid, powder or embedded in matrix. The precise meaning can be determined by the shape as well as the diameter of the NPs measured. The morphologies might differ extensively at the nanoscale. For instance, fullerene are spherical whereas single-wall-carbon nanotubes (SWCNTs) are cylindrical (Warheit, 2006; Tanta and Cumpson, 2007; Aihong *et al.,* 2008; Kaiser *et al.,* 2008; Witzmann and Monteriro-Riviere, 2008).

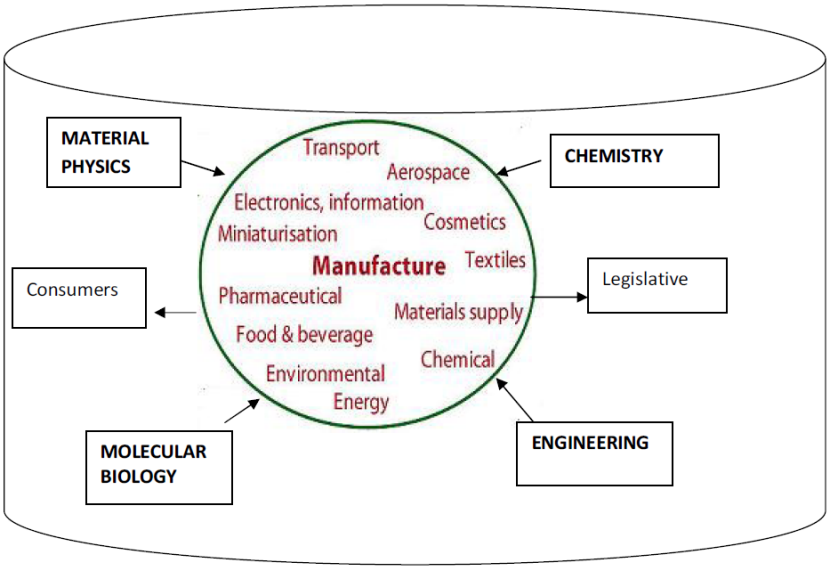


Fig. 2: A perspective of nanotechnology applications as related to discipline.

The scarcity of scientific data obliges us to face uncertainty of the risks arising from NPs. Therefore, the processes of generating nanoscale materials in the gas phase, or using or producing nanoscale materials as powders or solutions pose the risk for releasing NPs (Oberdorster *et al.,* 2005). Potential exposure may arise during their production, development, use, or discarding (Stern and McNeil, 2008). Also there is likely exposure to NPs if it involves disturbing deposited nanoscale material. There are likely possibilities that the resultant environment may increase NPs hazards to:

- Working with ultrafine particles in solution without adequate protection (gloves, gowns, masks) will increase the risk of skin exposure.
- Working with nanoscale materials in solution during pouring or mixing operations, where a high degree of agitation is involved, will lead to an increase possibility of inhaling droplets being formed.
- Generating NPs in the gas phase in non-enclosed systems will enhance the likelihood of aerosol expose to the workplace.
- Using ultrafine powders will lead to the risk of aerosolization.
- Maintenance on equipment and processes used to produce or fabricate nanosize materials or the clean-up of spills or waste material will pose a potential for exposure to workers performing these tasks (Cross *et al.,* 2007).
- Cleaning of dust collection systems used to capture NPs can pose a potential for both skin and inhalation exposure.
- Machining, sanding, drilling, or other mechanical disruptions of materials containing nanoscale materials can potentially lead to aerosol of NPs.
- The transfer of nanomaterials in open systems is likely to increase exposure potentials even for relatively hydrophobic NPs (Lam *et al.,* 2006). Open systems during NPs processing may increase exposure to human beings.

Ultrafine particles

Ultrafine particles are not purposefully manufactured nor are they necessarily of a constant composition or size although they are less than 100nm, so they are nano-sized. The ultrafine particles have been used to define aerosol and airborne particles less than 100 nm in diameter. There is no clear distinction between ultrafine particles and nanoparticles. The two terms are used so as to make a distinction between engineered (nanoparticle) and incidental (ultrafine) nanoscale particles (Nemmar *et al.,* 2001; Jacobson and Seinfelf, 2004; Beck-Speier *et al.,* 2005; IRSST, 2006). However, this does not imply that significant differences exist among their properties in relation to measurement, risk assessment, and control of exposures.

According to Borm and Kreyling (2004) the effects of ultrafine particles absorbed by inhalation were influence by dose, deposition, dimension, durability and defence mechanisms. Toxicity of NPs often will depend on the efficiency of these mechanisms. The dose, deposition, dimension, and durability link toxicity to surface concentration. On the other hand, the T and B lymphocytes at the respiratory site help to reduce the potential effect of the surface concentration and toxicity of NPs (IRSST, 2006).

Engineered nanoparticles

Engineered NPs are nanoscale particles which are products of processes involving combustion and vaporization which are designed with very specific physical and chemical properties that make them very attractive for commercial development (Medina *et al.,* 2007). They have found applications in cosmetics, clothes electronics, biomedicine, aerospace and computer industry. Due to their small size and large surface area, engineered NPs may have a high rate of pulmonary deposition and, translocation ability to travel from the lung to systemic sites, penetrate dermal barriers, and a high inflammatory potency per mass (Medina *et al.,* 2007). Therefore, engineered NPs just like ultrafine particles need to be studied to determine if they pose health risks similar to those that have been associated with the ultrafine particles. In biomedical field, engineered NPs application as drug delivery system is on the increase due to the vast physiochemical properties that make them very accessible to be conjugated with various drugs and molecules (Ngoy *et al.,* 2011). However, the NPs bio-conjugates cellular toxicity needs to be validated before human applications.

Nano-aerosol

Aerosol is a suspension of fine solid particles or liquid droplets in a gas. It includes smoke, air pollutants, and perfume spray. A nanoaerosol, therefore, comprise of NPs suspended in a gas, and may be present as discrete particles, or as clusters of NPs (Heim *et al.,* 2005). These assemblies may have diameters larger than 100 nm. In the case of an aerosol consisting of micrometer-diameter particles formed as assemblies of NPs, the definition of nanoaerosol is open to interpretation. It is generally accepted that if the nanostructure associated with the NPs is accessible through physical, chemical, or biological interactions, then the aerosol may be considered a nanoaerosol. However, if the nanomaterial within individual micrometer-scale diameter does not directly influence particle activity, the aerosol would not be described as aerosols (Dreher, 2004). There are not many literatures on health implication of gaseous nanoparticles, however studies are needed to identify mechanisms by which nano-aerosols induce cellular damage and generate oxidative stress (Quadros and Marr, 2010). Airborne such as silver nanoparticles exert toxic effects mainly through

the aqueous phase and the complication depend on the particle size and the concentration of the silver particles. Although National Institute for Occupational Health has set airborne at concentration of 10 mg $m^{-3,}$ inhaled Silver nanoparticles have been found to show inflammatory reaction of the respiratory and cardiovascular systems, leading to asthma complications, chronic bronchitis (Quadros and Marr, 2010). According to Quadros and Marr, (2010) NPs are capable penetrating more into the cellular tissues of the respiratory tract causing intracellular reactions and oxidative stress.

Nano-agglomerate

Agglomerate is a group of coarse accumulations of material particles held together by weak forces such as van der Waals forces, electrostatic forces and surface tension (Ku and Maynard, 2005; ISO, 2006). These agglomerates of NPs have the potential to enter the body or skin if they are in the form of airborne. The agglomerate are usually deposited according to their diameter (18), resulting in inflammation and later the development of interstitial fibrosis and granulomas (Jacobson and Seinfeld, 2004; Seaton and Donaldson, 2005; Warheit, 2006). However, more research needs to be done in order to ascertain other health effects of these NPs.

Nano-aggregate

Unlike nano agglomerates, nano aggregates are heterogeneous particles held together by relatively strong forces, and as a result cannot easily breakup (IRSST, 2006; ISO, 2006). Furthermore, these aggregates can adhere to each other through Van der Waals forces to form agglomerates (Friedlander and Pui, 2003). For example, Murr *et al* (2004) clearly showed that airborne particles are agglomerates of aggregates of aerodynamic diameters ranging from a few nanometres to several micrometres.

Aggregates NPs have been shown to cross the cellular membrane barrier causing inflammatory and cytokines activities, however, their activities have not been able to display serious cytotoxicity effect to cells (Peter *et al.,* 2004). Furthermore, aggregates of SWCNTs have been found to induce dose interstitial inflammation in mice (Maynard and Kuempel, 2005). The health effect of these carbon nanotubes however, depends on the concentration and size of the clumps or aggregates of the NPs. The aggregate NPs when in the lungs have the ability to disaggregate and form smaller particles. The hazards created by the small particles in the lugs can catalysts inflammatory responses (Maynard and Kuempel, 2005).

Nano-portal routes

Due to the increased use of nanomaterials, and the envisaged potential risks associated with exposure, it is inevitable that the potential routes of entry are well understood. As alluded to earlier, the main routes of entry are through the skin, lungs or intestinal tract causing adverse biological effects (Peter *et al.,* 2004; Warheit *et al.,* 2004; Oberdorster *et al.,* 2005; Davoren *et al.,* 2007; Li *et al.,* 2007). Other potential routes of NPs in the case of biomedical applications include parental administration such as intravenous, intradermal and peritoneal exposures into experimental Stern and McNeil, 2008; De Jong *et al.,* 2008). Factors that my influence NPs entry includes size, charge, surface area and shape (Auffan *et al.,* 2008). According to Auffan *et al* (2008) nanosize particles have an elevated surface/volume ratio of approximately 35-40% of atoms localized at the surface of a 10nm NPs compared with less than 20% for particles larger than 30nm. These NPs represent a target for the potential toxicity. The toxicity of these materials depends on their persistence or clearance from the different organs due to the immune response of the host (Jeffrey *et al.,* 2008).

Nano-respiratory route

Much research has been done with NPs toxicity of the respiratory tract. These nanomaterials can be inhaled (Peter *et al.,* 2004; Warheit *et al.,* 2004; Oberdorster *et al.,* 2005) naturally in the form of aerosol, powders or artificially by instillation into the respiratory tract for toxicity studies. For instance, findings by Warheit *et al* (2004) and Li *et al* (2007) found that NPs can be instilled via intratracheal, oropharyngeal and intrapharyngeal respectively when determining the toxicity of NPs in animals respiratory tract. The respiratory system is the part of the organs that deal with the process of respiration that is, moving from the nose through the trachea to the bronchioles (fig. 3). The system is responsible for taking in and sending out air from living animals. The lungs are part of the respiratory tract responsible for exchange of gases.

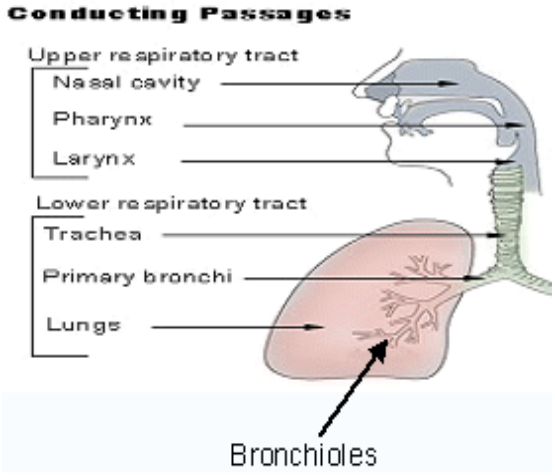


Fig. 3: Passage way of NPs of the lungs. http://en.wikipedia.org/wiki/Upper_respiratory-tract

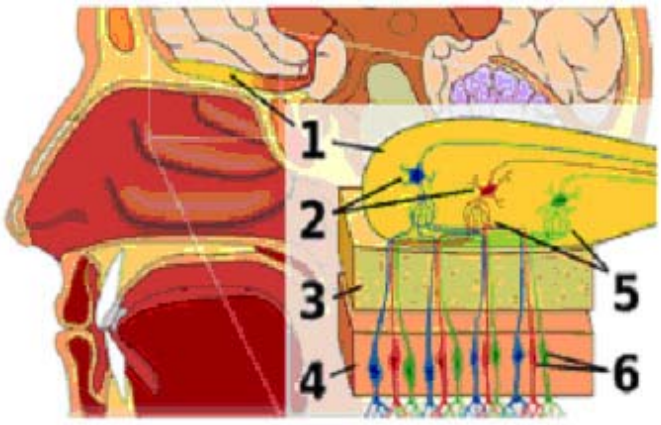
Inhalation is the most common route of exposure to NPs in the workplaces. Once inhaled, these materials are carried by electrostatic force of the air from the upper to

the lower respiratory tract (Oberdorstern *et al.,* 2005; Cross *et al.,* 2007). The particles are usually inhaled in the form of airborne NPs, systemic administration of drugs, chemicals and other compounds to the lungs through direct cardiac output to the pulmonary arteries (Jeffrey *et al.,* 2008). Immediately the NPs are in the pulmonary sites, translocation to blood circulation through the lymphatic pathways can occur depending on the nanomaterial size. Earlier reports by Berry *et al* (1977) described the translocation of 30nm gold NPs across the alveolar epithelium of rats by interstitial instillation. This report was further supported by Ballou *et al* (2004) when they showed quantum dots (10nm) in liver, lymph and bone marrow of experimental mouse. Also when the NPs are deposited in the alveolar, they are usually attacked by the process of phagocytosis. This also led to chemotactic activities which trigger the complement system cascade and the inflammatory cell response to the site of NPs. According to Oberdorster *et al* (2005) the effect of the inflammatory and the complement cascade may take upto two month 10 days in rat and roughly two years in humans to be cleared. According to earlier reports of Borm and Kreyling (2004) the interstitial translocation of NPs across the lung alveolar cells are more common in primate's species but they assumed that the high translocation can occur in humans. Gwinn and Vallyathan (2006) reported that inhaled nanosize particles may trigger phagocytosis and cause systemic health effects in experimental animals. Other animal studies, have also shown that discrete NPs may enter the body from the lungs and translocate the bloodstream to other organs (Obordorster *et al.,* 2005; Nemmar *et al.,* 2001; Das *et al*., 2007).

There are also reports that nanoscale viruses (30nm) such as the polio virus found in the lungs can enter the sensory nerve endings of the olfactory organ (Yakovenko *et al.,* 2009). The discrete NPs that are deposited in the nasal region have been found to enter the brain by translocation to the olfactory nerve of experimental animals (Flesken *et al.,* 2007). Other reports by Oberdorster *et al* (2005) confirmed that inhaled MnO_2 NPs (30nm) can be translocated from the lungs into the olfactory organ after a 7 day post exposure in experimental rats. The olfactory system is the sensory organ used for olfaction, or the sense of smell (fig. 4), the prominent part of the face of mammals.

It receives stimuli interpreted as odours from volatile and soluble substances and lies in the upper part of the nasal cavity, and that forms a mucous membrane continuous with the rest of the lining of the nasal cavity. This reveals that the nerve endings of the nasal olfactory mucosa are portal entry of nanomaterials into the host. According to earlier findings by De Lorenzo (1970) silver coated colloidal gold particles of upto 50nm can be transported through olfactory nerves as well as across the synapses of the dendrite cells. Taking into cognizance the nano-respiratory tract toxicity studies from non human animals there is likely possibility that these translocation pathways can exist in human highly dependent on the chemical and physical properties of the NPs. It can be concluded therefore, the unbridled growth and use of ultrafine particles (carbon nanotubes) in medical and human health could possibility become the "asbestos" of the 21st century.

The deposition of NPs in the respiratory tract is influenced by the particle's size. According to NIOSH (2006) agglomerates of NPs can be deposited in the respiratory tract according to the diameter of the agglomerate. In addition, reports by ICRP (1994) have shown that discrete NPs are deposited in the lungs to a greater extent than larger particles. Studies by Daigle *et al* (2003) have shown that increase in breathing rate and mechanism (e.g., nasal or mouth) affects the rate of NPs



The olfactory system
1. Olfactory build, 2. Mittral cells, Bone, 4. Nasal epithelium, 5. Glomerulus, 6. Olfactory receptor cells.

Fig. 4: The olfactory nerve passage of nanoparticles. http://en.wikipedia.org/wiki/File:Olfactory_system.svg

deposition. Persons with existing lung diseases or conditions are also susceptible to increase in NPs deposition.

Nano-gastrointestinal route

The gastrointestinal tract is the system of organs in animals that takes in food substances, and expels the remains as waste (fig. 5). The functions are for digestion, absorption and defecation and differ from species to species. Fig. 5 below demonstrates a simple illustration of primates' intestinal tract.

Ingestion is another route whereby NPs may enter the body. Most of the toxicity studies pertaining to NPs are focused mainly on respiratory tract (RT) exposures with few studies describing the gastrointestinal tract (GI) exposures. The gastrointestinal tract (GI) exposures usually occur either unintentional from hand to mouth transfer or from traditional materials. Furthermore, it could occur during handling of the materials that contain the NPs. Other possible gastrointestinal tract (GI) exposures may come from particles cleared from the respiratory system through the mucociliary escalator (Obordorster *et al.,* 2005; Chen *et al.,* 2006; Li *et al.,* 2007). Nanomaterials can also be exposed into the GI viz water, food, cosmetics, drugs, drug delivery devices. Some studies have investigated the potential intestinal absorption and the translocation of NPs and generally found uptake within the GT. Studies by Jani (1990) have showed that gastrointestinal absorption of titanium particles ranging from 150-500 nm are larger than those typically used in sunscreen into the lymph, liver and spleen. More critical findings concerning the fate of ingested NPs can be viewed from radioactive metal studies, where NPs have been shown to translocate from the gastrointestinal tract to other organs (Borm and Kreyling, 2004).

Furthermore, NPs administered orally are usually absorbed, through the epithelial cells of the Peyer's patches in the gut-associated lymphoid tissue (GALT) and also through the gut enterocytes (Chen *et al.,* 2006; Alexander, 2005). Earlier reports by Jani *et al* (1990) indicated that oral administration of NPs can be absorbed

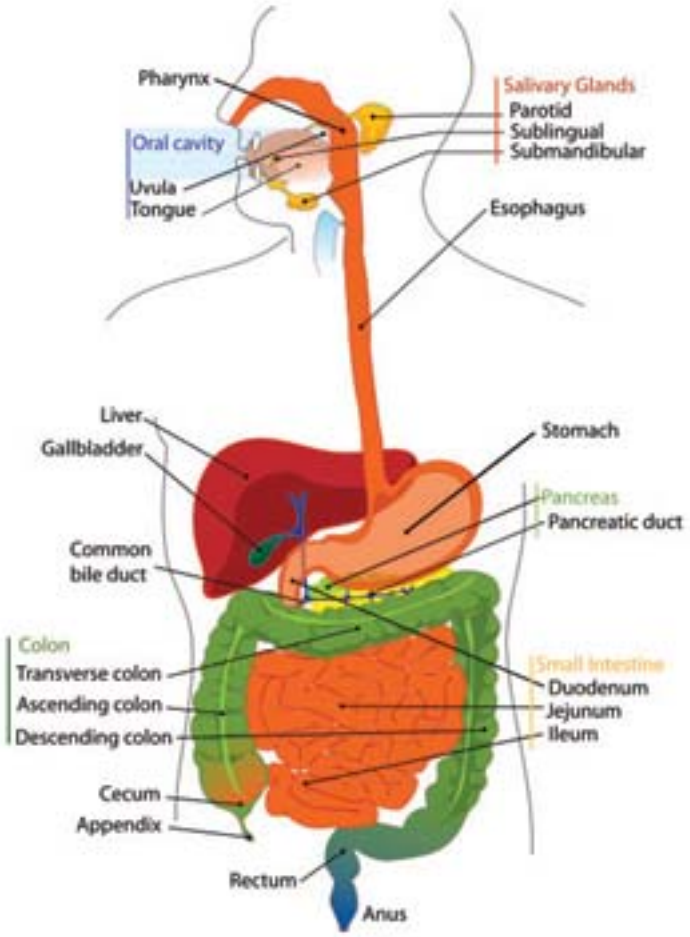


Fig 5: Intestinal passage of nanoparticle orally and by gavage feeding respectively (http://en.wikibooks.org/wiki/File:Digestive_system_diagram_en.svg

across the GI tract via the lymph nodes to the liver and spleen. Reports of Yoshifumi (2002) showed that NPs substances are easily taken up by the recticuloendothelial cells during drug transfer. The uptake of these particles of different sizes can lead to different toxicological effects. Studies on polystyrene latex NPs in the range of 3 µm to 50 nm have shown that maximal absorption can occur with particle size of approximately 50-100 nm in diameter (Hussain *et al.,* 2001). However, further studies by Hussain *et al* (2001) found that even latex particles above 1 mm can be retained in the Peyer's patches. The ingestion of ultrafine particles by the GI tract can stimulate phygaocytosis at the GI mucosa and cause antigen-antibody mediated reactions and inflammatory responses and from there systematically to other organs of the body (Hussain *et al.,* 2001).

Studies by Chen *et al* (2006) have shown that copper NPs can cytotoxically trigger injuries on the lymph, payer's patches, liver, spleen and kidney of experimental animals. In these studies by Chen *et al* (2006) after gavaging mice with copper NPs, they discovered that the GI tract toxicity belongs to Hodge and Sterner Scale (3 classes of moderately toxicity). Other symptoms associated with the toxicity were alimentary canal include loss of appetite, vomiting and diarrhea. Others included hypopnea, tremor and arching of the back.

Nano-dermal route

The dermal organ is the outer surface covering of the skin (epidermis and dermis) and the largest organ of the body. It guards the underlying internal organs (figs. 6a and 6b). Apart from its interface with the environment, it also protects the body against external interferences and acts as an insulator and synthesis of vitamin D.

Skin barrier alterations (such as the wounds, scrapes, or dermatitis) could act as exposures routes to NPs into the body and should not be overlooked. Debilitated skin represents a good channel for entry of finer and even larger particles (0.5-7µm) as reported by Blundell *et al* (1989). These studies found large accumulation of soil particles in lymph nodes of bear footed human associated with elephantiasis. Findings by North Carolina State University have shown that quantum dot NPs skin penetration was as a result of skin abrasion therefore raising the workers safety issues arising from workplace activities especially of those involved with quantum dot manufacturing and others NPs (Hagens *et al.,* 2007; Monteiro-Riviere *et al.,* 2008). Earlier reports by Kim *et al* (2004) showed that mice injected intradermally with quantum dots can localize in the lymph nodes and can systematically spread to other organs as previously described. The United Kingdom Royal Society and Royal Academy of Engineers have reported that NPs of titanium dioxide used in sunscreens do not go through the skin especially beyond the epidermis (The Royal Society, 2004). However, the societies have made several recommendations concerning further and more information on dermal nanoparticles skin penetration. Findings by Tinkle *et al* (2003) have shown that NPs less than 1 μm in diameter may penetrate skin mechanically. Recent studies by Zhang *et al* (2008) reported the penetration of quantum dot (QD621) NPs (i.e., NPs containing cadmium and selenium core with cadmium sulphite) when topically applied to weaning porcine skin (of Yorkshire pigs). The same group also used the same QD621 and found that the quantum dot could penetrate neonatal human epidermal kerationcytes leading to inflammatory responses. The QD621 were depicted in the intercellular lipid bilayers of the stratum corneum by transmission electron microscopy (Zhang *et al.,* 2008) by elevating cytokines (interleukin-6 and interleukin 8). Monteiro-Riviere (2008) reported that quantum dot penetration was a function of intercellular lipid structure or hair follicle density which could modify these penetration processes. Previous studies by Ryman-Rasmussen *et al* (2007) showed that quantum dot could penetrate through the epidermal layers synthesized with the same core/shell and with similar surface coatings

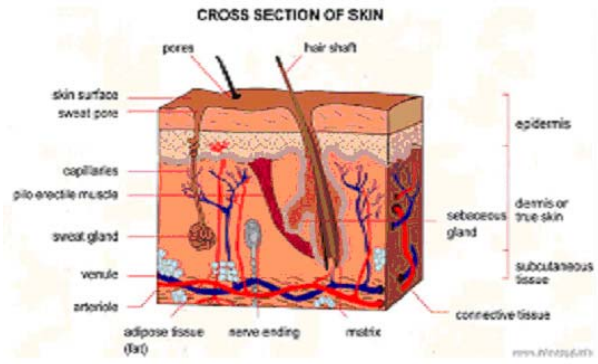


Fig. 6a: Cross section of the skin.

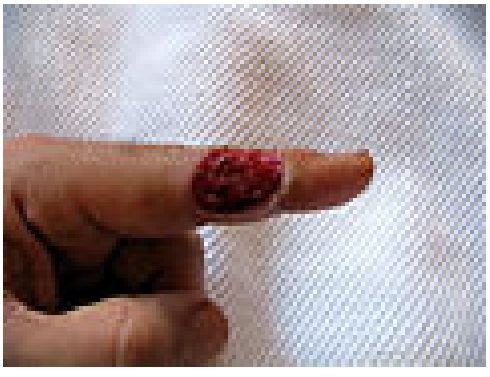


Fig. 6b: Debilitated tissue

Fig. 6: Dermal passage of nanoparticles showing the anatomy of the skin (a) and debilitated skin (b) respectively. http://www.essentialdayspa.com/Skin_Anathomy_and_Physiology.htm, http://en.wikipedia.org/wiki/Wound

having similar hydrodynamic diameters but different penetration rates. Apart from quantum dot, Zhang *et al* (2008) also showed the interactions of functionalized SWCNTs in human epidermal keratinocytes stimulating lymphokines and cytokines.

Other NPs such as TiO_2 and ZnO have also been reported as key particles that are capable of penetrating the skin when applied topically to human skin *in vitro* (Friedlander and Pui, 2003). Studies by Tan *et al* (1996) showed absorption of titanium through human skin with micro fine TiO_2, while studies with microfine zinc and TiO_2 particles applied to porcine skin did not show penetration. This is because pig porcine skin has collagenate (Mediskin), which can provide limited adhesion of NPs. Other studies by Rodney and Barbara (1972) have shown that porcine skin has limited wound adhesion and therefore, limited bacterial infection. Other studies have shown that quantum dots of different sizes, shapes, and surface coatings have varying physicochemical properties when they do penetrate intact skin of pigs (Ryman-Rasmussen *et al.,* 2007). The mode of the NPs penetration was mediated by passive diffusion and was found localize within the dermal layers of the stratum corenum within 8 to 24 hours. Another experiment carried out by Baroli (2008) with excised human ski healthy female abdominal skin samples, exposed to NPs for a maximum of 24 hours showed that penetration of NPs to the skin occurred through the stratum corneum lipidic matrix and hair follicle orifices, allowing NPs to reach the deepest layers of the stratum corneum, hair follicles and the stratum granulosum. He also showed that in some exceptional cases, the NPs were found in viable epidermis (Baroli, 2008).

In vitro studies concerning cultured human skin cells have shown that both SWCNT and MWCNT can enter cell membranes and trigger the release of pro-inflammatory molecules (lymphokines cytokines) resulting into oxidative stress, and decreased viability (Monteiro-Riviere *et al.,* 2008). Actually there no clear cut NPs adverse effect studies on experimental animals yet. Studies on the dermal exposure of NPs are still ongoing and it is still unknown.

Most of the penetration and distribution of nanomaterials in skin and toxicity are minimal and limited to the uppermost stratum corneum layers and areas near hair follicles. This usually led to irritation of the inflammation area in experimental animals. This is because the stratum corneum is the primary barrier for skin and that any type of perturbations to the skin such as an open wound, cut, or alteration to this skin barrier could expose NPs to viable skin cells (Cross *et al.,* 2007; Zhang *et al.,* 2008). Therefore, more toxicological assessment such as abrasion should be conducted to determine if penetration to this barrier would allow an enhancement of absorption of nanomaterials. This raises the question whether nanomaterials could penetrate the dermis, be eventually absorbed systemically, and be responsible for an acute/chronic and local/systemic potential health risk. We already know that the skin is nanoporous at the nanoscale, having orifices of hair follicles and glands open on skin surface therefore, providing alternative entrance routes.

Nano-ocular route

The eyes are used to detect light and sending of signals along the optic nerve to the visual areas of the brain (Frentiu and Adriana, 2008) as shown in fig.7

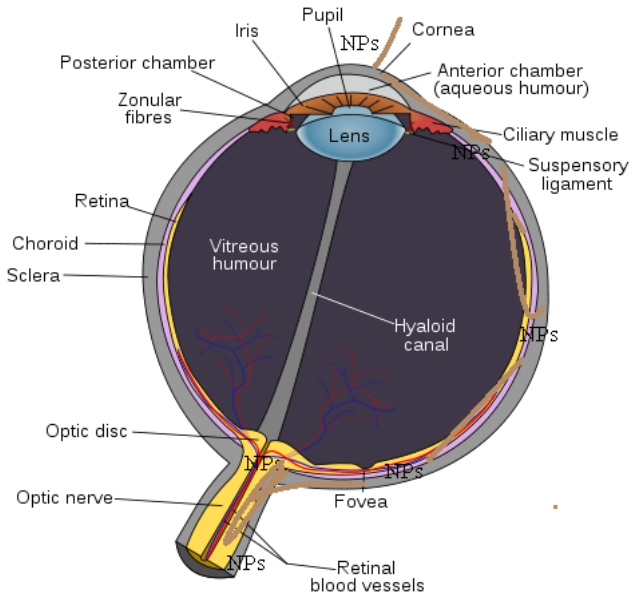


Fig. 7: Optical passage of nanoparticle showing the anatomy of an the eye
http://commons.wikimedia.org/wiki/File:Schematic_diagram_of_the_human_eye_no.svg

The eye is divided into the anterior and posterior segments. There are few literatures reports in indicating that the eye as route of entry of NPs into animals. Drug delivery is achieved through topical application (Aniruddha *et al.,* 2008). However, topical treatment of posterior eye infection is not effective due to the rapid precorneal elimination due to solution drainage, long diffusional path length, induced lacrimation, and corneal epithelial impermeability (Jarvinen *et al.,* 1995).

However, NPs have generated considerable interest for drug delivery into the eye (Aniruddha *et al.,* 2008). According to Herrero-Vanrell and Refojo (Herrero-Vanrell and Refojo, 2001), intravitreally administrations of NPs have shown to sustain drug delivery to the eye. The subconjunctival administrations of Fluorescent NPs (Fluospheres TM, 20 nm) to male Sprague-Dawley rats containing sodium fluorescein, NPs, were detected within

15 minutes. Recent reports by Farjo *et al* (2006) explained how DNA NPs can be implored to transfer genes into the mouse retina. Jani *et al* (2007) reported that albumin NPs encapsulating pCMV.Flt23K when injected into the corneas of uninjured mice the NPs were detected in the corneal keratocyte cytoplasm. The albumin NPs can be used to express intraceptors for extended periods that are effective in suppressing injury-induced corneal neovascularization. The highly efficient transfer of the reporter gene into photoreceptor cells could lead to effective treatments for conditions such as retinitis pigmentosa. Therefore, by modifying the properties of NPs, they could be made to target specific organs.

Nano-auditory route

The ear is the organ that detects sounds and plays a major role in the sense of balance and body position (fig. 8). It is part of the auditory system. Vertebrates have a pair of ears, which are symmetrically placed on opposite sides of the head. Their arrangement and the ability to localize sound sources (waves) can passively facilitate the entry of NPs into the inner ear and to the other vital parts of the body via blood.

However, very few researches have been made public that the auditory pathway is a channel for NPs transport into the ear. This is due the complex natures of the anatomies of the ears which contains hollow channels filled with fluid, and have sensory cells that are studded with hair cells. The microscopic hairs structural protein filaments that project out into the fluid medium and reduce NPs chances of penetrating the ear. Some preliminary reports by Mamedova *et al* (2005) of the Hough Ear Institute showed that superparamagnetic NPs can be used as drug delivery into the inner ear of guinea pigs and into the prilymphatic fluid. Another pilot report by Xianxi *et al* (Xianxi *et al.,* 2007) of San Diego CA also showed that polylactic/glycolic acid (PLGA) polymer coated with iron oxide NPs, applied to the round window membrane of chinchillas, induced by magnetic field can enter the inner ear and will be found in multiple locations within the cochlea tissue. According to the recent work by Barnes *et al* (2007) to compare two different physiological studies that involve magnetic acceleration of superparamagnetic nanoparticles (SPION) through two round window membrane (RWM) models. Through electron microscopy studies, they were able to confirm that SPION were pulled through the RWM of anesthetized guinea pigs.

Nano-intravenous routes

In biological assessment, intravenous administration of NPs is very important route used in determining toxicological assessment in experimental animals. In the study of De Jong *et al* (2008) to determine particle size-dependent organ distribution of gold NPs they intravenously injected experimental rat in the tail vein with gold NPs with diameters of 10, 50, 100 and 250 nm, respectively. Their results gave an oxidative stress in the rat's liver cells. The 10nm gold NPs showed the most widespread presence in the various organ systems

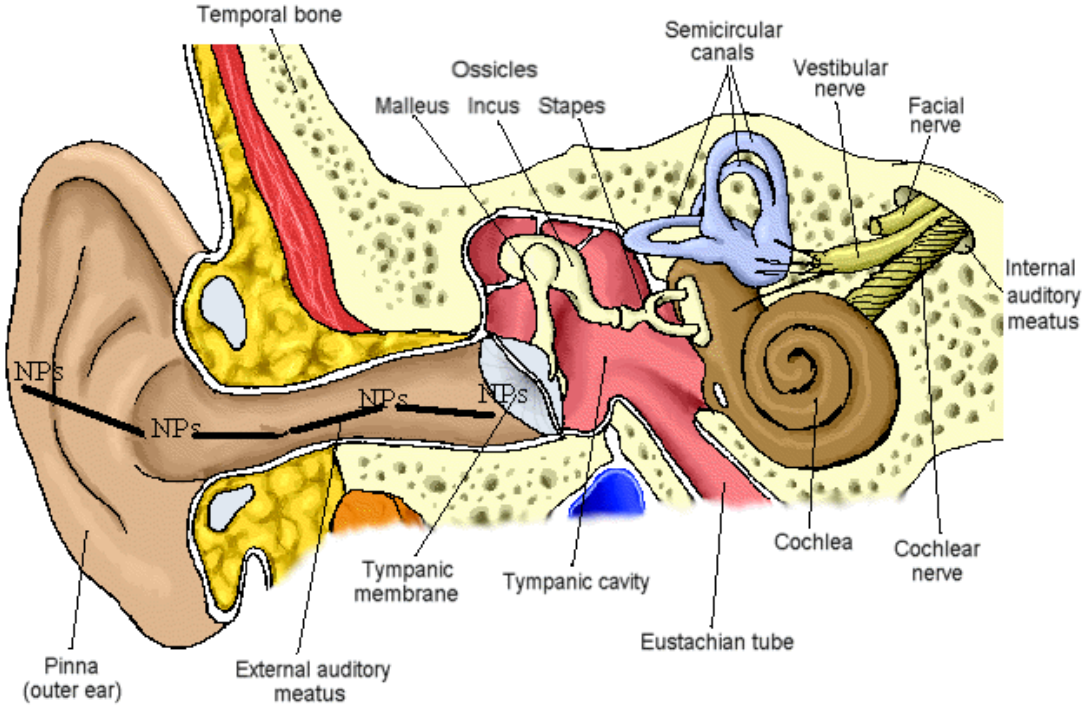


Fig. 8: Auditory passage of nanoparticles showing the anatomy of the ear.
http://www.music.sc.edu/fs/bain/vc/musc726a/MUSC%20726%20Lecture/more%20ear-brain/01-ear.gif

including brain, heart, kidneys, lungs, testis and thymus (DeJong *et al.,* 2008). Also in order to test the toxicity and biomedical imaging of layered nanohybrids consisting of magnesium/aluminium core, Flesken *et al* (2007) subjected the NPs subcutaneously, intra-peritoneally and intravenously injections to mice. Their histological findings showed inflammatory lesions in the lungs and dermis after intravenous and subcutaneous administration respectively. Early experimental studies by Rocio *et al* (1997) also administered intravenously NPs at single doses of 20 and 100 mg/kg for 14 days. In these studies, the liver was seen as a passive target tissue for NPs if given intravenously, due to the phagocytosis by Kupffer cells. Indeed, intravenous administration of NPs is followed by inflammatory responses, characterized by an increased synthesis and secretion of cytokines. Experimental animals absorb NPs from the site of injection into the lymphatic system (Thanos *et al.,* 1999) as shown in fig. 9. The subcutaneous route involves a complex sequence of nanoparticle movement, mostly involving lymph and blood. The relevance of intravenous administration of NPs into experimental animals studies to humans have been questioned not only in drug delivery but also in vaccination, a modality which requires systematic absorption of the encapsulated active drug to achieve a biological response (Rocio *et al.,* 1997) as shown in fig. 9.

Nano-mucus route

The nano-mucus membrane pathway is the lining of most endodermal cells that cover the epithelium and are involved in absorption and secretion. They line various body cells and cavities that are exposed to the external environment and internal organs. It is continuous with the skin, nostrils, lips, ears, the genital and the anus. NPs deposited on the various mucus tissues pathway, encounter mucus or epithelial lining fluid. This may trigger phagocytosis or contact fibroblasts B cells or endothelial cells resulting into the NPs removal (Brayden, 2001; Obordorster *et al.,* 2005a). The mucus membrane is the first barrier that confronts NPs that are deposited in the conducting epithelium. Other reports by Moghimi *et al* (2001) have shown that NPs can be translocated through the mucosal lining and epithelial cells of the intestine and do associate with the GALT and the blood circulatory system. According to Umamaheshwari *et al* (2004) NPs deposited on the mucus membranes might lead to various types of interaction forces between mucoadhesive nanomaterials and the mucus surface. These forces include weak Van der Waals forces, strong hydrogen bonding, electrostatic attraction, mechanical interpenetration, and entanglement. Furthermore, methods such as fluorescence probe have been used to evaluate these interactions *in vitro* and *in vivo* to measure mucoadhesive capacity (Umamaheshwari *et al.,* 2004). Depending upon the specific mucus membrane application, NPs exposure may translocate and impart a cytological toxic effect depending on the factors earlier reported.

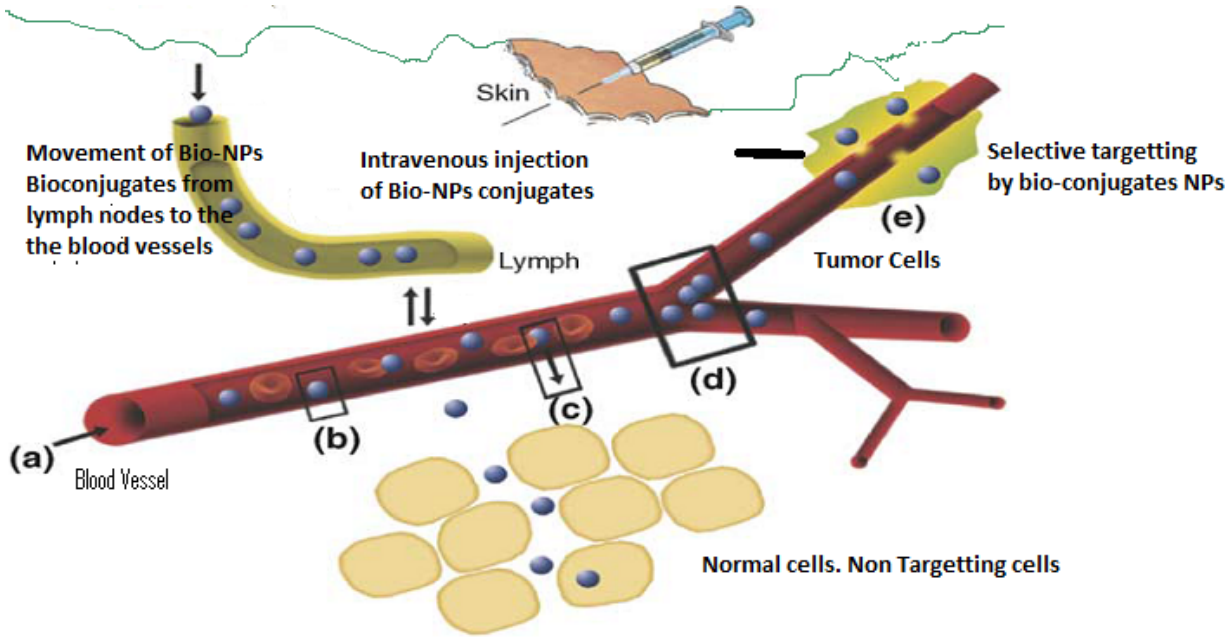


Fig. 9: Intravenous injection and transport of NPs to key and target organs (tumor cells). (a) blood vessel; (b) adhesion to capillary wall; (c) extravagation and flow in tissue; (d) flow at vessel junction; (e) movement to target tumours cells.

The effect of Nanomaterial ligands and cell membrane interactions

The small size of NPs and their 'needle-like' penetrating ability into cells have made NPs excellent carriers in biomedical and molecular biology techniques (Yum *et al.,* 2010). The needle like feature has been reported by De Jong *et al* (2008) as a means for ease of absorption, penetration, circulation and distribution of NPs in bio-systems. This is due to the large surface area to volume ratio that contributes to cell membrane interactions (Van-Doren *et al.,* 2011), thus providing easy penetration and reactivity in biological system than bulk material. For example, De Jong *et al* (2008) revealed that 10 nm was the most widespread in the system after 24 h of AuNPs. This shows that AuNPs circulations in the system are highly size dependent. Apart from size, ligands attached to NP modify their surface activities thus increasing the NP hydrophilicity and biocompatibility properties. This facilitates NPs ease of penetration and membrane cells interaction (Verma and Stellacci, 2010) as shown in fig 10.

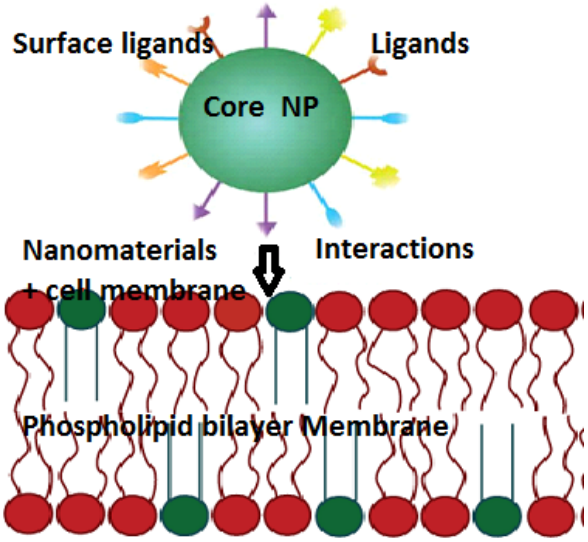


Fig. 10: Ligands surface interaction of nanomaterials with cell membranes (Verma and Stellacci, 2010).

Of these ligands, PEG has gained popularity as a modifying agent due to its amphiphilic and solubility characteristics (Niidome *et al.,* 2006; Patra *et al.,* 2010). This increases the circulation period of the NPs in the blood stream. A study investigating the bio-distribution of PEG modified and non-modified gold nanorods in mice reported a larger percentage of modified NPs in the blood in contrast to unmodified particles over the same time period (Patra *et al.,* 2010). Earlier studies Pan *et al* (2007) revealed that 1.4 nm AuNPs functionalized with TPPMS could enter the cells very easily and bind on dsDNA major groove and disrupt cellular function. Surface modification of NPs goes with surface charge modification as well. Surface charge is the measure of the zeta potential, and is a major physical characteristic influencing NPs cellular activities (Cho *et al.,* 2009). It determines the properties and functions of NPs, i.e. how negatively or positively NPs surfaces are charged. This makes them either very highly reactive and receptive to cell surfaces due to either cations or anions interaction, thus, creating a net surface charge (Patra *et al.,* 2010).

It is important to note that modifications to the NP surfaces may cause undesirable ionic interactions with biological systems (Verma *et al.,* 2010) due to changes in surface charges. Many NPs are stabilized with surface charges to prevent aggregation via electrostatic repulsion (Alkilany and Murph, 2010), thus playing a significant role in membrane cell interactions. For example, Schaeublin *et al* (2011) found both cationic and anionic NPs responsible for mitochondrial membrane potential disruption suggesting that both charged ligands conjugated on NPs can lead to cellular disorders. Apart from that, the issue of NPs translocation into host tissues and their toxicokinetics *in vivo* is an important underlying principle in understanding NPs-cellular interactions. This bio-distribution of NPs can serve as basses for assessing health impact due to surface charges depending on the route (nasal, oral or dermal) of exposure to the NPs. These biodistribution processes after exposures are all tied down to the surface physio-chemical properties that make them chemically more reactive upon interaction with biological systems (Gosens *et al.,* 2010). Therefore, the attributes of NPs and its coated surface charges must be examined with care to ascertain cellular activities.

CONCLUSION

The safety issues derived from NPs routes of entry and their potential bio-distribution are governed by surface area, shape, agglomeration, aggregation solubility and size with protein (opsonisation) interactions within the host (Poland *et al.,* 2008). The size fractions in the nanoscale range have greater lung deposition and rapid systemic translocation having various inflammatory, oxidative and cytotoxic effects on experimental animals than larger particles (Andrew *et al.,* 2004; Obordorster *et al.,* 2005a). With these discussed possible potential routes of NPs, nanotechnology research should proceed with caution. The combination of hazard and production should go hand in hand so as to reduce potential acquisition of NPs through the International Standards Organisation (ISO), good manufacturing practices (GMP), and good laboratory practice (GLP). Suitable quality control procedures should be part of the process so as to ensure NPs product safety and quality and hence part of the company quality assurance scheme. Also the manufacturing industries of nanotechnology should work hand in hand with the health and hazard risk assessment so as to establish a lower health risk of any type emanating from the production and used of NPs. Though there is limited toxicological data available at the present, with the current review, there is hope to increase the

awareness and safety issues of concerning the development of nanotechnology.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from the Department of Science and Technology (DST), South Africa.

REFERENCES

Aihong L, Kangning S, Jiafeng Y and Dongmei Z (2008). Toxicological effects of multi-wall carbon nanotubes in rats. *J. Nanopart. Res.,* **10**: 1303-1307.

Alexander TF (2005). Nanoparticle uptake by the oral route: Fulfilling its potential? Drug Discovery Today: Technologies / *Drug Delivery / Formulation and Nanotechnology*, **2**(1): 75-81.

Alkilany AM and Murph CJ (2010). Toxicity and cellular uptake of gold nanoparticles: What we have learned so far? *J. Nanopart. Res*., **12**: 2313-2333.

Andrew EP, Sadaf S, Edith BG, Joan SV and James KG (2004). Local nanomechanical motion of the cell wall of *Saccharomyces cerevisiae. Science,* **305**(20):1147-1150.

Aniruddha CA, Surya PA and Uday BK (2008). Ocular distribution of intact nano- & microparticles following subconjunctival & systemic routes of administration. *Drug Delivery Technology,* **3**: 2.

Auffan M, Achouak W, Rose J, Chane´C, Waite DT, Masion A, Woicik J,Wiesner MR and Bottero JY (2008). Relation between the redox state of ironbased nanoparticles and their cytotoxicity towards *Escherichia coli*. *Environ. Sci. Technol.,* **42**(17): 6730-6735.

Ballou B, Lagerholm BC, Ernst LA, Bruchez MP and Waggoner AS (2004). Non-invasive imaging of quantum dots in mice. *Bioconjugate Chem.,* **15**: 79-86.

Barnes AL, Wassel RA, Mondale F, Chen K, Dormer KL and Kopke RD. (2007). Magnetic characterization of superparamagnetic nanoparticles pulled through model membranes. *Biomagn Res Technol.,* **5**: 1 doi:10.1186/1477-044X-5-1

Baroli B. (2008). Nanoparticles and Skin Penetration. Are there any potential toxicological risks? *J. Verbr. Lebensm.,* **3**: 330-331.

Beck-Speier I, Dayal N, Karg E, Maier KL, Schumann G, Schulz H, Semmler M, Takenaka S, Stettmaier K and Bors W (2005). Oxidative stress and lipid mediators induced in alveolar macrophages by ultrafine particle. *Free Radical Biol. Med.*, **38**: 1080-1092.

Berry JP, Arnoux B, Stanislas G, Galle P and Chretien J (1977). A microanalytic study of particles transport across the alveoli: Role of blood platelets. *Biomedicine*. **27**: 354-357.

Blundell G, Henderson WJ and Price EW (1989). Soil particles in the tissue of the foot in endemic elephantiasis of the lower legs. *Ann. Trop. Med. Parasitol.,* **83**(4): 381-385.

Borm PJ and Kreyling W. (2004). Toxicological hazards of inhaled nanoparticles – Potential implications for drug delivery. *J. Nanosci. Nanotechnol.,* **4**: 521-531.

Bottini M, Bruckner S, Nika K, Bottini N, Bellucci S, Magrini A, Bergamaschi A and Mustelin T (2006). Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicol. Lett.,* **160**: 121-126.

Brayden D (2001). Oral vaccination in man using antigens in particles: Current status. *Eur. J. Pharm. Sci.,* **14**: 183-189.

Chen Z, Huan M, Gengmei X, Chunying C, Yuliang Z, Guang J, Tiancheng W, Hui Y, Feng Z, Zhifang C, Chuannfeng Z, Xiaohong F, Baocheng M and Lijun W (2006). Acute toxicological effects of copper nanoparticles *in vivo. Toxicol Lett.,* **163**: 109-120.

Cho WS, Cho M, Jeong J, Choi M, Cho HY, Han BS, Kim SH, Kim HO, Lim YT, Chung BH and Jeon J (2009). Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles. *Toxi. Appl. Pharmacol.,* **236**(1): 16-24.

Cross SE, Innes B, Roberts MS, Tsuzuki T and Robertson TA (2007). McCormick P. Human skin penetration of sunscreen nanoparticles: *In vitro* assessment of a novel micronized zinc oxide formulation. *Skin Pharmacol. Physiol*., **20**: 148-154.

Daigle CC, Chalupa DC, Gibb FRMorrow PE, Oberdorster G, Utell MJ and Frampton MW (2003). Ultrafine particle deposition in humans during rest and exercise. *Inhalation Toxicol.,* **15**(6): 539-552.

Das M, Patil S, Bhargava N, Kang JF, Riedel LM, Seal S and Hickman JJ (2007). Auto-catalytic ceria NPs offer neuroprotection to adult rat spinal cord neurons. *Biomaterials.,* **28**(10): 1918.

Davoren M, Herzog E, Casey A, Benjamin C, Gordon C, Hugh JB and Fiona ML (2007). *In vitro* toxicity evaluation of single walled carbon nanotubes on human A549 lung cells; *Toxicology in Vitro.,* **21**: 438-448.

De Jong WH, Hagens WI, KrystekP, Burger MC, Sips AJAM and Geertsma RE. (2008). Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomater*., **29**: 1912-1919.

De Lorenzo AJD (1970). The olfactory neuron and the blood–brain barrier. *In*: Wolstenholme, GEW, Knight, J (Eds.), Taste and Smell in Vertebrates. CIBA Foundation Symposium Series. J & A Churchill, London, pp.151-176

Deendayal M, Mark EB, Debabrata M, Gobinda S and Priyabrata M. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. *Appl. Microbiol. Biotechnol*., **69**: 485-492.

Donaldson K, Aitken R, Lang T, Vicki S, Rodger D, Gavin F and Andrew A (2006). Carbon Nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol. Sci.*, **92**(1): 5-22.

Dreher KL (2004). Health and environmental impact of nanotechnology: Toxicological assessment of manufactured NPs. *Toxicol. Sci.*, **77**: 3-5.

Farjo F, Skaggs J, Quiambao AB, Cooper MJ and Naash MI. (2006). Efficient non-viral ocular gene transfer with compacted DNA nanoparticles. *PLoS ONE,* **1**: e38.

Flesken AN, Toshkov IN J, Katherine MT, Rebecca MW, Warren RZ, Giannelis MP and Nikitin AY (2007). Toxicity and biomedical imaging of layered nanohybrids in the mouse. *Toxicol. Pathol*.,**35**:804-810.

Frentiu FD and Adriana DB. (2008). A butterfly eye's view of birds. *BioEssays,* **30**: 1151.

Friedlander SK and Pui DYH (2003). National Science Foundation Workshop Report on "Emerging Issues in Nanoparticle Aerosol Science and Technology (NAST)". Los Angeles, Univ. of Calif., p.125.

Giles J (2003). Nanotechnology: What is there to fear from something so small? *Nature,* **426**: 750.

Gosens I, Post JA, de la Fonteyne LJJ, Jansen EHJM, Geus JW, Cassee FR and de Jong WH (2010). Impact of agglomeration state of nano- and submicron sized gold particles on pulmonary inflammation. *Part Fibre Toxicol*., **7**: 37.

Gwinn MR and Vallyathan V (2006). Nanoparticles: Health Effects – Pros and Cons. *Environ. Health Perspect.*, **114**(12): 1818-1825.

Hagens WI, Agnes GO, Wim H, de Jong Flemming RC and Adrie¨nne JAMS (2007). What do we (need to) know about the kinetic properties of nanoparticles in the body? *Regul. Toxicol. Pharm.,* **49**: 217-229.

Heim M, Mullins B, Wild M, Meyer J and Kasper G. (2005). Filtration Efficiency of Aerosol Particles Below 20 Nanometers.*AerosolSci.Technol.*,**39**:782-789.

Herrero-Vanrell R and Refojo MF. (2001). Biodegradable microspheres for vitreoretinal drug delivery. *Adv. Drug Delivery Rev.,* **52**(1): 5-16.

Hussain N, Vikas J and Alexander TF (2001). Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Adv. Drug Delivery Rev.,* **50**: 107-142.

ICRP (1994). Human respiratory tract model for radiological protection. Oxford, England: Pergamon, Elsevier Science Ltd., International Commission on Radiological Protection, Publication No.66.

IRSST (2006). Nanoparticles. Actual knowledge about occupational health and safety risks and prevention measures. R-470.

ISO (2006). Workplace Atmospheres – Ultrafine, nanoparticle and nano-structured aerosols – Exposure characterization and assessment. Geneva, Switzerland. International Standards Organization. 2006. Document No. ISO/TC 146/SC 2/WG1 N324, p.32.

Jacobson MZ and Seinfeld JH (2004). Evolution of nanoparticle size and mixing state near the point of emission. *Atmospheric Environment.* **38**: 1839-1850.

Jani PD, Singh N, Jenkins C, Raghava S, Mo Y, Amin S, Uday BK and Balamurali KA (2007). Nanoparticles Sustain Expression of Flt Intraceptors in the Cornea and Inhibit Injury-Induced Corneal Angiogenesis. *Invest. Ophthalmol. Vis. Sci.,* **48**: 2030-2036.

Jani PU, Halbert GW, Langridge J and Florence AT (1990). NPs up take by the rat gastrointestinal mucosa: quantitation and particle size dependency. *J. Pharm. Pharmacol.,* **42**: 821-826.

Jarvinen K, Jarvinen T and Urtti A (1995). Ocular absorption following topical delivery. *Adv. Drug Del. Rev.,* **16**: 3-19.

Jeffrey WC, Zeldin DC, Bonner JC and Nestmann RE (2008). Pulmonary applications and toxicity of engineered nanoparticles. *Am. J. Physiol. Lung Cell Mol. Physiol*., **295**(3): 1-55.

Jiale H, Qingbiao L, Daohua S, Yinghua L, Yuanbo S, Xin Y, Huixuan W, Yuanpeng W, Wenyao S, Ning H, Jinqing H and Cuixue C (2007). Biosynthesis of silver and gold NPs by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology,* **18**(10): doi:10.1088/0957-4484/18/10/105104

Kaiser JP, Wick P and Manser P (2008). Single walled carbon nanotubes (SWCNT) affect cell physiology and cell architecture. *J. Mater. Sci.- Mater. Med.,* **19**: 1523-1527.

Kim S, Lim YS, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG and Frangioni JV (2004). Near infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat. Biotechnol.,* **22**(1): 93-97.

Ku BK and Maynard AD (2005). Comparing aerosol surface-area measurements of monodisperse ultrafine silver agglomerates by mobility analysis, transmission electron microscopy and diffusion charging. *J. Aerosol Sci.,* **36**: 1108-1124.

Lam CW, James JT, McCluskey R, Arepalli S and Hunter RL (2006). A review of carbon nanotube toxicity and assessment of potential and environmental health risks. *Crit. Rev. Toxicol.,* **36**: 189-217.

Li Z, Hulderman T, Salmen R, Chapman R, Stephen SL, Shih-Houng Y, Shvedova A, Luster MI and Simeonova PP (2007). Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. *Environ. Health Perspect.,* **115**(3): 377-382.

Mamedova N, Dormer K, Kopke R, Chen K, Liu J, Ronald J, Costello M. Gibson D and Mondalek F (2005). Feasibility of superparamagnetic NPs for drug delivery to the inner ear. 2005. http://www.nanobmi.com/images/Abstract_Nano_2005.pdf

Mayank DB and Mansoor MA. (2007). Gastrointestinal distribution and *in vivo* gene transfection studies with NPs-in-microsphere oral system (NiMOS). *J. Control Release,* **119**: 339-348.

Maynard AD and Kuempel E (2005). Airborne nanostructured particles and occupational health. *J. Nanopart. Res.,* **7**: 587-614.

Medina C, Santos-Martinez MJ, Radomski A and Corrigan OI (2007). Radomski MW. Nanoparticles: Pharmacological and toxicological significance. *British J. Pharmacol.,* **150**: 552-558.

Moghimi SM, Hunter AC and Murray JC (2001). Long-circulating and targetspecific NPs: theory to practice. *Pharmacol. Rev.,* **53**: 283-318.

Mohanpuria P, Rana NK and Yadav SK. (2008). Biosynthesis of NPs: technological concepts and future applications. *J. Nanopart. Res.,***10**: 507-517.

Monteiro-Riviere NA. *In*: Zhai H, Wilhelm KP, Maibach HI (Eds.). (2008). Anatomical factors that affect barrier function. CRC Press, New York, pp.39-50.

Murr LE, Esquivel EV and Bang JJ (2004). Characterization of nanostructure phenomena in airborne particulate aggregates and their potential for respiratory health effects. *J. Mater. Sci.-Mater. Med.,* **15**: 237-247.

National Institute For Occupational Safety and Health (NIOSH) (2006). Approaches to safe nanotechnology. www.cdc.gov/niosh

Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PHM, Verbruggen A and Nemery BS (2001). Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am. J. Respir. Crit. Care Med*., **164**(9): 1665-1668.

Ngoy JM, Iyuke SE, Neuse WE and Yah CS (2011). Covalent Functionalization for Multi-Walled Carbon Nanotube (*o-MWCNT*) -Folic Acid bound bioconjugate. *J. Appl. Sci.,* **11**(15): 2700-2711.

Niidome T, Yamagata M, Okamoto Y, Akiyama Y, Takahashi H, Kawano T, Katayama Y and Niidome Y (2006). PEG-modified gold nanorods with a stealth character for *in vivo* applications. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere NA, Warheit D, Yang H. (2005a). A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity, Screening Working Group Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part. Fibre Toxicol.,* **2**: 8.

Oberdörster G, Oberdörster E and Oberdörster J (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.,* **113**(7): 823-839.

Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, Schmid G, Brandau W and Jahnen-Dechent W (2007). Size-dependent cytotoxicity of gold nanoparticles. *Small,* **3**: 1941.

Patra CR, Bhattacharya R, Mukhopadhyay D and Mukherjee P (2010). Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer. *Advanced Drug Delivery Reviews*. **62**: 346-361.

Peter HH, Irene BH and Oleg VS (2004). Nanoparticles – known and unknown health risks. *J. Nanobiotechnocol.,* **2**: 12.

Poland AC, Rodger D, Kinloch I, Maynard A, Walace WAH, Seaton A, Stone V, Brown S, MacNee W and Donalson K (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos like pathogenicity in pilot study. *Nat. Nanotech. Lett.,* **3**: 423-428.

Quadros ME and Marr LC (2010). Environmental and human health risks of aerosolized silver nanoparticles, *J. Air & Waste Manage. Assoc.,* **60**: 770-781.

Rocio FU, Fattal E, Fbger J, Couvreur P and Thond P (1997). Evaluation of hepatic antioxidant svstems after intravenous ahministration of polymeric NPs. *Biomoteriols*, **18**: 511-517.

Roco MC and Bainbridge W (2005). Societal implications of nanoscience and nanotechnology. *J. Nanopart. Res.,* 7: 1-13.

Rodney FS and Barbara LE (1972). Bacteria of porcine skin, xenografts, and treatment with neomycin sulfate. *Appl. Microbiol.,* **23**(2): 293-297.

Ryman-Rasmussen J, Riviere JE and Monteiro-Riviere NA (2007). Surface coatings determine cytotoxicity and irritation potential of quantum dot NPs in epidermal keratinocytes. *J. Invest. Dermatol*., **127**: 143-153.

Sadowski Z, Maliszewska IH, Grochowalska B, Polowczyk I and Koźlecki T (200). Synthesis of silver NPs using microorganisms. *Mater. Sci.-Poland.,* **26**(2): 419-424.

Schaeublin NM, Braydich-Stolle LK, Schrand AM, Miller JM, Hutchison J, Schlagera JJ and Hussain SM (2011). Surface charge of gold nanoparticles mediates mechanism of toxicity. *Nanoscale,* **3**: 410-420.

Seaton A and Donaldson K (2005). Nanoscience, nanotoxicology, and the need to think small. *Lancet.* **365**: 923-924.

Simate GS, Iyuke SE, Ndlovu S, Yah CS and Walubita LF (2010). The production of carbon nanotubes from carbon dioxide – challenges and opportunities. *JNGC.,* **19**(5): 453-460

Stern ST and McNeil ES (2008). Nanotechnology safety concerns revisited. *Toxicol. Sci.,* **101**(1): 4-21.

Tan MH, Commens CA, Burnett L and Snitch PJ (1996). A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. *Australas J. Dermatol.,* **37**: 185-187.

Tantra R and Cumpson P (2007). The detection of airborne carbon nanotubes in relation to toxicology and work place safety. *Nanotoxicol.,* **1**(4): 251-265.

Thanos C, Sandor M, Jong Y, Jacob J, Yip KP, Harper J, Morrell C, Scherer J and Mathiowitz E (1999). Inter-species uptake of polymeric particles. *Mater. Res. Soc. Symp. Proc.,* **550**: 65-70.

The Royal Society. The Royal Academy of Engineering. (2004). Nanoscience and nanotechnologies. London,

UK: The Royal Society and The Royal Academy of Engineering, www.nanotec.org.uk/finalReport.htm

Tinkle SS, Antonini JM, Rich BA, Robert JR, Salmen R, DePree K and Adkins EJ (2003). Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ. Health Perspect.,* **111**(9): 1202-1208.

Umamaheshwari RB, Ramteke S and Jain NK (2004). Anti-*helicobacter pylori* effect of mucoadhesive nps bearing amoxicillin in experimental gerbils model. *AAPS PharmSciTech*., **5**(2):32.

Van Doren EAF, Temmerman PRHD, Francisco AD and Mast J (2011). Determination of the volume specific surface area by using transmission electron tomography for characterization and definition of nanomaterials. *J. Nanobiiotechnology,* **9**: 17.

Verma A and Stellacci F (2006). Effect of surface properties on nanoparticles cell interactions. *Small.,* **6**(1): 21-21.

Warheit B (2004). What is currently known about the health risks related to carbon nanotubes exposure? *Carbon.,* **44**: 1064-1069.

Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM and Webb TR (2004). Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol Sci.,* **77**: 117-125.

Witzmann F and Monteiro-Riviere NA (2008). Malti-Walled carbon nanotubes exposure alters protein expression in human keratinocytes. *Nanomed: Nanotechnol, Bio Med.,* **2**: 158-168.

Xianxi G, Ronald J, Jianzhong L, Balough B and Hoffer ME (2007). Distribution of polylactic/glycolic acid (PLGA) NPs in Chinchilla Cochlea. The 13th Annual Midwinter Research Meeting of the Association for research In otolaryngology, Denver, Colorado, USA, February, pp.10-15.

Yah CS, Iyuke SE, Simate GS, Unuabonah EI, Bathgate G, Matthews G and Cluett JD (2011a). Continuous synthesis of multiwalled carbon nanotubes from xylene using the swirled floating catalyst chemical vapor deposition technique. *J. Maters Res.,* **26**(5): 623-632.

Yah CS, Simate GS, Moothi K and Maphuta S (2011b). Synthesis of large carbon Nanotubes from Ferrocene: The chemical vapour deposition technique. *Trends Appl. Sci. Res.,* **6**(11): 1270-1279.

Yakovenko ML, Ekaterina AK, Olga EI, Tatyana PE, Samoilovich E, Iryna U, Gene VG and Vadim IA (2009). Evolution of the sabin vaccine into pathogenic derivatives without appreciable changes in antigenic properties: Need for improvement of current poliovirus surveillance. *J. Virol.,* **83**(7): 3402-3406.

Yoshifumi T (2002). Lipid formulation as a drug carrier for drug delivery. *Curr. Pharm. Des*., pp.467-474.

Yum K, Wang N and Yu MF (2010) Nanoneedle: A multifunctional tool for biological studies in living cells. *Nanoscale*, **2**: 363-372

Zhang WL, Yu WW, Vicki LC and Monteiro-Riviere NA. (2008). Biological interactions of quantum dot NPs in skin and in human epidermal keratinocytes. *Toxicol. Appl. Pharmacol*., **228**: 200-211.

Zhang Z, Kleinstreuer C, Donohue JF and Kim CS (2005). Comparison of micro- and nano-size particle depositions in a human upper airway model. *J. Aerosol Sci.,* **36**(2): 211-233.