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REVIEW ARTICLE NANOTECHNOLOGY

# NANOTECHNOLOGY A PATH TO NANOVACCINE

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## ABSTRACT

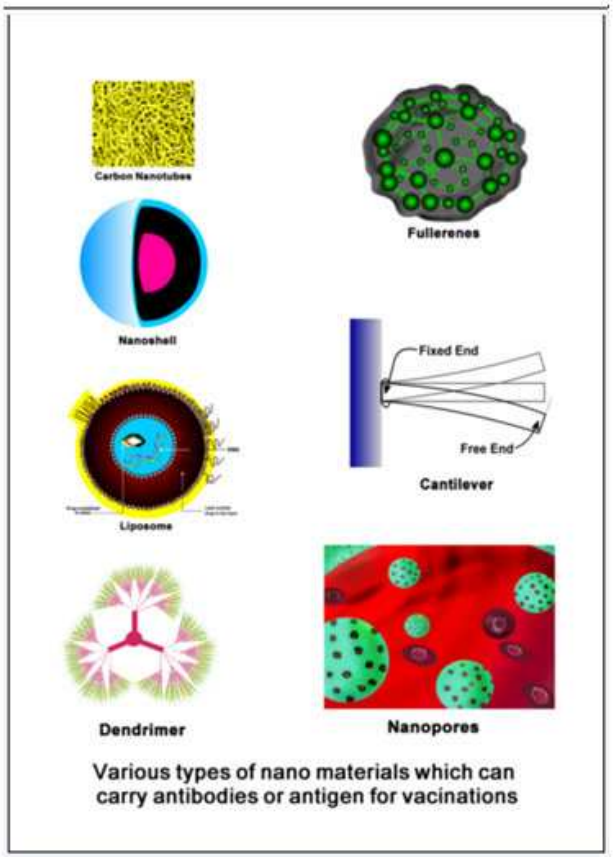
Nanotechnology has gained importance in medicine and is referred to as nanomedicine.Various tools of nanomaterials have been investigated for their application in imaging, diagnosis and therapy; these are carbon nanotubes, fullerenes, cantilevers, liposomes, quantum dots, nanoshells, nanoparticles, dendrimers etc. Antigens/epitopes can be tagged to nanomaterials for immunization/vaccination. Different routes of administration have been tried for nanovaccines which include oral, nasal, intradermal and microneedle patches. Due to their small size and large surface area these nanoparticles are useful as nanovaccines. To enhance the immunogenicity of small peptides/epitopes in nanovaccines polymers, immune stimulating complexes (ISCOMs), virus-like particles (VLPs), nanobeads and nanocarriers have been used as adjuvants. These adjuvants are safeand enhance the immune response. Although nanoparticles have numerous advantages, they have certain drawbacks as well. Therefore, study of toxicity of nanoparticles is essential. In vitro systems and in vivo models have been developed to answer the safety of the nanoparticles/nanodevices.

## KEY WORDS

nanobeads; adjuvants; nanotoxicity; cancer

## INTRODUCTION

Nanotechnology is the creation of very small particles, devices or systems. Nanomaterials are designed and applied for diagnostics, therapeutics for the benefit of human health and as biomedical tools for research [1]. Nanotechnology is gaining importance in medicine due to its small size and targeted effects. Nanoparticles are 100-10,000 times smaller than a human cell. Due to their small size and large surface area, nanoparticles can readily interact with biomolecules such as receptors present on the surface of a cell or inside a cell. A number of drug delivery systems for nanomaterials have been reported and has been reviewed recently by Surendiran et al [2], these are carbon nanotubes, nanocrystals, nanoparticles, nanoshells, cantilevers, dendrimers (Figure 1) etc. Each of these has certain advantages and products made from these tools can be used for diagnosis and for therapy of a disease by conjugating proteins, antibodies or DNA.




Various types of nano materials which can carry antibodies or antigen for vacinations

***Liposome***:

Liposomes are spherical nanoparticles made of lipid bilayer membranes with an aqueous interior. They are synthesized from cholesterol and nontoxic phospholipids in the body. As these are natural materials, liposomes are considered harmless drug delivery carriers. Cancer chemotherapeutic drugs and other toxic drugs

like anphotericin and hamycin when used as liposomal drugs produce much better efficacy and safety as compared to conventional preparations.

The major limitation of the liposome is its rapid degradation and clearance by the liver macrophages [3]. Immunoliposomes are the liposomes conjugated with an antibody directed towards a tumour antigen. These immunoliposomes when injected into the body reach the target tissue and are accumulated at the site of action. This reduces the adverse effects of the drug and increases the drug delivery to the targeted site therapy enhancing its efficacy and safety [4]. The antibody directs the enzyme to the target tissue where it activates the prodrug selectively. Thus the drug does not act on normal tissues and the toxicity of the drug is minimized [5-7]. This antibody directed enzyme prodrug therapy is tried with epirubicin and doxorubicin [6,8].

***Fullerenes**:*

Fullerenes are carbon allotropes also called as bucky balls. The common form of fullerene is buckminster fullerene which has 7 $A^0$diameter with 60 carbon atoms arranged in a shape of truncated icosahedrons resembling a soccer ball [9]. Fullerenes have a potential to stimulate host immune response and production of fullerene specific antibodies. Highly purified fullerenes are expensive restricting its application in medicine [10]. However, fullerenes unlike nanotubes do not induce platelet aggregation in rats and are therefore they are more safe.

***Nanotubes**:*

Carbon nanotubes are tubular structures capped at one or both ends by bucky balls. Cell specificity can be achieved by conjugating antibodies to carbon nanotubes with fluorescence or radiolabelling [11] Nanotubes enter the cell by endocytosis or by insertion through the cell membrane. DNA can be attached to the tips of nanotubes or can be incorporated within the tubes either for gene therapy or for delivering antibodies. Further, carbon nanotubes can be bound with a peptide to produce a high immunological response.

***Quantum dots***:

Quantum dots are nanocrystals which can be made to fluorescence when stimulated by light. Their structure consists of an inorganic core and shell and an aqueous organic coating to which biomolecules can be conjugated. In the experimental model for prostate cancer developed in male mice, accumulation of quantum dots probe has been shown by enhanced permeability and retention as well as by antibody directed targeting [12]. However, the application of quantum dots in clinical practice has limitations because of its elimination factors.

***Nanoshells**:*

Nanoshells consist of nanoparticles with a core of silica and a coating of thin metabolic shell. These can be targeted to a tissue by using immunological methods, to release the drug at the site of the tumor. Nanoshells are being developed for the treatment of micrometastasis of tumors and of Diabetes [13-14].

***Paramagnetic nanoparticles***:

Paramagnatic nanoparticles can be used for both diagnostics as well as therapeutic purpose. Leuschner et al reported [15] the use of supra paramagnetic iron oxide nanoparticles conjugated to LH releasing hormone (LHRH) for the early detection of breast cancer metastasis. Monoclonal antibodies can also be conjugated to these iron nanoparticles to target specific antigen on the tumor. Triton Biosystems have designed magnetic nanoprobes for solid tumors to be used in clinical trials.

***Dendrimers**:*

Dendrimers are nanomolecules with regular branching structures. The branches being spherical, the cavities formed can be used for drug transport. Dendrimers can be used for targeting, diagnosis of disease state, delivery of drug and imaging.

***Cantilevers**:*
Cantilevers are tiny bars anchored at one end and can be engineered to bind to biomolecules. If antibodies are attached to the free end of cantilever, the specific antigen on a cell or a tumor can be diagnosed. It can also bind to antigen, inactivate it and act as a therapy to suppress the tumor.

The most promising application of nanotechnology in medicine is of drug delivery, focus being on targeted drug delivery and controlled drug release. The drugs can be loaded by encapsulation, surface attachment or end mapping. Drugs are normally targeted or implanted so as to obtain controlled drug release for few days or a couple of months. Water solubility and size of the nanoparticles are equally important. Bioavailability and solubility usually go hand in hand with efficacy of the drug. Large particles of a drug are unable to enter tumor pores or a cell while nanoparticles (40-100 nm) can easy pass through the pores or cell membrane which is useful for the treatment of cancer and other disorders. Due to these advantages of nanoparticles, in the last five years a number of investigators [reviewed by 16] have explored the possibility of conjugating drugs/antigens/epitopes to nanoparticles for vaccination against infectious diseases caused by bacteria or viruses.

***Vaccination:***
Immunization, also called as vaccination, is a method of stimulating resistance in the body to specific diseases using microorganisms – bacteria or viruses – that have been killed or modified. Vaccination strategy has been developed for a number of diseases utilizing antigenic protein or DNA encoding the protein which is responsible for causing the disease. This leads to both humoral and cell mediated immune responses. Active immunization is one method of vaccination while in passive immunization the antibodies usually monoclonal to specific antigen / epitope are employed to block the action of the antigen causing the disease.

***Veterinary vaccines:***
Due to genetic diversity of the species, the diseases in different species of animals vary and therefore vaccine requirements differ among the species. In wildlife species knowledge of targeting molecules is limited while the regulatory requirements for biodegradable are much stricter for animals bred for human consumption than those used as pets [17]. The route of delivery of the vaccine can vary from oral administration in pets to long distance ballistic intramuscular delivery in wild animals. The size of the particles can also range from a few nanometers (microparticles 1-100 um) to cm (bio bullets 1-2 cm).

***Therapy in humans for prevention or cure of diseases*:**
In human, vaccines are used for prevention of infectious diseases due to bacteria or viruses such as diphtheria, poliomyelitis, HIV- AIDS etc. Targeted therapy is the most attractive area in the treatment of cancer due to the broad therapeutic options available for the approach. Drugs can be specifically formulated to attack tumor cells and destroy these without affecting the normal cells. Recent advances in the formulation of nanoparticles and nanostructures for targeted therapy emerge as a challenge in designing and delivery of these drugs. Different routes of administration have been sought by various investigators for delivery of drugs in the form of nanomaterials such as oral, nasal and intradermal.

***Routes of administration: Oral Route:***
Polymer based nanoparticles are used for the delivery of oral DNA vaccines by Bhavsar and Aniji [18]. However higher concentration of peptide / protein antigen may be required for its efficacy due to dilution during the passage through gastro- intestinal tract.

***Nasal route:***

Adjuvants such as alum used in conventional vaccine cause irritation, pain and redness. Baker's group reported [19] use of nanoemulsion with hepatitis B antigen to be non-toxic, pain free and effective. This nanoemulsion was made of soyabean oil, alcohol, water and detergents emulsified into droplets of 40 nm. The strategy of novel nanoemulsion for small pox, influenza, anthrax and HIV vaccines showed promising results. These studies can lead to development of nanoemulsion vaccines for various viral proteins.

Unit or duo – dose nasal spray devices are small, disposable devices, delivering limited number of sprays. Such an easy to use ready to use device Flumist for influenza vaccine has been marketed [20]. The use of bidirectional nasal delivery devices for vaccination against influenza and diphtheria has shown promising results [21].

The main problems with the nasal delivery of antigens are 1. The free antigen is readily cleared from the nasal cavity 2. Poor absorption by the nasal epithelial cells 3. Generate low immune response. To overcome these problems, encapsulation of antigen into bioactive nanoparticles is a promising approach to nasal vaccine delivery [22].

***Intradermal route****:*

Intradermal route of vaccination is the most common method for the delivery of drugs and genes. The dose is injected in the outer layer of the skin to reach epidermis, the layer which is immunogenically sensitive. The drug is emulsified with an adjuvant to form a depot so as to allow slow release of the drug over a long period.

Bal et al [23] reported that both soluble TMC/ antigen mixtures and THC nanoparticles are able to induce DC maturation and enhance immune responses after intradermal injection. This demonstrated that TMC functions as an immune potentiator for antigens delivered via the skin.

Badea et al [24] reported that intradermal injection of hen egg lysozyme (HEL)/CpG (cytosine-guanine tandems) nanoparticles induced a more pronounced Th1 immune response compared with the HEL and HEL/CpG topical formulations. This demonstrated the advantage of co-delivery of HEL/CpG s by gemini nanoparticles intradermally.

Recently, covalently stabilized nanoparticles loaded with ovalbumin (OVA) were prepared by Verheul et al [25]. In both the nasal and intradermal immunization study, OVA loaded stabilized thyolated N-Trimethyl Chitosan (TMC)-Hyluronic acid (HA) particles demonstrated superior immunogenicity compared to non-stabilized particles indicated by higher IgG titers. Thus, stabilization of the TMC/HA particulate system greatly enhances the immunogenicity of OVA in nasal and intradermal vaccination.

***Microneedle Patches:***

In the conventional methods, the delivery of vaccine is by injection of the antigen intradermally but the antigen may not reach the Langerhans cells of the epidermis to induce immune response. Therefore vaccine is administered by intramuscular route, but with DNA the immune response is modest as it acts indirectly on dendritic cells. Further, needle prick can be painful and irritating. Therefore microneedle patches applied to the skin can be one of the least invasive methods for the delivery of molecules less than 500 Da. A set of needles of microscale length with nanoscale tips are coated with drug such as plasmid DNA or protein and applied to the skin as a small patch which can deliver biomolecules to epidermis efficiently and safely and can be used in future as therapy for curing diseases [26]. Use of microneedle patches to the skin for vaccination against influenza has recently been reported by Quan et al [27] and Pearton et al [28].

Cui et al [29] reported that a novel nanoparticle-based DNA vaccine delivery system by administering it intradermally into mouse skin

using Biojector 2000, a needle-free jet injection device. For pDNA alone, jet and SC injection did not result in significant differences in IgG titer. For pDNA-coated nanoparticles, jet injection led to as high as a 20-fold enhancement in IgG titer over SC injection. In addition, jet injection of pDNA-coated nanoparticles enhanced the IgG titer by more than 200-fold over jet injection of pDNA alone. Also, jet injection of pDNA-coated nanoparticles resulted in significantly enhanced specific serum IgA titer. Thus a combination of a novel cationic nanoparticle-based DNA delivery system with ID jet injection led to enhanced antibody production, Th-1/Th-2 balanced cytokine release, and enhanced splenocyte proliferation.

Uniform 150nm diameter PLGA nanoparticles containing Nile red were prepared by Donnelly et al [30]. They employed polymeric microneedles (MN) to deliver a model lipophilic dye, Nile red, into excised porcine skin. Tissue penetration studies revealed that high tissue concentrations of Nile red were observed following MN delivery which is a one-step strategy for the local delivery of highly hydrophobic agents, an advantage over the current delivery strategies.

***Adjuvants:***

To enhance the immune response, adjuvants are used such as oils (particulate), saponins (non particulate) or immuno-stimulating complexes (ISCOMs, combined compositions). Sinyakov et al [31] reported that use of biodegradable magnetic nanoparticles were superior to mono disposed polyacrolein nanoparticles as adjuvants, covalently bound to bovine serum albumin and were comparable to conventional adjuvant alum. A number of adjuvants have been reported such as polymers, ISCOMs, virus-like particles (VLPs), nano beads and colloidal nanocarriers.

***Polymer****:* Encapsulation of antigen with a polymer can help in slow release and provide protection from degradation. Nanoparticles prepared from biodegradable polymer polylactide-co-glycolide acid (PLGA) have been used for encapsulating peptides [32]. Polymers such as polylactide, polyacrylate can be coated with nanoparticles or nanobeads and can be used as drug delivery devices. Recently our group has formulated nanoparticles with a peptide using a polymer PLGA and shown that the bioactivity of the peptide was retained when injected to mice [33]. Further studies have also revealed that when these nanoparticle-peptides were administered in marmosets, the new world monkeys, a controlled release of the peptide could be observed for 8 days (unpublished data).

***VLPs:*** Particulate structures hold great promise for the development of effective and affordable recombinant prophylactic as well as therapeutic vaccines. Different types of particulate structures including VLPs have been developed depending on the nature of the viral pathogen to be targeted and the type of immune response (humoral or cellular) to be elicited. VLPs can be inserted or fused with foreign antigen sequences, resulting in chimeric particles delivering the foreign antigen which can trigger immune response [34]. VLPs are promising vaccine technology as they are safe & can elicit strong immune responses.

A single intramuscular vaccination with $H_5N_1$ influenza VLPs provided complete protection against lethal challenge [35]. Their group further reported [28] that use of microneedle vaccination of mice in the skin with a single dose of $H_1N_1$ influenza VLPs conferred protection superior to that with intramuscular injection. A single dose of recombinant influenza VLPs resulted in complete virus clearance in the ferret lung [36].

Pearton et al [28] have reported that microneedle facilitated delivery of influenza VLP vaccines targeting dendritic cells in the skin generating immune response. The experiments in mice suggest its possible application in human for preclinical testing of intradermal vaccines.

Novel mammalian and insect suppression cell line systems for the efficient production of rift

valley fever (RVF) virus – like particles based vaccines were tested in a lethal challenge model which showed full protection, demonstrating RVF – VLPs as promising vaccine candidate [37].

A multivalent vaccine candidate against hepatitis B & C viruses was constructed on the basis of HBV core virus like particles as carriers. The presence of two different foreign epitopes within the HBV core molecule did not interfere with its VLP forming ability as HBV pre S, epitope was expressed on the surface while the HCV core epitope was buried within the VLPs [38].

The VLPs of DNA encoding porcine encephalomyocaditis viral (EMCV) antigen as vaccine showed cell mediated as well as humoral responses in mice suggesting a novel vaccine strategy for the control of EMCV infection in pig farms [39].

VLPs have been used for vaccination against cancer polyomarvirus not only in mice with a well functionary immune system but also in immune suppressed mice [40].
Immunization of human MUC1 transgenic mice, where MUC1is a self antigen with the VLP vaccine has been reported to induce MUC1 specific CTL, delay the growth of MUC1 transplanted tumors and elicit complete tumor rejections in some animals [41].

Recombinant proteins specially single domain or peptides are poorly immunogenic and require conjugation to carrier protein for enhancing immune response. VLPs are efficient conjugation system for complex and long epitopes of up to 55 amino acid residues and induce high immunogenicity [42].

***ISCOMs:***

The immune stimulating complex (ISCOM) is a 40 nm nanoparticle used as delivery system for vaccine antigens for both parental and mucosal administration. The ISCOM is made up of saponins and lipids (cholesterol) which are compulsory elements and hold the antigens usually by hydrophobic interaction. Without the antigen ISCOM- MATRIX is formed. A large number of ISCOM vaccines have been experimentally tested and protection has been reported against a number of pathogens including chronic and persistent infections exemplified by HIV-1 and -2, simian immuno deficiency virus in primates, rabbies in mice, the latter being more efficient than the conventional one. It is also possible to use the ISCOM adjuvant in a mixture of live and killed vaccine antigen, thus opening another approach [43].

The serum titer after 3 high doses of ISCOM incorporating recombinant hepatitis B surface antigen (HBsAg) by nasal route was comparable with the conventional subcutaneous administration of alum – HBsAg [44]. They earlier reported [45] that although the confirmation of HBsAg was moderately altered, yet due to strong adjuvant ability, it was highly immunogenic by subcutaneous route also.

The vaccine formulation prequenza consists of influenza antigen along with the second generation ISCOM-MATRIX as an adjuvant. The vaccine is found to be protective and safe for the last 2 years as a commercial vaccine in Europe and has been shown to be safe in pregnant mares as well as in foals [46].

ISCOMs have the advantage of particulate carrier system with the presence of in built adjuvant and have been found to be more immunogenic, while removing its hemolytic activity of the saponin producing less toxicity. ISCOM vaccines have been shown to induce strong antigen – specific cellular or humoral immune responses to a broad range of antigens of viral bacterial, parasite origin or tumor in a number of species including primates and human [47].

***Nanobeads:***

Solid inert beads with surface-adsorbed antigen have been shown to stimulate CD8 T cell-mediated responses [48]. The antigen

conjugated to beads and size of the bead play a major role in eliciting humoral or cell-mediated immunity. The nanobeads are localized to dentritic cells in the draining lymph nodes and have been reported to be the best adjuvants for induction of CD8 T cell-mediated responses with whole antigen such as ovalbumin [49]. A single dose of antigen-conjugated beads protected mice from tumors and caused rapid clearance of established tumors. Thus nanobeads are effective as immunogens for therapeutic as well as preventive purposes [48]. Greenwood et al [50] reported that combining several peptides in the peptide nanobead-based vaccine approach can improve immunogenicity and may prove beneficial, especially for highly variable pathogens such as foot-and-mouth disease viruses.

The alpha fetoprotein-derived growth inhibitory peptide (GIP-34) and its segment GIP-8 have been characterized as anticancer therapeutic peptides [51]. GIP conjugated to doxorubicin underwent tumor cell uptake and induced cytotoxic cell destruction indicating its utility as a cancer therapeutic agent. Kim et al [52] reported the direct detection of HIV particles using broadly HIV-1 neutralizing gp120 monoclonal antibody-conjugated magnetic beads and fluorescent nanosized polymeric beads can be a specific, rapid and convenient diagnostic system.

***Colloidal Nanocarriers***

Colloidal nanocarriers (NCs) in various forms can provide targeted delivery of therapeutic and diagnostic agents. Misra and his group [53] have addressed NCs potential application specifically to oncology and suggested that similar approach can be applicable to other conditions as well.

NCs are colloidal systems having droplet size of 500nm. Their application can be oral, parenteral or transdermal. For this purpose NCs are developed as microemulsion, vesicular or particulate forms and has been reviewed by Neubert [54].

Antigen loaded NCs have shown to be capable of actively taken up by antigen presenting cells (APCs) and have shown promising potential in cancer immunotherapy. They could simultaneously co-deliver adjuvants with the antigens to enhance APCs activation and maturation. These colloidal and particulate nanoscale systems can also enhance accumulation in solid tumours and thereby have potential to translate into clinical cancer vaccines [55].

Clawson et al [56] reported that because of the capacity to activate dendritic cells( DCs), Hp 91 conjugated to the surface of NPs, was ~20 fold more potent than free Hp 91 in initiating immune response. Thus, such systems are promising as delivery vehicles for subunit vaccines against infectious diseases or cancer.

Cruz et al [57] also agree that DCs are key players in initiation of adaptive immune responses and exploited for immunotherapy against cancer and infectious diseases. They generated nanovaccine carriers made of biodegradable poly (d,l-lactide-co-glycolide) harbouring supramagnetic iron oxide particles and fluorescently labelled antigen in a single particle. Incorporation of two imaging agents within a single carrier allowed tracking of nanovaccines on a subcellular cellular and possibly organism levels thereby facilitating rational design of in vivo targeted vaccination strategies. Recently, Bedi et al [58] reported a novel approach for intracellular delivery of siRNA into breast cancer cells through their encapsulation into liposomes targeted to the tumor cells with preselected intact phage proteins. They claim that it has potential for development of new anticancer siRNA-based targeted nanocarriers in medicine.

***Nano Regulations****:*

Nanomaterials are micro formulations, therefore manufacture of nano drugs and control of contamination are the major issues in nano regulation. In addition to this, adequate training of workers and also of supervisors in manufacturing process is essential for good manufacturing practice and quality control of the nano drug products. It is expected that the staff of the regulatory administration is sufficiently

trained and updated with the recent developments in nanomedicine to evaluate the nanomaterials [59].

***Nanotoxicity:***

Nanoparticles due to their small size can reach various tissues as well as cells of the body. Administration through nasal route can cause respiration problem, oral route can affect gastrointestinal system and intradermal injections can lead to dermatological problem. Parenteral route can cause cardiovascular disorders. While passing the blood brain barrier can bring about damage to the brain. Platelet aggregation and acceleration of vascular thrombosis in rats may be another problem of nanoparticles as reported by Radomski et al [60]. Humans and animals are exposed to tiny particles from dust, storms, volcanic ash and other natural processes or particles originating from human activities e g smoke from combustion, industrial waste etc. Environmental exposure to nanomaterials is thus inevitable as it has become a part of our daily life and as a result, nanotoxicity research is gaining attention [61]. The combination of a reliable design and documentation and sufficient characterization of nanomaterial is essential to assess the quality of nanotoxicity studies [62]. In vitro systems and in vivo models have been designed by various investigators for assessing nanomaterial toxicity.

***In Vitro Systems:***

An array biosensor capable of performing multiple cytotoxicity simultaneously was designed by Hondroulis et al [63] to address the need for a consistent method to measure real time assessments of toxicity. Advantages of this method are its easy usage, multiple sample analysis and monitoring kinetic effect continuously over a desired time frame.

Using luminous bacteria as a reporting agent Zheng et al [64] developed toxicity test to determine EC (50) of different nanomaterials. They claim that this luminescent bacteria test is simple rapid and sensitive and has the potential to be developed as a general test of toxicity for a wide variety of nanomaterials.

Nanoproperty quantifiable sensors or sensors used to measure nanoscale properties can enhance our understanding of the potential toxic effects of emerging pollutants from nanomaterials. These nanosensors are of critical interest to nanotoxicology, detection and risk assessment as well as monitoring of environmental or biological exposure. Sadik et al [65] have attempted to combine existing analytical techniques with conventional cytotoxicity methods for development of nanosensors for fullerenes, nanoparticles and quantum dots.

Braydich-Stolle [66] assessed toxicity of nanoparticles on male germ line in vitro. Their results demonstrated that silver particles were the most toxic while molybdenum trioxide was the least toxic. Thus the cell lines provide a valuable system to assess cytotoxicity of nanoparticles on germ cell line in vitro.

As the particles or fibres of nanomaterials are less than 1 micron in diameter, they may be respirable in humans. Based on their geometry, composition, size, concentration and transport in the body, they have potential to cause adverse effects on human health. In vitro assays include cell culture assays for cytotoxicity, proliferation, genotoxicity and altered gene expression [67]. They suggested that intra thoracic or intra pleural injection of nanomatrials in rodents can be misleading because they bypass normal clearance mechanisms and non-pathogenic fibers and particles can test positive in these assays.

In vitro nanotoxicity data needs to be translated to in vivo effects to define nanoparticle exposure risk. In vitro effects of aluminum (80 nm) nanoparticle exposure and its impact on a population of rat alveolar macrophages have been reported by Wagner et al [68]. The model

demonstrate how in vitro results can be used within a simulation setting of in vivo cell dynamics and suggest that physiologically-based pharmacokinetic models can interpret nanotoxicity data, guide in vivo study design and accelerate nanoparticle risk assessment [69].

***In Vivo Models:***

As the biosafety of nanomaterials becomes a growing concern, in vivo nanotoxicity is a necessity. Xie et al [70] investigated long-term (30 days) tissue and subcellular distribution and potential toxicity of intravenously administered Silica nanoparticles in mice using radiolabeling, radioactive counting, transmission electron microscopy and histological analysis. Their results indicated that Silica particles mainly accumulate in lungs, liver and spleen and are retained for over 30 days in the tissues due to endocytosis by macrophages and could possibly cause liver injury.

Although in vitro cell cultures are simple and cost-effective systems for screening toxicity of nanomaterials, unfortunately these fail to simulate the in vivo models, small animals are therefore most commonly used for assessing toxicity and biodistribution of nanomaterials in humans. Zebrafish can prove to be a quick, cheap and facile model for nanotoxicity studies [71]. Using Zebrafish model King-Heiden et al [72] were able to distinguish toxicity intrinsic to quantum dots from that caused by released metal ions such as Cadmium. They conclude that Zebrafish model provides a rapid, low-cost approach for assessing structure toxicity relationships of nanoparticles.

Chen et al [73] compared copper nanoparticles with micro copper and cupric ions in mice. While nanoparticles induced heavy injuries in kidney, liver and spleen but microparticles did not on mass basis.

Animal and human studies show that inhaled nanoparticles are less efficiently removed than the large particles by macrophages in the lungs, causing lung damage. Nanoparticles can translocate through circulatory, lymphatic and nervous systems to many tissues. These can also pass through blood brain barrier exhibiting their toxic effects in brain [74].

Thus, prior to the use of nanoparticles for vaccination, detailed study of characterization of nanoparticles, their efficacy in vitro and in vivo in smaller animals and their safety by evaluating these with a battery of tests for toxicity [75] is essential for their clinical application.

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