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Transmission Electron Microscope: Determining Potential Risks of Nanomaterials to Human Health at Microscopic Level

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Abstract: Human beings have been subjected continually to naturally occurring as well as anthropogenic nanoparticles from theprehistoric era. The persistent growth in the engineering of nanomaterials has immensely led to a polluted environment triggered by lack of monitoring the safety, beginning from the production process to the disposal of nanomaterial waste causing various health concerns. There are several researches that prove that the pollution caused by nanomaterials have effects on the respiratory and cardiovascular systems thereby causing higher death rate. Due to this issue, relevant studies have been carried out to understand the field of nanotoxicology and epidemiology that covers the wide research on the cell culture of human beings and animals. Studies have perceived that the nanoparticles penetrate the skin of human beings and can travel quickly to any organ through blood circulation. In this study, we explore pioneering ways of using transmission electron microscopy in diagnostic pathology to determine potential risks of nanomaterials to human health.

Keywords: Nanomaterials, Transmission Electron Microscope, human health, nanoparticles, risk, toxicity.

1. Introduction

Due to technological advancement, devices are shrinking in size and despite being small, their performance is highly boosted. Several devices are miniaturized to nanometre size on the basis of requirement which may be a concern in order to accomplish goals of particular functionality. Nanotechnology is the design, fabrication and application of nanostructures or nanomaterials and the fundamental understanding of the relationships between physical properties or phenomena and material dimensions. In simpler terms, Nanotechnology is basically a discipline of operating matter at a miniature level. Nanotechnology has helped in the development of innumerable products which are highly used in our day to day routine, for example, in the manufacture of dyes, paints, cosmetics, packaging material and the like. With high development in nanotechnology, producing nanomaterials of metastable state characterized by unpredictable factors such as magnetism and superconductivity is highly conceivable.

Due to the incessant growth in the field of nanotechnology, the miniaturization of devices such as sensors, surgical instruments and various other machineries has influenced the world widely. For instance, nanotechnology has led to the development of small sized computers with high computation power that pose the functionality of a human brain, there are biosensors that provide us with the status of a fatal disease that can save numerous lives, and nanorobots that have the ability to heal the internal injuries and further eliminate the chemical toxins inside the human bodies, and nanoscaled electronic devices that keep track of the environmental changes.

2. Nanomaterials

Nanomaterials are the particles (crystalline or amorphous) of organic or inorganic materials having sizes in the range of 1-100 nm (Edelstein, 1999). Nanomaterials are classified into nanostructured materials and nanophase/ nanoparticle materials. Nanomaterial refers to consolidated mass that are composed of particles of grain sizes in the nanometre size range while the nanoparticles are generally the diffused particles (Zhang, Lagally, 1997). To differentiate nanomaterials from the bulk material, it is crucial to determine the unique characteristics of nanomaterials and their potential impacts.

Nanomaterials are classified into metals (e.g. iron, gold, silver, platinum), metal oxides (zinc oxide, titanium

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dioxide, aluminium oxide), carbon-based nanomaterials (fullerenes, carbon nanotubes), and hybrid structures such as quantum dots, core-shell structures, functionalised materials that combine nanomaterials into intricate engineered structures for the purpose of presenting specific characteristics (Christian, 2008). The contrived structures further add concerns in evaluating the possible toxicity produced, apart from those introduced by the nanomaterial used itself.

Nanomaterials have various characteristics that are considerably diverse and substantially enhanced comparative to the coarser-grained nanomaterials. The variations in characteristics of the nanomaterials result from the small grain sizes, the large percentage of their atoms in large grain boundary environments and the interaction between the grains. Examination on the assortment of chemical, mechanical and physical properties is starting to produce a gleam of understanding of the interactionthat establishes itself in the properties of these new materials. In general, one can have nanoparticles of metals, semiconductors, dielectrics, magnetic materials, polymers or other organic compounds. Semiconductor hetero structures are usually referred to as one-dimensional artificially structured materials composed of layers of different phases/ compositions. The semiconductor hetero structured material is the optimum candidate for fabricating electronic and photonic nano devices (Jayadevan, Tseng, 2004)**.**

3. Nanotoxicology

Nanotoxicology is a branch of toxicology that focuses on the investigation of the toxicity of nanomaterials, which are further classified as toxics obtained from ignition such as wastes from diesel, production processes such as spraying and the befall of natural disasters and geological processes such as volcanos.

Quantum dots are basically semiconductors of nanoparticles. The main characteristic of Quantum Dots is their luminosity. This luminousness is due to the central arrangement, size and covering. The existence of Quantum Dots is based on several aspects such as their physicochemical properties and the ecological surroundings they come across (Hardman, 2006).

For the study of nanotoxicology, nanomaterials are further classified based on their location and form (Hansen, 2008). Due to this classification it is expedient to understand that the same material relocates to several locations and reforms into many forms. Consequently, it is vital to conclude that the toxicity of nanomaterials is based on different points in the product's life cycle. For example, surface bound materials are having reduced toxicity in their proposed usage than airborne particles that are easily inhaled. Conversely, nanomaterials in consumer item salter their toxicity level during usage.

Nanoparticles that are present in paint such as titanium dioxide are typically suspended as a liquid; nonetheless convert into a solid state when the paint has dried out. The consumer is subjected to minimal toxicity after the paint has dried. As the paint wears out with time, the nanomaterial penetrates into the environment causing toxicity exposure risks for the environment as well as human beings and animals.

The study of the classification strategy mentioned above that is used for nanomaterials, when applied to the consumer products list, depicts 45 per cent of the products are exposed to consumers. Metal oxides such as Zinc oxide, gold and titanium dioxide were the probable outcomes of the exposure. Also, there are several products that do not specify about the categories of nanomaterials used which increases concern regarding the harm of the potential risk of exposure (Hansen, 2008).

4. Environmental Exposure

The growth of industries has led to growth of combustion engines due to which a considerable rate of environmental pollution has occurred in urban areas covering a substantial part of the earth. The climatic and environmental impacts caused by nanomaterials to a certain extent still remain unknown. Also, the future of nanomaterials is not definite. The nanoparticle wastes are disposed directly through dumping in unprofitable areas used specifically for waste disposal or recycling stations, burning of the material and during waste water treatment. At least 95 per cent of nanoparticles that are used in the consumer products eventually runoffor wear out after usage, which finally drain down to the waste water(Mueller and Nowack, 2008). Solid consumer products such as plastics, sport gears and gadgets, which are composed of nanomaterials such as carbon nanotubes persist to stay together until disposed. Recycling for these products is an enduring objective, but the rationality of this possibility is however unknown.

5. Human Exposure

Nanomaterials are expected to come in contact with the environment or human beings at different levels of their life cycle. Human beings are subjected to nanomaterial toxicity via inhalation through respiration and absorption through skin, eyes and gastrointestinal tracts. After the penetration, the nanomaterial toxins are scattered through the circulatory system, broken down and absorbed. Even a fetus inside the womb is subjected to these toxins(Takeda, 2009).Likewise, a nanomaterial which may be comparatively safe, may act as a virus after a more toxic material attaches to it. This may also lead to penetration into the human body and further causing more damage (Ashwood, Thompson, Powell, 2007). Furthermore, people already suffering from respiratory diseases and diabetes are more susceptible to the toxicity caused by nanoparticles. The response mechanisms of certain genes are likely to cause health issues as well.

6. Transmission Electron Microscope

The Transmission Electron Microscope (TEM) is a prevailing tool in nanotechnology. TEM offers the most powerful magnification, potentially over one million times or more. Firstly, a light emission is shone through a dainty specimen. The electrons and other particles of this emission are associated for observing several features such as, of a crystal whose features like dislocations and the grain boundaries.

Chemical analysis of the nanomaterials can also be done. TEM can be employed in researching the development of layers, their structure and shortcomings in semiconductors. High resolution of TEM is used to examine the quality, shape, size and density of quantum wells, wires and dots. Additionally, TEM is used in a range of scientific fields for applications in cancer research, virology, and materials science.

Alike a light microscope, TEM works on the similar basic principles but in TEM electrons are substituted for light, as the electrons have smaller wavelength, due to which the ideal resolution obtained is higher. Subsequently TEM helps in the study of the internal structures of nanomaterials.

A schematic view of the TEM is shown in figure 1.

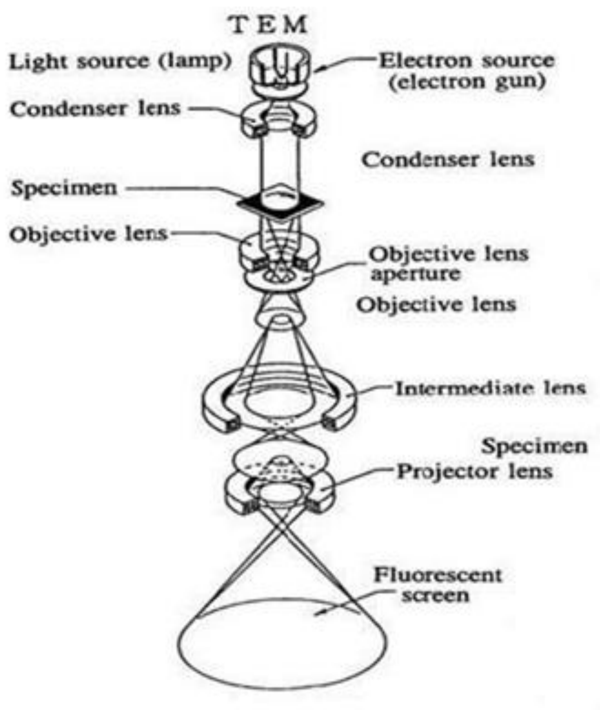

Figure 1: A schematic representation of TEM

6.1 Imaging

An electron gun emits a beam which is absorbed as a dainty and intelligible beam by using a condenser lens. The condenser opening limits the beam is limited as it rejects high angle electrons. When this beam falls on the specimen, the beam is scattered and part of it is distributed on the basis of the specimen's thickness and electron transparency. The distribution of the scattered beam is observed by the objective lens into an image on either a phosphor screen or a charge coupled device camera. The image can be enhanced by obstructing high angled diffracted electrons. This image is enlarged by further passing down via the intermediate and projector lenses.

Light is produced when the image falls on the phosphor screen which displays the image to the user. Certain parts of this image are darker that signifies that lesser number of electrons have been transmitted. Whereas in the lighter part of the image, electron transmission is higher.

6.2 Diffraction

The figure 2illustrates the pathway of an electron beam as seen from a TEM, located above the specimen and down the column to the phosphor screen. The beam passes over the specimen, is dispersed, that makes the electrons scatter due to the electrostatic potential which is established by the specimen's composed elements. The beam then passes over the electromagnetic objective lens. This lens is required to concentrate on the scattered electrons in the image plane. As depicted in figure 1, the electrons that flow through the same path are gathered into a single point. This is the back focal plane of the objective lens and is where the diffraction pattern is formed (Leadley, 2010).

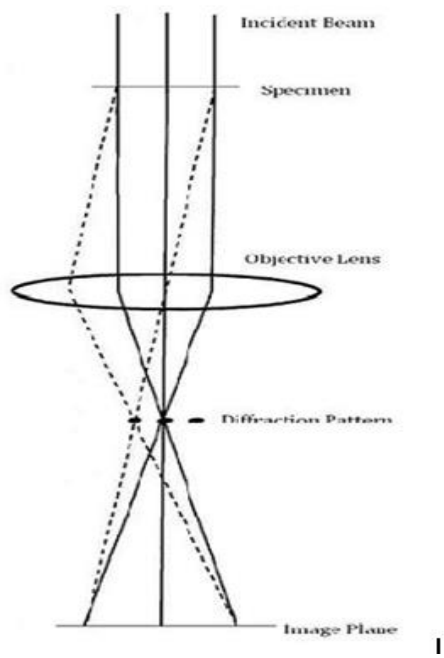

Figure 2: Diffraction mechanism of TEM

Efficient imaging of nanoparticles using TEM is based on the contrast of the specimen compared to the background.

For the purpose of imaging, Specimens are arranged by drying nanoparticles on a copper grid that is coated with a thin layer of carbon. Materials with electron densities that are considerably greater than amorphous carbon are effortlessly imaged. Figure 3 describes TEM image of silica coated gold nanoparticles. The gold cores appear specifically darker than the silica shells because of higher electron density (Nanocomposix, 2012).

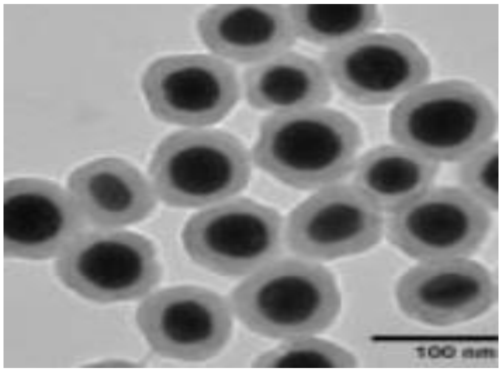

Figure 3: Image of silica coated gold nanoparticles using TEM

The purpose of this study is to review various latent health risks due to the usage of nanomaterials in our day to day lives and the study of these risks must be carried out by deploying a TEM. A comprehensive literature review is presented followed by a brief discussion on the prospects of using a TEM to determine the impact of nanomaterials on human health.

7. Literature Review

Electrons are required to image the tissues at a higher amplified rate in TEM than a light microscope that employs photons. This feature gives us the ability to visualize the finest details of the structures of nanomaterial. TEM has aided in several areas of tumour genesis, embryology, transplantation biology, nerve regeneration, and colonic and pancreatic stem cell research that hasled to several noteworthy contributions (MGH, n.d).

Amid the materials detected in the beginning of rise of Electron Microscopy (EM), there existed sub-microscopic pathogens, i.e., virus particles (tobacco virus) and bacteria. The amplification power of EM and the opportunity to classify morphologically virus particles and the virus family was a critical element in the outline of EM in virology. Currently the abilities and characteristics of EM in this field, remain exactly equivalent. As mentioned earlier, the preparation method developed faster and was further enhanced. These conditions along with a chain of instrument developments make EM an indispensable practice in the diagnosis of infectious diseases (Pedro, 2008).

Translocation of nanoparticles to lymph nodes has been immensely researched for drug delivery and tumour imaging. Progression of many cancers such as lung, oesophageal, esothelioma, and so on, and their status i.e. in case of tumours, spread of tumour cells to local lymph nodes are studied and diagnosis is made accordingly(Liu, Wong, Moselhy, Bowen, Wu, Johnston, 2006).

In another study, nanoparticles are frequently found in colon tissue of people suffering from cancer, Crohn's disease, and ulcerative colitis; however inhealthy individuals, nanoparticles were absent (Gatti, 2004).

Latest epidemiological researches have revealed that a strong association between particulate air pollution levels, respiratory and cardiovascular diseases, various cancers, and mortality. Adverse effects of nanoparticles on health are based on distinct aspects such as genetics and existing disease, as well as exposure, and nanoparticle chemistry, size, shape, agglomeration state, and electromagnetic properties (Buzea, Pacheco, Robbie, 2007).

According to the study conducted by Xia, Li and Nel (2009), along with workers' exposure due to their professions, personal exposure has been high due to the use of nanomaterials for drug therapy, imaging, and gene delivery. While the exposure may raise several concerns, it is uncertain that the toxic potential of the nanomaterials on the well-being of human beings. However, more research is required in the understanding of nanotoxicology, and the research must be carried out assuming no impact has occurred on human health due to nanomaterials. Granting clinical toxicity by engineered nanomaterials has not been recognized, the literature on particles and fibres', including ultra- fine particles (UFPs), diesel exhaust particles (DEPs), quartz, and asbestos (51, 58), specifies a history of adverse health impacts. It is a conceivable thought that engineered nanomaterials and fibres may cause similar threats. The unique physicochemical properties of the engineered materials may present novel mechanisms of injury and toxicological paradigms.

Respiratory ailments such as Asthma and lung damage are the major concerns due to the toxicity of nanomaterials. Researches that have examined nanomaterials validate that the toxicity of nanomaterials transfer to the lungs. Additionally, there are concerns over the possible harm to other organs. In this study, the researchers have illustrated that, through a number of independent trials, several types of Polyamidoamine (**PAMAM**) dendrimers destroyed human lung cells in the lab. There was no indication that the cells were dying by apoptosis, a common type of programmed cell death. However, they found that the particles generated autophagic cell death through the Akt-TSC2-mTOR signalling pathway. Autophagy is a process that damages injured materials in a cell and plays a standard part in cell growth and renewal, however scientists have found that occasionally an over activity of the destruction process leads to cell death (Oxford University Press, 2009).

A ground-breaking research by scientists at Trinity College Dublin (2012) has found that exposure to nanoparticles can have a serious impact on health, linking it to rheumatoid arthritis and the evolution of other serious autoimmune diseases. Ecological pollution by carbon particles discharged by car exhaust, smoking and long term inhalation of dust of various back ground have been accepted as risk factors producing chronic inflammation of the lungs.

A research on rodents established the fact that despite the process by which Carbon Nanotubes (CNT) were synthesized and the categories and quantities of metals they composed, CNTs can cause inflammation, epithelioid granulomas, fibrosis, and biochemical and toxicological changes in the lungs. The hair-like shape of CNTs is similar to asbestos fibres, raising the concern that prevalent use of CNTs may cause pleural mesothelioma, a cancer of the lining of the lungs, or peritoneal mesothelioma, a cancer of the lining of the abdomen (Whytock, 2014).

Although extensive research is carried out on Transmission Electron Microscopy of nanomaterials, and various pathological studies separately, literature is nil for the determination of health risks caused by nanomaterials using a TEM. Therefore through this review we will recognize and discuss the prospects of implementing a distinct methodology of ascertaining nanomaterial threat to human health using a TEM.

8. Discussion and Conclusion

While the literature review does not show substantial research on the usage of TEM to determine health risks of nanomaterials, we can however develop an innovative idea of collaborating the medical applications of TEM to determine the possible health risks caused by nanomaterials. This is practically a new segment in the fields of nanotechnology and medical research.

TEM has been practically used in medical research particularly in pathology. TEM is greatly efficient in assessment of single cells or more refined structures, such as organelles, cytoskeleton and other sub-cellular elements. For example, for tumours that are challenging to be identified, a precise diagnosis and typification has to be accomplished by means of well-selected set of antibodies.

It is compelled to understand that TEM is extremely helpful in the examination of clinical diagnostics for renal diseases, tumour related diseases, storage disorders and the identification of several viruses.

Chemical fixation is a typical practice employed by innumerable laboratories. Usage of resins for implanting objects and the process of dehydration are also used. For delicate objects such as semi-thin and ultra-thin materials, a qualified pathologist must follow an approach of correlative microscopy, through which valuable information for a significant amount of cases can be attained.

Much could be learnt from this study, however, as this is a novel idea, more precision is required in employing the proposed scheme. From the view of profession, the study of nanotoxicology is a well-developed discipline that has led to extensive perception into particle and fibre injury. Further adding the application of TEM to this study would amplify the research potential in this sector. Fresh design and efforts must emphasizeon boosting performance escalate the scope and association in research of TEM to establish a standard in diagnosing health risks due to nanomaterials and as the applications expand, many proponents are positioning nanotechnologies as part of a greener, more sustainable future.

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