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Nanocytotoxicity

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Abstract: Nanotechnology is widely used in our daily life including its use in medicine and dentistry. While choosing the nanoparticle for the use in the field of nano dentistry its chemical, physical, along with the biological aspect of nanostructures should be taken into consideration. A nanoparticle is a small particle that ranges between 0.1 to 100 nanometres in size.
Nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts. Some nanoparticles are used for oral disease preventive drugs, prostheses and for teeth implantation. Nanomaterials further deliver drugs, preventing and curing some oral disease (oral cancer), tooth anti-sensitivity and enamel surface polishing.

Keywords: *Nancytotoxicity,nanoparticle,greennanotechnology,physicochemical factors,surface charge,how to reduce nanocytotoxicity.*

I. Introduction

Nanoparticle cytotoxicity is defined as the extent to which the interaction of nanoparticles with cells disrupts cellular structures and/or processes essential for cell survival and proliferation.(1)

Nanotoxicology focuses on determining the adverse effects of nanomaterials on human health and the environment.(2)

I. Routes of nanoparticles entry

Nanoparticles may be high risk both for patient and staff. In addition to patients, the workers who most likely come into contact with dental nanomaterials in the production, research, and development are in the risk of nanomaterial's toxicity.

Nanomaterials may introduce to the staff body through inhalation. The nanomaterials may enter the body then into the bloodstream (or lymph fluid) via absorption through oral mucosa. They may also enter through the digestive tract after swallowing. They can be distributed to different organs (liver, spleen, kidneys, heart, lungs, and brain) by systematic pathway. They may also directly translocate to the brain via nerves.(3)

I.1. Injection Route

In drug delivery purposes, by injection NPs can enter the systemic circulations where they affect the circulatory system, central nervous system. (4)

I.2. Dermal Route

Formulations that contain the NPs such as cosmetic preparations and wound dressings which contains NPs of silver, titanium serve as a dermal route entry of NPs. Skin toxicity

of nanoparticles is yet controversial. It was found that the adverse health effects for the topical application of sunscreens containing TiO_2 nanoparticles are not found, while other studies confirm adverse effects of nanosized particles and human dermal cells. (4)

I.3. Inhalation Route

As most of the dental nanomaterials are directly applied in the oral cavity or maxillofacial region during polishing or mechanical grinding. Due to their smaller size, NPs could penetrate the respiratory system and enter systemic circulation. They cause various lung diseases such as asthma, emphysema or even lung cancer, based on the concentration and physicochemical properties of NPs. From lung they travel to other organs such as bone marrow, brain or heart and lead to more severe diseases including Alzheimer, Parkinson or cardiac malignancies. (4)

I.4. Oral Route

Oral ingestion of NPs from dental fillings contain nanoparticles can cause brain damage. Titanium dioxide NPs could cause various pathological effects in a dose-dependent manner, such as blood-brain barrier destruction, cellular oedema and brain tissue necrosis.(4)

II.The physicochemical factors governing nano-cytotoxity:

1. Size

Cytotoxicity induced by nanomaterials results from the interaction between the nanomaterial surface and cellular components. As the diameter decreases, the surface area of the particle increases exponentially. Thus, even when particles have the same composition, they can have significantly different levels of cytotoxicity depending on both particle size and surface reactivity. Additionally, particle size induces significant differences in the cellular delivery mechanism and distribution in vivo. Size affects absorption, distribution and cellular uptake of nanoparticles. (5)

1.1. Absorption

To generate cytotoxicity and inflammatory response in animal models, it is essential that the nanoparticles should migrate across the epithelial barrier. In this respect, the size of the nanoparticles plays a key role in cytotoxicity. Different sized nanoparticles show specific distribution patterns in the respiratory tract. Cytotoxicity induced by inhaled silver nanoparticles of different sizes were assessed; 18, 34, 60, and 160 nm. It was found that silver nanoparticles in sizes of 18 and 34 nm induced lactate dehydrogenase (LDH) expression, which is a marker of cell damage, in a dose dependant manner. Meanwhile, there was no dose-dependent cell damage when 60 and 160 nm nanoparticles were used. It was justified that the increased surface area of the NPs was the most likely factor contributing to the toxicity of the silver nanoparticles(5).

1.2. In Vivo distribution and Clearance:

The distribution of nanoparticles in vivo, or pharmacokinetics, is also an important consideration in assessing cytotoxicity. Nanoparticles with a diameter greater than 6 nm cannot be excreted by the kidneys and accumulate in specific organs, such as the liver and spleen, until clearance by the mononuclear phagocyte system causing serious side effects. This pharmacokinetic characteristic of nanoparticles is dependent on particle size and surface chemistry.

The in vivo distribution of gold nanoparticles according to size was evaluated. The sizes used were from 10 to 250 nm. The in vivo distribution after intravenous injection in a rat model was assessed. It was found that 10 nm nanoparticles were distributed differently than their larger counterparts. 10 nm NPs were found in almost all organs, including the blood, liver, spleen, kidneys, testes, thymus, heart, lungs and brain. Meanwhile, most nanoparticles larger than 50 nm were detected only in the blood, liver and spleen.

1.3. Cellular Uptake:

One of the major factors determining cellular uptake efficiency and mechanism is nanoparticle size. With respect to particle size, nanoparticles are internalized into the cell through various pathways, such as phagocytosis and pinocytosis. Sizes suitable for uptake range from 10 to 500 nm. Large particles are most likely to be engulfed via macropinocytosis.

Gold nanoparticles typically form a surface coated layer with serum proteins when incubated with cells. Serum-layered gold nanoparticles usually induce receptor-mediated endocytosis, which is dependent on particle size. The uptake efficiency of gold nanoparticles as a function of size was evaluated. Gold nanoparticles ranging from 1 to 100 nm were incubated with Hela cells, and the 50 nm nanoparticle showed maximal uptake efficiency by receptor-mediated endocytosis. (5)

A similar experiment using ligand-coated gold nanoparticles showed that a diameter of 40–50 nm was the critical cutoff point for receptor-mediated nanoparticle internalization. This phenomenon is tightly related to the nanoparticle's binding and its cellular surface receptors.(5)

2. Surface

2.1. Surface Area

A larger surface area may cause higher reactivity, resulting in possibly harmful effects when used in fillers, cosmetics, and as drug carriers. Smaller particles occupy less volume, such that a larger number of particles can occupy a unit area, resulting in increased pathophysiological toxicity mechanisms, for instance oxidative stress, ROS generation and mitochondrial perturbation. It has yet to be determined what features of nanoparticles cause such biological toxicity. It was found that the size of the nanoparticle alone may not be responsible for toxicity, but that the total number per unit volume may be important.

The relationship between a nanoparticle's surface area and its biological toxicity was assessed with different nanoparticle surface areas and it was found that the total surface area played a critical role in lung inflammation rather than the size.(5)

2.2. Effect of Surface Charge:

Surface charge also plays an important role in toxicity of nanoparticles as it largely defines their interactions with the biological systems. Various aspects of nanomaterials such as selective adsorption of nanoparticles, plasma protein binding, blood-brain barrier integrity, and transmembrane permeability are primarily regulated by surface charge of nanoparticles.

Positively charged nanoparticles show significant cellular uptake compared to negatively charged and neutral nanoparticles, owing to their enhanced opsonization by the plasma proteins. Moreover, they have also been shown to induce hemolysis and platelet aggregation owing to which causes severe toxicity to the system.

For example, positively charged Si nanoparticles have been shown to be more cytotoxic compared to neutral and negatively charged Si nanoparticles which display minimal to no cytotoxicity issues. Neutral nanoparticles show limited cellular uptake and are useful in applications where nonspecific interactions with cells and the cellular uptake is not desired and could be done by modifying the nanoparticle surface with hydroxyl group to produce neutral charge. (6-8)

2.3. Effect of Surface Coating and Surface Roughness:

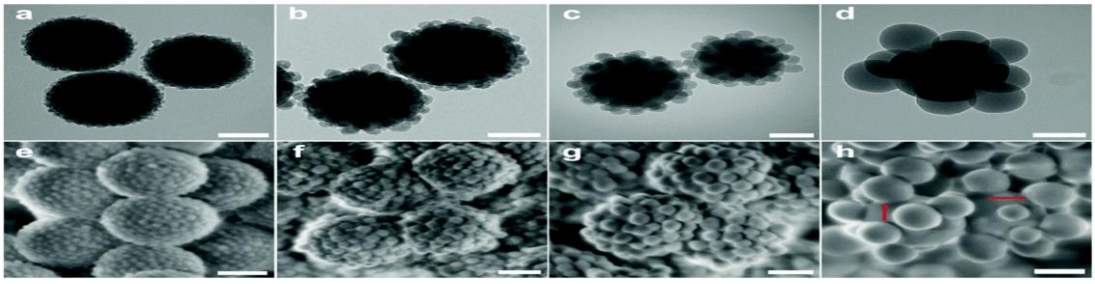
Surface coating can affect the cytotoxic properties of nanoparticles by changing their physicochemical properties such as magnetic, electric, and optical properties and chemical reactivity and can alter the pharmacokinetics, distribution and accumulation of nanoparticles.

It has been known that the presence of oxygen, ozone, oxygen radicals and transition metals on nanoparticle surfaces leads to the generation of ROS and the induction of inflammation.

However, on the other hand, surface coating could also be employed to reduce the toxicity of the nanoparticles. In general, surface coating can eliminate the adverse effects of nanoparticles. In particular, proper surface coating can lead to stabilization of nanoparticles as well as preventing release of toxic ions from nanomaterials.

Surface coarseness dictates the strength of nanoparticle-cell interactions and promotes cell adhesion. Pore structure is critical in cell-nanoparticle interactions. It has been demonstrated that size dependent hemolysis effect of mesoporous silica nanoparticles is only observed when the nanoparticles have porous structure. (6)

- The rough silica nanoparticles demonstrate a high efficiency of intracellular delivery of therapeutic proteins in cancer cells, causing significant cell inhibition, so controlled surface roughness could be used for the delivery of therapeutic proteins. Using “neck-enhancing” approach to synthesize stable rough silica nanoparticles with controllable surface roughness. By increasing the shell particle size from 13 to 98 nm while fixing the core size at 211 nm. Shell nanoparticles with the mean sizes of 28, 54, 98, 135 and 175 nm were fabricated using the Stöber method by reacting at 70, 60, 50, 40 and 30 °C, respectively. The reactions were first carried out for 20 minutes for the formation of shell particles (28, 54, 98 and 135 nm). For the shell particle of 175 nm. Absolute ethanol (50 ml) was mixed with deionized (DI) water (3.8 ml) and ammonium hydroxide solution (2 ml) at 25 °C. Then, TEOS (3 ml) was added to the solution. After 6 h, the as-synthesized nanoparticles were separated by centrifugation and washed with ethanol. The final product was obtained by drying at 100 °C overnight. After that, aminosilane was grafted to create positively charged surfaces. (9)



3. Morphology

Nanomaterials come in varied shapes including fibres, rings, tubes, spheres, and planes. Basically, shape dependent nanotoxicity influences the membrane wrapping processes *in vivo* during endocytosis or phagocytosis. It has been observed that endocytosis of spherical nanoparticles is easier and faster as compared to rod shaped or fibre nanoparticles and more importantly spherical nanoparticles are relatively less toxic irrespective of whether they are homogenous or heterogeneous. Non-spherical nanomaterials are more disposed to flow through capillaries causing other biological consequences. (6)

Study conducted on spherical and rod-shaped gold NPs demonstrated that rod-shaped NPs undergo lower cellular uptake than spherical NPs. There were two possible explanations: first, membrane wrapping for rod-shaped NPs takes longer than for spherical NPs; second, surfactant molecules adsorbed onto the longitudinal axis of nanorods impinge upon the ligand binding on the NP surface that facilitates cellular uptake.(8)

4. Aggregation Status

Aggregation could be a potent inducer of inflammatory lung injury in humans. For certain types of chemicals, exposure at higher levels has been shown to lead to serious chronic diseases such as fibrosis and cancer. It is still under consideration to figure out what features are inducing such toxicological effect in a living organism. Aggregated carbon nanotubes have more toxic effects than well-dispersed carbon nanotubes and enhance pulmonary fibrosis(5)

Basically, the aggregation states of NPs depend on size, surface charge, and composition. It has been observed that carbon nanotubes are mainly accumulated in liver, spleen, and lungs without manifesting any acute toxicity but induce cytotoxic effects mostly because of accumulation of aggregates for longer periods. (10)

5. Effect of Aspect Ratio:

Moreover, it has also been observed that the higher the aspect ratio, the more the toxicity of particle. In case of asbestos induced toxicity, it was observed that asbestos fibres longer than 10 microns caused lung carcinoma while fibres >5microns caused mesothelioma and fibres >2microns caused asbestosis as longer fibre will not be effectively cleared from the respiratory tract due to the inability of macrophages to phagocytise them.The toxicity of fibres with long aspect is closely related to their plasma shelf life. The fibres that are sufficiently soluble in lung fluid can disappear in a matter of months, while the insoluble fibers are likely to remain in the lungs indefinitely.

It was also observed that long-aspect ratio particles (SWCNTs) produce significant pulmonary toxicity compared to spherical particles.(6)

6. Effect of Composition and Crystalline Structure.

Although it has been emphasized that particle size plays significant role in deciding toxicity of nanoparticles, we cannot simply ignore studies comparing toxicities for diverse nanoparticles chemistries having the same dimensions. These studies highlight that the composition and crystalline structure

of nanoparticles also influence their toxicity issues. In a study it was observed that nanosilver and nanocopper with their soluble forms caused toxicity in all tested organisms,

whereas TiO_2 of the same dimensions did not cause any toxicity issues, thus emphasizing role of compositions in determining the toxicities of NPs.(6)

Crystal structure also influences the toxicity of nanoparticles and it has been observed that rutile TiO_2 nanoparticles induce oxidative DNA damage, whereas anatase nanoparticles of the same size and chemical composition did not.

7. Effect of Concentration:

Moreover, generally, it has been observed that with increase in the concentration of nanoparticles, the toxicity increases. At a concentration of 100 μg/ml NPs decreasing the cell survival by 20% only. The NPs do not cause apoptosis, ROS generation, or serious morphological changes in cells at concentrations lower than 100 μg/ml. (8)

8. Effect of Solvents/Media:

Medium/solvent conditions have been known to affect particle dispersion and agglomeration state of nanoparticles, which in turn have effect on their particle size, thereby influencing the toxicity associated with nanoparticles.

It has been observed that particles of TiO_2, ZnO, or carbon black have significantly greater size in PBS than in water; Accordingly, the same nanoparticles exhibit different toxic manifestations when dissolved in different mediums. Although, the dispersing agent may improve the physicochemical and solution properties of nanomaterials formulations, they may also adversely affect the toxicity of nanomaterials.(6)

III. Methods for Reducing the Toxicity of Drug Carriers Based on Nanoparticles

There are many methods to prevent or limit the toxic effects of metallic nanoparticles and metal oxides. Studies have shown that changing the shape and size of particles, and methods to modify their surface, can lead to the formation of nanoparticles with the desired properties, but without a toxic effect. (11)

Nanoparticles used as drug carriers are exposed to a physiological medium consisting of high levels of salt and various proteins. Both of these factors affect the stability of nanoparticles. High salt concentration reduces electrostatic repulsion between nanoparticles, leading to their aggregation, while proteins are adsorbed on the surface of nanoparticles and change the size of particles and surface charge. (11)

III.1. Methods for the Synthesis of Metal and Metal Oxide Nanoparticles

At the stage nanoparticles are obtained it is possible to limit their potential toxic properties. The methods of producing nanoparticles allow products of various shapes and sizes to be obtained. Factors that affect these properties include process temperature, pH of the reaction, form of energy supply, reagents, and reaction environment. (11)

Nanoparticles can be obtained by chemical, physical, and biological methods. In chemical processes, nanoparticles are most often obtained in simple precipitation reactions. Initially, it leads to the formation of metallic particles, which stick together to form agglomerates. To inhibit the agglomeration process, stabilizing substances are introduced into the system or the temperature and pH of the system are controlled. (11)

In the case of metal nanoparticles intended for drug carriers, stabilizers perform two functions. First, they stabilize and protect nanoparticles against further agglomeration.

Secondly, they change the nature of the surface of nanoparticles, so the joining of nanoparticles with a drug become easier. (11)

Compounds containing sulfur, nitrogen, or oxygen easily react with metal ions initiating their reduction causing simultaneous stabilization of freshly formed nanoparticles. An example of obtaining metallic nanoparticles by the biological method is the synthesis of nano gold modified with para-aminobenzoic acid-quat-pullulan (PABA-QP) as a carrier of doxorubicin. Due to surface modification, higher drug loading was possible.(11)

III.2. Morphology of Metal and Metal Oxide Nanoparticles

By changing the method of synthesis, process parameters used, it is possible to obtain nanoparticles with spherical, elongated, cubic, triangular, and many other shapes. This results in compounds with different surface to volume ratios.

The relationship between the influence of the shape of AgNPs on cell toxicity was demonstrated and it was found that platelet-shaped AgNPs showed greater toxicity epithelial cell lines compared to spherical and wire-shaped nanoparticles. The concentrations of the analyzed nanoparticles were tested in the range of 1–300 μM. The results showed a low cytotoxicity profile of spherical nanoparticles, especially at lower concentrations.

Another study compared the shape effect of ZnO NPs on their toxicity and showed that nanowire-shaped particles had higher toxicity compared to spherical and cubic particles. (11)

III.3. Protective Coatings:

A different approach to modify the properties of metallic nanoparticles limiting their toxic effects, is to use appropriate surface modifications.

The main task of using coating compounds is to improve the stability of nanoparticles by preventing the release of ions from inside, preventing oxidation of the surface of nanoparticles and inhibiting agglomeration of nanoparticles. (11)

It was found that coating of AgNPs with a thin layer of SiO_2 minimized their toxicity by blocking the release of ions and contact of bacteria and/or cells. Natural compounds such as saccharides, hydrocolloids, and polyphenols can be effectively used as factors improving the biocompatibility of metal nanoparticles.

AuNPs were stabilized with karaya gum, which were used as the carrier of the anti-cancer drug. Also, rubber stabilized nanoparticles have been found to be biocompatible during cytotoxic studies and hemolysis because it acts as a reducing agent and gives nanoparticles colloidal stability. (11)

III.4. Surface Functionalization

An important method is to functionalize the surface of nanoparticles by introducing appropriate functional groups. Depending on the properties of the nanoparticles, their future use or the drug to be combined with the nanocarrier, a variety of ligands are used. In the case of drug delivery systems, such surface modifications allow the creation of appropriate mechanisms for loading and releasing the drug into target cells, changing their character to hydrophilic/hydrophobic, which improves drug solubility in the system and improves penetration through well-defined membranes. The most important groups that can be used as surface modifiers of metal nanoparticles are disulfide, amine, thiolate and

dithioline, carboxylate, and phosphine groups. It was found that by modifying the surface of ZnO NPs with polyethylene glycol, the cytotoxicity was reduced and increasing their cell compatibility. The use of polyethylene glycol reduced the formation of protein crowns, which led to lower cytotoxicity compared to pure ZnO NPs .

Due to the need to supply drugs to both hydrophilic and hydrophobic environments, it may be necessary to change the nature of the carrier surface. The increase of hydrophilic properties most often occurs by attaching carboxyl groups (-COOH). Using the addition of N-vinylpyrrolidone in the preparation of Ag NPs, it was possible to obtain a carrier for hydrophobic drugs in the aqueous medium(11).

Green nanotechnology:

Green nanotechnology is a branch of green technology that utilizes the concepts of green chemistry and green engineering, where the word "green" refers to the use of plant products. It reduces the use of energy and fuel by using less material and renewable inputs wherever possible. Green nanotechnology significantly contributes to environmental sustainability through the production of nanomaterials and nanoproducts, without causing harm or cytotoxicity to human health or the environment. The rationale behind the utilization of plants in nanoparticle formulations is that they are easily available and possess a broad variability of metabolites, such as vitamins, antioxidants, and nucleotides. For instance, gold (Au) nanoparticles, titanium dioxide and zinc oxide nanoparticles are also important metal oxide nanomaterials that have been synthesized from a number of plant extracts.(12)

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