We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,500
Open access books available

176,000
International authors and editors

190M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Nanotoxicological Assessments of Upconversion Nanoparticles

Dalia Chávez-García and Karla Juarez-Moreno

Abstract

Upconversion nanoparticles (UCNPs) are highly efficient luminescent nanomaterials with emission in the visible spectra while being excited by near-infrared region light (NIR). With their unique properties such as high luminescence intensity, sharp emission peaks with narrow bandwidth, large anti-Stokes' shift, and sizes smaller than 100 nm, UCNPs have emerged as promising candidates for diverse biomedical applications such as cancer detection and therapy, fluorescence imaging, magnetic resonance imaging (MRI), and drug delivery. The UCNPs are composed of a crystalline matrix doped with lanthanide ions that can absorb NIR light (~980 nm) and upconvert it to visible light. However, to achieve successful biomedical applications, proper functionalization, target-specific cell interaction, and biocompatibility are critical factors that must be considered. Additionally, a comprehensive nanotoxicological assessment is necessary to ensure that UCNPs are not cytotoxic or genotoxic. This assessment is particularly important for long-term studies of nanoparticles' tracking *in vivo*. Therefore, this chapter aims to provide an in-depth evaluation of the nanotoxicological issues related to nanoparticles (NPs) and UCNPs in biomedical applications, and ensure their safety and efficacy as bioimaging and chemotherapeutic delivery tools.

Keywords: cytotoxicity, nanoparticles, upconversion, nanotoxicological, luminescent

1. Introduction

The toxicity assessment of nanoparticles (NPs) is a relevant issue since many researchers are using, specially, luminescent nanoparticles for various applications, such as bioimaging or drug delivery for *in vivo* and *in vitro* applications [1–3]. In this chapter, we will analyze how the approach in this analysis has been carried out for upconversion luminescent nanoparticles (UCNPs), which are a special type of NPs since they can receive energy in the near-infrared region (NIR) and emit in the visible or NIR spectrum. These NPs are composed of a matrix cell that can be made of oxides, oxysulfides, oxyhalides, phosphates, molybdates, tungstates, gallates, vanadates, and fluorides. The UCNPs are doped with lanthanide elements such as: Yb^{3+}, Er^{3+}, Tm^{3+}, and Ho^{3+}, among others. It is common in the upconversion process to have lanthanide elements co-doped to bring about a photon transfer between energy levels. For example, for the doping of Yb/Er, the Yb^{3+} absorbs NIR radiation at 970–980 nm of

wavelength in its base state ($^2F_{7/2}$–$^2F_{5/2}$), then this energy is transferred to Er^{3+} and the electron is populated to level $^4I_{11/2}$, then, a second photon is absorbed and by Yb^{3+} and it is transferred to Er^{3+}, so the electron is raised to level $^4F_{7/2}$. From this state, it decays rapidly to $^4S_{3/2}$, and the green emission happens ($^4S_{3/2}$–$^4I_{15/2}$), and this process is called the APTE (Addition de photons par transfert d'énergie, i.e., photon energies by adding transfers), as can be seen in **Figure 1**. There are more upconversion processes with different doping combinations and concentrations of the ions, where the percentage of doping directly affects the color of emission [4].

The UCNPs have emerged as a promising nanomaterial for identifying specific cells and for drug delivery. Unlike other dyes, UCNPs exhibit stable emission if the source of excitation is maintained, making them more reliable. There are other types of upconversion processes such as: two-step absorption, cooperative sensitization, cooperative luminescence, the second harmonic generation, and two-photon absorption [4].

One crucial aspect of using UCNPs in biomedical applications lies in ensuring their biocompatibility on cells and or organisms. To achieve this, UCNPs must be functionalized with different ligands that specifically target the desired cells and organs. Several chemical groups, including polyethylene glycol (PEG) [5], polyethyleneimine (PEI) [6], polyvinylpyrrolidone (PVP) [7], polyacrylic acid (PAA) [8], and silica [6], have been used for this purpose. However, it is important to highlight that the toxicology of UCNPs depends on their physicochemical and physiological properties. Physicochemical properties include size, shape, surface area, and chemical composition, while physiological properties refer to the disease conditions, genetics, and other factors [9]. The recommended size for optimal penetration of NPs is below 100 nm. However, this size may also pose a risk of toxicity due to their potential to penetrate cellular structures and organs via the circulatory system. Moreover, UCNPs may generate reactive oxygen species (ROS) that can induce DNA damage, which not only affects the cell growth by means of protein oxidation, but also impacts mitochondrial respiration [10].
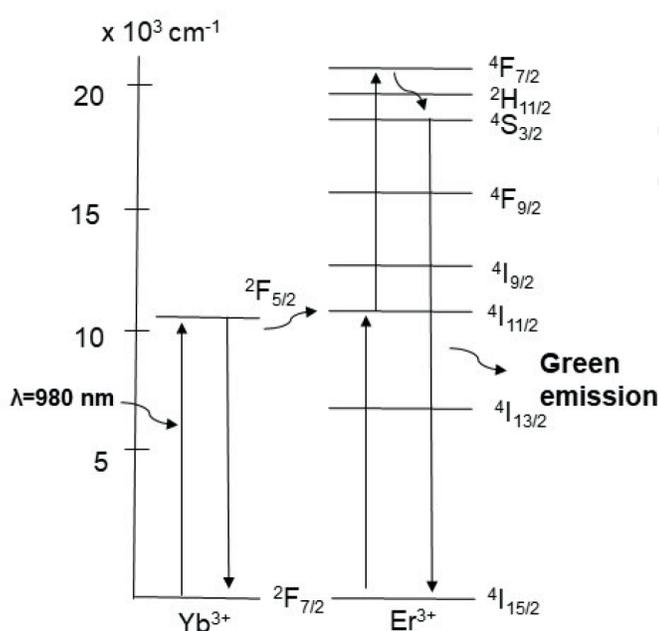
Figure 1.
Upconversion process between Yb^{3+} and Er^{3+} ions.

Several toxicological studies have been conducted on both *in vivo* and *in vitro* human cell lines and organs to assess the potential harmful effects of UCNPs. These studies have evaluated the effects of gene expression, growth, and reproduction of the organisms. It is crucial to continue monitoring and evaluating the toxicity of UCNPs as their use becomes more prevalent in biomedical applications.

2. Biocompatibility of nanoparticles

This section will provide an overview of different methods that researchers use to achieve biocompatibility of UCNPs. **Figure 2** depicts the common way to coat and functionalize UCNPs for several researches, as generally, the UCNPs or NPs need to be coated to ensure biocompatibility and they need functional groups to attach to several types of ligands that can bind to the surface of the targeted cells, as depicted.

2.1 Polyethylene glycol

The most used method to achieve biocompatibility is through PEGylation, which is both effective and straightforward. Although the specific approach may vary among different authors, PEGylation generally refers to the covalent conjugation of PEG to other molecules. This process enhances the physicochemical properties of the molecules, leading to reduce the immunogenicity and improve solubility, electrostatic binding, and hydrophobicity of a given biomolecule [11]. Overall, PEGylation represents a valuable tool for improving the biocompatibility of drugs and biomolecules, allowing for safer and more effective biomedical applications.

The first polymer conjugation was developed by Abuchowski et al. in 1977 [12], and various authors have developed different PEGylation methods for diverse applications, ranging from biocompatibility to trimodal fluorescence. For instance, Zeng et al. [13] developed PEG-modified BaGdF$_5$:Yb/Er UCNPs for multimodal fluorescence/CT (computed X-ray tomography)/magnetic bioimaging applications, which exhibited low cytotoxicity and long circulation time. Similarly, Maldiney et al. [14]

Layer: PEG, Silica, PVP
PEI, PAA, etc.
FG=Functional groups such as amino,
 carboxyl, hydroxyl, etc.

FL=Functional ligands such
as genes, folic acid,
antibodies, proteins, etc.
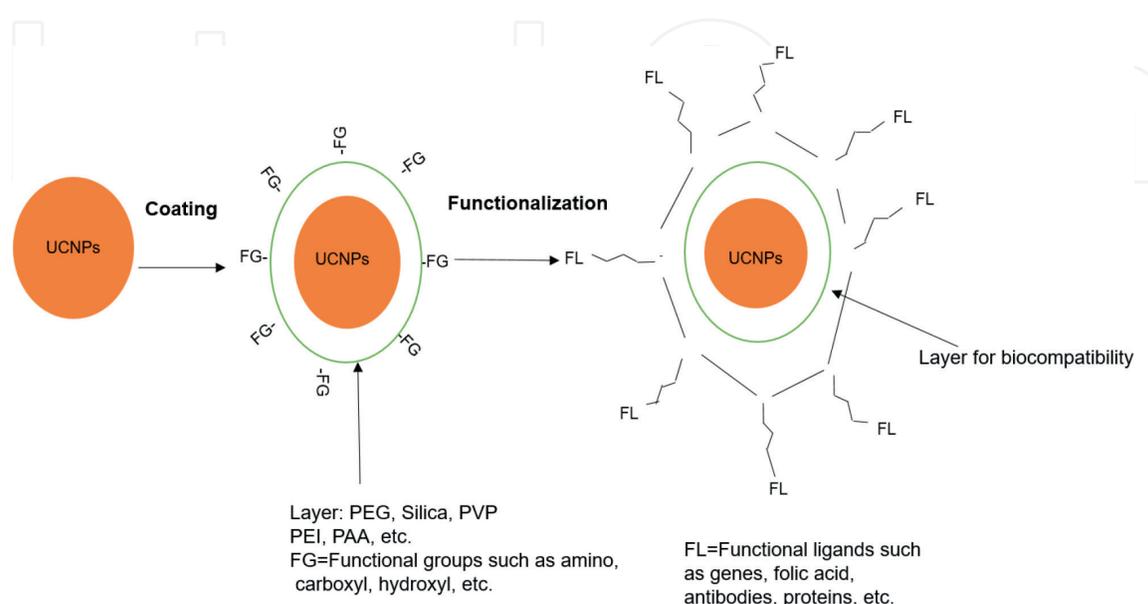
Figure 2.
Biocompatibility and functionalization of several types of UCNPs.

utilized luminescent NPs emitting in the near-infrared spectra, with two types of mice: healthy and tumor carrier mice. They reported that PEG coating enabled the formation of stealthy particles that were more uniformly distributed throughout the animal. It is important to note that PEGylation tends to increase the diameter of the NPs by about 10 nm, similar to other conjugation methods. However, an essential aspect of PEGylation is the characterization of NPs, and the dynamic light scattering (DLS) is a crucial technique that can provide three critical parameters: size; zeta potential that measures the surface charge of the NPs and determines their colloidal stability (values between −10 and +10 mV are neutral, while values greater than +30 mV or less than −30 mV are considered strongly cationic and strongly anionic, respectively), and size distribution [15]. The selection of ligands to bind the PEGylated-NPs may vary depending on the application. The purpose of having PEGylated-NPs with ligands is to target specific receptors on the surface of cancer cells and to allow for retention in the area due to the enhanced permeability and retention effect (EPR). A variety of ligands can be used, including molecules, peptides, proteins, antibodies, aptamers, among others [12, 16–19].

However, PEG may undergo degradation due to light, stress, or heat. Some authors have addressed this issue by combining PEG with copolymers such as PVP and poly(lactic-co-glycolic acid) or PLGA [20]. With these challenges, research with PEG continues to be relevant, as it has proven to be an important tool for achieving the biocompatibility of NPs.

2.2 Polyethyleneimine

Polyethyleneimine is a very versatile aliphatic polymer that contains primary, secondary, and tertiary amino groups, with a ratio of 1:2:1 [21]. It has found numerous applications in non-viral gene delivery and therapy for *in vitro* and *in vivo* models. In addition, PEI has been used for non-pharmaceutical applications, such as water purification and shampoo manufacturing. For instance, Ge and collaborators [22] developed near-infrared emitting nanoparticles coated with PEI and gold nanorods coated with dithiothreitol to detect arsenic (III), while Pan et al. [23] synthesized PEI-coated upconversion nanoparticles for use as an optical probe to determine the water content in organic solvents.

Polyethyleneimine-modified nanoparticles have also been explored for various biomedical applications. Mi et al. [24] developed luminescent NPs coated with PEI that can bind to antibodies through their amino groups, resulting in tunable colors. Xu et al. [25] functionalized NPs with folic acid and polycaprolactone/PEI for *in vivo* drug delivery in SKOV-3 cancer cells. Their results showed that their method was more effective in killing cancer cells than free doxorubicin. PEI-NPs have also been used for pulmonary gene delivery. Bivas-Benita et al. [26] developed a PLGA-PEI-NP that can deliver genes to the lung epithelium using Calu-3 cells. Huh et al. [27] used PEI-NPs composed with glycol chitosan and encapsulated with siRNA, which significantly inhibited red fluorescent protein (RFP) gene expression in B16-F10-bearing mice cells.

PEI nanoparticles represent an important tool especially for drug delivery of anticancer drugs and also gene therapy applications, among others.

2.3 Polyvinylpyrrolidone

PVP is commonly used as a coating for silver NPs and as a drug carrier [25, 28, 29]. However, several authors have also used PVP as a coating for UCNPs [25, 30–34].

PVP is a versatile coating because it can work as a NP dispersant or as a surface stabilizer, and it also has reducing properties. Its functional groups, which include C=O, C–N, and CH$_2$, enable it to control the growth of certain aspects by binding onto others, providing biocompatibility to the NPs [29, 35].

Johnson et al. [34] synthesized β-NaYF$_4$:Yb^{3+}/Er^{3+} UCNPs and used PVP to replace the oleate surface ligands. This modification makes the UCNPs water-dispersible, which is crucial for *in vivo* applications. Additionally, PVP is biocompatible, has a prolonged blood circulation time, and shows low accumulation in vital organs. Zou et al. [33] prepared UCNP NaYF$_4$:Yb^{3+}/Er^{3+} embedded into PVP nanotubes using the electrospinning method, resulting in an intense emission of the UCNPs compared to bare UCNPs. Due to their biocompatibility, these modified NPs may have important applications in biomedicine.

2.4 Polyacrylic acid

PAA is a hydrophilic and pH-responsive polymer that can replace hydrophobic ligands on the surface of NPs, making it an excellent candidate for *in vivo* and *in vitro* applications [36]. Its biocompatibility and other desirable qualities make it an attractive coating option for various types of NPs [37–41].

Hilderbrand et al. [42] synthesized UCNPs coated with PAA and linked amino-PEG to the carboxyl groups of the PAA. The resulting modified UCNPs were non-cytotoxic and displayed good NIR emission. Wang et al. [41] also prepared UCNPs YF$_3$:Yb^{3+}/Er^{3+} with NIR emission and coated with PAA, resulting in strong luminescence. In a study by Xiong et al. [40], PAA-coated UCNPs were shown to have excellent biodistribution and cellular uptake in mice, with no observed toxicity, suggesting that these NPs could be used for long-term therapy and bioimaging studies *in vivo*. Additionally, Jia et al. [36] investigated the effects of doxorubicin hydrochloride (DOX) and PAA-coated UCNPs (DOX@PAA-UCNPs) on HeLa cells and found that the UCNPs were biocompatible and effective as a drug carrier.

In summary, PAA is a very versatile polymer that can be used to coat on various types of NPs for a wide range of biomedical applications.

2.5 Silica

Silica (SiO$_2$) is a commonly used coating material for various types of NPs due to its favorable properties, including biocompatibility, thermodynamic stability, low toxicity, colloidal stability, ease synthesis, and scalability. Two main methods are generally used for producing the coating: sol-gel in a reverse micelle nanoreactor and the Stöber method [43, 44]. However, achieving a complete and homogeneous coating is a significant challenge, and Ureña-Horno et al. [45] developed a method for coating UCNPs with silica. By determining the optimal concentration of nanoparticles, they were able to achieve high yields of homogeneous functionalization and prevent agglomeration.

Hlaváček et al. [46] employed agarose gel electrophoresis for the purification of silica-coated UCNPs and for the separation of the protein-UCNPs from surplus reagents. This work represents a significant advancement in nanoparticle separation and measurement of their size and surface charge. In another study, also, Gnanasammandhan et al. [47] used silica-coated UCNPs for photoactivation in two specific applications: photodynamic therapy (PpDt) and photoactivated control of gene expression. The UCNPs were coated with PEG and functionalized with FA to target specific tumors, and their protocols for photoactivation therapy are valuable for future studies.

Overall, the efficient coating and functionalization of nanoparticles with silica are vital for their successful use in various applications, and these studies provide important insights and protocols for achieving these goals.

3. Toxicology of nanoparticles

The study of the toxicological effects elicited by NPs on cells and organisms is crucial in biomedical-nanotechnology applications. Thus, it is important to ensure that NPs are not cytotoxic or genotoxic. **Table 1** summarizes various approaches used by different authors for the toxicological assessment of nanoparticles.

3.1 Cytotoxicity assays

Assessing the cytotoxicity of new agents or nanomaterials is a crucial step in evaluating their potential biomedical applications. *In vitro* cell culture tests are preferred over *in vivo* animals test for ethical, speed, and cost reasons. However, cell cultures tend to be susceptible to various environmental factors, such as pH, nutrients, and temperature, which may interfere with the interpretation of the results. Therefore, it is important to ensure that the observed cell viability is observed solely due to the toxicity of the nanomaterials being tested, rather than environmental factors. Performing a range of tests with different concentrations of NPs and consistent experimental conditions enhances the validity of results [56, 57].

The MTT assay, based on the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide by dehydrogenase enzymes, is one of the most common methods to assess cell viability, as it measures mitochondrial activity in living cells [58–60]. This assay detects living cells, and the results are easily read using a multiwell scanning spectrophotometer (ELISA plate reader). Several authors have successfully used this assay, including those listed in **Table 1** [48, 50, 55].

Another variation of the MTT assay is the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay, which uses MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium], and phenazine ethosulfate, instead of MTT. Bahadar et al. [61] used both methods to evaluate the cytotoxicity of different metallic and non-metallic NPs on cells.

Other methods for measuring cell viability include the trypan blue and neutral red assays, which detect dead cells based on dye penetration into cell membrane. Ramírez-García et al. [62] used the trypan blue assay to measure the cell viability of zinc-gallium luminescent NPs; also, Zairov et al. [63] used gadolinium-based luminescent NPs with PC12 cells for obtaining low cytotoxicity, and the viability of the living cells was measured with a hemocytometer.

Live/dead viability assay, which measures the number of damaged cells, uses calcein acetoxymethyl (calcein AM) and ethidium homodimer. This method was mostly used to test the cytotoxicity exerted by gold nanoshells, silver, silica NPs, or fullerenes on cells [64]. The water-soluble tetrazolium (WST-1) assay is another method that measures mitochondrial activity by transforming the light-red tetrazolium salt into dark-red formazan salt due to the mitochondrial activity in living cells. Braun et al. [65] evaluated silica NPs with C2C12 cells using MTT and WST assays, and described that the MTT assay overestimated the low and medium cytotoxicity of the NPs, while the WST assay underestimates the particle concentrations studied.

NPs	Cytotoxicity assay	ROS quantification	Genotoxicity/gene expression	Stability or distribution	Cells/organism	References
Y_2O_3:Eu and Yb/Er	MTT assay	Comet assay	Comet assay	—	HeLa, MCF-7 cells	[48, 49]
$NaYF_4$:Yb/Er	MTT assay	—	—	—	RAW 264.7 cells	[50]
$NaYF_4$: Yb/Er	—	Oxidative stress assay	—	Stability assay/ biodistribution studied	Fetal bovine serum/ mice	[51, 52]
NPs-PEI-SiRNA	RFP	—	RFP expressing B16F10 cells	—	Murine melanoma/RFP/ B16-F10 cells	[27]
Yb_2O_3:Gd	A. cepa chromosomal aberration assay method	—	A. Cepa genotoxic studies	-	*E. coli* and *S. aureus*	[53]
Gd_2O_2: Yb/Tm	*In vitro* biodegradation assay	—	—	Biodistribution and toxicity in organs studied	Mice *in vivo*/blood *in vitro*	[54]
Y_2O_3	MTT assay	—	—	—	Human breast cancer	[55]

Table 1.
Toxicological assessment of NPs described by different authors.

There are alternative approaches to assess the cytotoxicity of NPs: For instance, Das et al. [66] carried out a study on the toxic effects of three types of functionalized UCNPs: oleate ligands-NPs, PEG-NPs, and bilayer PEG-oleate-NPs. They employed the calcein and propidium iodide viability assay and concluded that the bilayer NPs exhibited significant toxicity due to functionalization. In another study, Malvindi et al. [67] evaluated the cytotoxicity of silica-coated iron oxide NPs using the WST-8 ([2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] assay and lactate dehydrogenase release (LDH assay) to analyze cell viability and cell membrane integrity. The NPs demonstrated good internalization in HeLa cells with no observed toxicity. Meindl et al. [68], on the other hand, assessed the cytotoxicity of NPs by measuring intracellular calcium levels, providing an example of an alternative approach for toxicity evaluation. It is important to select an appropriate method for assessing cytotoxicity, with suitable experimental parameters and consistent concentrations of NPs and exposition times across different studies. Otherwise, non-toxic NPs may yield misleading results due to factors such as cellular senescence.

3.2 Reactive oxygen species/reactive nitrogen species

The production of reactive nitrogen species (RNS), such as nitric oxide (NO), is closely associated with inflammatory responses and can react with oxygen to produce ROS. When NPs interact with cells, they may induce cell death by triggering the production of NO. The production of RNS is regulated by the enzyme nitric oxide synthase (NOS), while ROS production is regulated by NAD(P)H oxidase isoforms. Excessive ROS production can cause oxidative stress, leading to damage in the cell membrane, proteins, lipids, or DNA. However, low or moderate concentrations of ROS/RNS are beneficial, as they can help to defend against infections [69–71].

Several studies have demonstrated that metal and silica nanoparticles can induce oxidative stress and inflammation. The reactivity at the target sites and the surface area are two crucial factors affecting these outcomes. In a study conducted by Tran et al. [72], the effects of nanoparticles' surface area on lung health were investigated. They demonstrated that NPs with a higher surface area tend to be retained and accumulate in the lungs, reaching a saturation point where they become less susceptible to phagocytosis and exhibit reduced mobility. This overload effect stimulates macrophages, leading to the production of inflammatory responses, including tumor necrosis factor.

In a recent study, Wang [73] investigated the use of ROS probes to detect and visualize ROS production in living cells. The most commonly used ROS include H_2O_2, $^1O_2$, $O_2^{\bullet-}$, ClO−, ONOO−, and •OH. Luminescent NPs were found to be effective probes for detecting H_2O_2 and other ROS forms in living cell systems. The authors suggest that these nanoprobes may have promising therapeutic applications for sensing ROS.

3.3 Genotoxicity

When conducting deeper cytotoxicity studies, determining the genotoxic potential of NPs is often necessary. Various authors have employed different methods to ensure single- and double-stranded DNA breakage caused by NPs exposure. One of the most used methods is the flow cytometry that differentiates among various cell populations, between cell size, and complexity (granularity) through a laser beam [74]. Intercalating dyes such as propidium iodide can be used to measure DNA

damage by counting apoptotic cells as the dye fluoresces in proportion to the increase in cell membrane permeability of damaged cells, and the presence of cell death-associated molecules in the cell membrane [56].

The comet assay, or alkaline single cell gel electrophoresis assay, is another widely used method, in which agarose gels are suspended and then lysed, electrophoresed, and stained with a fluorescent DNA-binding dye [75]. DNA damages can manifest in various forms, such as mutations, carcinogenesis, oxidative stress, or damage to the mitotic spindle and its components [76]. However, the assay has limitations in terms of processing multiple samples, as the electrophoresis can hold only 20 slides per run and generally each slide has one or two gels. Some researchers have attempted to increase throughput by increasing the size of the gels. For instance, Azqueta et al. [77] compared standard, medium- and high-throughput comet assays, by increasing throughput by an increase in the size of the gels to analyze more samples, however, scoring the results can be time-consuming.

There are still some issues with the analysis of the DNA damage that need to be addressed, such as the potential for NPs to induce additional DNA breaks during the assay, leading to false high damage results, or interfering with the scoring of the DNA-heads, resulting in less intensity.

3.4 Apoptosis/necrosis

Cell death, whether induced by apoptosis or necrosis, can be measured using flow cytometry. Upon injection of NPs into the bloodstream, the kidneys play a key role in clearing the NPs. However, depending on the timeg duration of therapy, NPs can potentially cause nephrotoxicity. The surface charge of NPs is an important factor in their physical stability. Positively charged NPs tend to interact more strongly with blood components and are easily cleared from the circulatory system. Conversely, NPs with a more negative surface charge exhibit lower interaction with plasma proteins [78].

In vivo studies have investigated the toxicity and DNA damage induced by various types of NPs, including metal, silver, and gold NPs. Some findings suggest that oxidative stress may be a mechanism of cytotoxicity and apoptosis induced by the NPs [78, 79]. Zhao et al. [80] conducted a study on nickel NPs in mouse epithelial (JB6) cells, revealing high cytotoxicity and apoptosis resulting from NPs interactions.

Moreover, Wang and Cho [81] discovered that the nuclear factor kappa B (NF-κB) plays a crucial role in regulating inflammatory responses that may induce DNA damage. In the process of evading apoptosis, reduced tissue capability to eliminate damaged cancer cells can occur. Therefore, NPs that cause DNA damage can lead to unwanted inflammatory responses.

One approach utilized in certain studies is the utilization of apoptosis as a targeted cancer treatment through customized NPs designed for this purpose. In some instances, these NPs have been observed to induce morphological changes and trigger autophagy through toxicity, such as cerium oxide NPs or iron oxide NPs [82, 83].

Necrosis, on the other hand, refers to the premature cell death caused by injury. Mohammadinejad et al. [84] conducted a study on the apoptotic, necrotic, and autophagic effects of several types of NPs. They concluded that during necroptosis, which is a regulated necrosis, the cell receptor apoptotic signaling pathway detects different stimuli, leading to a complex process. For instance, Schaeublin et al. [85] demonstrated that charged gold NPs induced apoptotic death in human keratinocyte cells (HaCaT), while non-charged NPs induced necrosis. Therefore, it is crucial to address the study of the effects of different types of NPs on cell death.

When evaluating cell death, it is important to assess the specific mode of death rather than solely relying on cell viability. This is because apoptosis, in some cases, may be followed by secondary necrosis, which can lead to inconclusive or erroneous results.

3.5 Hemocompatibility

The hemolysis test is employed to assess the acute hemolytic activity of nanomaterials intended for prolonged contact with soft tissue and cells [86]. Additionally, the hemocompatibility assay evaluates the effect on the blood components resulting from contact with certain materials, including NPs. *In vivo* assays are specifically designed to simulate clinical conditions, including flow dynamic and the geometry factors. The ISO 10993-4 is the standard that serves as the governing norm that establishes the parameters that must be fulfilled when conducting tests involving blood contact.

Liang et al. [87] developed $NaYF_4$:Yb, Er-FA UCNPs and tested their biocompatibility in L929 fibroblast cells using the hemolysis and coagulation tests. They found good compatibility and conducted additional tests using blood from rats stabilized with heparin sodium. Fresh plasma was obtained from the sample via centrifugation. T prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured to evaluate the effects of the UCNPs on blood coagulation. The researchers detected almost no hemolysis effect due to the UCNPs interaction.

The response of organism in the bloodstream may vary depending on the exposure to NPs, but the liver and spleen are the most common sites of NPs accumulation. Some studies have suggested that quantum dots (QDs) tend to accumulate in the lymph nodes. The Bakalova [88] used polymersomes as carriers for lymph node mapping, employing QDs as a contrast agent. Other authors have investigated the effects of metal-NPs on blood, and their findings indicate primary accumulation in the liver [89, 90]. In a study conducted by Balasubramanian et al. [91], they examined the biodistribution of gold NPs in rats and observed a significant biodistribution after two months following a single intravenous injection, along with gene expression changes in organs such as the kidney, liver, and spleen.

Mehrizi studied the effects of polymeric, metallic, and nonmetallic NPs on red blood cells (RBCs), and multiple studies converge on the conclusion that PEGylated forms of NPs and negatively charged dendrimers exhibit the best hemocompatibility on RBCs [92]. Smaller NPs, higher concentrations, and longer exposure times tend to induce hemolysis. NPs can compromise RBCs integrity through hemolysis or hemagglutination, which refers to the agglutination of the RBCs. Colorimetric assays detecting the release of hemoglobin can be used to evaluate these issues, with the standard assay being conducted according to ASTM-F756 guidelines. According to Nemmar et al. [93], silica NPs exhibit a dose-dependent hemolytic behavior. Hemagglutination of RBCs can be measured using DLS. The Lima group employed this assay to evaluate chitosan-NPs in human erythrocytes [94]. Other authors have evaluated the hemagglutination in other types of NPs, such as gold, iron oxide, silver, or carbon, with results strongly dependent on the size, type, or the functionalization of a given nanoparticle [95–97].

Thrombogenicity is another assessment that can be conducted on certain types of NPs can trigger clotting in the blood. T platelet activation assays (flow cytometry), thrombin generation (computed tomography assay), platelet aggregation (light aggregometry), or clinical coagulation assays, such as thromboelastographic aPTT, can be employed to evaluate this property in accordance with ASTM standard ASTM F2382-18 [98].

Additionally, Saha et al. discussed NPs approved by the US Food and Drug Administration for cancer treatment, specifically protein-bound NPs, raising concerns about their hemocompatibility. These concerns include anemia, neutropenia, thrombocytopenia, bradycardia, hyper/hypotension, and the possibility to undergo severe cardiovascular events [98]. Nonetheless, further studies are necessary to understand the behavior of NPs in the bloodstream and their potential long-term consequences.

4. Conclusions

The impact of NPs on different cellular components, plasma, organelles, or membranes may elicit a variety of responses in both *in vitro* and *in vivo* studies. Therefore, nanotoxicological assessments must be conducted for each type of NP, cell, or organism, and at different doses, concentrations, and exposition times, to guarantee their safety. Some research groups have reported negligible or low toxicity at low doses of NPs, while others have found toxicity at high doses and long incubation times. Although these factors have varied depending on the type of NP used, as some nanomaterials tend to be more toxic, such as QDs or silver nanoparticles. Another crucial factor is that some NPs can induce the overproduction of ROS, also cellular uptake of NPs has been implicated in cellular toxicity, and significant changes in cellular responses including cell morphology and differentiation processes.

The function of the NPs relies on their matrix cell and the doping elements, which make them functional, biocompatibility is crucial to guarantee their safe use in cells and organisms, and most NPs require some coating to fulfill biocompatibility. Generally, the *in vitro* toxicity studies reported that NP exposure is toxic and causes cell death. Still, on some occasions, there may not be cell death, representing toxicity that is not correctly interpreted. Only a few groups have reported studies on cell signaling using genomic and proteomic array tests. The Coto-García et al. [99] investigated the bioanalysis for proteomics and genomics of several types of NPs and concluded that there is still a need for further discussion to approach the nanotoxicological effects of the different NPs used.

Nanoparticles can elicit various effects on cells, including cell proliferation, differentiation, cell cycle regulation, DNA damage, and cell death via apoptosis or necrosis. These effects are contingent upon factors such as nanoparticle type, size, surface charge, shape, and functionalization. Consequently, the assessment of NP toxicity encompasses a complex process that necessitates addressing the multitude of casual factors and interpreting the results. However, there are existing gaps in our understanding due to some inconsistencies among different studies, hindering conclusive determinations regarding the impact of NPs on living organisms. Furthermore, a standardized and systematic approach to testing has yet to be established.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

Author details

Dalia Chávez-García[1*] and Karla Juarez-Moreno[2]

1 Centro de Enseñanza Técnica y Superior (CETYS), Ensenada, Mexico

2 Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, México

*Address all correspondence to: dalia.chavez@cetys.mx

IntechOpen

References

[1] Henri RB. In: Taylor & Francis Group L, editor. Biomedical application of nanoparticles. 1st ed. Taylor & Francis Group, LLC: Boca Raton, FL; 2018. p. 321

[2] Chatterjee K, Sarkar S, Jagajjanani Rao K, Paria S. Core/shell nanoparticles in biomedical applications. Advances in Colloid and Interface Science. 2014;**209**:8-39

[3] Su Y, Xue T, Liu Y, Qi J, Jin R, Lin Z. Luminescent metal nanoclusters for biomedical applications. Nano Research. 2019;**12**:1251-1265. DOI: 10.1007/s12274-019-2314-y

[4] Blasse G, Grabmaier BC, Blasse G, Grabmaier BC. Luminescent Materials. Berlin Heidelberg: Springer-Verlag; 1994. p. 230. DOI: 10.1021/ja965667t

[5] Manson J, Kumar D, Meenan BJ, Dixon D. Polyethylene glycol functionalized gold nanoparticles: the influence of capping density on stability in various media. Gold Bulletin. 2011;**44**(2):99-105

[6] Buchman YK, Lellouche E, Zigdon S, Bechor M, Michaeli S, Lellouche JP. Silica nanoparticles and polyethyleneimine (PEI)-mediated functionalization: a new method of pei covalent attachment for siRNA delivery applications. Bioconjugate Chemistry. 2013;**24**(12):2076-2087

[7] Gao L, Ge X, Chai Z, Xu G, Wang X, Wang C. Shape-controlled synthesis of octahedral α-NaYF4 and its rare earth doped submicrometer particles in acetic acid. Nano Research. 2009;**2**(7):565-574

[8] Liu C, Wang Z, Wang X, Li Z. Surface modification of hydrophobic NaYF4:Yb,Er upconversion nanophosphors and their applications for immunoassay. Science China. Chemistry. 2011;**54**(8):1292-1297

[9] Kang H, Mintri S, Menon AV, Lee HY, Choi HS, Kim J. Pharmacokinetics, pharmacodynamics and toxicology of theranostic nanoparticles graphical abstract HHS public access. Nanoscale. 2015;**7**(45):18848-18862

[10] Kumar V, Sharma N, Maitra SS. In vitro and in vivo toxicity assessment of nanoparticles. International Nano Letters. 2017;**7**(4):243-256

[11] Veronese FM, Pasut G. PEGylation, successful approach to drug delivery. Drug Discovery Today. 2005;**10**:1451-1458

[12] Abuchowski A, Van Es T, Palczuk NC, Davis FF. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. Journal of Biological Chemistry. 1977;**252**(11):3578-3581

[13] Zeng S, Tsang MK, Chan CF, Wong KL, Hao J. PEG modified BaGdF5:Yb/Er nanoprobes for multi-modal upconversion fluorescent, in vivo X-ray computed tomography and biomagnetic imaging. Biomaterials. 2012;**33**(36):9232-9238

[14] Maldiney T, Richard C, Seguin J, Wattier N, Bessodes M, Scherman D. Effect of core diameter, surface coating, and PEG chain length on the biodistribution of persistent luminescence nanoparticles in mice. ACS Nano. 2011;**5**(2):854-862

[15] Jokerst JV, Lobovkina T, Zare RN, Gambhir SS. Nanoparticle PEGylation for imaging and therapy. Nanomedicine. 2011;**7**:715-728

[16] Yang L, Peng XH, Wang YA, Wang X, Cao Z, Ni C, et al. Receptor-targeted nanoparticles for in vivo imaging of breast cancer. Clinical Cancer Research. 2009;**15**(14):4722-4732

[17] Smith BR, Cheng Z, De A, Koh AL, Sinclair R, Gambhir SS. Real-time intravital imaging of RGD-quantum dot binding to luminal endothelium in mouse tumor neovasculature. Nano Letters. 2008;**8**(9):2599-2606

[18] Lee ALZ, Wang Y, Ye WH, Yoon HS, Chan SY, Yang YY. Efficient intracellular delivery of functional proteins using cationic polymer core/shell nanoparticles. Biomaterials. 2008;**29**(9):1224-1232

[19] Zhang P, Liu S, Jiang S. Anti-PEG antibodies in the clinic: current issues and beyond PEGylation. Journal of Controlled Release. 2016;**244**:184-193. DOI: 10.1016/j.jconrel.2016.06.040

[20] Cheng J, Teply BA, Sherifi I, Sung J, Luther G, Gu FX, et al. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. Biomaterials. 2007;**28**(5):869-876

[21] Vicennati P, Giuliano A, Ortaggi G, Masotti A. Polyethylenimine in medicinal chemistry. Current Medicinal Chemistry. 2008;**15**(27):2826-2834

[22] Ge K, Liu J, Wang P, Fang G, Zhang D, Wang S. Near-infrared-emitting persistent luminescent nanoparticles modified with gold nanorods as multifunctional probes for detection of arsenic(III). Microchimica Acta. 2019;**186**(3)

[23] Pan Z, Wen Y, Wang T, Wang K, Teng Y, Shao K. One-step synthesis of hollow PEI-NaBiF4:Yb3+/Er3+ upconversion nanoparticles for water-responsive luminescent probe. Journal of Rare Earths. 2020;**38**(4):362-368

[24] Mi CC, Tian ZH, Han BF, Bin MC, Xu SK. Microwave-assisted one-pot synthesis of water-soluble rare-earth doped fluoride luminescent nanoparticles with tunable colors. Journal of Alloys and Compounds. 2012;**525**:154-158

[25] Xu P, Van Kirk EA, Zhan Y, Murdoch WJ, Radosz M, Shen Y. Targeted charge-reversal nanoparticles for nuclear drug delivery. Angewandte Chemie. 2007;**119**(26):5087-5090

[26] Bivas-Benita M, Romeijn S, Junginger HE, Borchard G. PLGA-PEI nanoparticles for gene delivery to pulmonary epithelium. European Journal of Pharmaceutics and Biopharmaceutics. 2004;**58**(1):1-6

[27] Huh MS, Lee SY, Park S, Lee S, Chung H, Lee S, et al. Tumor-homing glycol chitosan/polyethylenimine nanoparticles for the systemic delivery of siRNA in tumor-bearing mice. Journal of Controlled Release. 2010;**144**(2):134-143

[28] Bharali DJ, Sahoo SK, Mozumdar S, Maitra A. Cross-linked polyvinylpyrrolidone nanoparticles: a potential carrier for hydrophilic drugs. Journal of Colloid and Interface Science. 2003;**258**(2):415-423

[29] Li J, Inukai K, Takahashi Y, Tsuruta A, Shin W. Effect of PVP on the synthesis of high-dispersion core–shell barium-titanate–polyvinylpyrrolidone nanoparticles. Journal of Asian Ceramic Societies. 2017;**5**(2):216-225

[30] Kormshchikov ID, Voronov VV, Burikov SA, Dolenko TA, Kuznetsov SV. Study of stability of luminescence intensity of β-nagdf4:Yb:Er nanoparticle colloids in aqueous solution. Nanosystems: Physics, Chemistry, Mathematics. 2021;**12**(2):218-223

[31] Zhang J, Mi C, Wu H, Huang H, Mao C, Xu S. Synthesis of NaYF4:Yb/Er/Gd up-conversion luminescent nanoparticles and luminescence resonance energy transfer-based protein detection. Analytical Biochemistry. 2012;**421**(2):673-679

[32] Chen H, Zhai X, Li D, Wang L, Zhao D, Qin W. Water-soluble Yb3+, Tm3+ codoped NaYF4 nanoparticles: aynthesis, characteristics and bioimaging. Journal of Alloys and Compounds. 2012;**511**(1):70-73

[33] Zou P, Hong X, Ding Y, Zhang Z, Chu X, Shaymurat T, et al. Up-conversion luminescence of NaYF 4:Yb 3+/Er 3+ nanoparticles embedded into PVP nanotubes with controllable diameters. Journal of Physical Chemistry C. 2012;**116**(9):5787-5791

[34] Johnson NJJ, Sangeetha NM, Boyer JC, Van Veggel FCJM. Facile ligand-exchange with polyvinylpyrrolidone and subsequent silica coating of hydrophobic upconverting β-NaYF4:Yb 3+/Er3+ nanoparticles. Nanoscale. 2010;**2**(5):771-777

[35] Koczkur KM, Mourdikoudis S, Polavarapu L, Skrabalak SE. The versatile role of PVP in nanoparticle synthesis polyvinylpyrrolidone (PVP) in nanoparticle synthesis. Dalton Transactions. 2015;**44**:17883-17905. DOI: 10.1039/C5DT02964C

[36] Jia X, Yin J, He D, He X, Wang K, Chen M, et al. Polyacrylic acid modified upconversion nanoparticles for simultaneous pH-triggered drug delivery and release imaging. Journal of Biomedical Nanotechnology. 2013;**9**(12):2063-2072

[37] Shen J, Zhao L, Han G. Lanthanide-doped upconverting luminescent nanoparticle platforms for optical

imaging-guided drug delivery and therapy. Advanced Drug Delivery Reviews. 2013;**65**:744-755

[38] Lahtinen S, Lyytikäinen A, Sirkka N, Päkkilä H, Soukka T. Improving the sensitivity of immunoassays by reducing non-specific binding of poly(acrylic acid) coated upconverting nanoparticles by adding free poly(acrylic acid). Microchimica Acta. 2018;**185**(4):220

[39] Luo Y, Li H, Cai M, Liu Y, Chen L, Xu S, et al. Designing polyacrylic acid capped luminescent rare earth core–shell nanoparticles for simultaneous Cu(II) and temperature sensing. Materials and Design. 2022;**224**:111405

[40] Xiong L, Yang T, Yang Y, Xu C, Li F. Long-term in vivo biodistribution imaging and toxicity of polyacrylic acid-coated upconversion nanophosphors. Biomaterials. 2010;**31**(27):7078-7085

[41] Wang L, Zhang Y, Zhu Y. One-pot synthesis and strong near-infrared upconversion luminescence of poly(acrylic acid)-functionalized YF3:Yb3+/Er3+ nanocrystals. Nano Research. 2010;**3**(5):317-325

[42] Hilderbrand SA, Shao F, Salthouse C, Mahmood U, Weissleder R. Upconverting luminescent nanomaterials: application to in vivo bioimaging. Chemical Communications. 2009;**28**:4188-4190

[43] Liu JN, Bu WB, Shi JL. Silica coated upconversion nanoparticles: a versatile platform for the development of efficient theranostics. Accounts of Chemical Research. 2015;**48**(7):1797-1805

[44] Stöber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. Journal of Colloid and Interface Science. 1968;**26**(1):62-69

[45] Ureña-Horno E, Kyriazi ME, Kanaras AG. A method for the growth of uniform silica shells on different size and morphology upconversion nanoparticles. Nanoscale Advances. 2021;**3**(12):3522-3529

[46] Hlaváček A, Sedlmeier A, Skládal P, Gorris HH. Electrophoretic characterization and purification of silica-coated photon-upconverting nanoparticles and their bioconjugates. ACS Applied Materials and Interfaces. 2014;**6**(9):6930-6935

[47] Gnanasammandhan MK, Idris NM, Bansal A, Huang K, Zhang Y. Near-IR photoactivation using mesoporous silica-coated NaYF4:Yb, Er/Tm upconversion nanoparticles. Nature Protocols. 2016;**11**(4):688-713

[48] Chávez-García D, Juárez-Moreno K, Campos CH, Alderete JB, Hirata GA. Upconversion rare earth nanoparticles functionalized with folic acid for bioimaging of MCF-7 breast cancer cells. Journal of Materials Research. 2018;**33**(2):191-200

[49] Chávez-García D, Juarez-Moreno K, Calderón-Osuna I, Navarro P, Hirata GA. Nanotoxicological study of downconversion Y2O3:Eu3+luminescent nanoparticles functionalized with folic acid for cancer cells bioimaging. Journal of Biomedical Materials Research. Part B, Applied Biomaterials 2020;(December 2019):1-11

[50] Kembuan C, Oliveira H, Graf C. Effect of different silica coatings on the toxicity of upconversion nanoparticles on RAW 264.7 macrophage cells. Beilstein. Journal of Nanotechnology. 2021;**12**:35-48

[51] Zhou M, Ge X, Ke DM, Tang H, Zhang JZ, Calvaresi M, et al. The bioavailability, biodistribution, and toxic effects of silica-coated upconversion nanoparticles in vivo. Frontiers in Chemistry. 2019:7. DOI: 10.3389/ fchem.2019.00218

[52] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials [Internet]. 2006;**27**(15):2907-2915

[53] Chaudhary S, Kumar S, Chaudhary GR. Tuning of structural, optical and toxicological properties of Gd3+ doped Yb2O3 nanoparticles. Ceramics International. 2019;**45**(15):19307-19315

[54] Santelli J, Lechevallier S, Calise D, Marsal D, Siegfried A, Vincent M, et al. Multimodal gadolinium oxysulfide nanoparticles for bioimaging: a comprehensive biodistribution, elimination and toxicological study. Acta Biomaterialia. 2020;**108**:261-272

[55] Isacfranklin M, Ameen F, Ravi G, Yuvakkumar R, Hong SI, Velauthapillai D, et al. Y2O3 nanorods for cytotoxicity evaluation. Ceramics International. 2020;**46**(12):20553-20557

[56] Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanopartides. Small. 2008;**4**:26-49

[57] Weyermann J, Lochmann D, Zimmer A. A practical note on the use of cytotoxicity assays. International Journal of Pharmaceutics. 2005;**288**(2): 369-376

[58] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;**65**(1-2):55-63

[59] Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT

assay. Cold Spring Harbor Protocols. 2018;**2018**(6):469-471

[60] Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. In vitro evaluation of cell/biomaterial interaction by MTT assay. Biomaterials. 1993;**14**(5):359-364. DOI: 10.1016/0142-9612(93)90055-7

[61] Bahadar H, Maqbool F, Niaz K, Abdollahi M. Toxicity of nanoparticles and an overview of current experimental models. Iran Biomed J [Internet]. 2016;**20**(1):1-11

[62] Ramírez-García G, Martínez-Alfaro M, d'Orlyé F, Bedioui F, Mignet N, Varenne A, et al. Photo-stimulation of persistent luminescence nanoparticles enhances cancer cells death. International Journal of Pharmaceutics. 2017;**532**(2):696-703

[63] Zairov R, Mustafina A, Shamsutdinova N, Nizameev I, Moreira B, Sudakova S, et al. High performance magneto-fluorescent nanoparticles assembled from terbium and gadolinium 1,3-diketones. Scientific Reports. 2017;**16**:7

[64] Sayes CM, Fortner JD, Guo W, Lyon D, Boyd AM, Ausman KD, et al. The differential cytotoxicity of water-soluble fullerenes. Nano Letters. 2004;**4**(10):1881-1887

[65] Braun K, Stürzel CM, Biskupek J, Kaiser U, Kirchhoff F, Lindén M. Comparison of different cytotoxicity assays for in vitro evaluation of mesoporous silica nanoparticles. Toxicology in Vitro. 2018;**52**:214-221

[66] Das GK, Stark DT, Kennedy IM. Potential toxicity of up-converting nanoparticles encapsulated with a bilayer formed by ligand attraction. Langmuir. 2014;**30**(27):8167-8176

[67] Malvindi MA, De Matteis V, Galeone A, Brunetti V, Anyfantis GC, Athanassiou A, et al. Toxicity assessment of silica coated iron oxide nanoparticles and biocompatibility improvement by surface engineering. PLoS One. 2014;**9**(1):e85835

[68] Meindl C, Kueznik T, Bösch M, Roblegg E, Fröhlich E. Intracellular calcium levels as screening tool for nanoparticle toxicity. Journal of Applied Toxicology. 2015;**35**(10):1150-1159

[69] Hsiao IL, Hsieh YK, Wang CF, Chen IC, Huang YJ. Trojan-horse mechanism in the cellular uptake of silver nanoparticles verified by direct intra- and extracellular silver speciation analysis. Environmental Science & Technology. 2015;**49**(6):3813-3821

[70] Liang H, Nacharaju P, Friedman A, Friedman JM. Nitric oxide generating/releasing materials. Future Science OA. 2015;**1**(1):FSO54

[71] Wink DA, Hines HB, Cheng RYS, Switzer CH, Flores-Santana W, Vitek MP, et al. Nitric oxide and redox mechanisms in the immune response. Journal of Leukocyte Biology. 2011;**89**(6):873-891

[72] Tran CL, Buchanan D, Cullen RT, Searl A, Jones AD, Donaldson K. Inhalation of poorly soluble particles. Influence of particle surface area on inflammation and clearance. Inhalation Toxicology. 2008;**12**(12):1113-1126. DOI: 10.1080/08958370050166796

[73] Wang HS. Development of fluorescent and luminescent probes for reactive oxygen species. TrAC, Trends in Analytical Chemistry. 2016;**85**:181-202

[74] Kumar A, Pandey AK, Singh SS, Shanker R, Dhawan A. A flow cytometric method to assess nanoparticle

uptake in bacteria. Cytometry. Part A. 2011;**79**(9):707-712

[75] Speit G, Hartmann A. The Comet Assay. In: Henderson DS. (eds) DNA Repair Protocols. Methods in Molecular Biology. Humana Press. 2016;**314**. DOI: 10.1385/1-59259-973-7:275

[76] Karlsson HL, Di Bucchianico S, Collins AR, Dusinska M. Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity?. Environ. Mol. Mutagen. 2015;**56**:82-96. DOI: 10.1002/em.21933

[77] Azqueta A, Gutzkow KB, Priestley CC, Meier S, Walker JS, Brunborg G, et al. A comparative performance test of standard, medium- and high-throughput comet assays. Toxicology in Vitro. 2013;**27**(2):768-773

[78] Wu W, Wu Z, Yu T, Jiang C, Kim WS. Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. Science and Technology of Advanced Materials. 2015;**16**(2):023501

[79] Magaye R, Zhao J, Bowman L, Ding M. Genotoxicity and carcinogenicity of cobalt-, nickel- and copper-based nanoparticles (review). Experimental and Therapeutic Medicine. 2012;**4**(4):551-561

[80] Zhao J, Bowman L, Zhang X, Shi X, Jiang B, Castranova V, et al. Metallic nickel nano- and fine particles induce JB6 cell apoptosis through a caspase-8/AIF mediated cytochrome c-independent pathway. J Nanobiotechnology. 2009;**7**:2

[81] Wang H, Cho CH. Effect of NF-B Signaling on Apoptosis in Chronic Inflammation-Associated Carcinogenesis. 2010

[82] Wu QH, Jin RR, Feng T, Liu L, Yang L, Tao YH, et al. Iron oxide nanoparticles and induced autophagy in human monocytes. International Journal of Nanomedicine. 2017;**12**:3993-4005

[83] Hussain S, Al-Nsour F, Rice AB, Marshburn J, Yingling B, Ji Z, et al. Cerium dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes. ACS Nano. 2012;**6**(7):5820-5829

[84] Mohammadinejad R, Moosavi MA, Tavakol S, Vardar DÖ, Hosseini A, Rahmati M, et al. Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. Autophagy. 2019;**15**:4-33

[85] Schaeublin NM, Braydich-Stolle LK, Schrand AM, Miller JM, Hutchison J, Schlager JJ, et al. Surface charge of gold nanoparticles mediates mechanism of toxicity. Nanoscale. 2011;**3**(2):410-420

[86] Niom H-P. Comparison of the methods available for assessing cytotoxicity. International Endodontic Journal. 1988;**21**:89-99

[87] Liang X, Fan J, Wang Y, Zhao Y, Jin R, Sun T, et al. Synthesis of hollow and mesoporous structured NaYF4:Yb,Er upconversion luminescent nanoparticles for targeted drug delivery. Journal of Rare Earths. 2017;**35**(5):419-429

[88] Bakalova R. Lymph node mapping using quantum dot-labeled polymersomes designing new generation anticancer agents by theranostic functionalization view project multifunctional Magneto-DDS for single drug-based anticancer effect view project. General Physiology and Biophysics. 2015;**34**(4):393-398. DOI: 10.4149/gpb_2015007

[89] Ballou B, Andreko SK, Osuna-Highley E, McRaven M, Catalone T, Bruchez MP, et al. Nanoparticle transport from mouse vagina to adjacent lymph nodes. PLoS One [Internet]. 2012;**7**(12):e51995. DOI: 10.1371/journal.pone.0051995

[90] Paek HJ, Lee YJ, Chung HE, Yoo NH, Lee JA, Kim MK, et al. Modulation of the pharmacokinetics of zinc oxide nanoparticles and their fates in vivo. Nanoscale. 2013;**5**(23):11416-11427. DOI: 10.1039/C3NR02140H

[91] Balasubramanian SK, Jittiwat J, Manikandan J, Ong CN, Yu LE, Ong WY. Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials. 2010;**31**(8):2034-2042

[92] Mehrizi TZ. Hemocompatibility and hemolytic effects of functionalized nanoparticles on red blood cells: a recent review study. Nano [Internet]. 2021;**16**(08):2130007. DOI: 10.1142/S1793292021300073

[93] Nemmar A, Beegam S, Yuvaraju P, Yasin J, Shahin A, Ali BH. Interaction of amorphous silica nanoparticles with erythrocytes in vitro: role of oxidative stress. Cellular Physiology and Biochemistry. 2014;**34**(2):255-265

[94] Lima JM De, Sarmento RR, Souza JR De, Brayner FA, Feitosa APS, Padilha R, et al. Evaluation of hemagglutination activity of chitosan nanoparticles using human erythrocytes. BioMed Research International 2015;**2015**:247965-247971

[95] Choi J, Reipa V, Hitchins VM, Goering PL, Malinauskas RA. Physicochemical Characterization and in vitro hemolysis evaluation of silver nanoparticles. Toxicological Sciences. 2011;**123**(1):133-143

[96] Love SA, Thompson JW, Haynes CL. Development of screening assays for nanoparticle toxicity assessment in human blood: preliminary studies with charged Au nanoparticles. Nanomedicine [Internet]. 2012;**7**(9):1355-1364. DOI: 10.2217/nnm.12.17

[97] Aseichev AV, Azizova OA, Beckman EM, Skotnikova OI, Dudnik LB, Shcheglovitova ON, et al. Effects of gold nanoparticles on erythrocyte hemolysis. Bulletin of Experimental Biology and Medicine. 2014;**156**(4):495-498. DOI: 10.1007/s10517-014-2383-6

[98] Saha AK, Zhen MYS, Erogbogbo F, Ramasubramanian AK. Design considerations and assays for hemocompatibility of FDA-approved nanoparticles. Seminars in Thrombosis and Hemostasis. 2020;**46**(5):637-652

[99] Coto-García AM, Sotelo-González E, Fernández-Argüelles MT, Pereiro R, Costa-Fernández JM, Sanz-Medel A. Nanoparticles as fluorescent labels for optical imaging and sensing in genomics and proteomics. Analytical and Bioanalytical Chemistry. 2011;**399**:29-42