Chapter 12

Review of *In vitro* Toxicity of Nanoparticles and Nanorods: Part 1

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Abstract

The specific use of engineered nanostructures in biomedical applications has become very attractive, due to their ability to interface and target specific cells and tissues to execute their functions. Additionally, there is continuous progress in research on new nanostructures with unique optical, magnetic, catalytic, and electrochemical properties that can be exploited for therapeutic or diagnostic methods. On the other hand, as nanostructures become widely used in many different applications, the unspecific exposure of humans to them is also unavoidable. Therefore, studying and understanding the toxicity of such materials is of increasing importance. Previously published reviews regarding the toxicological effects of nanostructures focuses mostly on the cytotoxicity of nanoparticles and their internalization, activated signaling pathways, and cellular response. Here, the most recent studies on the *in vitro* cytotoxicity of NPs, nanowires, and nanorods for biomedical applications are reviewed and divided into two parts. The first part considers nonmagnetic metallic and magnetic nanostructures. While part 2 covers carbon structures and semiconductors. The factors influencing the toxicity of these nanostructures are elaborated, to help elucidating the effects of these nanomaterials on cells, which is a prerequisite for their save clinical use.

Keywords: nanoparticles, nanowires, nanorods, biocompatibility, cytotoxicity, nanomedicine

1. Introduction

Nanostructured materials are defined as possessing one of their dimensions in the range of 1–100 nm, according to the American Society for Testing and Materials (ASTM) international

standards definition [1]. For nanoparticles (NPs), which can be of more or less spherical or cubical shape, two dimensions are required to be within this range. In contrast, the shape of nanorods (NRs) is in one dimension much larger than in the others. For a small aspect ratio (<10), both their length and diameter are in the nanoscale, whereas NRs with a large aspect ratio (>10) only have their diameter within this scale, and are often called "nanowires" (NWs). Nanostructures within this specific size scale show unique size-dependent optical, magnetic, catalytic, and electrochemical properties, among others, as well as high surface to volume ratios. Moreover, their shape, surface chemistry, and chemical composition can be used to tailor specific properties, making nanostructures highly versatile for different applications [2, 3].

The size scale of nanostructures is within the range of several biomolecules, such as proteins and antibodies, allowing specific interactions to occur between them. This, when coupled with the high surface to volume ratios and tunable sizes and properties, makes nanostructures prime candidates for biomedical applications such as imaging, drug delivery, and therapy [4–6]. Examples of applications include the use of NPs as magnetic resonance imaging (MRI) contrast agents [7, 8], tissue engineering [9–11], as well as the recent focus on hyperthermia and cancer cell eradication with the use of NPs and NRs [12–17]. Such applications, if they are aimed for a clinical setting, ultimately require a direct NP/NR exposure in the form of ingestion or intravenous delivery into the body. Naturally, there is a rigorous testing required before any new drug formulation is approved for the clinical use in order to ensure their safety and effectiveness. Currently, very few NP-based drugs have been approved by the Food and Drug Administration and are commercially available. Examples include GastroMARK, used as an MRI contrast agent to enhance the delineation of the bowel, and ferumoxytol, an iron-replacement formulation approved for adults with chronic kidney disease with an iron deficiency [18].

Within this scope, biocompatibility and cytotoxicity data are of paramount importance to evaluate the potential of nanostructures for biomedical applications. Nanostructures are normally engineered to interface and target specific cells or tissues to execute their functions, raising questions about their toxicological effects. For instance, there are several characteristics involved in the toxicity of fiber-like nanomaterials, such as shape, length, chemical composition, agglomeration, and purity, making them suitable to fit the "fiber toxicological paradigm" according to the World Health Organization (WHO) criteria used to describe the toxicity of asbestos fibers [19]. Further, nanostructures are usually tuned for biocompatibility on top of the desired biomedical function, with the most relevant aspects that influence their toxicity being the material [20], size and shape [21], surface charge [22], and surface functionalization [23]. *In vitro* studies, while not able to give a complete insight into the biocompatibility of nanostructures, have a high importance, due to their easy implementation, and provide valuable cytotoxicology data regarding the safety of the use of nanostructures in biomedical applications. Previously published reviews regarding the biosafety of nanostructures include that of Lewinski et al. [24] and Zhao et al. [25]. The former focuses mostly on the cytotoxicity of NPs of different materials, whereas the latter is a more in depth review of the internalization, activated signaling pathways, and cellular response of different kinds of NPs.

Here, we review relevant studies assessing the *in vitro* cytotoxicity of both nanoparticles (NPs) and nanowires (NWs)/nanorods (NRs) with the potential to be used in biomedical applications. Due to their prevalence within the applied nanomaterials in biomedicine, this review

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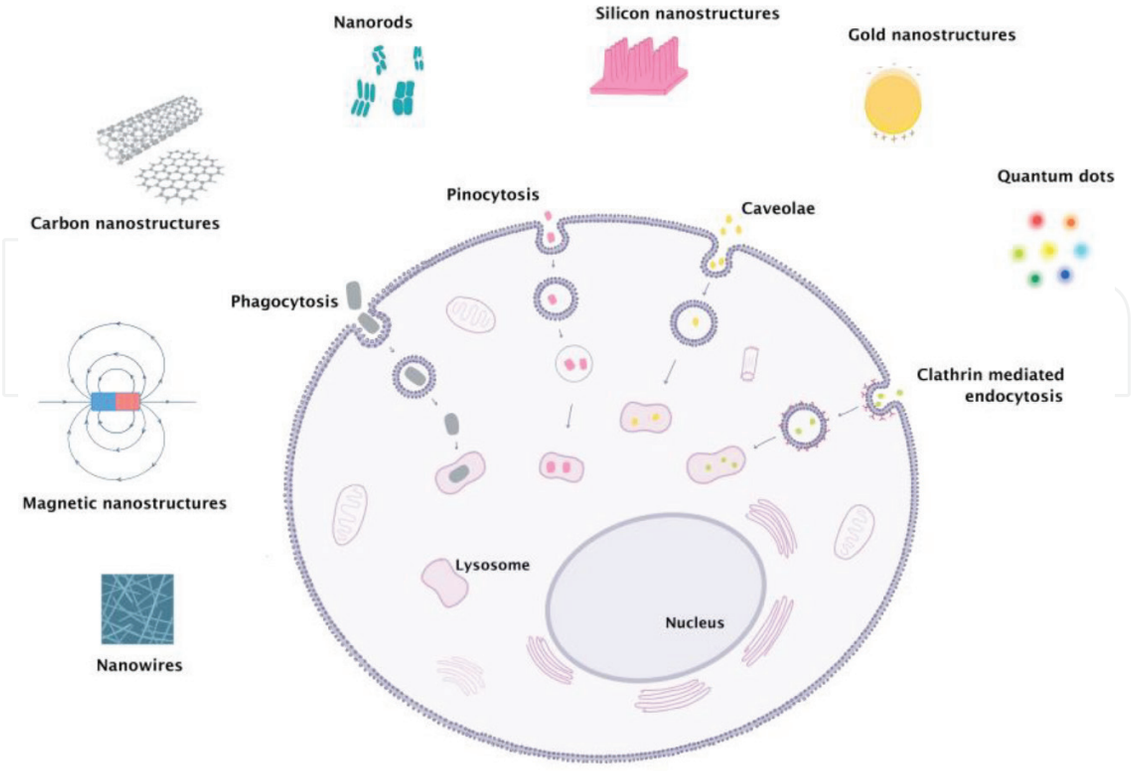

Figure 1. Schematic of the pathways for intracellular uptake of different materials and structures.

covers various materials from four different classes (on Scopus almost 50% of all publications related to cytotoxicity, since the year 2000, fall within these materials) that are typically considered in the context of nanomaterials for biomedical applications. The first part of this review covers nonmagnetic metals and magnetic materials, while the second part covers carbon structures and semiconductors. An overview of the materials and structures covered, together with the various intracellular uptake mechanisms, is given in **Figure 1**.

2. Nonmagnetic metallic nanostructures

2.1. Gold nanoparticles

Gold (Au) NPs are some of the most heavily used nanostructures in biomedical applications, most notably in medical imaging and therapy. The absorbance and fluorescence of Au NPs are higher than that of bulk Au and they can be finely tuned from the visible spectrum to the near infrared by changing their size and morphology [26]. Au can also readily bind different kinds of functionalizing molecules, giving them great versatility [27]. These properties make Au nanostructures popular candidates for X-ray-based imaging and radiotherapy, as well as photothermal therapy, when coupled to their ability to transform absorbed light into heat [26–30].

Two of the first cytotoxicity studies with Au NPs were performed by Tkachenko et al. [31]. In their first approach, they found that NPs conjugated with bovine serum albumin (BSA) and different peptides could enter the cell cytoplasm via an energy-dependent, receptor-mediated

endocytosis pathway, with a decrease in cell viability of only around 5%, using the lactate dehydrogenase (LDH) colorimetric assay. The following year, they tested the same principle in three different cell lines and found that the uptake of Au NPs, as well as their ultimate fate (endosomal or nuclear), was cell dependent [32]. Later, Goodman et al. tested the effects of cationic and anionic Au NPs in the metabolic activity of red blood COS-1 cells, and showed that the former are more cytotoxic, probably due to them being drawn by the cell membrane's negative charge and then taken up [33]. A different approach using human leukemic K562 cells was performed by Connor et al. using different surface modifiers and the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay, concluding that, despite the Au fabrication precursor being cytotoxic, Au NPs are inherently not, even though they are internalized and engulfed in endocytic vesicles [34]. This last finding was also confirmed by Chan and colleagues, who reported a size-dependent, clathrin-mediated uptake of citric acid, and transferrin-coated NPs in HeLa cells (**Figure 2**) [35, 36]. Similarly, size-dependence cytotoxicity was subsequently reported by Pan et al. [37] They showed that fibroblasts, epithelial cells, macrophages, and melanoma cells incubated with small (1.4 nm) Au NPs for 2 days have an IC_{50} ranging from 30 to 56 μM, whereas the same cells can tolerate concentrations 60-fold higher, when changing the particle diameter to 15 nm. The same group then went on to confirm the size-dependent cytotoxicity of Au NPs coated with TPPMS, now extending their findings to elucidate a necrotic death pathway in HeLa cells, due to oxidative stress, intracellular formation of reactive oxygen species (ROS) and a compromised mitochondrial activity [38].

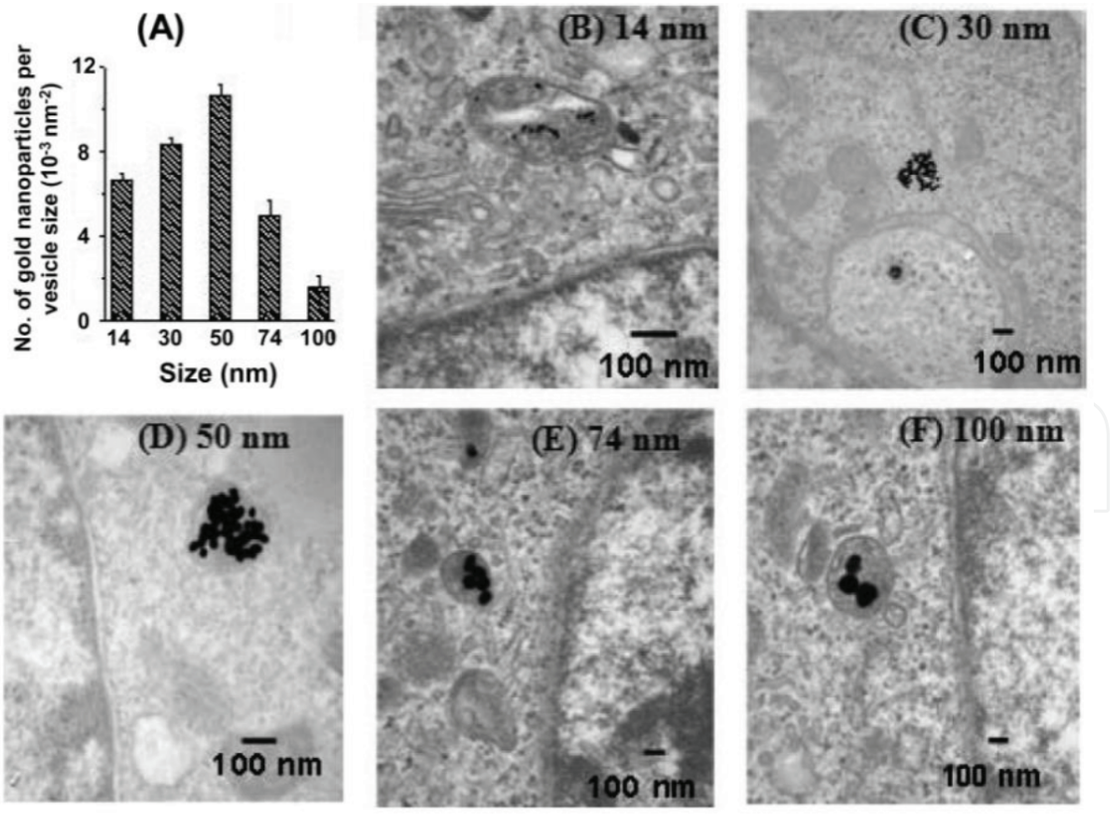

Figure 2. Size-dependent uptake of Au NPs of different sizes in HeLa cells at different positions from B-F, as observed by TEM. While (A) Quantify the number of each Au NPs per vesicle. Adapted with permission from Chithrani et al. [35]. Copyright 2006 American Chemical Society.

The cytotoxicity of Au NPs has also been tested in other relevant cell lines, such as the alveolar type II-like A549 and NCIH441 [39]. Using a combination of metabolic activity, cell proliferation, and release of LDH assays, it was determined that 9.5 nm Au NPs, though internalized and stored in endocytic vesicles, do not have an effect on either of the cell lines' metabolic activity. A 50% decrease in cell proliferation was detected for A549 cells after 24 hours of exposure, decreasing further as time progressed. A mild dose-dependent LDH release was also reported for 24 and 48 hours for Au NP concentrations up to 0.7 mM, although after 72 hours, the release was significantly higher for this concentration, at around 35 and 90% for A549 and NCHIH441 cells, respectively. Macrophages have also been subjected to cytotoxicity assessments using Au NPs [40]. The results in this case are particularly relevant, due to their shedding light on a potential immunological response. Au NPs of three different sizes (2–4, 5–7, and 20–40 nm) were tested in macrophage J774 A1 cells at two concentrations, 1 and 10 ppm. Whereas only the small and medium-sized NPs produced a slight decrease in cell proliferation for the lower concentration, NPs of all three sizes decreased cell proliferation to around 30–40% for the 10 ppm concentration. Au NPs were shown to be inside vesicles in the cytoplasm, as was reported for human leukemic [34], HeLa [35], and alveolar type II-like cells [39]. Additionally, it was reported that Au NPs upregulate the expression of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF-α). This data show that macrophages, being one of the principal immune effector cells, activate a pro-inflammatory response, when in the presence of Au NPs of either size.

Cho et al. looked closely at the effects of different surface functional groups on the uptake and cell membrane adsorption of Au NPs by breast cancer SK-BR-3 cells [41]. They tested Au NPs of two sizes (15 and 45 nm) with three different surface groups: poly(ethylene glycol) (PEG), anti-HER2, and poly(allylamine hydrochloride) (PAH). The smaller NPs were found to be more readily internalized than the larger ones, in contrast with previous findings [35, 36]. The PAH-modified NPs showed the greatest amount of internalization, followed by anti-HER2 and then PEG NPs. Using inductively coupled plasma mass spectrometry (ICP-MS) and an etching method to remove the NPs adsorbed to the cell membrane [42], they were able to distinguish between internalized nanoparticles to those that remained attached to the cell membrane and found that PEG-modified NPs had the lowest adsorption rate, thereby internalizing most of the NPs that came in contact with the cells. PAH-modified NPs had a very strong affinity to the cell membrane, probably due to their electrostatic interactions.

A more recent study compared NPs of 10, 25, and 50 nm and found that larger NPs are more readily taken up by NRK cells [43]. It was also reported that Au NPs enter the cells through endocytosis, ultimately accumulating in lysosomes and impairing their degradation capacity through alkalinization: Au NPs cause the dissociation of the V1 protein from the acidification-regulator complex H+(V)-ATPase, down-regulating its activity. The impairment of lysosomal function thus reduced the turnover of autophagosomes, carriers of intracellular content to their final degradation in lysosomes, leading to their accumulation in the cell cytoplasm.

2.2. Gold nanorods and nanowires

Gold NRs and NWs, unlike NPs, possess a transverse and a dominant longitudinal plasmon [44]. This intense absorption band is near the infrared region, which biological tissue hardly absorbs, thus making these Au nanostructures attractive in the biomedical field. Early

cytotoxicity data showed that HeLa cells incubated with PEG-stabilized NRs at concentrations up to 0.5 mM survived with more than 90% viability after 24 hours, as per MTT assay [45]. Takahashi et al. observed similar viability effects under the same conditions, but using NRs modified with phosphatidylcholine [46]. Chithrani et al. reported that an increased uptake was observed for NRs in terms of the number of particles per cell with NRs with smaller aspect ratios coated with citric acid ligands [35] and transferrin [36]. Similarly, NRs of varying surface charges provided by layers of polyelectrolyte coatings seem to maintain a cell viability of around 90%, with an increased cellular uptake observed with NRs with a positive surface charge [47]. These results were later confirmed using NRs coated with the polyelectrolytes polyacrylic acid (PAA) and PAH, with human colon cancer HT-29 cells showing 90% viability after incubation with 0.4 nM Au NRs with either coating [48]. Cell growth was also reported not to be impaired, when compared with control cells, although higher internalization numbers were found for PAH-coated NRs compared to PAA ones. This is in agreement with previous findings [47]: positively charged particles (PAH-coated) are more readily taken up, when compared to negatively charged ones (PAA-coated), possibly due to the cell membrane's negative charge attracting the positively charged particles, leading to a higher membrane adsorption, as was observed with Au NPs [33, 41].

More recent studies have continued the analysis of the toxicological properties of Au NRs with a higher aspect ratio, or Au NWs, as they provide enhanced properties such as absorption and scattering, due to their increase in length [49]. One of the first approaches aimed to compare the cytotoxic effects of Au NRs with Au NWs with an aspect ratio 10 times larger [50]. Both instances of particles were coated either with tannic acid (TA) or carboxylated PEG (PEG-COOH) and the cytotoxicity to human keratinocyte cells was evaluated using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) assay, an indicator of mitochondrial metabolic activity similar to the MTT assay. Following the same trend as previous cytotoxic data with surface-modified Au NRs, both TA and PEG-COOH-coated Au NRs showed a cell viability of up to 90% after 24 hours of incubation and for concentrations as high as 100 μg/mL; however, in the case of Au NWs, TA decreased the cell viability to around 70% at the 50 μg/mL concentration, whereas PEG-COOH-Au NWs maintained the viability above the 90% mark. These results indicate that, as Au NRs, Au NWs show very low cytotoxic potential, though specific surface coatings may elicit a toxic response. The authors also reported an increased uptake for Au NWs, when compared with NR independent of the surface coating. However, the values are reported in mass of Au per cell, which could be explained by the amount of material, due to the increase in size, and not be a clear representation of the amount of particles internalized.

2.3. Silver nanoparticles and nanowires

Silver (Ag) NPs have proven themselves greatly useful for their antimicrobial activity [51] and they are widely used in therapeutics and as a treatment for burns [52]. Naturally, a plethora of toxicity assessment studies has been carried out in order to understand the potential side effects of these nanostructures. The first cytotoxicity studies observed the effects of NPs on metabolic activity and membrane damage through LDH leakage, as well as the dependence

of the generation of ROS on particle size and the evaluation of the inflammatory response [53–55]. In all three studies, a dose-dependent decrease in mitochondrial metabolic activity and an increase in LDH leakage were reported for Ag NPs of 15 nm in rat liver BRL 3A cells, C18-4 germline stem cells, and macrophages for doses up to 75 μg/mL. Changes in cell morphology and uptake of NPs were also reported, with low levels of apoptosis. Interestingly, larger NPs (55 nm) induced a lesser cytotoxic response in macrophages, a result that can be attributed to the larger agglomerates not being easily internalized. Additionally, Ag NPs significantly impacted ROS generation and the release of inflammatory mediators including TNF-α, MIP-2, and IL-1β in macrophages for doses starting at 5 μg/mL. In contrast, Yen et al. reported no upregulation of the pro-inflammatory genes TNF-α, IL-1, and IL-6, though the doses tested were considerably higher [40].

It was later confirmed by Miura et al. that the ROS-related genes ho-1 and mt-2A are upregulated in HeLa cells [56], further cementing its role in the cytotoxic response. On the other hand, Autrup and co-workers showed that polyvinylpyrrolidone (PVP)-coated Ag NPs of 70 nm in diameter seemed to elicit both an apoptotic and necrotic response in THP-1 monocytes [57] and human alveolar A549 cells [58]. Although necrosis was markedly higher, the effect could be a progression from an early apoptotic stage to a late apoptotic/necrotic one.

Other parameters, such as genotoxicity, have also been studied. Using human lung fibroblast IMR-90 cells and glioblastoma U251 cells as test models, AshaRani et al. concluded that starch-coated Ag NPs (6–20 nm in size) are taken up and reside inside the mitochondria and nucleus and, on top of generating ROS and reducing the metabolic activity and cell viability (**Figure 3**), they also reduced the ATP content of the cells and induced DNA damage and chromosomal aberrations in a dose-dependent manner. The latter resulted in cell cycle arrest in the G_2/M phase with no significant cell death observed, possibly due to the repair of DNA damage [59]. On the other hand, RAW 264.7 macrophage cells exposed to increase concentrations of 70 nm Ag NPs showed a significant increase in TNF-α, protein, and gene levels. The secretion of nitric oxide, a second messenger in the inflammatory response, as well as

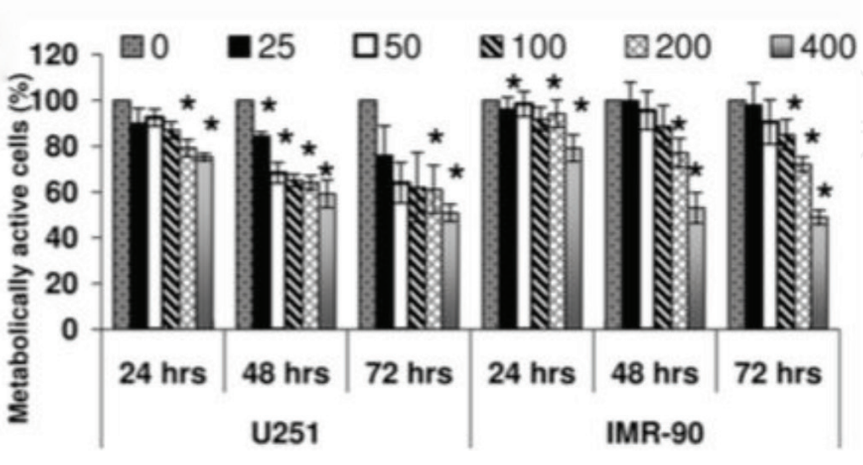

Figure 3. Cytotoxicity of Ag NPs on U251 glioblastoma and IMR-90 fibroblast cells. Different concentrations of NPs with sizes ranging from 6 to 20 nm in diameter were cocultured with the cells for incubation times up to 72 hours. Adapted with permission from AshaRani et al. [59]. Copyright 2009 American Chemical Society.

the gene expression of matrix metalloproteinases (MMPs) MMP-3, MMP-11, and MMP-19, which play a key role in extracellular matrix degradation and can be activated through ROS were also reported [60]. Further, another parameter that has been observed is the effect of Ag NPs of different sizes on the differentiation of embryonic stem cells [61]. Differentiation into cardiomyocytes was inhibited in a dose-dependent manner, with Ag NPs of 20 nm having a stronger effect compared to larger ones.

In recent studies, it has been proposed that the intracellular release of Ag ions from the NPs is one of the causes of their cytotoxicity. Singh et al. showed that, after being taken up through scavenger receptor-mediated phagocytosis in macrophages, intracellular dissolution of Ag NPs had a 50 times faster rate than in water, at around 5% of the total dose being dissolved [62]. It was suggested that Ag ions are a cytotoxic response initiator in human lung BEAS-2B cells [63].

Ag NW cytotoxicity, on the other hand, has not been extensively studied. In a comparative study of Ag NWs with a diameter of around 100 nm and lengths of 3, 5, 10, 14 and 28 μm, it was found that only the wires with a length of 28 μm could elicit a significant decrease in cell proliferation and membrane instability in THP-1 cells [64]. Using light microscopy and back-scattered electron imaging, it was also proven that NWs of 14 and 28 μm are not properly internalized, resulting in a frustrated phagocytosis, or an inability to engulf its target, which is in turn an initiator of the inflammatory response. In a different approach, red blood cells exposed to Ag NWs of 2 μm in length and a diameter of 40 nm were confirmed to suffer structural changes, aggregation, and hemolysis in a dose-dependent manner [65].

3. Magnetic nanostructures

3.1. Magnetic nanoparticles

Magnetic NPs are usually made of a magnetic core bound within a shell that allows them to be functionalized with relevant ligands and gives them stability in solution [66]. Their main advantage is their ability to be manipulated using an external magnetic field, making them attractive for different biomedical applications. These include cell labeling and MRI contrast agents [67, 68], targeted drug delivery [69, 70], and cancer cell eradication [71, 72]. Iron oxide NPs such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most widely used magnetic NPs [73]. Sufficiently small Fe oxide NPs exhibit superparamagnetism: NPs become magnetized under an external magnetic field, but loose do not possess any remanent magnetization once the field is removed [74]. Superparamagnetic Fe oxide NPs (SPIONs) can be manipulated and guided by a magnetic field without losing the stable colloidal suspension, when there is no field applied, a quality that is attractive for biomedical applications.

3.2. Superparamagnetic iron oxide nanoparticles

Initial cytotoxicity studies compared the cytotoxic effects of bare magnetite NPs with PEG-coated ones in primary human fibroblast hTERT-BJ1 cells, and found that, whereas cells treated with 40–50 nm PEG-coated NPs for 24 hours remained 100% viable for concentrations

as high as 1 mg/mL, uncoated, 10–15 nm NPs reduced the viability to around 70% at a concentration of 250 μg/mL [75]. Similar results were observed with pullulan-coated and uncoated NPs within the same size range [76]. There, it was also shown that bare NPs significantly reduce cell attachment and disrupt the distribution of actin filaments and microtubules, while also being taken up at a higher rate compared to the coated ones. Uncoated NPs were also reported to have cytotoxic effects only at higher doses (100–250 μg/mL) in terms of cell viability and LDH leakage in rat liver BRL 3A cells [53]. In agreement with these findings, hydroxy-tetramethylammonium-coated SPIONs at higher concentrations (23 mM) did not induce a reduction inviability of kidney COS-7 cells, though the time of incubation tested was only of 4 hours [77].

Ma et al. studied the uptake of 30 nm aminosilane-coated NPs by human lung cancer SPC-A1 and human lung WI-38 cells and found that the intracellular Fe content was 15 times higher for the cancerous cells compared to normal counterparts [78]. As with other NPs, they are likely endocytosed through phagocytosis and found within endosomes and lysosomes. Human monocytes-macrophages were also found to endocytose SPIONs and retained them inside lysosomes, remaining highly viable with no apparent activation of pro-inflammatory cytokines for up to 14 days following the incubation with 0.4 mg/mL SPIONs [79].

Using bare 20–30 nm magnetite NPs, Karlsson et al. tested other parameters of cytotoxicity in the human alveolar A549 cells, such as DNA damage and intracellular ROS [80]. No DNA damage or intracellular ROS were found for doses up to 40 μg/cm^2, although a slight oxidative DNA lesion was found at this dose. In contrast, another study showed that uncoated SPIONs elicited a significant level of apoptosis on mouse fibroblasts (L929), whereas PVA-coated ones did not show a loss of cell viability, apoptosis, necrosis, or cell cycle arrest for up to 72 hours of incubation and concentrations up to 200 mM [81]. However, an increase in the concentration to 400 mM did induce apoptosis and cell cycle arrest, possibly due to DNA damage through oxidative stress. Naqvi et al. obtained similar results for Tween 80-coated NPs in macrophage J774 A1 cells: >95% cell viability for low concentrations (25–200 μg/mL) and low incubations times, with a decrease to 55–65% for higher concentrations (300–500 μg/mL) associated to an apoptotic death pathway through ROS generation [82].

In contrast to previous findings, both citric acid and dextran-coated NPs were found to produce a dose-dependent cytotoxicity in human umbilical vein endothelial cells (HUVECs) [83]. Concentrations as low as 0.1 nM decreased the cell viability for both NPs to around 80% after 24 hours and increasing the value to 20 nM would decrease the cell viability to less than 15%. Additionally, as shown by Soenen et al. [84], actin filaments and microtubules appeared disrupted, thinner, and less organized and vinculin adhesion points were diminished. Further, NPs also reduced the migration and vasculogenesis capabilities of HUVECs. Similar results regarding cell attachment and cytoskeleton morphology were also reported in a multiparametric study with NPs with different coatings on various cell lines [84].

With an aim to understand the differences in cytotoxicity between the charges provided by different coatings on SPIONs, a study showed that when different functional groups were added in order to provide either a positive or negative charge on SPIONs, cell viability and cell membrane integrity remained above 85% up to 24 hours for doses as high as 1000 ppm

on L929 fibroblasts for all the coatings tested [85]. As observed for other types of NPs, the positively charged NPs were more readily taken up than negatively charged ones. Similarly, ROS generation was not significantly different. However, the positively charged and highest negatively charged NPs showed DNA damage starting from concentrations of 200 ppm. In agreement with this, another study using HCM (heart), BE-2-C (brain), and 293T (kidney) cell lines reported similar results [86]. There, bare, positively, and negatively charged NPs all showed a dose-dependent response for doses up to 36 mM, with positively charged NPs being more cytotoxic for the three cell lines, suggesting a cell-specific response. Gene expression analysis showed that genes that were mainly altered were those related to apoptosis, cell cycle, and cell proliferative responses, most probably due to ROS.

More recent studies have focused on looking at other parameters to better understand the cytotoxic response. A size-dependent response was observed for uncoated NPs of 5 and 30 nm, with only the latter inducing a significant increase in ROS generation, whereas dextran-coated and PEG-coated did not have an effect on ROS levels [87]. Khan et al., on the other hand, studied the effects of SPIONs on autophagy, a homeostasis mechanism used to degrade proteins and organelles for multiple functions [88]. They proved that ROS induces autophagy through the mTOR pathway only on human alveolar cancer A549 cells, and not on normal human lung fibroblast IMR-90 cells, while the authors attributed the autophagy to be involved with cell death. Lastly, Singh et al. observed a different cytotoxic response and uptake related to the Fe redox state (magnetite vs. maghemite) in human lymphoblastoid MCL-5 cells [89]. While no significant difference was found between these two states in terms of uptake, a decrease in the serum concentration drastically increased the uptake for dextran-coated maghemite NPs and this specific kind of NPs was the only one reported to elicit a genotoxic response.

3.3. Magnetic nanowires

Magnetic NWs possess tunable lengths and diameters and can be functionalized to provide specific targeting and biocompatibility. Depending on the fabrication method and its parameters, as well as precursor materials, the magnetic properties of NWs can be finely modulated [66, 90]. Magnetic NWs have anisotropic structures with high aspect ratios, which allow them to exert torques when under a magnetic field [91]. Additionally, they possess higher magnetization values per unit of volume when compared to NPs, allowing them to exert larger forces [92]. These qualities have made magnetic NWs prime candidates for different biomedical applications, including cell separation and guidance [91–93], targeted drug delivery [94, 95], and cell eradication [15, 16, 96, 97].

The cytotoxicity of Fe NWs was first characterized by Song et al. on HeLa cells [98]. Using uncoated NWs of <10 μm in length and 50 nm in diameter, they determined that Fe NWs have no significant effect on the cell viability and proliferation for concentrations up to 10,000 NWs per cell and for incubation times up to 72 hours. Fe NWs were internalized either as single NW, bundles or as aggregates, mainly localizing in the cytoplasm and inside vesicles, but not inside cell nuclei. Later, Safi et al. proved the same intracellular distribution in fibroblast NIH/3T3 cells using maghemite NWs of <15 μm and identified such vesicles as late

endosomal or lysosomal endosomes (**Figure 4**) [99]. It was concluded that NWs are degraded by cells and cut into shorter pieces, possibly by the decrease in pH occurring in lysosomal compartments [100]. Along with no significant decrease in cell viability, no ROS were found after 4 hours of incubation for doses up to 170 NWs per cell. In a recent study, NWs with iron core and iron oxide shell were compared to pure iron NWs and tested on HCT 116 cells. The experiments confirmed the high cell viability values found for iron NWs before and revealed even higher values for the core/shell NWs [101]. An additional advantage of the core/shell NWs is the possibility to tune their magnetic properties to the specific requirements of various applications.

Studies with Ni NWs first showed a similar distribution to that of Fe NWs: Ni NWs of 20 μm in length and 200 nm in diameter activated cell membrane receptors associated with metalloproteins, thereby being internalized, triggering lysosomal function in the process and localizing inside them around the cell nucleus [102]. Lamellipodium extensions, due to cell tethering and re-alignment, were also a consequence of Ni NW internalization, possibly due to a cell stiffening response. The same group then studied the biocompatibility of Ni NWs on human monocyte THP-1 cells using high content analysis [103]. Measuring cell viability

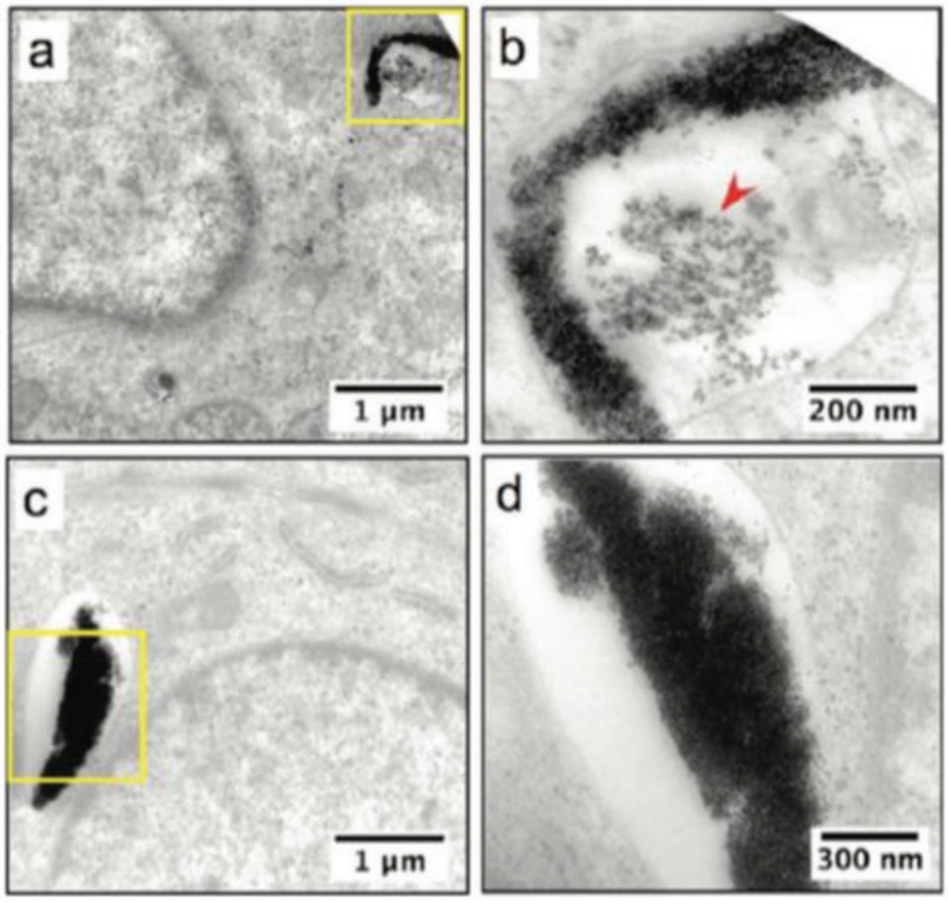

Figure 4. NIH/3T3 fibroblasts incubated with maghemite (γ-Fe_2O_3) NWs during 24 hours. The NWs were found inside membrane-bound compartments, identified as late endosomal or lysosomal endosomes, b) and d) are zoomed images from a) and b) respectively. Adapted with permission from Safi et al. [99]. Copyright 2011 American Chemical Society.

and membrane permeability, they found Ni NWs to be nontoxic for low incubation times (<10 hours) and concentrations (<100 NWs per cell). Hossain and Kleve then investigated the effects of Ni NWs on human pancreatic adenocarcinoma Panc-1 cells [104]. Using NWs of around 6.5 μm in length and 215 nm in diameter, a dose-dependent cytotoxic response was shown, including ROS generation and the induction of apoptosis and cell cycle arrest in the G_0/G_1 phase. A similar response was then reported for HeLa cells, along with mitochondrial membrane depolarization [105].

We reported the cytotoxicity of Ni NWs in human fibroblast WI-38 cells [106] and human colorectal carcinoma HCT 116 cells [107]. Whereas WI-38 cells showed no significant decrease of cell viability up to doses of 120 μg/mL for 24 hours of incubation and the viability of HCT 116 cells decreased significantly at the same incubation time for doses as low as 5 μg/mL. For both cell lines, NWs were internalized and appeared in the cytosol inside membrane-bound compartments, possibly lysosomes, as shown previously [102], with the internalization in HCT 116 cells taking place through the phagocytosis pathway. Apoptosis was also confirmed to be the cell death pathway, which would later progress into secondary necrosis and induce cell membrane instability and LDH leakage. Lastly, it was also confirmed that Ni^{2+} is released intracellularly following NW uptake, due to the acidic pH inside the lysosomes. Although the percentage of this intracellular dissolution is low compared to the total dose, it is plausible that the leeched Ni^{2+} contributes to the cytotoxic effects observed. It should be mentioned that Au-coated Ni NWs showed an improved biocompatibility, possibly due to the Au reducing the degree of dissolution, while also providing a functionalization layer [108, 109]. Similarly, we have reported the stabilization of Fe NWs by coating with a poly(MPC) homopolymer in order to increase dispersion and biocompatibility [110].

4. Conclusion

Recent studies on the *in vitro* cytotoxicity of nonmagnetic and magnetic structures in biomedical applications were reviewed, taking into account nanoparticles and nanowires/nanorods. A summary of the results of representative studies is provided in **Table 1**.

Comparisons between the cytotoxicities of those different nanomaterials are generally difficult to make, due to the vast range of methods, concentrations, dimensions, cell lines, etc. For instance, the concentrations reported in the different studies were typically evaluated using either ICP or cryogenic TEM. However, the concentration or dose of the nanomaterial plays a significant role in the cytotoxic response as well as the biomedical applications.

While the concentrations and exposure times are critical factors, the toxicity of these nanostructures is also material dependent. These relations can be seen in **Figure 5**, which presents the average values reported for the cell viabilities (ignoring differences in concentrations, incubation times, etc.), when exposed to the nanomaterials in the studies covered in **Table 1**. Fe nanomaterials showed higher cell viabilities than Au ones.

Nanostructure type	Surface coating	Nanostructure concentration	Average size	Cell line	Cell viability	Viability test	References
Au NPs	BSA	–	20 nm	HepG2	95%	LDH assay	[31]
Au NPs	–	1 and 10 ppm	2–4, 5–7 and 20–40 nm diameter	J774 A1 macrophages	Cell proliferation decreased to 30–40% for all three sizes at 10 ppm	Multisizer quantification	[40]
Au NRs	PEG	0.5 mM	65 nm length and 11 nm width	HeLa cells	>90%	MTT assay	[45]
Ag NWs	–	4 μg/cm^2	100 nm diameter and 28 μm length	THP-1 cells	Significant decrease in cell proliferation and increase of membrane instability	Alamar Blue, LDH assay	[64]
SPIONs	PEG	1 mg/mL for 40–50 nm NPs, 250 μg/mL for 10–15 nm NPs	40–50 nm diameter, 10–15 nm diameter	hTERT-BJ1	100% for 40–50 nm NPs, 70% for 10–15 nm NPs	MTT assay	[75]
SPIONs	–	100–250 μg/mL	47 nm	BRL 3A rat liver cells	70%	MTT, LDH assay	[53]
Fe NWs	–	10,000 NWs per cell	10 μm length and 50 nm diameter	HeLa cells	No significant effect	MTT assay	[98]
Ni NWs	–	5 μg/mL	5.4 μm length and 33 nm diameter	HCT 116 cells	<80%	MTT, LDH assay	[107]

Table 1. Summary of ***in vitro*** cytotoxicity studies with different kinds of nanoparticles (NPs) and nanowires (NWs), NWs with aspect ratio < 10 are often called nanorods (NR), SPION referred to superparamagnetic iron oxide NPs.

In addition, the particle size plays a major role in the cytotoxic properties of the nanostructure, whereby both the cellular uptake efficiency and pathway are affected, with smaller particles being internalized faster than larger ones.

The induction of ROS after dissolving the nanostructures in the lysosomes was shown to be the primary underlying cause of the toxicity in several cases, leading to cell death through the apoptotic pathway, due to ROS generation and mitochondrial damage. The acidic condition inside the lysosome increases the digestion of the particles, enhancing the release of ions that

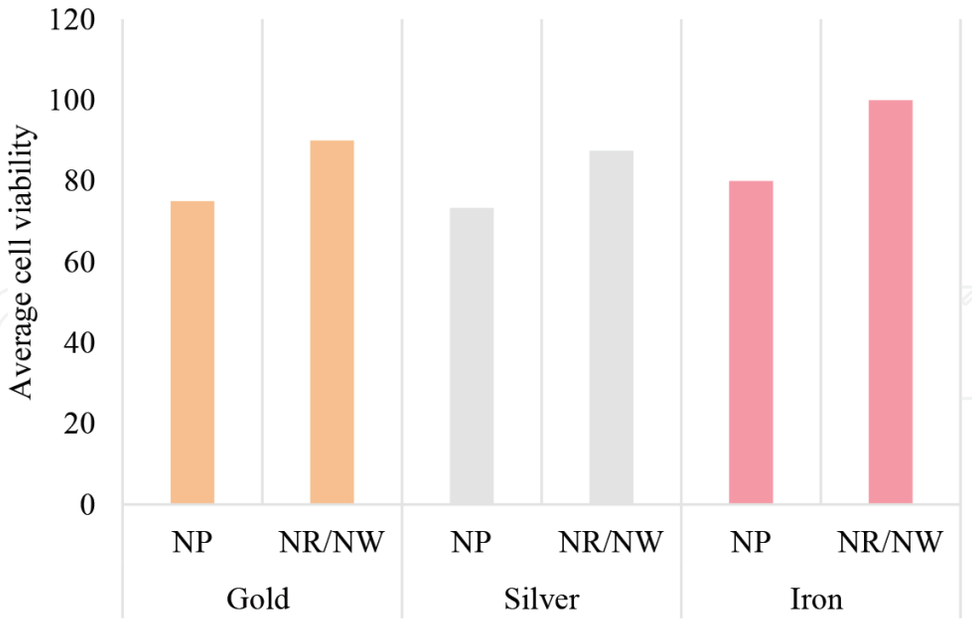

Figure 5. Average cell viability when exposed to nanomaterials reported in **Table 1**, considering nanoparticles (NP), nanorods (NR), and nanowires (NW).

affect the viability of the cells. However, the oxidation of Fe nanostructures did not decrease the cell viability.

Adding a coating to the nanostructure typically affected both the toxicity and the surface charge of the nanostructure, where cationic surfaces are more toxic than anionic. For instance, coating Au with TA coating led to the decrease of the cell viability to around 70%, whereas with a PEG coating, the viability was maintained above 90%.

The cytotoxicity of the nanomaterial depends also on the nanostructure's shape. In this regard, several advantages have been reported for NWs over NPs. For instance, they enhance the drug-loading capacity, due to their large surface area. Moreover, magnetic NWs, due to their higher magnetization, can be better manipulated by the use of the magnetic field than NPs. An interesting observation from **Figure 5** is that NWs/NRs are, on average, less cytotoxic than NPs. This was attributed to the increased interaction of the nanomaterial with the cells, due to the large surface area.

While all these studies contributed to obtain a better picture of the cytotoxicity of nanomaterials and the underlying mechanisms, it is a persisting issue that a consistent measurement and reporting system will be needed for future studies. This will not only enable performing more accurate comparisons of the toxicological characteristics of nanostructures but also to better evaluate the potential of using them for biomedical applications.

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