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Impact of gold nanoparticle exposure on genetic material

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ABSTRACT

Metal nanoparticles, with gold nanoparticles (AuNP) at the forefront, have gained immense attention due to their unique properties. At the nanoscale, gold exhibits remarkable physical, chemical, optical, and electronic features, making it ideal for a plethora of applications, including bioimaging, sensing, diagnostics, and drug delivery. Despite their promising utility, concerns have arisen regarding the potential adverse effects of AuNP on human health. Research has indicated that these nanoparticles can accumulate in vital organs and interact with proteins and cellular structures, potentially leading to diverse toxicological manifestations. The precise understanding of these nano-bio interactions is further complicated by the varied physicochemical properties of AuNP that influence their biological effects. This review aims to consolidate the current knowledge on the genotoxic effects of AuNP, shedding light on the underlying mechanisms and factors affecting their toxicity. The search was conducted in PubMed and Web of Science databases. Eventually, 32 studies focusing on the genotoxic effects of AuNP were included in the review. *In vitro* and *in vivo* findings revealed that AuNP can induce primary DNA damage, oxidative DNA damage, chromosomal damage, alterations in gene expression, and effects on epigenetic regulation. These effects were found to be influenced by various factors, including nanoparticle size, shape, and surface coating. However, the existing literature also highlights the challenges associated with assessing the genotoxicity of nanomaterials (NM), emphasizing the need for standardized and adapted testing protocols. The interference of nanoparticles with conventional toxicity assays may lead to unreliable results; thus, specific methodologies tailored for NM evaluation must be implemented. In conclusion, while AuNP hold tremendous potential for innovative applications, their safety profile remains a critical concern. Continued research is imperative to elucidate the mechanisms of AuNP induced genotoxicity and develop robust testing protocols, ensuring their safe and effective use in diverse applications.

1. Introduction

Nanotechnology has been considered as one of the most important industries and research fields of the 21st century [1]. The prefix "nano" is referred to a Greek prefix meaning "dwarf" or something very small, so structures or molecules with at least one dimension between 1 and 100 nm can be considered nanomaterials (NM) [1,2]. Therefore, the simplest definition of nanotechnology may be "technology at the nanoscale", but a more elaborated definition by The National Nanotechnology Initiative in the Unites States is "a science, engineering and technology conducted at the nanoscale, where unique phenomena enable novel applications in a wide range of fields, from chemistry,

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physics and biology, to medicine, engineering and electronics" [3,4].

Engineered NM increased massively about two decades ago revolutionizing most of the technological and scientific fields [5]. Among the highly promising NM, metal nanoparticles (NP) have gained significant attention due to their particular properties [6]. They are very interesting materials, easy to synthesize and manipulate, offering precise control over the particle physicochemical properties, and functionalisation with a variety of functional groups, such as peptides, antibodies, RNA, and DNA, to target different cells along with potential biocompatible polymers, for example, polyethylene glycol (PEG) [7]. Among them, gold nanoparticles (AuNP) are some of the most deeply investigated [5,8].

At the nanometric scale, gold has fascinating physical, chemical, optical, and electronic properties, explained by a combination of the electronic structure with other effects due to the extremely small dimensions [5,9]. It exhibits advantageous features such as simple synthesis with tunable sizes and shapes, easy modification, and unique optical properties, particularly, their surface plasmon resonance (SRP) [10,11]. Surface plasmons are oscillating electrons in a metallic nanostructure that obtain a frequency oscillation equal to the irradiating electromagnetic field. The SPR wavelength of AuNP can be tuned from visible to near-infrared by changing the size and/or shape of the NP [12]. Therefore, these NP can absorb energy from a light source and produce light scattering, making them attractive for a variety of biological applications, such as bioimaging, nanosensing, and molecular diagnostics [1].

Because of these special features, AuNP are widely used for numerous applications in different fields, ranging from clinical, pharmaceutical, and biomedical uses to their utilization in microelectronics and food packaging [8]. Above all, these NP have gained significant attention in biomedicine, where they can be used in targeted drug delivery, gene therapy, medical imaging as contrast agent, biosensing and diagnosis, and as counter cancer and antimicrobial agents, among others [13,14].

Many previous studies have reported that AuNP accumulation may occur in vital organs, such as the liver and the spleen [15–17]. AuNP can also interact with proteins and subcellular organelle structures leading to neurotoxicity, genotoxicity and immunotoxicity [14]. Consequently, even though their unique properties make AuNP technologically and biomedically interesting, these properties raise also alarm bells about their possible adverse effects on health and the environment; nevertheless, the information about their short- and long-term effects on living organisms is still very limited [13].

Additionally, physicochemical properties of AuNP, in particular, were shown to complicate the prediction of their biological effects [18], limiting the understanding of their nano-bio interactions, hampering their use and commercialization [1]. This is aggravated by the fact that conventional toxicity assays may not be appropriate for nanotoxicity testing due to NP interference with assay components or detection methods [19]. This interference is frequently not properly addressed often leading to unreliable results in biocompatibility studies and over- or under-estimation of the damage induced.

In this context, the main objective of this work was to gather the main genotoxic effects associated with AuNP exposure reported so far in the literature, as well as the factors and physical-chemical properties influencing these effects, and to shed light on the action mechanism of these NP on the genetic material. The current gaps and future needs of proper standardised techniques for nanogenotoxicity evaluation are also addressed and discussed.

2. Search strategy

Studies included in this review were identified by a bibliographic search using PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Web of Science (WoS, all databases, all collections) (https://www.webofscience.com), updated to March 2024. The search strategy comprised two terms that were intersected using the Boolean term "AND". The search term included as first descriptor was "gold nanoparticles", and the second one was related to genotoxic effects ("genotox*") or epigenetic alterations (epigenetic*). Initial screening was focused on Topic (including title and abstract,) in both PubMed and WoS. Although language restriction was not applied, all articles found were written in English. The initial search retrieved a total of 175 manuscripts. Reviews, book chapters, conference papers, articles just addressing gold nanoparticle synthesis or characterization, or those where no genotoxicity or epigenetics assessment was conducted were all discarded for the final revision. At the end, 32 studies addressing potential genotoxic effects or epigenetic modifications induced by different AuNP were included in the present review.

3. Effects of gold nanoparticles on the genetic material

Several direct and indirect mechanisms through which nanomaterials could potentially cause DNA damage are reported in the literature. Direct mechanisms would involve interaction with DNA molecule, while indirect mechanisms would take place without physical interaction with the genetic material, through their ability to generate free radicals and cause oxidative stress. Alternatively, if NM are able to accumulate within cells, but not necessarily gain access to the nucleus, they may still come into direct contact with DNA during mitosis, when the nuclear membrane breaks down, possibly resulting in DNA aberrations. Due to the variety of mechanisms involved in genotoxicity induction, a battery of assays is required to evaluate the genotoxic potential of a nanomaterial [20]. The most common types of genotoxic damage caused by NM include primary DNA damage, chromosomal aberrations, adduct formation, alterations in gene expression and in epigenetics [21]. AuNP, in particular, were found to interact with DNA in many different ways causing a number of genetic injuries (Fig. 1).

The following sections describe the different types of genotoxic effects induced by AuNP exposure on biological systems (Tables 1–4).

3.1. Primary DNA damage

Primary DNA damage, including single-strand breaks (SSB), double-strand breaks (DSB), alkali-labile sites, and incomplete excision repair sites, can be detected by the alkaline comet assay. Due to its sensitivity, simplicity and versatility, this assay is one of the most widely used methods for assessing the genotoxicity of NM [22,23].

Although biological behaviour of NP can be modulated by several factors such as size, shape and functionalisation, most studies with different types of AuNP have detected primary DNA damage using this technique, both *in vitro* and *in vivo* (Table 1). Plotnikov et al. [24] and Bin-Jumah et al. [25] studied the genotoxicity of large AuNP (> 50 nm) in albino mice and in human healthy (Chang) and cancer liver (HuH-7) cells, respectively. They detected primary genetic damage following a dose-response pattern, with notable damage at the highest concentrations tested. Similar effects were observed in other studies using smaller AuNP of 3–4 nm in human lung carcinoma cells (A549) [11], of 7 nm in the gilt-head bream (*Sparus aurata*) [26] and of 9 and 14 nm in Chinese hamster ovarian cells (CHO) [20,27].Also using comet assay, Cardoso et al. [28] found that acute and chronic exposure to AuNP (10 and 30 nm) induced DNA damage in cerebral cortices isolated from brain of exposed rats, although a higher damage was observed after chronic exposure.

Nevertheless, some studies do highlight the importance of the AuNP properties on the genotoxicity results. In the work by Lebedová et al. [29], significant DNA damage in human bronchial epithelial cells (HBEC) was only detected in the smallest diameter AuNP (5 nm), but not in the 50 nm ones. Furthermore, different functionalisations have also been reported to influence AuNP-induced genotoxicity. The work by Rogers et al. [30], testing human kidney epithelial cells (HK-2), showed that certain coatings may play a protective role; e.g., PEG-coated AuNP did not produce DNA damage, while uncoated AuNP did. The absence of

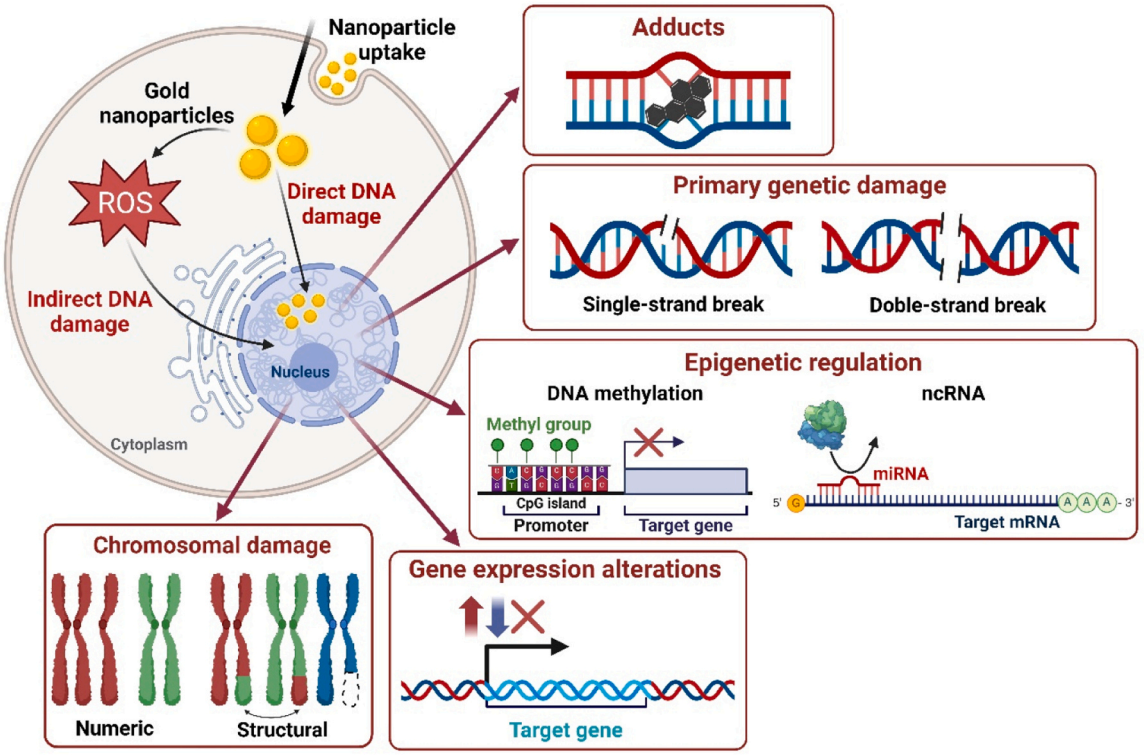

Fig. 1. Types of genetic damage induced by AuNP reported in the literature. Created in BioRender. Fernández Bertólez, N. (2024) BioRender.com/w62z785.

genetic damage induced by PEG-coated AuNP was corroborated by Mulder et al. [31] in a study in human hepatocellular carcinoma cells (HepG2), where it was also observed that damage caused by AuNP functionalised with certain molecules [bovine serum albumin (BSA), poly(sodium 4-styrene sulfonate) (PSSNA), polyvinylpyrrolidone (PVP) and glutathione (GSH)] can be repaired to some extent over time. This repair of genetic damage was also observed in the work by Khan et al. [32], who tested AuNP in the grass carp (*Ctenopharyngodon idella*) and found that blood cells damage increased with concentration at 14 days of exposure, while it decreased after 28 days of exposure.

In addition, the simultaneous effect of both properties – size and surface coating – was observed in other studies, such as the one by Vales et al. [33], where two differently sized AuNP (5 and 20 nm) with three coatings (PEG, carboxylate and ammonium) were tested in transformed human bronchial epithelial cells (BEAS-2B), and DNA damage was only observed in the 5 nm PEG-coated AuNP and in the 20 nm ammonium-coated AuNP.

To complement the information about primary DNA damage obtained with the alkaline comet assay, May et al. [11] performed the fluorometric detection of alkaline DNA unwinding (FADU) assay and the γH2AX assay after exposure of A549 lung cells to 3–4 nm AuNP. The FADU assay, used also to detect DNA strand breaks, showed lower DNA damage than the alkaline comet assay, leading to the conclusion that these NP mainly induce alkali-labile sites and, to a lesser extent, SSB and DSB. Moreover, the lack of positive results in γH2AX assay (which indirectly detects DSB) at concentrations lower than 100 μg/mL would indicate that most DNA breaks induced by AuNP are SSB. Other techniques, such as random amplified polymorphic DNA (RAPD) or apurinic/apyrimidinic (AP) sites detection, can also be employed to assess primary DNA damage. Mahaye et al. [34] studied the genotoxicity of 5, 20 and 40 nm AuNP coated with citrate and branched polyethylamine (BPEI) in the freshwater green algae *Pseudokirchneriella subcapitata*. The RAPD profiles, which allow for an estimation of genomic template stability, showed a different size range of genomic bands in samples exposed to citrate-coated AuNP compared to controls. Additionally, a significant increase in the content of AP sites was observed in treated samples irrespective of the AuNP size and coating type, demonstrating their ability to induce genotoxic effects.

A current issue in nanotoxicology is that most of the available studies focus on the effects of high concentrations of NM, while the effects of lower concentrations, most likely representative of a realistic human exposure scenario, have been neglected. NM are highly reactive and very prone to aggregation, which might explain that most of the available studies on the effects induced by the NM show a 'all-or-none' response rather than a gradual, concentration-dependent trend. An unusual inverse relationship between the toxic effects and NM concentration has been reported, both in vitro and in vivo [35]. In this regard, Fraga et al. [36] found that low concentrations of citrate-coated AuNP (22 nm) induced higher DNA damage on human hepatocyte HepG2 cells than high concentrations after 24 h of exposure.

Moreover, it should be noted that some of the studies mentioned have been carried out *in vivo* in both murine and fish models, and others *in vitro* using different cell lines. Therefore, AuNP would produce primary DNA damage in most eukaryotic cells, regardless of cell type, depending on their features such as size and surface modifications.

3.2. *Oxidative DNA damage*

Oxidative stress is the action mechanism underlying NP toxicity most frequently reported (reviewed in Horie and Tabei [37]). High ROS levels induced by many NM can damage cells by producing lipid peroxidation, mitochondrial damage, DNA disruption, gene transcription modulation, and protein oxidation, which would eventually result in cell apoptosis/death [38–40]. DNA is prone to oxidative stress-related lesions. They can cause mutagenic events [41] and can also be responsible for loss in epigenetic information, probably due to an impairment in CpG island methylation asset in gene promoters [42].

Due to its versatility, the combination of the comet assay with different enzymes allows the evaluation of specific nucleobase damages, such as oxidative damage, since these enzymes specifically detect

Table 1
Studies assessing primary and oxidative DNA damage induced by AuNP.

Study	NP tested	Technique	Cell/organism	Conditions	Results
In vitro					
Bin-Jumah et al. [25]	Spherical 55 nm green gold nanoparticles (gAuNP)	Comet assay	Chang and HuH−7 cells	Exposure to 10–700 μg/mL for 24 h	Concentration-dependent DNA damage in both cell types, greater in HuH−7 cells
Di Bucchianico et al. [43]	Spherical 5 and 15 nm citrate-coated AuNP (Cit-AuNP)	Comet assay EndoIII- and Fpg-modified comet assay	Human primary lymphocytes and Raw264.7 cells	Exposure to 0.1–100 μg/mL for 2 and 24 h	Dose-dependent DNA damage in both cell types Dose-dependent oxidation of pyrimidines (detected by EndoIII), but not purines (detected by Fpg)
Fraga et al. [36]	Spherical 22 nm Cit-AuNP and 33 nm 11-mercaptoundecanoic acid (MUA)-AuNP	Comet assay	HepG2 cells	Exposure to 0.1–100 μM for 24 h	Only Cit-AuNP were genotoxic in an inverse concentration-dependent manner
George et al. [20]	Spherical 14 nm Cit-AuNP	Comet assay	CHO cells	Exposure to 6.2–50 mg/mL for 20 h	Dose-dependent genotoxicity, significant at all conditions tested.However, the presence of interference was detected in this assay
Levedobá et al. [29]	Spherical 5 and 50 nm Cit-AuNP	Comet assay	HBEC cells	Exposure to 1–20 μg/mL for 48 h	Only 5 nm NP were genotoxic at the different concentrations tested
May et al. [11]	3 spherical 3–4 nm AuNP (positively charged AuNP I and negatively charged AuNP II and III)	Comet assay	A549 cell line	Exposure to 0–100 μg/mL for 3 and 24 h	Genotoxicity after 24 h exposure to AuNP I and AuNP II from 40 μg/mL onwards, and to AuNP III only at 100 μg/mL
Mulder et al. [31]	Spherical 18 nm AuNP biofunctionalized with serum albumin (BSA), poly(sodium 4-styrene sulfonate) (PSSNA), Citrate (Cit), mercaptoundecanoic acid (MUA), glutathione (GSH), polyvinylpyrrolidone (PVP) and polyethylene glicol (PEG)	Comet assay	HepG2 cells	Exposure to IC_{30} concentrations (pM): BSA (9); Cit (146); GSH (202); MUA (22); PEG (95); PSSNA (99) and PVP (138) for 3 and 24 h	After 3 h, PVP-AuNP had the greatest genotoxic effect, and PEG-AuNP the lowest. After 24 h, MUA-AuNP induced the greatest DNA damage Cells exposed to BSA-, PSSNA-, PVP- and GSH-AuNP repaired the induced DNA damage after 24 h
Rogers et al. [30]	Uncoated and PEG-coated AuNP, with 26 and 35 nm diameter, respectively. Both negatively charged	Comet assay	HK−2 cells	Exposure to 50–200 μM for 24 h	Concentration-dependent genotoxicity only for uncoated AuNP, significant at all the concentrations tested
Santos et al. [27]	9 nm gAuNP	Comet assay	CHO-K1 cells	Exposure to 0.0368–3.68 ng/mL for 24, 48 and 96 h	Genotoxicity at highest concentration after 48 h and from 0.368 ng/mL onwards after 96 h
Vales et al. [33]	AuNP (5 nm and 20 nm core sizes), functionalized with carboxylate (negative surface charge), ammonium (positive surface charge), and PEG (neutral surface charge)	Comet assay	BEAS−2B cells	Exposure to 1–250 μg/mL for 24 h	Only 5 nm PEG-AuNP induced genotoxicity at the highest concentration tested Only 20 nm ammonium AuNP induced DNA damage at 5 and 10 μg/mL
In vivo					
Barreto et al. [26]	Spherical 7 nm Cit-AuNP and PVP-AuNP, both negative charged	Comet assay Fpg-modified comet assay	Peripheral blood cells from *Sparus aurata*	Exposure to 4–1600 μg/L for 96 h	Dose-dependent genotoxicity, significant at all conditions tested No significant oxidative DNA damage was found
Cardoso et al. [28]	Spherical 10 and 30 nm negatively charged AuNP	Comet assay	Cerebral cortices isolated from brain of adult male Wister rats	Exposure to 70 mg/L for 24 h and 28 days	Both acute and chronic exposure induced DNA damage, although a higher damage was observed after chronic exposure
Khan et al. [32]	gAuNP	Comet assay	Erythrocytes of *Ctenopharyngodon idella*	Exposure to 20–40 mg/mL for 14 and 28 days	Damage increased with concentration at 14 days of exposure, while damage decreased at 28 days, possibly due to DNA repair mechanisms
Plotnikov et al. [24]	57 nm AuNP with a predominantly non-spherical shape	Comet assay	Mouse blood leukocytes of adult male BALB/c mice	Exposure to 0.01–1 mg/mL for 30 min	Genotoxicity at concentrations of 0.3 and 1 mg/mL

particular lesions that convert into breaks [23]. A relatively recent work by Barreto et al. [26] tested 7 nm AuNP with citrate and PVP coatings in *Sparus aurata*, and found no significant oxidative DNA damage in peripheral blood cells, as detected by the enzyme formamidopyrimidine DNA glycosylase (Fpg)-modified comet assay, under any of the conditions tested (4, 80 and 1600 μg/L of AuNP for 96 h) (Table 1). Di Bucchianico et al. [43] evaluated the oxidative DNA damage induced by AuNP (5 nm and 15 nm) by applying the comet assay modified with endonuclease-III (EndoIII) and Fpg enzymes. Significant dose-dependent oxidation of pyrimidines (detected by EndoIII), but not purines (detected by Fpg), was obtained in peripheral blood lymphocytes and murine macrophages (Raw264.7 cells) after AuNP treatments for 2 and 24 h.

Table 2
Studies assessing chromosomal aberrations induced by AuNP.

Study	NP tested	Technique	Cell/organism	Conditions	Results
In vitro					
Di Bucchianico et al. [52]	AuNP with an average size <10 nm prepared in deionized water or acetone by pulsed laser ablation. 3 different AuNP: - B1: 532 nm laser ablation in pure acetone and subsequently transferred to water. - B2: 1064 nm laser ablation in pure water. - B3: 1064 nm laser ablation in acetone.	CBMN (cytokinesis-block micronucleus assay)	A549 cells	Exposure to 0.0046–4.6 μg/mL for 48 h	Significant increase of MN (micronuclei) after exposure to the two highest concentrations of B1, and the highest one of B3 Nucleoplasmic bridges (NPB) frequency increased after all B1 tested concentrations, and at 2.3 μg/mL B2 exposure Nuclear buds (NBUD) were induced after all B1 exposures except the lowest concentration, and at 2.3 μg/mL B2 exposure
George et al. [20]	Spherical 14 nm citrate-coated AuNP (Cit-AuNP)	CA (Chromosomal aberrations) CBMN	CHO cells	Exposure to 6.2–50 mg/mL for 20 h	Non statistically significant damage was observed
Lebedová et al. [29]	Spherical 5 and 50 nm Cit-AuNP	Micronuclei by flow cytometry	HBEC cells	Exposure to 1–20 μg/mL for 48 h	No effects were observed
Magogotya et al. [53]	14 nm PEG-liganded carboxyl AuNP (PEG-COOH14), PEG-liganded hydroxyl AuNP (PEG-OH14) and PEG-liganded amine AuNP (PEG-NH214), 14 nm (CITR14) and 20 nm (CITR20) citrate-stabilized AuNP	CBMN	Caco−2 and HaCaT cells	Exposure to 0.5–5 nM for 2 h	All AuNP induced concentration-dependent increases in MN and NPB frequency in Caco−2 cells. Only CITR14 AuNP induced NBUD at all concentrations All AuNP induced MN in HaCaT cells after exposure to the highest concentrations of NP. NPB were observed for cells treated with all concentrations of citrate AuNP, except for CITR20 0.5 nM. NBUD were induced by the PEG-$NH_2$14 at 2 nM and 5 nM
Rogers et al. [30]	26 nm uncoated AuNP and 35 nm polyethylene glycol-coated AuNP (PEG-AuNP), both negatively charged	CA	HK−2 cells	Exposure to 6.25–100 μM for 24 h	Only uncoated AuNP induced concentration-dependent genotoxic effects
Vales et al. [33]	AuNP (5 nm and 20 nm core sizes), functionalized with carboxylate (negative surface charge), ammonium (positive surface charge), and PEG (neutral surface charge)	CBMN	BEAS−2B cells	Exposure to 1–250 μg/mL for 48 h	Only 5 nm ammonium AuNP induced significant increases in MN frequency 20 nm PEG-AuNP induced MN at almost all doses tested, while carboxylated AuNP increased MN frequency only at the lowest concentration tested
In vivo					
Barreto et al. [26]	Spherical 7 nm Cit-AuNP and polyvinylpyrrolidone-coated AuNP (PVP-AuNP), both negatively charged	Erythrocytic nuclear abnormalities (ENA) assay	Peripheral blood cells from *Sparus aurata*	Exposure to 4–1600 μg/L for 96 h	All treatments, with the exception of 4 μg/L of Cit-AuNP, led to significantly higher ENA frequency. The lobed nuclei abnormality was the most commonly detected, followed by kidney-shaped nuclei. The segmented nuclei, micronuclei and vacuolated nuclei abnormalities were the less detected
Girgis et al. [44]	15 nm PVP-AuNP	CBMN	Adult male albino mice: PCE from bone marrow	Single daily oral dose of 80–320 mg/kg for 7 and 14 days	Significant increase of MN frequency at the highest dose tested for both exposure times
Jumagazieva et al. [50]	Spherical 16 and 55 nm PEG-AuNP	CBMN	White outbred male rats: polychromatic erythrocytes (PCE) from bone marrow	Single daily oral dose of 0.25 mg/kg for 7 days	No significant changes in MN frequency
Xia et al. [51]	Spherical 5, 20 and 50 nm AuNP	CBMN	Adult male Kunming mice: polychromatic erythrocytesfrom bone marrow	Single daily intravenously dose of 0.02, 0.1 and 0.5 mg/kg for 4 days	No significant changes in MN frequency
	Spherical 5 nm AuNP			Single daily intravenously dose of 0.17 and 0.5 mg/kg for 14 days	Dose-dependent increase of MN frequencies

Opposite results regarding oxidized pyrimidines were reported by Girgis et al. [44], who studied by high performance liquid chromatography (HPLC) the generation of the DNA adduct 8-OHdG in the hepatic mice genome after exposure to 15 nm PVP-coated AuNP. They observed a significant increase in 8-OHdG levels after treatment with the medium and high doses of AuNP tested (160 and 320 mg/kg, respectively) after 7 and 14 days of exposure.

3.3. Chromosomal damage

Chromosome aberrations can be caused by DNA or chromosomal damage and there are two types: chromosome structure changes,

Table 3
Studies assessing gene expression alterations induced by AuNP.

Study	NP tested	Technique	Cell/organism	Conditions	Results
In vitro					
Falagan-Lotsch et al. [59]	Nanospheres (18 nm) with citrate (Cit) or poly(acrylic acid) (PAA) coatings and nanorods (48 × 16 nm) with PAA or polyethylene glycol (PEG) coatings	RT-PCR of 72 genes related to stress and toxicity pathways	Human dermal fibroblasts (HDF)	Chronic: exposure to 0.1 nM continuously over 20 weeks Nonchronic: initial acute cell exposure to 0.1 nM, followed by 20 weeks in NP-free cell medium	Twenty-five out of 72 genes (~35 %) were significantly differentially expressed in at least one sample type (chronic or nonchronic, any NP type) Chronic exposure altered expression levels of 12 genes, and nonchronic of 19 genes (18 of them induced by PEG rods). A total of six genes differently expressed were common for both types of exposure
In vivo					
Girgis et al. [44]	15 nm polyvinyl pyrrolidone (PVP)-coated AuNP	RT-PCR of tumor-initiating genes (CYP3A, p53, and p27) and an antioxidant gene (GST)	Adult male albino mice: liver tissue	Single daily oral dose of 80–320 mg/kg for 7 and 14 days	Exposure to the highest dose for 14 days increased the expression of CYP3A, p53 and p27 genes and decreased the expression of GST
Kinaret et al. [58]	Spherical AuNP (5 and 20 nm average core) with NH_3^+, COOH, and PEG functional groups	Microarrays of genes expressed in airways	Mice: Bronchoalveolar lavage cells (BAL cells)	Oropharyngeal aspiration of 10 μg per day for 4 days	Only modest changes were observed (97 genes differentially expressed with 5 nm AuNP and 53 with 20 nm AuNP) NH_3^+-functionalized group induced the most changes, followed by carboxylation and then PEGylation
Vecchio et al. [56]	5 nm Cit-AuNP	RT-PCR of p53 gene	Wild-type *Drosophila melanogaster*	Third instar larvae extract of flies exposed to 3 μg/g per day	Three-fold overexpression of p53 gene
Wang et al. [57]	Spherical 26 nm citric acid-coated AuNP	RNA sequencing	*Caenorhabditis elegans*	Exposure to 5.9 mg/L for 12 h	Significant increase in around 800 genes and significant decrease in 270 genes Over 60 % of them were mainly functioning in the nervous system

including deletions, duplications, inversions and translocations of sections of chromosomes, and changes in chromosome number, namely, aneuploidy or polyploidy [22]. Chromosomal alterations are often linked to a number of human pathologies, developmental defects, and accelerated ageing [45,46].

Chromosomal damage is usually scored either in mitotic cells as chromosome aberrations (CA) or in interphase cells as micronuclei (MN). These MN may be analyzed by flow cytometry (MNcyt) or by microscopic scoring frequently using the cytokinesis-block micronucleus assay (CBMN), which allows the additional assessment of other damage estimation parameters (CBMN cytome assay), such as nucleoplasmic bridges (NPB), which originate from dicentric chromosomes formed by telomere end-fusions or mis-repair of DNA strand breaks, and nuclear buds (NBUD), which represent the process of elimination of amplified DNA, DNA repair complexes and possibly extra chromosomes of aneuploid cells [47–49].

In contrast to primary DNA damage, chromosome damage is not so frequently reported after exposure to different AuNP (Table 2). Jumagazieva et al. [50], George et al. [20] and Lebedová et al. [29] concluded that this type of damage was not observed in polychromatic erythrocytes of white outbred male rats, in CHO cells, and in human bronchial epithelial HBEC cells, respectively, exposed to AuNP, even though in the latter two studies genotoxicity was detected with the comet assay. Discordant comet assay and CBMN test results were also obtained by Vales et al. [33] in BEAS-2B cells, since the CBMN assay showed genotoxicity caused by 5 nm ammonium-coated AuNP and 20 nm PEGylated and carboxylated AuNP, while in the comet assay positive results were observed for 5 nm PEG-coated AuNP and 20 nm ammonium-coated AuNP. However, the results obtained by Rogers et al. [30] in human kidney HK-2 cells in the chromosome aberration test agreed with those of the primary DNA damage evaluation, where genotoxicity was only observed in cells exposed to the uncoated AuNP.

Barreto et al. [26], carried out the erythrocytic nuclear abnormalities (ENA) assay after exposure of *Sparus aurata* to 7 nm AuNP coated with citrate and PVP. All treatments, with the exception of 4 μg/L of citrate-coated AuNP, led to significantly higher ENA frequency. The lobed nuclei abnormality was the most commonly detected in all treatments, followed by kidney-shaped nuclei. The segmented nuclei, micronuclei and vacuolated nuclei abnormalities were the less detected. Xia et al. [51] found differences in MN induction in adult Kunming mice intravenously administered with AuNP depending on the NP size and the exposure time. In particular, AuNP (5, 20 or 50 nm) were found not to produce MN in erythrocytes of animals exposed for 4 days. However, when the exposure period was extended to 14 days, 5 nm AuNP caused significant clastogenic damage, with a dose-dependent increase of MN frequencies.

Other studies testing AuNP < 15 nm detected significant increases in MN frequency at high concentrations, both *in vivo* in albino mice at a dose of 320 mg/kg [44] and *in vitro* in A549 cells at doses of 2.3 and 4.6 μg/mL [52]. In addition, the latter one also showed a significant increase in the frequency of NPB and NBUD after AuNP exposure.

Additionally, Magogotya et al. [53] observed differences depending on the cell type when testing 14 and 20 nm AuNP differently coated. In human colorectal Caco-2 cells, a concentration-dependent increase in MN and NPB rates was observed for all AuNP, while only 14 nm citrate-coated AuNP induced NBUD production. In contrast, in HaCaT human keratinocytes, MN and NPB were just detected at the highest concentrations (2 and 5 nM) tested of all AuNP, and only 14 nm PEG-NH_2-coated AuNP induced NBUD production at the same concentrations.

3.4. Gene expression alterations

In addition to the more direct effects on the genetic material described in previous sections, the existing evidence demonstrates that NP are often able to modulate gene expression, showing both up- and down-regulation, even though the exact mechanisms are not known yet [54,55]. Accordingly, treatment with AuNP was also found to induce alterations in expression of some genes in a variety of *in vitro* and *in vivo* systems (Table 3).

Girgis et al. [44] analysed the expression of three tumour-initiating genes (CYP3A, p53 and p27) and one antioxidant gene (GST) in mice liver tissues after exposure to different doses of 15 nm PVP-coated AuNP. Exposure to the highest dose (320 mg/kg) increased the

Table 4
Studies assessing the effects on epigenetic regulation induced by AuNP.

Study	NP tested	Technique	Cell/organism	Conditions	Results
In vitro					
Brzóska et al. [62]	20 nm citrate-coated AuNP (Cit-AuNP)	miRNA expression analysis by real-time PCR Global DNA-methylation analysis	HepG2 cells	Exposure to 10 μg/mL for 6 h Exposure to 10 μg/mL for 24 h	Of 131 miRNAs related to inflammatory response and apoptosis, AuNP affected 4 of them (2 of them down-regulated and the other two up-regulated) No significant changes in global DNA methylation status
Huang et al. [63]	Spherical 22 nm AuNP	miRNA expression analysis by SOLiD sequencing technology	HDF cells	Exposure to 200 μM for 1, 4 and 8 h	Of 650 miRNAs, 202 were differentially expressed (109, 78 and 124 by 1, 4 and 8 h, respectively), mainly related to metabolic processes
Ng et al. [64]	20 nm AuNP	miRNA expression analysis by microarray and RT-PCR	Human MRC5 dermal fibroblasts	Exposure to 1 nM for 72 h	Significantly altered the expression of 19 genes, with up-regulation of 9 genes and down-regulation of 10 genes
Patil et al. [70]	Spherical 12–90 nm (mostly in the 23–30 nm range) AuNP	Global DNA-methylation analysis	HDF and A375 cells	Exposure to 62.5 μg/mL for 24 and 48 h	HDF cells: DNA hypermethylation after 24 h of treatment followed by DNA hypo-methylation at 48 h of AuNP exposure. A375 cells: DNA hypermethylation after 24 and 48 h of treatment
Smolkova et al. [68]	20 nm AuNP	Global DNA-methylation analysis. Gene-specific DNA-methylation analysis: 3 metastasis-associated genes (*TIMP3, ADAM23* and *BRMS1*)	SK-BR−3 cells	Exposure to 3 μg/mL for 24, 48 and 72 h	No significant changes neither global nor gene-specific DNA methylation
Sooklert et al. [67]	Spherical 23 nm AuNP	Global DNA-methylation analysis	HEK293 and HaCaT cells	Exposure to 100 μg/mL for 72 h	No significant changes in global DNA methylation status
In vivo					
Ali et al. [65]	Spherical 25 nm Cit-AuNP	miRNA expression analysis by quantitative real-time RT-PCR of miR−155 and two of its putative target genes (*PROS1* and *TP531NP1*)	Adult male Wister rats (*Rattus norvegicus*): lung tissue	Single intravenous injection of 0.015 mg/kg for 1 day, 1 week, 1 month and 2 months	Significantly increased expression of miR−155 and reduced mRNA expression of *TP53INP1* and *PROS1* genes at 1-day post-injection. In contrast, a significant down-regulation of miR−155 level of expression concurrent with up-regulation of expression level of *TP53INP1* and *PROS1* genes were shown after 1 week, 1 month and 2 months
Balansky et al. [66]	Spherical 40 and 100 nm AuNP	miRNA expression analysis by microarray	Swiss mice: lung and liver tissue of fetuses on day 18 of pregnancy	Intraperitoneal doses of 3.3 mg/kg on days 10, 12, 14 and 17 of gestation	Administration of AuNP−100 nm to pregnant mice alters miRNA expression by increasing the number of up-regulated miRNAs AuNP−40 nm do not produce adverse effects.
Tabish et al. [69]	5, 60 and 250 nm Cit- AuNP	Global DNA-methylation analysis Gene-specific DNA-methylation analysis: 17 genes involved in oxidative stress response, immune, cell cycle regulation and DNA methylation pathways	Male BALB/c mice: lung tissue Male BALB/c mice: lung tissue and blood	Single intra-tracheal instillation of 0.25 and 2.5 mg/kg for 48 h	No significant changes in global DNA methylation status 60 and 250 nm AuNP modified the methylation of genes, both hyper and hypomethylation, whereas 5 nm AuNP did not

expression of all three tumour-initiating genes after 7 and 14 days of exposure, while decreased the expression of GST at 14 days. The expression of p53 gene was also studied by Vecchio et al. [56] in the third instar larvae of the fruit fly (*Drosophila melanogaster*) and it was found to be approximately threefold overexpressed after treatment with 5 nm citrate-coated AuNP.

Wang et al. [57] addressed by RNA sequencing the alterations in gene expression after exposure of the nematode *Caenorhabditis elegans* to 5.9 mg/L 26 nm citrate-coated AuNP for 12 h, and observed over-expression of about 800 genes and underexpression of 270 genes; approximately 60 % of these genes function mainly in the nervous system.

Kinaret et al. [58] used microarrays to follow changes in the expression of genes expressed in the airways of mice after exposure to 5 and 20 nm AuNP coated with NH_3^+, COOH and PEG as functional groups. Ninety-seven differentially expressed genes were observed after the 5 nm AuNP treatment, and 53 genes for the 20 nm AuNP treatment. Regarding functionalisation, NH_3^+-capped AuNP group induced the most changes in gene expression, followed by COOH- and PEG-capped AuNP.

Genes related to stress and toxicity pathways have also been studied. The study by Falagan-Lotsch et al. [59] evaluated the effect of 18 nm gold nanospheres with citrate and poly(acrylic acid, sodium salt) (PAA) coatings and 48x16 nm gold nanorods with PEG and PAA coatings on 72 genes in human dermal fibroblasts after chronic and non-chronic exposure. About 35 % of the genes were differentially expressed in at least one experimental condition (chronic or non-chronic, any NP type). In the chronic exposure, 12 genes presented altered levels of expression, and 19 genes in the non-chronic exposure (18 of them induced by PEG-coated nanorods). A total of six genes differentially expressed were common between both types of exposure (*VEGFA, CCL2, PRDX1, EDN1, NQO1*, and *HSPA5*).

3.5. Effects on epigenetic regulation

Together with the genetic injuries previously mentioned detected by the most frequently employed cytogenetic assays, AuNP were also reported to induce some effects on epigenetic regulation (Table 4). Epigenetics refers to heritable changes in gene expression that occur without alterations in DNA sequence, including DNA methylation status, post-translational modifications of histones and non-coding RNAs (ncRNA), particularly microRNAs (miRNA) and long non-coding RNAs (lncRNA) [54,60]. Several studies have revealed that nanomaterials can induce epigenetic alterations, and these may occur even in the absence of cytotoxic and genotoxic effects [54,61]. The genes affected are mainly involved in the regulation of the epigenetic machinery itself, as well as in pathways related to apoptosis, cell cycle, DNA repair and

inflammation; hence, epigenetic modifications caused by nanomaterials should be considered when assessing their risk to human health [54,60].

One of the most frequently addressed epigenetic mechanisms is miRNA expression, and studies are fairly consistent in that AuNP alter their expression. Brzósca et al. [62], Huang et al. [63] and Ng et al. [64] evaluated *in vitro* the alteration of miRNA expression caused by AuNP of around 20 nm in human HepG2 hepatic cells, human NHDF dermal fibroblasts, and human MRC5 dermal fibroblasts, respectively. Despite the different genes studied, involved in diverse processes, and the different exposure times and doses of AuNP tested, they all demonstrated a significant alteration of the expression pattern, either up- or down-regulation. Similar effects were observed in some *in vivo* studies using AuNP of also 20 nm in Wister rats [65]and of 100 nm in Swiss mice [66]. However, the latter one found no effect after treatment with 40 nm AuNP.

Another common epigenetic outcome is the assessment of changes in DNA methylation, which can be evaluated globally or gene-specifically. Regarding global DNA methylation, Brzósca et al. [62], Sooklert et al. [67] and Smolkova et al. [68] studied the effect of AuNP (20 nm) in different cell lines, namely HepG2 cells, human HEK-293 embryonic kidney cells and human HaCaT keratinocyte cells, and human SK-BR-3 breast cancer cells, respectively. They concluded that AuNP do not affect the global methylation pattern, despite the different doses and exposure times (3–100 μg/L of AuNP for 24–72h). These results are supported by the *in vivo* study by Tabish et al. [69] where no alterations in DNA methylation were observed after treatment of BALB/c mice with 5, 60 and 250 nm AuNP for 48 h. Nevertheless, Patil et al. [70] observed modifications in DNA methylation, both hyper- and hypomethylation, in human NHDF and A375 skin melanoma cancer cells.

Gene-specific methylation effects have been barely addressed after AuNP exposure. Results obtained by Smolkova et al. [68] and Tabish et al. [69] are controversial. The first one showed that AuNP do not alter the methylation of some metastasis-associated genes (*TIMP3*, *ADAM23* and *BRMS1*), while Tabish et al. [69] demonstrated that 60 and 250 nm AuNP modify the methylation of genes related to oxidative stress response, immune, cell cycle regulation and DNA methylation pathways, whereas 5 nm AuNP do not.

4. Gaps and future needs

Due to their particular properties, AuNP are promising tools for a number of future biomedical applications. What enables AuNP to be fine-tuned for each application is that they present modifiable sizes and shapes, and straightforward surface modification [1]. However, these same properties may also affect their biocompatibility as well as make it difficult to establish their toxicological profile [8].

In particular, in this review, Au NP size, shape, or coating type were found as the physical-chemical properties affecting the most their biological behaviour. AuNP shape can be modelled into a variety of forms, such as spheres, rods, triangles, stars, boxes, clusters, etc. [8,71]. More atoms at angles and edges may cause stronger interactions with biomolecules; thus, higher toxicity is often observed for rods and stars than for spherical NP [8]. Furthermore, AuNP toxicity can be highly modulated by surface coatings or functionalisation due to the chemistry and charge of the groups [30,33]. Moreover, surface coating is often necessary for ensuring stability and biocompatibility of AuNP [53]. Conjugation with a large number of ligands such as polymers, peptides, nucleic acids, antibodies, and drugs has been used to design AuNP with the appropriate characteristics for particular applications [8]. Other variables such as AuNP surface charge or the exposed cell type/-organism, were also found to influence the effects observed after AuNP exposure.

Suitability of the traditional genotoxicity assays for NM assessment remains under debate, with more and more authors claiming for specific adaptations for NM testing, mainly due to their potential interference with assays when applying the usual test protocols [72–75]. A number of studies have shown that NM may interact with test components or equipment, leading to false positive/negative results. Therefore, it is imperative, if not to develop novel more appropriate specific assays, at least to test the possible interference of NM with the currently established methodologies to confirm the reliability of results obtained. Thus, together with the high variability in results from toxicity studies testing AuNP, data reported from many of these studies may be unreliable since potential interference was not discarded, or specific adaptation of the protocols to NM testing was not followed in most cases. This is particularly alarming in studies employing the comet assay, since NP interference with the comet assay protocol, not only due to direct contact between residual intracellular NP and DNA during the assay procedure but also with the repair enzymes employed in enzyme-modified versions, is well-established [20,22]. Similarly, in the CBMN assay, NP were found to induce interference with cellular uptake, due to the use of cytochalasin-B, leading to possible false negative results that need to be addressed [76,77]. Indeed, OECD testing guidelines for CA assay (OECD TG 473) and MN assay (OECD TG 487) (the latter recently updated in July 2023) included the following statement `*For manufactured nanomaterials, specific adaptations of this Test Guideline may be needed but are not described in this Test Guideline*', in allusion to the possible interference of NM with the traditional procedures and/or the necessity of specific protocols for NM testing.

Nevertheless, a recent analysis highlighted that the challenge of nanomaterial-induced interference was not explicitly addressed in more than 90 % of the nanotoxicity papers published up to 2014 [75]. Similarly, in the present review, only two studies assessed the presence of interference prior carrying out the genotoxicity testing [20,53]. George et al. [20] observed interference from AuNP in the comet assay, while no interference was obtained in the CA and MN assays, which may be the cause of the differences in results. The lack of interference in the micronucleus assay was also confirmed by Magogotya et al. [53]. Furthermore, Vales et al. [33] also suggested interference from AuNP as an explanation for the inconsistencies among genotoxicity results, highlighting the importance of conducting interference studies.

5. Conclusions

Most reviewed studies on AuNP genotoxicity reported induction of primary DNA damage and also chromosomal instability. This indicates that AuNP exposure induces a kind of damage of lower relevance that can be mostly easily repaired (e.g., SSB, abasic sites, alkali-labile sites). But, under certain circumstances, these NP may also cause damage with higher significance (e.g., DSB or chromosomal loss), which become fixed in the cells as chromosome alterations (i.e., CA or MN). Furthermore, alterations in epigenetic mechanisms, namely miRNA expression patterns and changes in gene-specific methylation levels, were also reported after exposure to AuNP. Still, further research on the specific action mechanisms underlying these effects as well as detailed studies addressing repair capacity after AuNP exposure are needed.

Physical-chemical factors, such as shape, size, surface charge or functionalisation, as well as biological factors, including cell/model type, were described to have prominent influence on the observed effects. Considering the influence of these physical-chemical properties on their biological impact, characterization of AuNP in both solvent and culture medium or biological fluids is highly encouraged in all further nanotoxicity studies.

More accurate protocols including proper interference testing, and international guidelines on nanogenotoxicity assessment are necessary in order to improve the reliability, comparability and reusability of toxicity results. Furthermore, to obtain more accurate and representative toxicological data on AuNP, there is an imperative need to increase the number of in vivo studies, particularly those testing doses adapted to real world scenarios, i.e. considering the doses that can be absorbed/introduced in the organism depending on the intended NP use, to develop more physiologically relevant *in vitro* models (3D cultures,

cocultures of several cell types), and to validate adequate assessment methods.

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CRediT authorship contribution statement

Assia Touzani: Methodology. **Natalia Fernández-Bertólez:** Writing – review & editing, Formal analysis. **Blanca Laffon:** Writing – review & editing, Formal analysis, Conceptualization. **Lucía Ramos-Pan:** Writing – original draft, Methodology. **Sónia Fraga:** Writing – review & editing, Conceptualization. **Vanessa Valdiglesias:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No new data was generated in this review

References

[1] N.H.A. Nguyen, P. Falagan-Lotsch, Mechanistic insights into the biological effects of engineered nanomaterials: a focus on gold nanoparticles, Int. J. Mol. Sci. 24 (2023) 4109, https://doi.org/10.3390/ijms24044109.
[2] B.A. Omran, Fundamentals of Nanotechnology and Nanobiotechnology, in: B. A. Omran (Ed.), Nanobiotechnology: A Multidisciplinary Field of Science, Springer International Publishing, Cham, 2020, pp. 1–36, https://doi.org/10.1007/978-3-030-46071-6_1.
[3] J. Ramsden, What is nanotechnology?. in: Nanotechnology: An Introduction Elsevier Inc, 2016, pp. 1–18, https://doi.org/10.1016/B978-0-323-39311-9.00007-8.
[4] S. Bayda, M. Adeel, T. Tuccinardi, M. Cordani, F. Rizzolio, The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine, Molecules 25 (2019) 112, https://doi.org/10.3390/molecules25010112.
[5] S. Medici, M. Peana, D. Coradduzza, M.A. Zoroddu, Gold nanoparticles and cancer: detection, diagnosis and therapy, YSCBI 76 (2021) 27–37, https://doi.org/10.1016/j.semcancer.2021.06.017.
[6] A.A. Yaqoob, H. Ahmad, T. Parveen, A. Ahmad, M. Oves, I.M.I. Ismail, H.A. Qari, K. Umar, M.N. Mohamad Ibrahim, Recent advances in metal decorated nanomaterials and their various biological applications: a review, Front. Chem. 8 (2020) 341, https://doi.org/10.3389/fchem.2020.00341.
[7] S. Fan, Y. Zheng, X. Liu, W. Fang, X. Chen, W. Liao, X. Jing, M. Lei, E. Tao, Q. Ma, X. Zhang, R. Guo, J. Liu, Curcumin-loaded PLGA-PEG nanoparticles conjugated with B6 peptide for potential use in Alzheimer's disease, Drug Deliv. 25 (2018) 1091–1102, https://doi.org/10.1080/10717544.2018.1461955.
[8] M. Kus-Liśkiewicz, P. Fickers, I. Ben Tahar, Biocompatibility and cytotoxicity of gold nanoparticles: recent advances in methodologies and regulations, Int. J. Mol. Sci. 22 (2021) 10952, https://doi.org/10.3390/ijms222010952.
[9] I. Ielo, G. Rando, F. Giacobello, S. Sfameni, A. Castellano, M. Galletta, D. Drommi, G. Rosace, M.R. Plutino, Synthesis, chemical–physical characterization, and biomedical applications of functional gold nanoparticles: a review, Molecules 26 (2021) 5823, https://doi.org/10.3390/molecules26195823.
[10] Y.-P. Jia, B.-Y. Ma, X.-W. Wei, Z.-Y. Qian, The *in vitro* and *in vivo* toxicity of gold nanoparticles, CCL 28 (2017) 691–702, https://doi.org/10.1016/j.cclet.2017.01.021.
[11] S. May, C. Hirsch, A. Rippl, N. Bohmer, J.-P. Kaiser, L. Diener, A. Wichser, A. Bürkle, P. Wick, Transient DNA damage following exposure to gold nanoparticles, Nanoscale 10 (2018) 15723–15735, https://doi.org/10.1039/c8nr03612h.
[12] X.-Y. Liu, J.-Q. Wang, C.R. Ashby, L. Zeng, Y.-F. Fan, Z.-S. Chen, Gold nanoparticles: synthesis, physiochemical properties and therapeutic applications in cancer, Drug Discov. Today 26 (2021) 1284–1292, https://doi.org/10.1016/j.drudis.2021.01.030.
[13] A. Sani, C. Cao, D. Cui, Toxicity of gold nanoparticles (AuNPs): a review, BB Rep. 26 (2021) 100991, https://doi.org/10.1016/j.bbrep.2021.100991.
[14] A.F. Burlec, A. Corciova, M. Boev, D. Batir-Marin, C. Mircea, O. Cioanca, G. Danila, M. Danila, A.F. Bucur, M. Hancianu, Current overview of metal nanoparticles' synthesis, characterization, and biomedical applications, with a focus on silver and gold nanoparticles, Pharmaceuticals 16 (2023) 1410, https://doi.org/10.3390/ph16101410.
[15] D.F. Báez, E. Gallardo-Toledo, M.P. Oyarzún, E. Araya, M.J. Kogan, The influence of size and chemical composition of silver and gold nanoparticles on in vivo toxicity with potential applications to central nervous system diseases, Int. J. Nanomed. 16 (2021) 2187–2201, https://doi.org/10.2147/IJN.S260375.
[16] T. Morais, M.E. Soares, J.A. Duarte, L. Soares, S. Maia, P. Gomes, E. Pereira, S. Fraga, H. Carmo, M. de L. Bastos, Effect of surface coating on the biodistribution profile of gold nanoparticles in the rat, Eur. J. Pharm. Biopharm. 80 (2012) 185–193, https://doi.org/10.1016/j.ejpb.2011.09.005.
[17] S. Fraga, A. Brandão, M.E. Soares, T. Morais, J.A. Duarte, L. Pereira, L. Soares, C. Neves, E. Pereira, M. de L. Bastos, H. Carmo, Short- and long-term distribution and toxicity of gold nanoparticles in the rat after a single-dose intravenous administration, Nanomedicine 10 (2014) 1757–1766, https://doi.org/10.1016/j.nano.2014.06.005.
[18] M. Xu, M.G. Soliman, X. Sun, B. Pelaz, N. Feliu, W.J. Parak, S. Liu, How entanglement of different physicochemical properties complicates the prediction of in vitro and in vivo interactions of gold nanoparticles, ACS Nano 12 (2018) 10104–10113, https://doi.org/10.1021/acsnano.8b04906.
[19] R. Guadagnini, B. Halamoda Kenzaoui, L. Walker, G. Pojana, Z. Magdolenova, D. Bilanicova, M. Saunders, L. Juillerat-Jeanneret, A. Marcomini, A. Huk, M. Dusinska, L.M. Fjellsbø, F. Marano, S. Boland, Toxicity screenings of nanomaterials: challenges due to interference with assay processes and components of classic in vitro tests, Nanotoxicology 9 (Suppl 1) (2015) 13–24, https://doi.org/10.3109/17435390.2013.829590.
[20] J.M. George, M. Magogotya, M.A. Vetten, A.V. Buys, M. Gulumian, From the cover: an investigation of the genotoxicity and interference of gold nanoparticles in commonly used in vitro mutagenicity and genotoxicity assays, Toxicol. Sci. 156 (2017) 149–166, https://doi.org/10.1093/toxsci/kfw247.
[21] R. Landsiedel, N. Honarvar, S.B. Seiffert, B. Oesch, F. Oesch, Genotoxicity testing of nanomaterials, WIREs Nanomed. Nanobiotechnol. 14 (2022) e1833, https://doi.org/10.1002/wnan.1833.
[22] Z. Magdolenova, A. Collins, A. Kumar, A. Dhawan, V. Stone, M. Dusinska, Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles, Nanotoxicology 8 (2014) 233–278, https://doi.org/10.3109/17435390.2013.773464.
[23] A. Collins, P. Møller, G. Gajski, S. Vodenková, A. Abdulwahed, D. Anderson, E. E. Bankoglu, S. Bonassi, E. Boutet-Robinet, G. Brunborg, C. Chao, M.S. Cooke, C. Costa, S. Costa, A. Dhawan, J. de Lapuente, C.D. Bo', J. Dubus, M. Dusinska, S. J. Duthie, N.E. Yamani, B. Engelward, I. Gaivão, L. Giovannelli, R. Godschalk, S. Guilherme, K.B. Gutzkow, K. Habas, A. Hernández, O. Herrero, M. Isidori, A. N. Jha, S. Knasmüller, I.M. Kooter, G. Koppen, M. Kruszewski, C. Ladeira, B. Laffon, M. Larramendy, L.L. Hégarat, A. Lewies, A. Lewinska, G.E. Liwszyc, A.L. de Cerain, M. Manjanatha, R. Marcos, M. Milić, V.M. de Andrade, M. Moretti, D. Muruzabal, M. Novak, R. Oliveira, A.-K. Olsen, N. Owiti, M. Pacheco, A.K. Pandey, S. Pfuhler, B. Pourrut, K. Reisinger, E. Rojas, E. Rundén-Pran, J. Sanz-Serrano, S. Shaposhnikov, V. Sipinen, K. Smeets, H. Stopper, J.P. Teixeira, V. Valdiglesias, M. Valverde, F. van Acker, F.-J. van Schooten, M. Vasquez, J.F. Wentzel, M. Wnuk, A. Wouters, B. Žegura, T. Zikmund, S.A.S. Langie, A. Azqueta, Measuring DNA modifications with the comet assay: a compendium of protocols, Nat. Protoc. 18 (2023) 929–989, https://doi.org/10.1038/s41596-022-00754-y.
[24] E. Plotnikov, S.P. Zhuravkov, A. Gapeyev, V. Plotnikov, D. Martemiyanov, Investigation of genotoxicity of gold nanoparticles prepared by the electric spark dispersion method, Adv. Mat. Res. 1040 (2014) 65–69, https://doi.org/10.4028/www.scientific.net/AMR.1040.65.
[25] M.N. Bin-Jumah, M. Al-Abdan, G. Al-Basher, S. Alarifi, Molecular mechanism of cytotoxicity, genotoxicity, and anticancer potential of green gold nanoparticles on human liver normal and cancerous cells, Dose Response 18 (2020), 1559325820912154. https://doi.org/10.1177/1559325820912154.
[26] A. Barreto, A. Dias, B. Duarte, E. Pinto, A. Almeida, T. Trindade, A.M.V.M. Soares, K. Hylland, S. Loureiro, M. Oliveira, Biological effects and bioaccumulation of gold in gilthead seabream (Sparus aurata) - Nano versus ionic form, Sci. Total Environ. 716 (2020) 137026, https://doi.org/10.1016/j.scitotenv.2020.137026.
[27] N.M. Santos, A.S. Gomes, D.G.S.M. Cavalcante, L.F. Santos, S.R. Teixeira, F. C. Cabrera, A.E. Job, Green synthesis of colloidal gold nanoparticles using latex from Hevea brasiliensis and evaluation of their in vitro cytotoxicity and genotoxicity, IET Nanobiotechnol. 13 (2019) 307–315, https://doi.org/10.1049/iet-nbt.2018.5225.
[28] E. Cardoso, G.T. Rezin, E.T. Zanoni, F. de Souza Notoya, D.D. Leffa, A.P. Damiani, F. Daumann, J.C.O. Rodriguez, R. Benavides, L. da Silva, V.M. Andrade, M.M. da Silva Paula, Acute and chronic administration of gold nanoparticles cause DNA damage in the cerebral cortex of adult rats, Mutat. Res. 766–767 (2014) 25–30, https://doi.org/10.1016/j.mrfmmm.2014.05.009.
[29] J. Lebedová, Y.S. Hedberg, I. Odnevall Wallinder, H.L. Karlsson, Size-dependent genotoxicity of silver, gold and platinum nanoparticles studied using the mini-gel comet assay and micronucleus scoring with flow cytometry, Mutagenesis 33 (2018) 77–85, https://doi.org/10.1093/mutage/gex027.
[30] C.R. Rogers, S. Dasari, A.K. Patlolla, P.B. Tchounwou, Physico-chemical characterization and assessment of cytotoxic and genotoxic effects of poly-ethylene-glycol coated and uncoated gold nanoparticles on human kidney (HK-2) cells, Austin J. Environ. Toxicol. 7 (2021) 1042.

[31] D. Mulder, C.J.F. Taute, M. van Wyk, P.J. Pretorius, A comparison of the genotoxic effects of gold nanoparticles functionalized with seven different ligands in cultured human hepatocellular carcinoma cells, Nanomaterials 12 (2022) 1126, https://doi.org/10.3390/nano12071126.
[32] A. Khan, M. Khan, N. Shah, M. Khan, A. Dawar, A.A. Shah, F. Dawar, M. Khisroon, Genotoxicity of copper, silver and green synthetic gold nanoparticles in fish (Ctenopharyngodon idella), Biol. Trace Elem. Res. (2023), https://doi.org/10.1007/s12011-023-03877-9.
[33] G. Vales, S. Suhonen, K.M. Siivola, K.M. Savolainen, J. Catalán, H. Norppa, Size, surface functionalization, and genotoxicity of gold nanoparticles in vitro, Nanomaterials 10 (2020) 271, https://doi.org/10.3390/nano10020271.
[34] N. Mahaye, S.K. Learend, N. Musee, Cytotoxicity and genotoxicity of coated-gold nanoparticles on freshwater algae Pseudokirchneriella subcapitata, Aquat. Toxicol. 236 (2021) 105865, https://doi.org/10.1016/j.aquatox.2021.105865.
[35] J.-A. Sergent, V. Paget, S. Chevillard, Toxicity and genotoxicity of nano-SiO2 on human epithelial intestinal HT-29 cell line, Ann. Occup. Hyg. 56 (2012) 622–630, https://doi.org/10.1093/annhyg/mes005.
[36] S. Fraga, H. Faria, M.E. Soares, J.A. Duarte, L. Soares, E. Pereira, C. Costa-Pereira, J.P. Teixeira, M. de Lourdes Bastos, H. Carmo, Influence of the surface coating on the cytotoxicity, genotoxicity and uptake of gold nanoparticles in human HepG2 cells, J. Appl. Toxicol. 33 (2013) 1111–1119, https://doi.org/10.1002/jat.2865.
[37] M. Horie, Y. Tabei, Role of oxidative stress in nanoparticles toxicity, Free Radic. Res. 55 (2021) 331–342, https://doi.org/10.1080/10715762.2020.1859108.
[38] M. Horie, H. Fukui, K. Nishio, S. Endoh, H. Kato, K. Fujita, A. Miyauchi, A. Nakamura, M. Shichiri, N. Ishida, S. Kinugasa, Y. Morimoto, E. Niki, Y. Yoshida, H. Iwahashi, Evaluation of acute oxidative stress induced by NiO nanoparticles in vivo and in vitro, J. Occup. Health 53 (2011) 64–74, https://doi.org/10.1539/joh.l10121.
[39] P. Chairuangkitti, S. Lawanprasert, S. Roytrakul, S. Aueviriyavit, D. Phummiratch, K. Kulthong, P. Chanvorachote, R. Maniratanachote, Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways, Toxicol. Vitr. 27 (2013) 330–338, https://doi.org/10.1016/j.tiv.2012.08.021.
[40] N. Fernández-Bertólez, C. Costa, M.J. Bessa, M. Park, M. Carriere, F. Dussert, J. P. Teixeira, E. Pásaro, B. Laffon, V. Valdiglesias, Assessment of oxidative damage induced by iron oxide nanoparticles on different nervous system cells, Mutat. Res. Gen. Tox En. 845 (2019) 402989, https://doi.org/10.1016/j.mrgentox.2018.11.013.
[41] N. Nishida, T. Arizumi, M. Takita, S. Kitai, N. Yada, S. Hagiwara, T. Inoue, Y. Minami, K. Ueshima, T. Sakurai, M. Kudo, Reactive oxygen species induce epigenetic instability through the formation of 8-hydroxydeoxyguanosine in human hepatocarcinogenesis, Dig. Dis. 31 (2013) 459–466, https://doi.org/10.1159/000355245.
[42] M. Yasui, Y. Kanemaru, N. Kamoshita, T. Suzuki, T. Arakawa, M. Honma, Tracing the fates of site-specifically introduced DNA adducts in the human genome, DNA Repair 15 (2014) 11–20, https://doi.org/10.1016/j.dnarep.2014.01.003.
[43] S. Di Bucchianico, M.R. Fabbrizi, S. Cirillo, C. Uboldi, D. Gilliland, E. Valsami-Jones, L. Migliore, Aneuploidogenic effects and DNA oxidation induced in vitro by differently sized gold nanoparticles, Int. J. Nanomed. 9 (2014) 2191–2204, https://doi.org/10.2147/IJN.S58397.
[44] E. Girgis, W.K.B. Khalil, A.N. Emam, M.B. Mohamed, K.V. Rao, Nanotoxicity of gold and gold-cobalt nanoalloy, Chem. Res. Toxicol. 25 (2012) 1086–1098, https://doi.org/10.1021/tx300053h.
[45] B.L. Daughtry, S.L. Chavez, Chromosomal instability in mammalian pre-implantation embryos: potential causes, detection methods, and clinical consequences, Cell Tissue Res. 363 (2016) 201–225, https://doi.org/10.1007/s00441-015-2305-6.
[46] C.V. Rao, A.S. Asch, H.Y. Yamada, Emerging links among chromosome instability (CIN), cancer, and aging, Mol. Carcinog. 56 (2017) 791–803, https://doi.org/10.1002/mc.22539.
[47] M. Fenech, Cytokinesis-block micronucleus cytome assay, Nat. Protoc. 2 (2007) 1084–1104, https://doi.org/10.1038/nprot.2007.77.
[48] M. Fenech, M. Kirsch-Volders, A.T. Natarajan, J. Surralles, J.W. Crott, J. Parry, H. Norppa, D.A. Eastmond, J.D. Tucker, P. Thomas, Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells, Mutagenesis 26 (2011) 125–132, https://doi.org/10.1093/mutage/geq052.
[49] M. Fenech, Cytokinesis-block micronucleus cytome assay evolution into a more comprehensive method to measure chromosomal instability, Genes 11 (2020) 1203, https://doi.org/10.3390/genes11101203.
[50] D.S. Jumagazieva, G.N. Maslyakova, L.V. Suleymanova, A.B. Bucharskaya, S. S. Firsova, B.N. Khlebtsov, G.S. Terentyuk, S.M. Kong, N.G. Khlebtsov, Mutagenic effect of gold nanoparticles in the micronucleus assay, Bull. Exp. Biol. Med. 151 (2011) 731–733, https://doi.org/10.1007/s10517-011-1427-4.
[51] Q. Xia, H. Li, Y. Liu, S. Zhang, Q. Feng, K. Xiao, The effect of particle size on the genotoxicity of gold nanoparticles, J. Biomed. Mater. Res. A 105 (2017) 710–719, https://doi.org/10.1002/jbm.a.35944.
[52] S. Di Bucchianico, L. Migliore, P. Marsili, C. Vergari, F. Giammanco, E. Giorgetti, Cyto- and genotoxicity assessment of Gold nanoparticles obtained by laser ablation in A549 lung adenocarcinoma cells, J. Nanopart. Res. 17 (2015) 213, https://doi.org/10.1007/s11051-015-3023-4.
[53] M. Magogotya, M. Vetten, M.P. Roux-van der Merwe, J. Badenhorst, M. Gulumian, In vitro toxicity and internalization of gold nanoparticles (AuNPs) in human epithelial colorectal adenocarcinoma (Caco-2) cells and the human skin keratinocyte (HaCaT) cells, MRGTEM 883–884 (2022) 503556, https://doi.org/10.1016/j.mrgentox.2022.503556.
[54] L. Moreira, C. Costa, J. Pires, J.P. Teixeira, S. Fraga, How can exposure to engineered nanomaterials influence our epigenetic code? A review of the mechanisms and molecular targets, MRR 788 (2021) 108385, https://doi.org/10.1016/j.mrrev.2021.108385.
[55] M. Kulasza, L. Skuza, Changes of gene expression patterns from aquatic organisms exposed to metal nanoparticles, Int. J. Environ. Res. Public Health 18 (2021) 8361, https://doi.org/10.3390/ijerph18168361.
[56] G. Vecchio, A. Galeone, V. Brunetti, G. Maiorano, L. Rizzello, S. Sabella, R. Cingolani, P.P. Pompa, Mutagenic effects of gold nanoparticles induce aberrant phenotypes in Drosophila melanogaster, Nanomedicine 8 (2012) 1–7, https://doi.org/10.1016/j.nano.2011.11.001.
[57] M. Wang, Z. Zhang, N. Sun, B. Yang, J. Mo, D. Wang, M. Su, J. Hu, M. Wang, L. Wang, Gold nanoparticles reduce food sensation in caenorhabditis elegans via the voltage-gated channel EGL-19, Int. J. Nanomed. 18 (2023) 1659–1676, https://doi.org/10.2147/IJN.S394666.
[58] P.A.S. Kinaret, J. Ndika, M. Ilves, H. Wolff, G. Vales, H. Norppa, K. Savolainen, T. Skoog, J. Kere, S. Moya, R.D. Handy, P. Karisola, B. Fadeel, D. Greco, H. Alenius, Toxicogenomic profiling of 28 nanomaterials in mouse airways, Adv. Sci. 8 (2021) 2004588, https://doi.org/10.1002/advs.202004588.
[59] P. Falagan-Lotsch, E.M. Grzincic, C.J. Murphy, One low-dose exposure of gold nanoparticles induces long-term changes in human cells, PNAS 113 (2016) 13318–13323, https://doi.org/10.1073/pnas.1616400113.
[60] M. Dusinska, J. Tulinska, N. El Yamani, M. Kuricova, A. Liskova, E. Rollerova, E. Rundén-Pran, B. Smolkova, Immunotoxicity, genotoxicity and epigenetic toxicity of nanomaterials: New strategies for toxicity testing? Food Chem. Toxicol. 109 (2017) 797–811, https://doi.org/10.1016/j.fct.2017.08.030.
[61] M. Pogribna, G. Hammons, Epigenetic effects of nanomaterials and nanoparticles, J. Nanobiotechnol. 19 (2021) 2, https://doi.org/10.1186/s12951-020-00740-0.
[62] K. Brzóska, I. Grądzka, M. Kruszewski, Silver, gold, and iron oxide nanoparticles alter miRNA expression but do not affect DNA methylation in HepG2 cells, Materials 12 (2019) 1038, https://doi.org/10.3390/ma12071038.
[63] Y. Huang, X. Lü, Y. Qu, Y. Yang, S. Wu, MicroRNA sequencing and molecular mechanisms analysis of the effects of gold nanoparticles on human dermal fibroblasts, Biomaterials 37 (2015) 13–24, https://doi.org/10.1016/j.biomaterials.2014.10.042.
[64] C.-T. Ng, S.T. Dheen, W.-C.G. Yip, C.-N. Ong, B.-H. Bay, L.-Y. Lanry Yung, The induction of epigenetic regulation of PROS1 gene in lung fibroblasts by gold nanoparticles and implications for potential lung injury, Biomaterials 32 (2011) 7609–7615, https://doi.org/10.1016/j.biomaterials.2011.06.038.
[65] G.E. Ali, M.A. Ibrahim, A.H. El-Deeb, H. Amer, S.M. Zaki, Pulmonary deregulation of expression of miR-155 and two of its putative target genes; PROS1 and TP53INP1 associated with gold nanoparticles (AuNPs) administration in rat, Int. J. Nanomed. 14 (2019) 5569–5579, https://doi.org/10.2147/IJN.S208372.
[66] R. Balansky, M. Longobardi, G. Ganchev, M. Iltcheva, N. Nedyalkov, P. Atanasov, R. Toshkova, S. De Flora, A. Izzotti, Transplacental clastogenic and epigenetic effects of gold nanoparticles in mice, Mutat. Res. 751–752 (2013) 42–48, https://doi.org/10.1016/j.mrfmmm.2013.08.006.
[67] K. Sooklert, S. Nilyai, R. Rojanathanes, D. Jindatip, N. Sae-Liang, N. Kitkumthorn, A. Mutirangura, A. Sereemaspun, N-acetylcysteine reverses the decrease of DNA methylation status caused by engineered gold, silicon, and chitosan nanoparticles, Int. J. Nanomed. 14 (2019) 4573–4587, https://doi.org/10.2147/IJN.S204372.
[68] B. Smolkova, S. Miklikova, V.H. Kajabova, A. Babelova, N.E. Yamani, M. Zduriencikova, I. Fridrichova, I. Zmetakova, T. Krivulcik, L. Kalinkova, M. Matuskova, L. Kucerova, M. Dusinska, Global and gene specific DNA methylation in breast cancer cells was not affected during epithelial-to-mesenchymal transition in vitro, Neoplasma 63 (2016) 901–910, https://doi.org/10.4149/neo_2016_609.
[69] A.M. Tabish, K. Poels, H.-M. Byun, K. Luyts, A.A. Baccarelli, J. Martens, S. Kerkhofs, S. Seys, P. Hoet, L. Godderis, Changes in DNA methylation in mouse lungs after a single intra-tracheal administration of nanomaterials, PLoS One 12 (2017) e0169886, https://doi.org/10.1371/journal.pone.0169886.
[70] Y.M. Patil, S.N. Rajpathak, D.D. Deobagkar, Characterization and DNA methylation modulatory activity of gold nanoparticles synthesized by Pseudoalteromonas strain, J. Biosci. 44 (2019) 15, https://doi.org/10.1007/s12038-018-9842-6.
[71] P. Talarska, M. Boruczkowski, J. Żurawski, Current knowledge of silver and gold nanoparticles in laboratory research-application, toxicity, cellular uptake, Nanomater. (Basel) 11 (2021) 2454, https://doi.org/10.3390/nano11092454.
[72] R. Elespuru, S. Pfuhler, M.J. Aardema, T. Chen, S.H. Doak, A. Doherty, C. S. Farabaugh, J. Kenny, M. Manjanatha, B. Mahadevan, M.M. Moore, G. Ouédraogo, L.F. Stankowski, J.Y. Tanir, Genotoxicity assessment of nanomaterials: recommendations on best practices, assays, and methods, Toxicol. Sci. 164 (2018) 391–416, https://doi.org/10.1093/toxsci/kfy100.
[73] R.K. Elespuru, S.H. Doak, A.R. Collins, M. Dusinska, S. Pfuhler, M. Manjanatha, R. Cardoso, C.L. Chen, Common considerations for genotoxicity assessment of nanomaterials, Front Toxicol. 4 (2022) 859122, https://doi.org/10.3389/ftox.2022.859122.
[74] S.H. Doak, C. Andreoli, M.J. Burgum, Q. Chaudhry, E.A.J. Bleeker, C. Bossa, J. Domenech, D. Drobne, V. Fessard, N. Jeliazkova, E. Longhin, E. Rundén-Pran, M. Stępnik, N. El Yamani, J. Catalán, M. Dusinska, Current status and future challenges of genotoxicity OECD Test Guidelines for nanomaterials: a workshop report, Mutagenesis 38 (2023) 183–191, https://doi.org/10.1093/mutage/gead017.
[75] N. El Yamani, E. Rundén-Pran, J. Varet, M. Beus, M. Dusinska, V. Fessard, E. Moschini, T. Serchi, M.R. Cimpan, I. Lynch, I. Vinković Vrček, Hazard assessment of nanomaterials using *in vitro* toxicity assays: Guidance on potential

assay interferences and mitigating actions to avoid biased results, Nano Today 55 (2024) 102215, https://doi.org/10.1016/j.nantod.2024.102215.

[76] L. Gonzalez, B.J.S. Sanderson, M. Kirsch-Volders, Adaptations of the in vitro MN assay for the genotoxicity assessment of nanomaterials, Mutagenesis 26 (2011) 185–191, https://doi.org/10.1093/mutage/geq088.

[77] N. Fernández-Bertólez, F. Brandão, C. Costa, E. Pásaro, J.P. Teixeira, B. Laffon, V. Valdiglesias, Suitability of the in vitro cytokinesis-block micronucleus test for genotoxicity assessment of TiO2 nanoparticles on SH-SY5Y cells, Int. J. Mol. Sci. 22 (2021) 8558, https://doi.org/10.3390/ijms22168558.