AIMS Biophysics

AIMS Biophysics, 11(3): 340–369.
DOI: 10.3934/biophy.2024019
Received: 19 July 2024
Revised: 23 August 2024
Accepted: 06 September 2024
Published: 11 September2024

http://www.aimspress.com/journal/biophysics

Review

Biophysical insights into nanomaterial-induced DNA damage: mechanisms, challenges, and future directions

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Abstract: Nanomaterials have garnered significant attention due to their unique properties and wide-ranging applications in medicine and biophysics. However, their interactions with biological systems, particularly DNA, raise critical concerns about genotoxicity and potential long-term health risks. This review delves into the biophysical mechanisms underlying nanomaterial-induced DNA damage, highlighting recent insights, current challenges, and future research directions. We explore how the physicochemical properties of nanomaterials influence their interaction with DNA, the pathways through which they induce damage, and the biophysical methods employed to study these processes.

Keywords: nanomaterials; DNA damage; dose enhancement; physicochemical property; Monte Carlo simulation; nanoparticles

1. Introduction

Nanomaterials are materials with at least one dimension in the nanometer scale (1–100 nm). At this size, they exhibit unique physical, chemical, and biological properties distinct from their bulk counterparts [1]. These properties arise from the increased surface area to volume ratio, quantum effects, and the predominance of surface atoms [2]. Nanomaterials can be composed of various substances, including metals, semiconductors, polymers, and ceramics, each offering specific functionalities [3]. Their tunable characteristics make them highly versatile for a broad range of applications across multiple fields such as wound healing [4−6]. In biophysics and medicine,

nanomaterials have revolutionized diagnostics and treatment methods. They are used in drug delivery systems to transport therapeutic agents directly to target cells, thereby minimizing side effects and improving efficacy [7]. Nanoparticles are also employed in imaging techniques to enhance the contrast and resolution of biological tissues, aiding in the early detection of diseases. Moreover, nanomaterials play a crucial role in the development of novel biomaterials for tissue engineering and regenerative medicine [8]. In the realm of electronics, nanomaterials contribute to the miniaturization and enhancement of devices. Carbon nanotubes and graphene are examples of nanomaterials that have significantly impacted the development of faster, smaller, and more efficient transistors and integrated circuits [9]. Moreover, they are key components in the creation of flexible and wearable electronic devices, which are becoming increasingly popular in consumer electronics and medical monitoring systems [10]. Environmental applications of nanomaterials are equally significant. They are utilized in water treatment processes to remove contaminants and pathogens through adsorption and catalytic degradation [11]. Nanomaterials also aid in air purification by capturing and neutralizing pollutants. In agriculture, they are used to develop smart delivery systems for fertilizers and pesticides, improving crop yield and reducing environmental impact [12]. Despite their numerous advantages, the widespread use of nanomaterials raises concerns about their potential impact on human health and the environment. Understanding how nanomaterials interact with biological systems, particularly their ability to induce DNA damage, is crucial [13].

The study of nanomaterial-induced DNA damage is important due to the critical role DNA plays in maintaining cellular function and genetic integrity. DNA damage can lead to mutations, which may cause various diseases, including cancer. As nanomaterials are increasingly integrated into medical treatments, consumer products, and industrial applications [14], understanding their potential genotoxic effects is essential to safeguard public health. Unlike traditional materials, the unique properties of nanomaterials may result in unforeseen interactions with cellular components, leading to novel mechanisms of DNA damage that are not yet fully understood. Moreover, the ability of nanomaterials to penetrate biological barriers and accumulate in tissues poses significant risks [15]. Their small size allows them to interact directly with cellular structures, including the nucleus where DNA resides. Various studies have shown that nanomaterials can generate reactive oxygen species (ROS), which are highly reactive molecules capable of causing oxidative stress and subsequent DNA damage [16−18]. Investigating these interactions helps in assessing the long-term safety of nanomaterials and developing guidelines for their safe use. Figure 1 illustrates the pathways through which various types of nanomaterials can cause DNA damage. The figure shows that nanomaterials can interact with DNA directly, generate ROS, and so on. These interactions lead to various forms of DNA damage, such as single-strand (SSBs) and double-strand breaks (DSBs), mutations, chromosomal aberrations, and, ultimately, cell death.

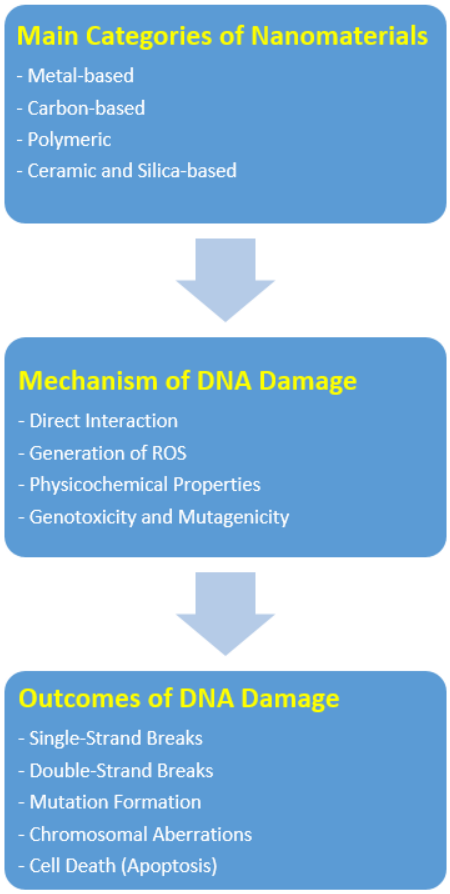

Figure 1. Block diagram illustrating the mechanisms of DNA damage induced by various types of nanoparticles.

Nanoparticle-enhanced radiotherapy (NPRT) represents a promising advancement in cancer treatment by leveraging the unique properties of nanoparticles to improve the efficacy of conventional radiotherapy [19]. Nanoparticles, such as gold and other high atomic number elements, enhance radiation therapy by increasing the local absorption of radiation, which leads to the production of secondary electrons and ROS that cause additional DNA damage in cancer cells [20]. The primary mechanism involves the generation of ROS, which induce oxidative stress, resulting in various forms of DNA damage, including SSBs, DSBs, and base modifications. DSBs are particularly detrimental and challenging to repair, making NPRT highly effective at destroying cancer cells [21]. In addition, nanoparticles selectively accumulate in tumor tissues due to the enhanced permeability and retention (EPR) effect, concentrating the radiation dose in the tumor and sparing healthy tissues [22]. However, the integration of nanoparticles in radiotherapy raises concerns about potential off-target effects and long-term safety, as increased ROS production and DNA damage in normal cells must be carefully managed [23]. Thus, while NPRT offers significant potential for more effective and targeted cancer therapies, ongoing research is essential to optimize nanoparticle formulations, dosages, and delivery methods to maximize therapeutic benefits while minimizing risks to healthy tissues.

A comprehensive review of nanomaterial-induced DNA damage due to nanomaterials based on

biophysics is crucial. First, it consolidates the existing knowledge, providing a clear picture of the current understanding of how different types of nanomaterials interact with DNA. This is particularly important given the rapid advancements in nanotechnology and the continuous development of new nanomaterials with diverse properties and applications [24]. A thorough review allows researchers and regulatory bodies to stay informed about potential risks and emerging trends in the field. Second, a review identifies gaps in the current research, highlighting areas that require further investigation. By synthesizing data from various studies, it can pinpoint inconsistencies and unresolved questions, thereby guiding future research efforts. This is essential for developing more accurate risk assessment and safety protocols for nanomaterial usage. Finally, a review on this topic serves as an educational resource for scientists, policymakers, and the public. It raises awareness about the potential health risks associated with nanomaterials and underscores the importance of ongoing research and regulation. As nanomaterials continue to be incorporated into numerous products and technologies, ensuring their safe application is a shared responsibility that relies on informed decision-making and evidence-based policies. This review aims to provide the necessary insights and direction to achieve these goals.

2. Mechanisms of DNA damage

2.1. Direct interaction with DNA

Nanomaterials can directly interact with DNA molecules, leading to various forms of damage. This interaction occurs through several mechanisms, including the physical binding of nanoparticles to DNA strands, which can disrupt the double-helix structure and interfere with replication and transcription processes [25]. Some nanoparticles may form covalent bonds with DNA bases, resulting in the formation of DNA adducts that can cause mutations if not properly repaired. Moreover, the physical presence of nanoparticles within the cellular environment can induce mechanical stress on DNA, leading to strand breaks and other structural alterations [26]. The extent and nature of these interactions depend on the specific characteristics of the nanomaterial, such as size, shape, surface charge, and chemical composition, highlighting the importance of understanding these properties to assess the potential genotoxic effects of nanomaterials.

2.2. Generation of ROS

One of the primary mechanisms by which nanomaterials induce DNA damage is through the generation of ROS. Upon exposure to nanomaterials, cells often experience oxidative stress due to an imbalance between ROS production and antioxidant defenses [27]. Many nanomaterials, particularly metal-based ones like titanium dioxide and zinc oxide, can catalyze the formation of ROS, including superoxide anions, hydroxyl radicals, and hydrogen peroxide [28]. These highly reactive molecules can cause various DNA lesions, including base modifications, SSBs, and DSBs. For instance, ROS can induce base modifications, such as 8-oxoguanine, leading to mispairing during DNA replication [29]. SSBs can occur when oxidative damage affects the DNA backbone, while DSBs represent a more severe form of damage that can result from the accumulation of multiple SSBs or direct interaction with ROS [30]. The oxidative damage to DNA compromises its integrity, leading to mutations, genomic instability, and potentially carcinogenesis if the damage is not adequately repaired [31]. Understanding the conditions and specific properties of nanomaterials that influence ROS generation

is crucial for evaluating the genotoxic risks associated with nanomaterial exposure. Figure 2 illustrates how nanomaterials cause DNA damage through both direct mechanisms (direct interaction with DNA) and indirect mechanisms (generation of ROS).

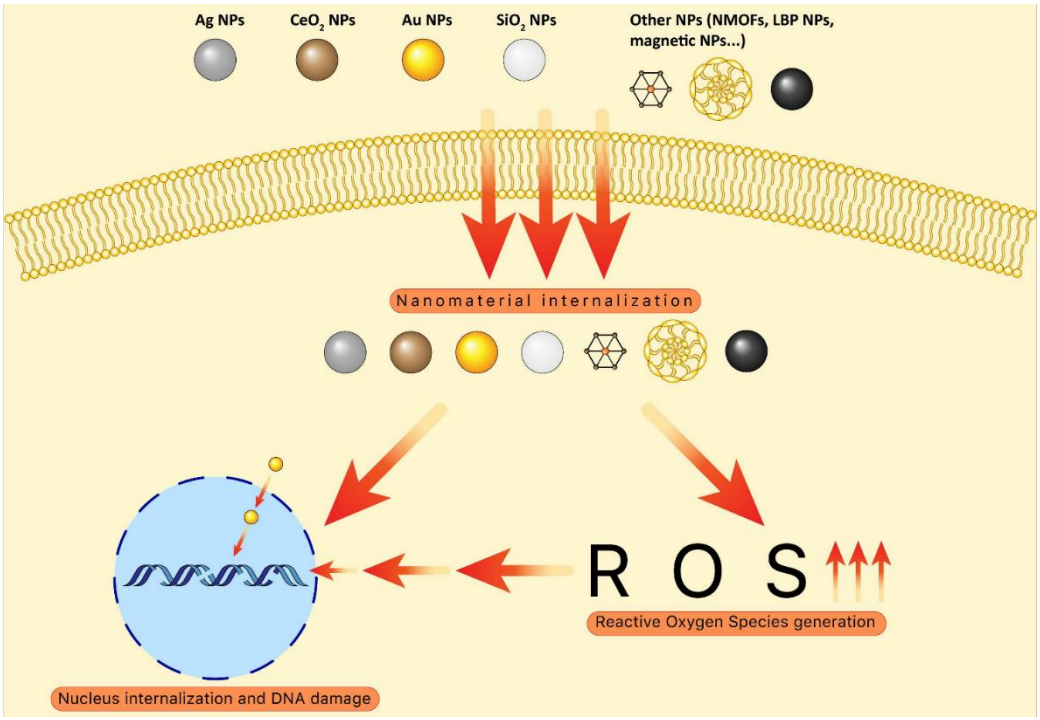

Figure 2. Direct and indirect effects of nanomaterials on DNA damage [32].

2.3. Nanomaterial physicochemical properties and DNA damage

The physicochemical properties of nanomaterials play a crucial role in determining their potential to cause DNA damage. Smaller nanoparticles typically exhibit higher reactivity and can more easily penetrate cellular membranes, increasing their likelihood of reaching and interacting with DNA [33]. The shape of nanomaterials can affect their cellular uptake and intracellular distribution, with certain shapes, such as rods or fibers, potentially causing more physical disruption than spherical particles [34]. Surface charge impacts the electrostatic interactions between nanomaterials and cellular components, with positively charged nanoparticles often showing greater cellular uptake and genotoxicity due to their attraction to negatively charged cell membranes and nucleic acids [35]. Additionally, surface coatings and functionalization can either enhance or mitigate DNA damage by altering the stability, solubility, and reactivity of nanomaterials [36]. Understanding these physicochemical properties is essential for predicting and controlling the genotoxic effects of nanomaterials, guiding safer design and application in various fields.

2.4. Genotoxicity and mutagenicity

The genotoxic and mutagenic effects of nanomaterials are critical aspects of their potential impact on human health. Genotoxicity refers to the ability of nanomaterials to cause damage to genetic

material, leading to mutations, chromosomal fragmentation, and alterations in gene expression. This can occur through direct interactions with DNA or indirectly via the generation of ROS and other free radicals. Mutagenicity, a subset of genotoxicity, specifically involves changes in the DNA sequence that can result in permanent alterations to the genetic code [37]. Various assessment methods, such as the comet assay, micronucleus assay, and the Ames test, are used to evaluate the genotoxic and mutagenic potential of nanomaterials [38]. These methods help identify the types and extent of genetic damage, providing insights into the mechanisms involved. The induction of mutations and chromosomal aberrations by nanomaterials can lead to long-term genetic consequences, including cancer and hereditary diseases, emphasizing the importance of thorough evaluation and regulation [39]. Understanding the genotoxic and mutagenic properties of different nanomaterials is essential for developing safer materials and mitigating potential health risks associated with their use.

3. Types of nanomaterials

3.1. Metal-based nanomaterials

Metal-based nanomaterials, including gold, silver, and iron oxide nanoparticles, are increasingly used in various fields such as biomedical applications, electronics, and environmental remediation, due to their unique physicochemical properties [40]. Their small size and high surface area-to-volume ratio enhance their interaction with biological systems, facilitating applications like targeted drug delivery, imaging contrast enhancement, and therapeutic treatments. However, these same properties also contribute to their potential to induce DNA damage, which is a critical concern for their safe application in biomedicine. For instance, gold nanoparticles are known for their biocompatibility and are widely used in drug delivery and cancer therapy. However, studies have shown that gold nanoparticles can generate ROS upon exposure to biological environments or external stimuli like light, leading to oxidative stress that results in DNA damage, such as SSBs and DSBs [41]. Additionally, silver nanoparticles, which are valued for their antimicrobial properties, have been observed to cause significant DNA damage through similar ROS-mediated mechanisms [42]. Silver nanoparticles can induce oxidative modifications of DNA bases, such as 8-oxoguanine, which can lead to mutations and genomic instability if not properly repaired [43]. Iron oxide nanoparticles, commonly used as contrast agents in magnetic resonance imaging (MRI) and for targeted drug delivery, can also catalyze the generation of ROS, resulting in oxidative DNA damage. Studies have reported that exposure to iron oxide nanoparticles can lead to significant levels of DNA strand breaks and chromosomal aberrations, further underscoring the need for careful assessment of their genotoxic potential in biomedical applications [44]. Furthermore, the direct physical interactions between metal nanoparticles and DNA molecules can disrupt the double-helix structure, potentially interfering with essential cellular processes and compromising genetic stability [44].

3.2. Carbon-based nanomaterials

Carbon-based nanomaterials, including carbon nanotubes (CNTs), graphene, and fullerenes, have garnered significant attention due to their exceptional mechanical, electrical, and thermal properties. CNTs, in particular, exhibit high tensile strength and electrical conductivity, making them ideal for applications in electronics, composites, and energy storage [45]. Graphene, a single layer of carbon

atoms arranged in a two-dimensional lattice, possesses remarkable strength and conductivity, influencing advancements in flexible electronics, sensors, and biomedical devices. Fullerenes, spherical carbon molecules like C60, are valued for their unique cage-like structure and ability to act as radical scavengers or photosensitizers in photodynamic therapy [46]. Despite their promising applications, carbon-based nanomaterials can also pose risks related to DNA damage. Studies suggest that CNTs and graphene can induce oxidative stress and inflammation, potentially leading to DNA strand breaks and mutagenesis [47].

3.3. Polymeric nanomaterials

Polymeric nanomaterials, including synthetic polymers like dendrimers and micelles, as well as natural polymers such as chitosan and albumin nanoparticles, offer versatile platforms for drug delivery, imaging, and tissue engineering [48]. Synthetic polymers are engineered to have precise surface functionalities, allowing for tailored interactions with biological systems and controlled release of therapeutic agents [49]. Natural polymers, derived from renewable sources, often exhibit biocompatibility and biodegradability, making them suitable for biomedical applications without significant toxicity concerns. However, both synthetic and natural polymeric nanomaterials can induce DNA damage through mechanisms such as oxidative stress from degradation products or interactions with cellular components [50].

3.4. Ceramic and silica-based nanomaterials

Ceramic and silica-based nanomaterials, such as titanium dioxide (TiO_2) and silica nanoparticles, are widely utilized in diverse fields ranging from catalysis and electronics to biomedical applications [51]. TiO_2 nanoparticles are known for their photocatalytic properties and are commonly used in environmental remediation and sunscreen formulations [52]. Silica nanoparticles are particularly valued for their biocompatibility, surface functionalization capabilities, and role as drug carriers in targeted therapies [53]. Notably, while these nanomaterials can induce DNA damage primarily through the generation of ROS upon exposure to UV radiation or cellular environments, studies indicate that their overall genotoxic effects tend to be minimal compared to other nanomaterials. ROS-mediated oxidative stress can lead to DNA strand breaks, base modifications, and genomic instability [54]. However, the physicochemical characteristics of ceramic and silica-based nanomaterials significantly influence their interactions with biological systems, suggesting that careful selection and characterization can mitigate potential genotoxic effects. Table 1 summarizes different nanomaterial types, their applications, and potential for DNA damage.

Table 1. Overview of nanomaterial types, their key properties, applications, and potential for DNA damage.

Nanomaterial type	Key properties	Applications	Potential for DNA damage	References
Metal-based nanomaterials	Small size, high surface area-to-volume ratio; efficient interaction with biological systems	Targeted drug delivery, imaging contrast enhancement, therapeutic treatments, electronics, environmental remediation	Catalyze ROS generation leading to oxidative stress, DNA strand breaks, base modifications; direct disruption of DNA structure	[40], [41], [42], [43], [44]
Carbon-based nanomaterials	Exceptional mechanical, electrical, and thermal properties; radical scavenging and photosensitizing abilities	Electronics, composites, energy storage, flexible electronics, sensors, biomedical devices, photodynamic therapy	Induce oxidative stress and inflammation, potentially causing DNA strand breaks and mutagenesis	[45], [46], [47]
Polymeric nanomaterials	Versatile platforms with precise surface functionalities, biocompatibility, and biodegradability	Drug delivery, imaging, tissue engineering	Oxidative stress from degradation products and interactions with cellular components leading to DNA damage	[48], [49], [50]
Ceramic and silica-based nanomaterials	Photocatalytic properties, biocompatibility, surface functionalization capabilities, minimal genotoxicity	Catalysis, electronics, biomedical applications, environmental remediation, sunscreen formulations	ROS generation leading to DNA strand breaks, base modifications, genomic instability; generally minimal genotoxic effects	[51], [52], [53], [54]

4. Physicochemical properties of nanomaterials

This section explores how the physicochemical properties of nanomaterials—such as size, shape, surface chemistry, aggregation behavior, and chemical composition—affect their interactions with DNA. Among these properties, size and surface chemistry are often the most dominant factors in inducing DNA damage.

4.1. Size and shape

The size and shape of nanomaterials are critical factors influencing their interaction with biological systems, including their potential to cause DNA damage [21]. Nanoparticles can vary widely in size, typically ranging from 1 to 100 nm. This size range allows them to interact uniquely with cells and biological molecules, crossing cellular membranes and even entering the nucleus [26]. Smaller nanoparticles often have a higher surface area-to-volume ratio, which can enhance their reactivity and increase their potential to induce oxidative stress, leading to DNA damage [1]. Moreover, the size of nanoparticles can influence their bio-distribution, cellular uptake, and clearance from the body. In radiotherapy, Monte Carlo simulations have been conducted to examine the impact of gold nanoparticle size on DNA damage. Various sizes of gold nanoparticles were simulated in association with DNA during electron beam irradiation (Figure 3). The results showed that reducing the nanoparticle size while maintaining the same mass of gold increases the total number of strand breaks [21].

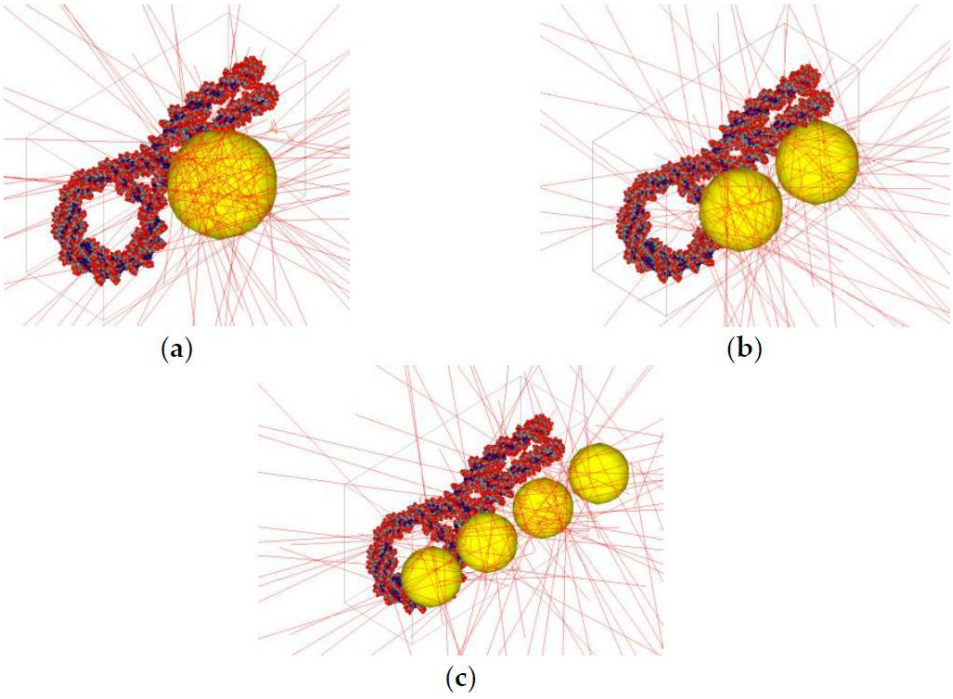

Figure 3. Visualization of (a) a single irradiated gold nanoparticle with a 5 nm radius interacting with a DNA molecule; (b) a single irradiated gold nanoparticle with a 3.97 nm radius interacting with a DNA molecule; (c) a single irradiated gold nanoparticle with a 3.15 nm radius interacting with a DNA molecule. The red tracks depict the paths of secondary electrons in the simulation [21].

Research has shown that both the size and shape of nanomaterials [55−57] can influence the generation of ROS, a primary mechanism of DNA damage [58]. Smaller nanoparticles can catalyze the production of ROS more efficiently, leading to oxidative damage of DNA [59]. This oxidative stress can result in a variety of DNA lesions, including SSBs and DSBs, base modifications, and cross-linking. Therefore, careful control and characterization of nanomaterials are imperative in assessing their safety and understanding their mechanisms of genotoxicity.

4.2. Surface chemistry and functionalization

Surface chemistry and functionalization of nanomaterials are pivotal in determining their interaction with biological systems, including their potential to cause DNA damage. The surface properties of nanomaterials, such as surface charge, hydrophobicity/hydrophilicity, and the presence of specific functional groups, dictate how these particles interact with cellular membranes, proteins, and nucleic acids [40,60]. For instance, nanoparticles with a positive surface charge are more likely to interact with the negatively charged cell membranes and DNA, potentially leading to increased cellular uptake and direct DNA binding [61]. This can enhance the genotoxic potential of the nanomaterials through direct physical interactions and disruption of the DNA structure.

Functionalization refers to the modification of the nanoparticle surface with various chemical groups, ligands, or biomolecules to improve their stability, biocompatibility, and targeting capabilities. Functionalization can significantly alter the biological behavior of nanomaterials [62]. For example, coating nanoparticles with polyethylene glycol (PEG) can reduce protein adsorption and prolong circulation time in the bloodstream, potentially leading to different bio-distribution and reduced non-specific interactions with cells [63]. Conversely, functionalizing nanoparticles with targeting ligands, such as antibodies or peptides, can increase their specificity toward certain cell types or tissues, including tumor cells, but may also enhance the localized DNA damage in those targeted cells.

The surface chemistry of nanomaterials also plays a crucial role in the generation of reactive ROS, which is a primary mechanism of DNA damage [64]. Surface functional groups can act as catalytic sites for ROS production, leading to oxidative stress within cells. For instance, nanoparticles with hydroxyl groups or other reactive moieties can facilitate the formation of ROS in the presence of cellular reducing agents or light, causing oxidative damage to the DNA [65]. Therefore, understanding how surface chemistry influences ROS generation is essential for predicting and mitigating the genotoxic effects of nanomaterials.

4.3. Aggregation and dispersion behavior

Aggregation and dispersion behavior of nanomaterials are critical factors influencing their interaction with biological systems and their potential to cause DNA damage [66]. Nanoparticles tend to aggregate due to van der Waals forces, magnetic interactions, and other inter-particle forces. The degree of aggregation can significantly impact the biological activity of nanoparticles, as aggregated particles may exhibit different cellular uptake pathways and reduced surface area compared to well-dispersed nanoparticles. Aggregation can affect the distribution, retention, and clearance of nanoparticles in biological environments, thereby influencing their overall genotoxic potential.

In biological media, the dispersion state of nanomaterials is influenced by several factors, including ionic strength, pH, and the presence of proteins and other biomolecules. For instance, high

ionic strength or low pH conditions can promote nanoparticle aggregation, reducing their availability for cellular interaction and uptake [67]. Conversely, the presence of proteins and other biomolecules can stabilize nanoparticles and prevent aggregation through steric and electrostatic stabilization mechanisms. The stability of nanoparticle dispersion in biological fluids is crucial for maintaining their reactivity and interaction with cellular components, including DNA.

Aggregation can influence the generation of ROS, a primary mechanism of DNA damage [68]. Well-dispersed nanoparticles have a larger surface area available for catalytic reactions, potentially leading to higher ROS production and oxidative stress. On the other hand, aggregated nanoparticles may exhibit reduced reactivity and lower ROS generation [69]. The extent of aggregation can also affect the localization of nanoparticles within cells, with well-dispersed particles being more likely to interact directly with the nucleus and DNA.

The behavior of nanoparticles in terms of aggregation and dispersion also impacts their interactions with other cellular components, such as proteins and lipids [70]. Aggregated nanoparticles may be more prone to opsonization and clearance by the immune system, reducing their effective concentration in target tissues and cells. In contrast, well-dispersed nanoparticles may have enhanced cellular uptake and intracellular trafficking, increasing their potential to induce DNA damage. The formation of a protein corona on the nanoparticle surface can further influence aggregation behavior and subsequent biological interactions [67]. The composition and dynamics of the protein corona are affected by the dispersion state of the nanoparticles, which in turn modulates their genotoxic potential [71].

4.4. Chemical composition and crystallinity

The chemical composition and crystallinity of nanomaterials are fundamental factors that determine their interaction with biological systems and their potential to cause DNA damage [72]. Nanomaterials can be composed of a wide variety of elements and compounds, including metals, metal oxides, carbon-based materials, and polymers. Each type of nanomaterial exhibits unique properties that influence its biological behavior and genotoxic potential. For example, metal nanoparticles such as gold, silver, and iron oxide have distinct chemical properties that affect their reactivity, cellular uptake, and mechanisms of DNA damage [73].

The chemical composition of nanomaterials influences their ability to generate ROS, a primary mechanism of DNA damage. Metal and metal oxide nanoparticles, such as titanium dioxide (TiO_2) and zinc oxide (ZnO), are known to catalyze ROS production under various conditions, leading to oxidative stress and DNA damage [74]. The presence of specific elements or compounds in the nanomaterial composition can also introduce additional mechanisms of genotoxicity, such as ion release and interaction with cellular enzymes.

Crystallinity, which refers to the degree of structural order in a solid, also plays a significant role in determining the biological interactions and genotoxic potential of nanomaterials. Nanomaterials can exist in different crystalline phases, each with distinct physical and chemical properties. For example, titanium dioxide can exist in anatase and rutile phases, with the anatase phase being more photocatalytically active and capable of generating higher levels of ROS under UV light exposure [75]. The crystallinity of nanomaterials affects their electronic structure, surface reactivity, and interaction with biological molecules, all of which contribute to their potential to induce DNA damage.

The crystallinity of nanomaterials can influence their mechanical properties and stability in

biological environments. Highly crystalline nanoparticles tend to be more stable and less prone to degradation, which can affect their persistence in biological systems and their long-term genotoxic effects [76]. Conversely, amorphous or less crystalline nanomaterials may degrade more easily, potentially releasing toxic ions or fragments that can interact with DNA and other cellular components. The crystallinity of nanomaterials also affects their interaction with cellular membranes, proteins, and DNA, influencing their uptake, intracellular localization, and mechanisms of genotoxicity [77].

5. Biophysical methods for studying DNA damage

5.1. Spectroscopic techniques

5.1.1. UV-visible spectroscopy

UV-visible spectroscopy is a fundamental technique in the study of DNA damage, offering insights into the structural and conformational changes in DNA upon interaction with nanomaterials [78]. By measuring the absorbance of UV and visible light by DNA samples, researchers can detect alterations in the DNA's electronic structure. These changes often manifest as shifts in the absorbance maxima or variations in absorbance intensity, which can indicate modifications such as strand breaks, cross-linking, or base modifications. UV-visible spectroscopy is particularly useful for quantifying the concentration of nucleic acids and assessing their purity, making it an essential tool for preliminary analysis in DNA damage studies.

5.1.2. Fluorescence spectroscopy

Fluorescence spectroscopy leverages the emission of light by fluorescent molecules to study DNA damage. When DNA interacts with certain nanomaterials, it can lead to the formation of fluorescent adducts or the quenching of inherent fluorescence signals. By labeling DNA with fluorescent probes, researchers can monitor real-time interactions and detect minute changes in the DNA structure. This technique is highly sensitive and allows for the detection of low levels of DNA damage [79]. In addition, fluorescence resonance energy transfer (FRET) can be used to study the proximity and interaction between DNA molecules and nanomaterials, providing detailed information on the spatial arrangement and conformational changes within the DNA [80].

5.1.3. Circular dichroism (CD) spectroscopy

Circular dichroism (CD) spectroscopy is a powerful method for examining the secondary structure of DNA and its changes upon interaction with nanomaterials. CD spectroscopy measures the differential absorption of left- and right-handed circularly polarized light, which is sensitive to the chiral nature of DNA molecules [81]. This technique can reveal alterations in the DNA helical structure, such as transitions between A-form, B-form, and Z-form DNA, as well as more subtle changes induced by nanomaterial binding. CD spectroscopy provides valuable information about the conformational integrity of DNA, helping researchers understand the extent and nature of structural perturbations caused by nanomaterial exposure.

5.2. Microscopy-based approaches

5.2.1. Atomic force microscopy (AFM)

AFM is a high-resolution imaging technique that provides detailed topographical maps of DNA molecules at the nanoscale. By scanning a sharp tip over the DNA sample's surface, AFM generates three-dimensional images that reveal structural changes in DNA induced by nanomaterials. This technique allows researchers to observe physical alterations such as strand breaks, cross-linking, and aggregation [82]. AFM is particularly advantageous for studying DNA in its native environment without requiring extensive sample preparation, thus preserving the integrity of the DNA and providing accurate insights into nanomaterial-induced damage.

5.2.2. Transmission electron microscopy (TEM)

TEM is another powerful imaging tool used to visualize DNA and its interactions with nanomaterials at an atomic level. TEM operates by transmitting a beam of electrons through a thin DNA sample, creating high-resolution images that can reveal detailed structural information. This technique is invaluable for identifying nanoscale structural damage, such as DSBs and the formation of DNA-nanomaterial complexes [83]. TEM's high resolution enables the detection of subtle changes in DNA morphology, making it a crucial method for studying the precise impact of nanomaterials on DNA integrity.

5.2.3. Confocal microscopy

Confocal microscopy offers a versatile and non-invasive approach to studying DNA damage in living cells. This technique uses laser scanning and fluorescence to create detailed, three-dimensional images of cellular structures, including DNA. By labeling DNA with fluorescent markers, researchers can track the localization and extent of DNA damage within the cellular context. Confocal microscopy is particularly useful for real-time monitoring of DNA repair processes and understanding how nanomaterials affect these dynamics [84]. The ability to visualize DNA damage in live cells provides critical insights into the biological consequences of nanomaterial exposure and the cellular responses to such damage.

5.3. Mass spectrometry

5.3.1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF mass spectrometry is a powerful analytical technique used to identify and characterize DNA damage at a molecular level [85]. By embedding DNA samples in a matrix and ionizing them with a laser, MALDI-TOF measures the mass-to-charge ratio of the resulting ions. This allows for the precise determination of molecular weights and the identification of DNA modifications, such as adducts formed by nanomaterial interactions. MALDI-TOF is particularly useful for detecting and characterizing bulky DNA adducts and oxidative lesions, providing valuable information on the

types and extent of damage induced by nanomaterials.

5.3.2. Electrospray ionization mass spectrometry (ESI-MS)

Electrospray ionization mass spectrometry (ESI-MS) is another highly sensitive technique for studying DNA damage [86]. ESI-MS ionizes DNA molecules in solution by applying a high voltage, producing ions that are then analyzed based on their mass-to-charge ratio. This technique is effective in identifying a wide range of DNA modifications, including small base modifications, cross-links, and strand breaks. ESI-MS is particularly advantageous for its ability to analyze intact DNA strands and their modifications, offering detailed insights into the specific sites and nature of damage. Its high sensitivity and accuracy make ESI-MS an essential tool for elucidating the molecular mechanisms of nanomaterial-induced DNA damage.

5.4. Electrophoretic techniques

5.4.1. Gel electrophoresis

Gel electrophoresis is a widely used technique for analyzing DNA damage, offering a straightforward and effective method for separating DNA fragments based on size. In this technique, DNA samples are loaded into a gel matrix, and an electric field is applied to drive the migration of DNA fragments through the gel. Damaged DNA, such as fragments resulting from strand breaks, migrates differently compared to intact DNA, allowing researchers to visualize and quantify the extent of DNA damage [87]. Techniques such as agarose gel electrophoresis and polyacrylamide gel electrophoresis (PAGE) are commonly employed to analyze the fragmentation patterns, providing insights into the types of damage induced by nanomaterials, including SSBs and DSBs.

5.4.2. Comet assay (single-cell gel electrophoresis)

The comet assay, also known as single-cell gel electrophoresis, is a highly sensitive method for detecting DNA damage at the single-cell level [88]. In this assay, cells are embedded in agarose gel on a microscope slide, lysed to release DNA, and subjected to electrophoresis. Damaged DNA migrates out of the cell nucleus, forming a "comet tail" when stained and viewed under a fluorescence microscope. The extent and intensity of the comet tail provide quantitative measures of DNA damage, such as strand breaks and alkali-labile sites. This technique is particularly useful for evaluating the genotoxic effects of nanomaterials in various cell types, allowing for the assessment of both initial DNA damage and repair capacity [89].

5.5. Nuclear magnetic resonance (NMR)

NMR spectroscopy is a powerful analytical technique used to investigate the structural and dynamic properties of molecules, including DNA [90]. NMR operates by exploiting the magnetic properties of atomic nuclei. When placed in a strong magnetic field and exposed to radiofrequency radiation, certain nuclei resonate at characteristic frequencies. By measuring these resonances, researchers can obtain detailed information about the molecular structure, dynamics, and interactions.

In the context of DNA, NMR can reveal important details about the helical structure, base pairing, and conformational changes induced by interactions with nanomaterials.

NMR spectroscopy is particularly valuable for studying the interactions between DNA and nanomaterials at a molecular level [24]. It provides insights into how nanomaterials influence the conformation and stability of DNA. For instance, NMR can identify binding sites of nanomaterials on the DNA molecule, detect changes in the DNA's secondary structure, and monitor the dynamics of these interactions over time [91]. This technique is sensitive to subtle structural perturbations, such as those caused by nanomaterial-induced oxidative damage or the formation of DNA adducts. By analyzing these interactions, NMR helps elucidate the mechanisms by which nanomaterials induce DNA damage, offering critical information for understanding their potential genotoxic effects.

5.6. Molecular dynamics (MD) and Monte Carlo (MC) simulation

5.6.1. Simulating DNA-nanomaterial interactions

MD simulations are particularly effective for exploring the interactions between DNA and nanomaterials at an atomic level [92]. By modeling these interactions, researchers can investigate how nanomaterials influence DNA structure, dynamics, and function. Simulations can reveal binding affinities, preferred binding sites, and the structural perturbations induced by nanomaterials. For example, MD simulations can show how nanomaterials might cause DNA bending, unwinding, or the formation of non-canonical structures. They can also simulate the potential generation of ROS by nanomaterials and their subsequent impact on DNA integrity [93]. Through these detailed simulations, researchers can gain a comprehensive understanding of the molecular mechanisms by which nanomaterials induce DNA damage.

5.6.2. Simulating DNA dosimetry and damage in nanoparticle-enhanced radiotherapy

MC simulations play a critical role in nanoparticle-enhanced radiotherapy, particularly for assessing and optimizing DNA dosimetry and damage [94,95]. This innovative therapeutic approach harnesses nanoparticles to augment radiation delivery to cancer cells, thereby enhancing treatment efficacy while minimizing harm to adjacent healthy tissues [96]. MC simulations enable researchers to model intricate interactions between DNA and nanoparticles when exposed to radiation. They accurately simulate the distribution and energy deposition of radiation within DNA, offering precise dosimetric insights [97]. By modeling the generation of secondary electrons and reactive species, MC simulations predict various forms of DNA damage, including SSBs and DSBs, as well as oxidative lesions [98]. This comprehensive molecular understanding elucidates how nanoparticles amplify radiation-induced DNA damage, informing refinements in therapeutic strategies. Moreover, MC simulations aid in designing nanoparticles with optimal attributes for enhancing radiotherapy effectiveness, thereby advancing targeted cancer treatments.

6. Experimental evidence

6.1. In vitro studies

In vitro studies on nanomaterial-induced DNA damage frequently employ a variety of cell culture models to elucidate the underlying mechanisms and effects. Commonly used cell lines include human epithelial cells, fibroblasts, and cancer cell lines such as HeLa and A549. These models provide a controlled environment to observe the direct interactions between nanomaterials and cellular components [99,100]. The primary mechanisms by which nanomaterials induce DNA damage in vitro include the generation of ROS and direct interactions with DNA. ROS generation can lead to oxidative stress, resulting in damage to cellular components including lipids, proteins, and DNA. In addition, direct interaction with DNA can cause physical disruptions such as strand breaks or the formation of DNA adducts, which interfere with normal cellular functions and can lead to mutagenesis [101,102].

Studies have identified several types of DNA damage induced by nanomaterials, including SSBs, DSBs, and the formation of DNA adducts. SSBs are the most common form of damage, often resulting from oxidative stress. DSBs, though less common, are more severe and can lead to chromosomal aberrations and cell death if not properly repaired [103,104]. Significant in vitro studies have demonstrated the genotoxic potential of various nanomaterials. For instance, silver nanoparticles have been shown to induce significant levels of oxidative DNA damage and apoptosis in human lung epithelial cells [105]. Similarly, titanium dioxide nanoparticles have been found to cause DNA strand breaks and oxidative damage in human keratinocytes [106].

6.2. In vivo studies

In vivo studies provide critical insights into the systemic effects of nanomaterials and their potential to cause DNA damage in living organisms. Commonly used animal models include rodents such as mice and rats, as well as zebrafish, which offer advantages in terms of genetic similarities to humans and ease of handling. These models allow researchers to study the bio-distribution, metabolism, and long-term effects of nanomaterials following different routes of exposure, such as inhalation, injection, and oral administration [107,108]. The mechanisms by which nanomaterials induce DNA damage in vivo are complex and multifaceted. Once inside the organism, nanomaterials can generate ROS, leading to oxidative stress and subsequent DNA damage. Additionally, nanomaterials can interact directly with cellular components in various tissues, causing physical disruptions and initiating inflammatory responses that contribute to DNA damage [109,110].

In vivo studies have reported various types of DNA damage caused by nanomaterials, including SSBs, DSBs, and chromosomal aberrations. For example, exposure to titanium dioxide nanoparticles has been shown to cause significant DNA damage in liver and lung tissues of mice, manifested as increased levels of SSBs and DSBs [111]. Similarly, C60 fullerenes and single-walled carbon nanotubes have been associated with DNA damage in rats, highlighting the systemic nature of nanomaterial-induced genotoxicity [112]. Significant findings from in vivo studies underscore the potential risks posed by nanomaterials. For instance, a study on the genotoxic effects of gold nanoparticles in mice demonstrated that chronic exposure led to DNA damage in the brain, liver, and spleen, with implications for long-term health risks [113]. Another study found that carbon nanotubes induced DNA damage and inflammation in the lungs of rats, raising concerns about their use in various

applications [114]. These studies highlight the importance of thorough in vivo testing to understand the full spectrum of nanomaterial-induced DNA damage and its implications for human health.

6.3. Nanomaterial characteristics influencing DNA damage

The size and shape of nanomaterials play crucial roles in determining their interactions with biological systems, including the mechanisms of DNA damage. Smaller nanoparticles have a larger surface area-to-volume ratio, which can lead to increased reactivity and greater potential for inducing oxidative stress and DNA damage. For instance, smaller gold nanoparticles have been shown to penetrate cellular membranes more easily, leading to increased intracellular ROS generation and subsequent DNA damage [115]. Similarly, the shape of nanoparticles can influence their cellular uptake and bio-distribution. Rod-shaped nanoparticles, for example, have been found to cause more significant DNA damage compared to spherical ones due to their ability to interact more extensively with cellular components [116].

Surface modifications on nanoparticles significantly influence their biocompatibility and potential to cause DNA damage. Surface chemistry can affect how nanoparticles interact with cells, including their ability to generate ROS or bind directly to DNA. For example, nanoparticles coated with positively charged groups tend to be more genotoxic because they can more readily interact with the negatively charged DNA backbone [117]. Moreover, the presence of functional groups such as carboxyl or amino groups on the nanoparticle surface can modulate the extent of oxidative stress and DNA damage. Studies have shown that surface-modified quantum dots exhibit different levels of DNA damage depending on their surface coatings, highlighting the importance of surface chemistry in nanoparticle-induced genotoxicity [118].

The composition of nanomaterials, whether metal-based, carbon-based, or composed of other elements, also plays a critical role in their genotoxic potential. Metal nanoparticles such as silver, gold, and titanium dioxide are known for their ability to generate ROS, leading to oxidative DNA damage [119]. Carbon-based nanomaterials, including carbon nanotubes and fullerenes, have been shown to cause DNA damage through both oxidative stress and physical interactions with cellular structures [120]. The stability of nanomaterials in biological environments is another important factor. Unstable nanoparticles can release toxic ions or degrade into smaller particles, both of which can enhance their genotoxic effects [121].

6.4. Genotoxicity assays

To detect DNA damage caused by nanomaterials, several genotoxicity assays are commonly employed, each with specific strengths and applications. The comet assay is widely used to assess DNA strand breaks at the single-cell level. In this assay, cells are embedded in agarose, lysed, and subjected to electrophoresis. DNA fragments migrate out of the cells, which is measured to determine the extent of DNA damage [122]. Another commonly used assay is the micronucleus test, which detects chromosomal damage by identifying micronuclei in the cytoplasm of interphase cells. Micronuclei are formed from acentric chromosomal fragments or whole chromosomes that fail to incorporate into daughter nuclei during cell division, serving as a marker for genotoxic events [123]. The γ-H2AX assay is another important method, detecting the phosphorylation of histone H2AX at the site of DNA DSBs. This is achieved through immunofluorescence, where the presence of γ-H2AX foci indicates

the occurrence of DSBs, providing a sensitive measure of genotoxic stress [124].

The sensitivity and specificity of genotoxicity assays vary, influencing their ability to detect nanomaterial-induced DNA damage. The comet assay is highly sensitive to detecting SSBs and alkali-labile sites. It can also detect DNA cross-links and oxidative base damage when modified with specific enzymes. However, it may not differentiate between different types of DNA damage [125]. In contrast, the micronucleus test is less sensitive than the comet assay for detecting SSBs but is specific for chromosomal damage. It is particularly useful for identifying clastogenic and aneugenic effects and is less labor-intensive, making it suitable for high-throughput screening [126]. The γ-H2AX assay is highly specific for DSBs and provides a quantitative measure of damage. It is more sensitive than the comet assay for detecting DSBs but requires sophisticated equipment and expertise for immunofluorescence imaging and analysis [127].

Studies employing these assays have provided significant insights into the genotoxic potential of nanomaterials. Comet assay results have shown significant DNA damage induced by nanomaterials. For instance, silver nanoparticles caused considerable DNA strand breaks in human lung fibroblasts, highlighting their genotoxic potential [128]. Similarly, titanium dioxide nanoparticles induced DNA damage in human bronchial epithelial cells [129]. The micronucleus test has demonstrated chromosomal damage induced by carbon nanotubes and fullerenes. One study reported that single-walled carbon nanotubes (SWCNTs) caused a significant increase in micronucleus formation in human lung cells, indicating clastogenic effects [130]. Additionally, fullerenes induced micronuclei in mouse midbrain cells [131]. The γ-H2AX assay has revealed the formation of DSBs following exposure to various nanomaterials. For example, zinc oxide nanoparticles caused DSBs in human dermal fibroblasts, as evidenced by γ-H2AX staining [132].

6.5. Nanoparticle-enhanced radiotherapy

NPRT leverages the unique properties of nanoparticles to improve the effectiveness of radiation therapy. MC simulations have been extensively used to model and predict the dose enhancement effects of nanoparticles in radiotherapy. These simulations provide detailed insights into the interactions between radiation and nanoparticles at the microscopic level, helping to optimize the use of nanoparticles for therapeutic purposes [133].

MC simulations have shown that the inclusion of high atomic number (Z) nanoparticles, such as gold nanoparticles, can significantly enhance the local radiation dose within tumor tissues. This dose enhancement occurs because high-Z nanoparticles have a higher probability of interacting with ionizing radiation, leading to the emission of secondary electrons (photoelectrons, Auger electrons) that deposit energy locally and cause increased DNA damage in cancer cells [134]. Studies using MC simulations have reported dose enhancement factors ranging from 1.1 to 4.0, depending on the concentration and distribution of nanoparticles, as well as the type and energy of the radiation used [135,136]. Cho et al. [137,138] utilized MC simulations to explore the effects of gold nanoparticles in brachytherapy, a form of internal radiotherapy. Their findings showed that AuNPs could significantly increase the dose delivered to the tumor while sparing surrounding healthy tissues, highlighting the precision and effectiveness of nanoparticle-mediated dose enhancement. Figure 4 presents the results of a Monte Carlo simulation where a gold nanoparticle associated with DNA was irradiated by photon beams in the presence of a magnetic field [97]. The simulation revealed that applying a magnetic field (0−2 T) led to only a slight dose enhancement of less than 3%, resulting in an insignificant increase in DNA damage. These findings are

particularly relevant for magnetic resonance-guided radiotherapy, where patients are irradiated under a magnetic field.

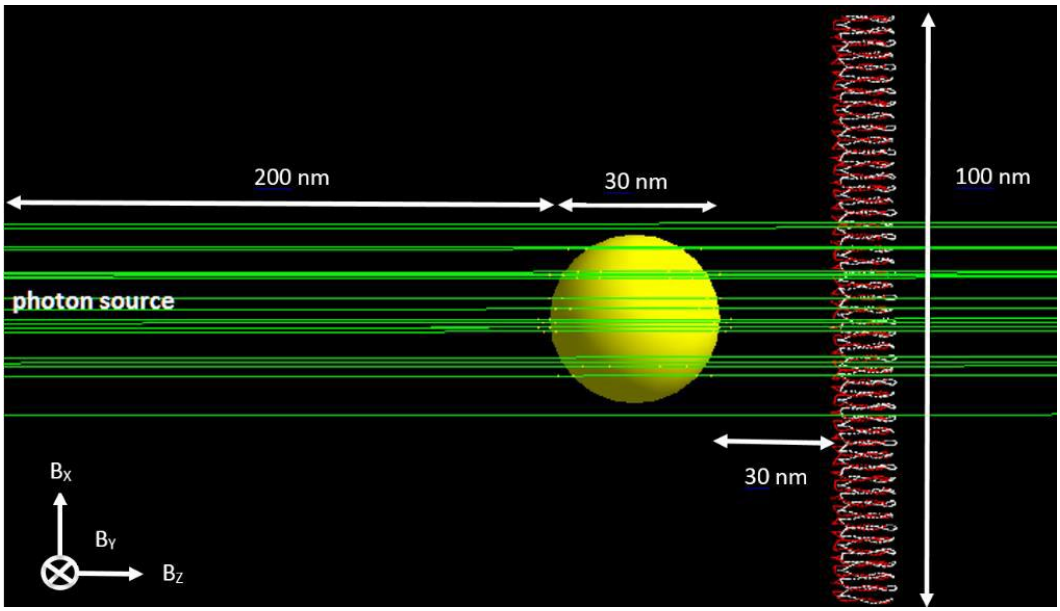

Figure 4. The Monte Carlo model geometry simulated in Geant4-DNA (not to scale) places a gold nanoparticle between the photon beam (green) and the DNA molecule. Nanoparticles with diameters of 30, 50, and 100 nm were used in the simulation [97].

7. Future directions

7.1. Identifying gaps in current research

Current research on nanomaterial-induced DNA damage has provided valuable insights but also revealed several gaps that need to be addressed. One significant gap is the limited understanding of the long-term biological effects and potential toxicity of nanoparticles. Most studies have focused on short-term impacts and acute toxicity, leaving the chronic effects and bioaccumulation of nanoparticles underexplored. Moreover, variability in nanoparticle size, shape, surface chemistry, and composition used in different studies complicates the comparison of results and hinders the establishment of standardized protocols for assessing DNA damage.

Another gap lies in the lack of comprehensive in vivo studies. While in vitro research has shed light on cellular responses to nanomaterials, translating these findings to complex living organisms remains challenging. The interaction of nanoparticles with various biological systems and their potential to induce systemic genotoxic effects are not fully understood. Furthermore, there is a need for more advanced and sensitive genotoxicity assays that can detect subtle and early DNA damage events, providing a more accurate assessment of nanomaterial-induced genotoxicity.

7.2. Suggesting future studies needed

Future studies should address these gaps through several key research directions. First, long-term

in vivo studies are essential to evaluate the chronic effects and potential toxicity of nanoparticles. These studies should investigate the bio-distribution, clearance, and bioaccumulation of nanoparticles, along with their long-term genotoxic impacts. Understanding these aspects is crucial for assessing the safety and risk associated with nanomaterial exposure.

Second, standardizing nanoparticle properties and experimental protocols is critical for enhancing the reproducibility and comparability of research findings. Researchers should focus on producing nanoparticles with uniform and well-characterized size, shape, surface chemistry, and composition. Establishing clear guidelines for nanoparticle synthesis, characterization, and testing in genotoxicity assays will facilitate consistent and reliable results across different studies.

Third, integrating advanced imaging and molecular techniques into genotoxicity studies could provide deeper insights into the mechanisms of DNA damage. Techniques such as high-resolution electron microscopy, single-molecule imaging, and next-generation sequencing can reveal the precise interactions between nanoparticles and DNA at the molecular level. Additionally, combining these techniques with traditional genotoxicity assays will offer a more comprehensive understanding of nanomaterial-induced DNA damage.

Fourth, interdisciplinary collaborations between materials scientists, biologists, toxicologists, and clinicians are essential for advancing research in this field. Such collaborations can foster the development of integrated models that combine physical, chemical, and biological data, improving our understanding of the complex interactions between nanomaterials and biological systems. These models can also aid in the design of safer and more effective nanoparticles for various applications.

Fifth, to address the variability in quantifying DNA damage caused by different nanomaterials, future studies should focus on developing standardized assays and protocols for assessing DNA damage. Establishing uniform methodologies will enable researchers to obtain comparable and reproducible data, facilitating accurate assessments of the impacts of various nanomaterials on DNA integrity. Comprehensive studies should utilize advanced techniques, such as comet assays and γ-H2AX foci formation, to quantify SSBs, DSBs, and other DNA lesions. By generating robust quantitative data, researchers can better contextualize the biophysical effects of nanomaterials and inform the development of safer nanomedical applications.

Lastly, exploring the potential of novel nanomaterials and hybrid systems for therapeutic purposes could open new avenues for cancer treatment and other medical applications. Investigating the use of nanoparticles in combination with existing therapies, such as radiation or chemotherapy, may enhance treatment efficacy and reduce side effects. Future research should focus on optimizing nanoparticle formulations and delivery methods to maximize their therapeutic potential while minimizing adverse effects.

8. Conclusions

Nanomaterial-induced DNA damage research has revealed crucial biophysical insights into how nanoparticle properties such as size, shape, surface chemistry, and composition influence genetic integrity. In vitro studies highlight that nanomaterials can cause DNA damage through direct interactions, ROS generation, and cellular disruption. Techniques like the comet assay, micronucleus test, and γ-H2AX assay have proven useful in detecting this damage, though their sensitivity and specificity vary. In vivo studies indicate that nanomaterials can induce systemic genotoxic effects, emphasizing the need for long-term safety evaluations.

MC simulations have shown the potential of nanoparticles to enhance radiotherapy efficacy, stressing the importance of nanoparticle properties in optimizing treatment outcomes. However, experimental validation and clinical studies are needed to translate these theoretical predictions into practice. Future research should focus on conducting long-term in vivo studies, standardizing nanoparticle properties and experimental protocols, and integrating advanced imaging and molecular techniques into genotoxicity assessments. Interdisciplinary collaborations will be key to developing comprehensive models that improve our understanding of nanomaterial interactions with biological systems. Furthermore, exploring novel nanomaterials and hybrid systems for therapeutic purposes could lead to safer and more effective treatments.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Acknowledgments

There is no financial support for conducting the research and preparing the article.

Conflict of interest

James C.L. Chow is an editorial board member for AIMS Biophysics and was not involved in the editorial review or the decision to publish this article. The author has no potential conflict of interest on financial or commercial matters associated with this study.

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