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Mechanism of Nanoparticle, Killing a Cell

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Outline

Introduction

With the evolution of bacteria and their property to develop resistance against the medicines used, it is becoming difficult to treat the infections with commonly available drugs, therefore, new generations of medicines are being developed and introduced [1-4]. The same is the case with cancers, for instance, there is a breast cancer type that is multi-drug resistant e.g., MCF-7, which means that it does not respond at all to various available drugs and spreads at will, resulting in risen number of deaths. According to a survey, in year 2020 alone, almost 10 million of the reported deaths were sue to some sort of cancer, while in 2019, almost 13.7 million deaths were caused by some sort of pathogenic infection. To bring these tolls down, scientists and researcher are focusing on developing a new range of medicines – the nanomedicines. In classical chemotherapy, medicinal agents employed for their chemotherapeutic efficacy have long been acknowledged for their cytotoxicity and potent anti-cancer properties [5]. However, the inherent cytotoxic nature of these drugs is associated with systemic toxicity. Chemotherapeutic agents, lacking target specificity, indiscriminately inflict damage upon both cancerous and normal healthy cell lines during the treatment process. In response to challenges posed by bacterial and fungal resistance as well as systemic toxicity, there has been a decade-long exploration of a novel class of therapeutic agents [6, 7]. Concurrently, pharmaceutical research endeavors to enhance drug availability and distribution, seeking improved therapeutic outcomes without resorting to elevated concentrations or more potent formulations. Addressing the limitations of conventional methodologies, a burgeoning field of research, known as nanomedicines, has emerged to provide potential solutions to these challenges. The nanomedicines are the use of nanoranged materials for medicinal purposes. There are 3 major factors that nanomedicines focus on, compared to the traditional drugs:

i. Reduced systematic toxicity or overall toxicity [8].
ii. Enhanced retention time and increased stability [9, 10].
iii. Enhanced bioavailability [10].

At the nanoscale, nanomaterials exhibit remarkable changes in their properties, contributing to the heightened efficacy and potency of nanomedicines against tested bacterial or fungal strains and cancer cell lines. Numerous studies substantiate the significant worth of nanomedicines, positioning them as a promising new generation of therapeutic agents for the treatment of critical diseases. In both in vivo and in vitro investigations, nanomedicines/nanoparticles showcase distinct advantages compared to classical drugs, renowned for their systemic potency. The considerable potential of nanomaterials has garnered attention from drug regulatory authorities, exemplified by the United States Food and Drug Administration (US FDA) approving various nano-related drugs. Notably, Droxil®, a nanomedicine, has secured US FDA approval [11]. **Figure 4.1** illustrates diverse types and shapes of nanoparticles with surface modifications, strategically employed for a spectrum of biomedical applications. This growing body of evidence underscores the transformative impact of nanomedicines in the realm of disease treatment.

Nanoparticles employ diverse routes and mechanisms to induce cell death, encompassing processes such as cell wall/membrane rupture, attachment to the cell wall/membrane, binding to the nucleus membrane, DNA rupture, interaction with cellular organelles, and the production of Reactive Oxygen Species (ROS). The prevailing consensus among researchers suggests that nanoparticle-mediated generation of ROS is a prominent mechanism for inducing cell death [2].

In this paper, we will provide a concise exploration of the various mechanisms and routes that nanoparticles may traverse to bring about cellular demise.

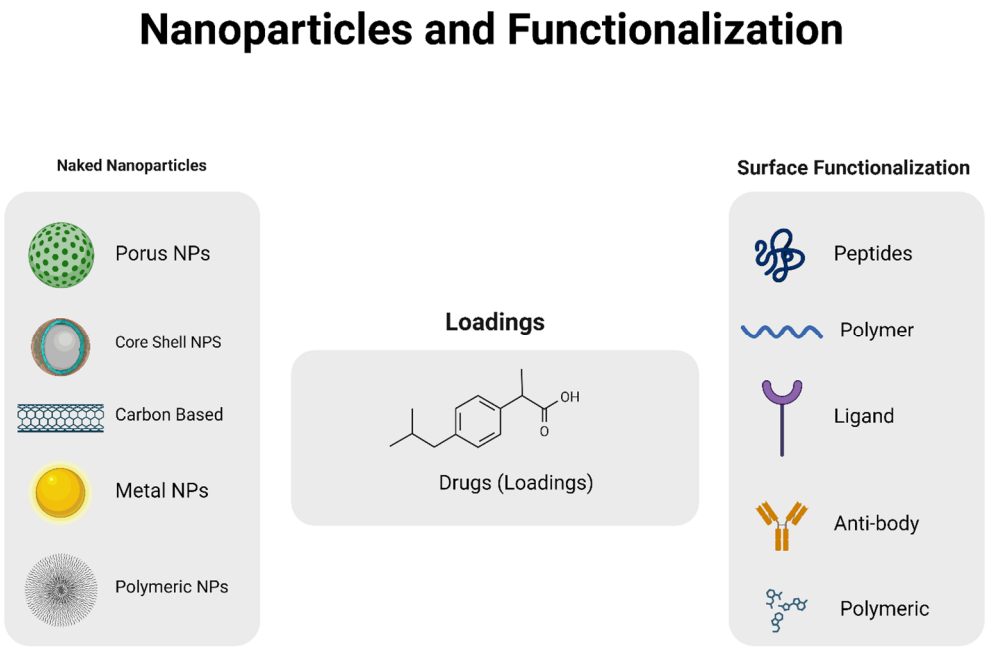

FIGURE 4.1
Types of Nanoparticles/Nanomedicines, Loadings, and Surface Functionalization.

Mechanisms Followed by Nanoparticles for Killing a Cell

Nanoparticles can adopt diverse routes and utilize various mechanisms to persuade cell death, regardless of whether the target is a bacterial cell, fungal cell, or cancer cell. Scientists have put forth several methods over time, and the routes and mechanisms involved may encompass:

i. Rapturing or blocking Cell wall/membrane.
ii. Rapturing/blocking the nucleus membrane.
iii. Interacting with cell organelles.
iv. Rapturing or interfering with DNA replication.
v. Generation of ROS.

The route or mechanism undertaken by nanoparticles critically depends on various factors, including but not limited to size, morphology, shape, and concentration. Additionally, surface modifications play a meaningful role in influencing the choice of nanoparticles for a particular application. Following we will be discussing these mechanisms and routes in a way that they are understandable and will use examples of mechanisms that have been proposed by various researchers.

Rapturing or blocking Cell wall/membrane

Exposure of nanoparticles (NPs) to bacterial cells can result in membrane damage, often initiated by NP adsorption and subsequent penetration into the cell [12, 13]. Numerous studies suggest that the primary mechanism of toxicity involves NP adsorption on the cell wall, leading to its disintegration [14, 15]. NP adsorption induces cell wall depolarization, causing a shift from the typical negative charge to a more permeable state. Observations using a laser scanning confocal microscope have reported a blurry appearance of the bacterial cell wall, indicating degradation following NP-induced changes [16]. Nanoparticle interaction with the cell membrane/wall can be broadly categorized into three major steps: a. endocytosis; pinocytosis; phagocytosis, b. rupture of the cell wall/membrane, and c. rupture of the cell membrane/wall. **Figure 4.2** provides a visual representation of the nanoparticle interaction with the cell membrane/wall.

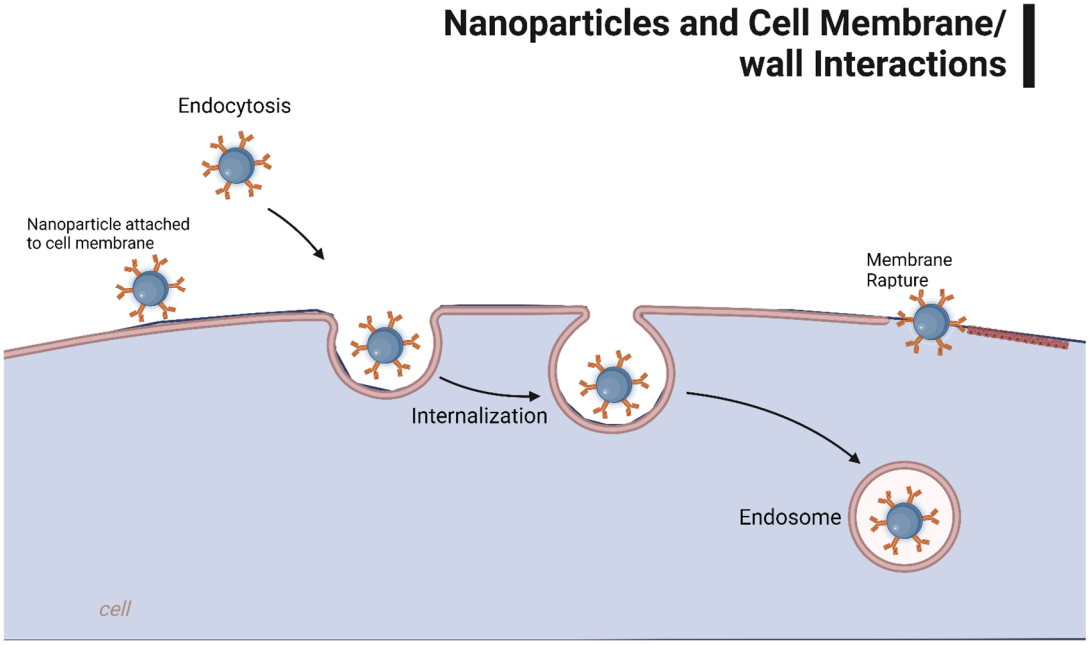

FIGURE 4.2
Pictorial Presentation between the Nanoparticle and Cell Membrane/Wall.

Endocytosis stands out as the predominant route for the transportation of nanomedicines across cellular membranes, typically categorized into pinocytosis and phagocytosis [17]. Phagocytosis, in the beginning identified in macrophages, involves the engulfment of large particles [18]. Pinocytosis, on the other hand, is a process present in all cell types and manifests in four distinct forms: caveolae-dependent endocytosis, clathrin-dependent endocytosis, clathrin- and caveolae-independent endocytosis, and macro-pinocytosis [19]. Phagocytosis, a distinctive endocytic pathway, is primarily observed in phagocytes such as neutrophils, macrophages, and monocytes [20]. This pathway is more inclined to accommodate relatively large particles. For nanoparticles to enter cells through

phagocytosis, they must first be recognized by opsonins, such as immunoglobulins (IgG and IgM), the blood serum proteins and accompanying components (C3, C4, and C5) [21]. Consequently, opsonized nanoparticles attach to the cell surface, initiating interactions with receptors that induce the formation of cup-shaped membrane extensions. These extensions envelop the nanoparticles, leading to their internalization and the formation of phagosomes, typically with a diameter ranging from 0.5 to 10 µm. Ultimately, the phagosomes migrate to fuse with lysosomes [22]. However, the cargo within the phagosomes undergoes degradation through enzyme-lysis and acidification in the lysosomes. Consequently, for nanomedicines to exert their desired effects, it is imperative to circumvent this route to prevent degradation. while Pinocytosis serves as a primary cellular pathway for the ingestion of fluids, suspensions, and solutes, comprising small particles. This process is cataloged into distinct forms, including caveolae-dependent endocytosis, clathrin-dependent endocytosis, clathrin- and caveolae-independent endocytosis, and macro-pinocytosis. The categorization is based on the specific proteins contained in each pathway [23]. In all these ways, nanoparticles can enter the cell and cause damage that it wills by following any pathway it follows.
Nanoparticles can be surface functionalized so that they can attach to the membrane pores, in that way they block the passage so that nothing (i.e., nutrition and all) passes inside the cell, and starves the cell, leading to cell death out of hunger. Lastly, the nanoparticles tend to damage the cell membrane/wall, and it causes the cell material to move out freely causing the cell to die.

Nanoparticles and Nucleus

Nanoscale materials, particularly nanoparticles (NPs), have garnered increased interest in the realm of biomedicine. A comprehensive understanding of the nanomaterials' behavior within living systems is crucial for the sketching of modified nanoparticles and the effective application of nano-based medicines already in use. Capitalizing on their elevated physical and chemical properties, nanomaterials offer notable gains in biomedical research. They exhibit preferential accumulation at the tumor growth site or the site of inflammation, facilitate rapid entry into cells through diverse mechanisms in comparison to small molecules, enable the delivery of various cargos to specific target sites, and can be easily modified to achieve desired functions [24]. In the case of nanoparticles, size emerges as a key parameter governing their in vivo and in vitro responses. Size-dependent variations have been observed in the distribution of nanoparticles within the organs of living mice in previous studies [25]. These nanoparticles are taken up into cells through distinct mechanisms [26] and follow size-specific pathways until they escape from the cells.
The nucleus stands out as the paramount organelle, playing a pivotal role in regulating cellular processes such as growth, metabolism, reproduction, and cell death via gene expression. The precise control of nuclear-driven processes has been a primary objective in the realm of nuclear-targeted treatment. However, a significant majority of nanomaterials predominantly flow into the cytoplasm, and limited knowledge is available regarding their uptake, infringement into the nucleus, and their role in controlling gene expression. In previous studies, gold nano-stars were employed for delivering drug molecules to the nucleus, inducing changes in apoptosis and nuclear phenotype [27]. Additionally, the utilization of nuclear-targeting gold nanoparticles (Au NPs) was narrated by Kang, B., disrupting the cancer cell division, selectively, with the execution of cytokinesis arrest [28]. Other studies demonstrated the attachment of reporter genes or therapeutics onto magnetic nanoparticles for targeted gene delivery through high-gradient magnets, resulting in *in vitro* transfection of various

cell lines [29, 30]. However, in these instances, nuclear targeting was achieved indirectly through the conjugation of nuclear-targeting peptides over nanoparticles or via magnetic targeting.

Various routes and mechanisms come into play when nanoparticles interact with the nucleus. One possible action involves the nanoparticle attaching itself to the nuclear membrane pores, preventing mRNA from issuing commands for the synthesis of specific proteins essential for cell functions. Given that the nucleus serves as the command-and-control center of a cell, hindering the passage of commands can lead to cellular dysfunction and potential demise. Additionally, nanoparticles may rupture the nuclear membrane, causing the release of nuclear material into the cytoplasm, ultimately resulting in cell death. Another prevalent mechanism involves nanoparticles attacking the DNA, impeding its translation and replication processes, thereby halting cell division. Notably, the effectiveness of these mechanisms is intricately linked to nanoparticle size. In the realm of gene therapy, there are numerous reports on the synthesis of ultra-small nanoparticles tailored for therapeutic purposes. These nanoparticles, often of minimal size, are designed to interact with DNA, aiming to modulate genetic processes and impede cell multiplication for therapeutic applications. For instance, Huo, S., and group's study focused on investigating the penetration capability of Au NPs, depending on the nanoparticle size, and explored the potential activity of ultrasmall Au NPs for intranuclear delivery and therapy. The research group synthesized Au NPs with varying diameters of 2, 6, 10, and 16 nm, assessing their intracellular distribution in MCF-7 breast cancer cells. Notably, nanoparticles smaller than 10 nm (2 and 6 nm) were observed to enter the nucleus, while larger ones (10 and 16 nm) were confined to the cytoplasm. The study further explored the use of ultrasmall 2 nm nanoparticles as carriers for nuclear delivery of a triplex-forming oligonucleotide (TFO) targeting the c-myc promoter. Comparative analysis revealed that the nanoparticle-conjugated TFO was more effective in reducing c-myc RNA and c-myc protein levels, consequently leading to a reduction in cell viability when compared to free TFO. The results underscored the critical size dependence for gold nanoparticles' entry into the cell nucleus. Significantly, the study proposed a strategy for modulating gene expression by directly delivering TFOs into the nucleus using ultrasmall gold nanoparticles. Moreover, the research provided valuable guidelines for selecting appropriate nanocarriers tailored to different biomedical purposes [30]. Figure 4.3(a) shows schematics of nanoparticles' interaction with cells and nucleus. In contrast, 4.3(b) shows BioTEM images of the nanoparticles with cells, at varied sizes (Figure is adopted from [30], Published under open access, Creative Commons (*CC*) License).

There have been other reports, like a report published by Dam, D. H. M., et. al., synthesized Au nano-constructs and loaded nucleolin-specified aptamer and studied their potential to interact with the nucleus. The synthesized Au nano-constructs had an average size of 25 nm, with aptamer AS1411 attached to the nano-construct surface. In summary, the researchers conducted a study wherein they directly observed the interaction between an aptamer-loaded Au nano-construct and the nucleus of cancer cells. They compared the observed deformations (morphological) in the Nuclear Envelope (NE) with enhanced efficacy. Leveraging the carrying protein nucleolin, the research group actively delivered the nano-construct to nanoscale locations in the vicinity of the nucleus, inducing intense invagination and deformation of the NE at the construct site. These morphological transformations were further intensified upon light-triggered, ultraquick release of the aptamer from the Au nano-constructs. The study suggested that the functionality of cancer cells can be linked to deformations and distortions in the nucleus, implying that challenges faced in nano-based nuclear therapy can be settled. For instance, complete internalization of nano-constructs within the nucleus may not be mandatory if prompted physical alterations in nuclear phenotype can disorder nuclear

functions. Moreover, since morphological effects are triggered by NPs outside the nucleus, there might be no limitation on the size or shape of NPs capable of achieving a similar response. These findings offer valuable insights for developing new strategies in the design of drug-loaded NPs with increased therapeutic efficacy [27].

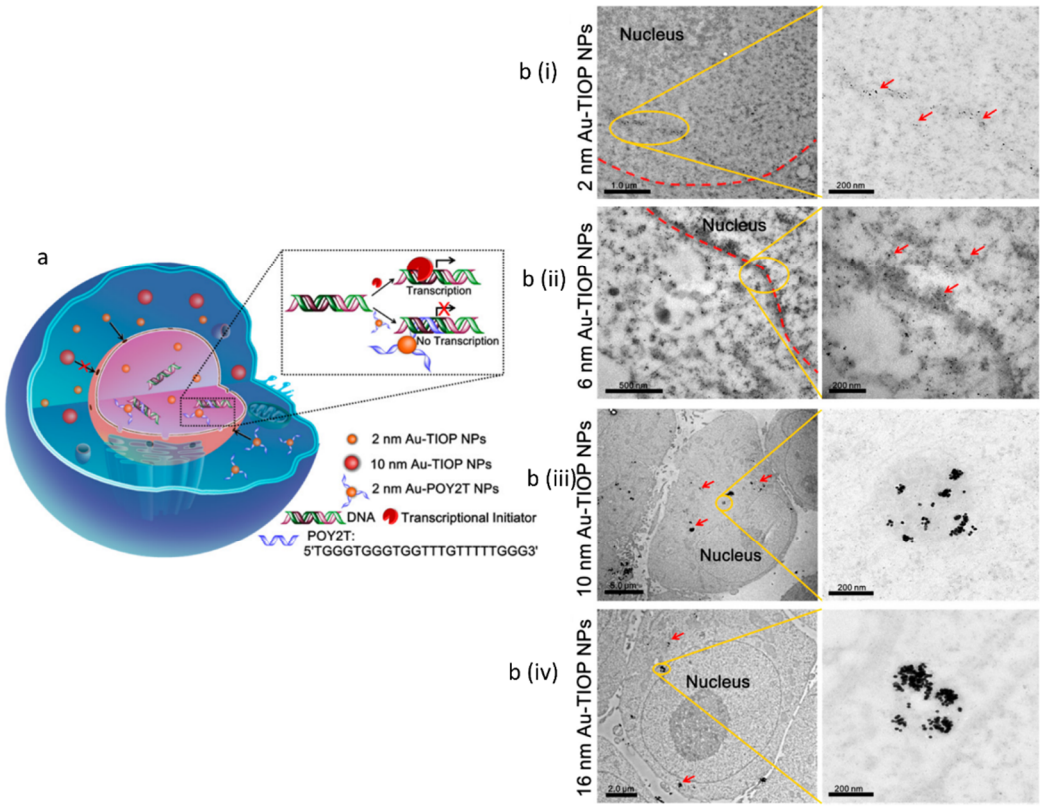

FIGURE 4.3
Nanoparticles' interaction with Nucleus. (a) Nanoparticle stopping replication of DNA. (b i) BioTEM image for 2 nm sized Nanoparticles interaction with nucleus, (b ii) BioTEM image for 6 nm sized Nanoparticles interaction with nucleus, (b iii) BioTEM image for 10 nm sized Nanoparticles interaction with nucleus, and (b iv) BioTEM image for 16 nm sized Nanoparticles interaction with nucleus (Figures adopted from [30], published under open access, Creative Commons (*CC*) License).

Nanoparticles and cell Organelles

The intricate and ever-changing interplay between the surface characteristics of nanomaterials and their interactions with biomolecules holds the promise of generating innovative and unforeseen effects on biological systems. Some of these effects may be strategically harnessed for diagnostic and therapeutic applications. A methodical approach has been employed to explore how nanoparticles engage with cells in vitro. These interactions involve mechanisms to traverse the plasma membrane and exploit cell-specific pathways for accessing subcellular organelles. Distinct pathways emerge depending on the surface chemistry and functionalization of nanoparticles, with biological outcomes influenced by both subcellular localization and nanoparticle surface properties.

The development of a biomolecular corona offers an avenue for manipulating highly toxic nanoparticles. In the absence of a protein corona, these nanoparticles may induce unregulated necrotic cell death. However, by leveraging the biomolecular corona, such nanoparticles can be delivered into cells, withholding their toxic potential until controlled apoptotic cell death is triggered upon degradation of the protein corona by lysosomal enzymes. Conversely, finely tailored engineering of nanoparticle surface components becomes crucial when the goal is to direct nanoparticles to a specific target organelle or subcellular compartment. This customization enables the nanoparticles to evade the endo-lysosomal pathway, ensuring precise delivery.

Lysosomal Rupture

Regardless of whether the uptake is specific or nonspecific, nanoparticles tend to accumulate in lysosomal compartments after traversing the endo-lysosomal pathways. Notably, it has been observed that cationic nanoparticles can induce swelling and eventual rupture of lysosomes upon uptake [31]. This phenomenon is attributed to the presence of positively charged amine groups on the surface of polystyrene nanoparticles. The swelling and rupture of lysosomes were experimentally demonstrated by labelling the biomolecular corona on the surface of the cationic particles [32]. The fluorescently labelled corona underwent monitoring throughout the nanoparticle uptake process, revealing colocalization with the nanoparticles upon reaching the lysosomes. Over time, the intensity of the labelled corona decreased in tandem with an observed increase in cathepsin activity. This suggests that lysosomal enzymes initiated the degradation of the biomolecular corona, exposing the amino groups on the particle's surface [33]. This indicates that highly positively charged polystyrene particles can enter cells by utilizing the strongly bound protein corona biomolecules, gaining access to the lysosomal pathway. Subsequently, lysosomal enzymes degrade the biomolecular protective layer, revealing the amino groups on the particle surface. The protonated amino groups, once exposed, lead to lysosomal compartment rupture, releasing enzymes and other lysosomal contents into the cytoplasm. This process ultimately induces cell death through apoptosis [34, 35].

Nanoparticle accumulation in Lysosomes

The recognition that mammalian cells in culture possess the ability to internalize substantial portions of the cell membrane through invagination pits, a process known as endocytosis, is well-established [36]. Endocytosis encompasses various mechanisms, including clathrin-dependent and clathrin-independent receptor-mediated endocytosis, pinocytosis, and phagocytosis [37, 38]. One extensively studied avenue of nanoparticle uptake involves receptor-mediated endocytosis, particularly through a clathrin-dependent mechanism. In this process, a high-density region of transmembrane receptors with high affinity for their corresponding ligands initiates the formation of an invagination pit on the cell membrane surface, facilitated by clathrin, a cytoplasmic membrane coating protein [39]. The resulting clathrin-coated vesicles, termed early endosomes, become fully enclosed within the cell and are present in the cytoplasm. The endosome containing nanoparticles typically follows the endo-lysosomal internalization pathway, progressing to mature acidic lysosomes. These lysosomes are designed to break down unwanted materials, utilizing lysosomal enzymes known as cathepsins. Lysosomes are often considered the 'graveyard' for nanoparticles, as cells seldom exhibit nanoparticle exocytosis or recycling back to the cell surface, a common occurrence in protein receptor recycling post endocytosis [40]. In rare instances, nanoparticles have demonstrated the

ability to escape the lysosomal pathway and access the recycling pathways of the endocytic process [41]. Moreover, it has been proposed that certain nanoparticles, regardless of their physical dimensions, shape, and surface modifications, can evade lysosomal confinement and be stored in non-lysosomal organelles [42].

Nanoparticles and Mitochondria

The mitochondrion stands as one of the largest organelles within human cells, encompassing approximately 25% of the cytoplasmic volume [43]. As an indispensable organelle for most eukaryotic cells, it plays a pivotal role in determining cell survival or death. Its primary function is renowned as the chief site for producing cellular adenosine triphosphate (ATP) [44], thereby serving as the hub for energy distribution throughout the cell. Beyond its role in ATP generation, mitochondria actively participate in critical cellular processes such as maintaining calcium homeostasis [45], generating reactive oxidation species (ROS) [46, 47], initiating apoptosis [48], and releasing metabolites that regulate cell fate and function [47]. Consequently, any impairment in mitochondrial function has the potential to significantly disrupt cell and tissue homeostasis. An increasing body of research indicates that mitochondrial dysfunction is a prevalent occurrence in both healthy aging and various diseases, with a particular emphasis on neurodegenerative diseases (NDs) [49]. In the context of diseases such as Alzheimer's disease (AD), mitochondrial dysfunction has been implicated in triggering the accumulation of phosphorylated tau (p-tau) and amyloid-beta (Aβ) [50]. Additionally, it is noteworthy that a significant number of familial Parkinson's disease (PD) loci have direct connections to mitochondrial functions [51].

NPs offer several advantages for the treatment of neurodegenerative diseases (NDs) [52]. Their adjustable size, appropriate charge, and lipophilic surface contribute to enhanced drug-loading capabilities due to the increased surface area to volume ratio of NPs. This, in turn, allows for the utilization of low drug doses in vivo, achieving both high efficacy and safety [53, 54]. Moreover, NPs can be customized by functionalization with polyethylene glycol (PEG) or specific targeting residues such as peptides, biotin, and folic acid [49]. These modifications serve to prolong circulation duration [55] or facilitate Central Nervous System (CNS) targeting, respectively [56, 57]. Notably, NPs serve as sustained and targeted drug delivery systems for addressing dysfunctional mitochondria in NDs . In the following, we introduce various types of NPs that hold promise for treating NDs associated with mitochondrial dysfunction. There have been various studies for the treatment of NDs by targeting mitochondria with the help of nanoparticles. For instance, In the treatment of a Huntington's Disease (HD) rat model, Solid Lipid Nanoparticles (SLN) encapsulated with thymoquinone (TQ-SLNs) have demonstrated efficacy. The HD condition is characterized by excessive hydroxyl radical production in the mitochondria, resulting in abnormal succinate dehydrogenase (SDH) activity. TQ-SLNs play a crucial role in enhancing the in vivo bioavailability of free TQ by fivefold. In comparison to the control group, the treatment with TQ-SLNs leads to a notable increase of 20% in SDH activity in the rat striatum. Moreover, there is a significant reduction in the degeneration of mitochondria observed 14 days after TQ-SLN treatment, indicating a positive impact on mitochondrial health [58]. Furthermore, Yang et al. conducted a study to explore the in vivo therapeutic effects of micelles for the treatment of Alzheimer's Disease (AD). They developed PEG-PLA micelles (CT-NM) conjugated with mitochondrial-targeting molecules, namely triphenylphosphonium (TPP) and a neural cell adhesion molecule mimetic peptide C3. The goal was to simultaneously target neurons and mitochondria in the treatment of AD. CT-NM, loaded with Cou-6, demonstrated significant cellular

accumulation and co-localization with mitochondria in merged images, with a co-localization coefficient value (R) of 0.81. This finding indicates that CT-NM exhibits superior targeting ability for neuronal mitochondria compared to other groups, including NM (PEG-PLA micelles), T-NM (micelles modified with TPP), and C-NM (micelles modified with C3). In comparison to the $A\beta_{25-35}$-treated group, CT-NM/Res (micelles loaded with a therapeutic agent, Res) showed a remarkable 63% reduction in mitochondrial reactive oxygen species (ROS) and restoration of mitochondrial membrane potential. These results suggest that CT-NM/Res effectively protects neuronal mitochondria *in vitro*. Figure 4.4 shows the targeting schematics of micelles towards mitochondria [47] (figure adopted from [47], published under Open access, Creative Commons (*CC*) License).

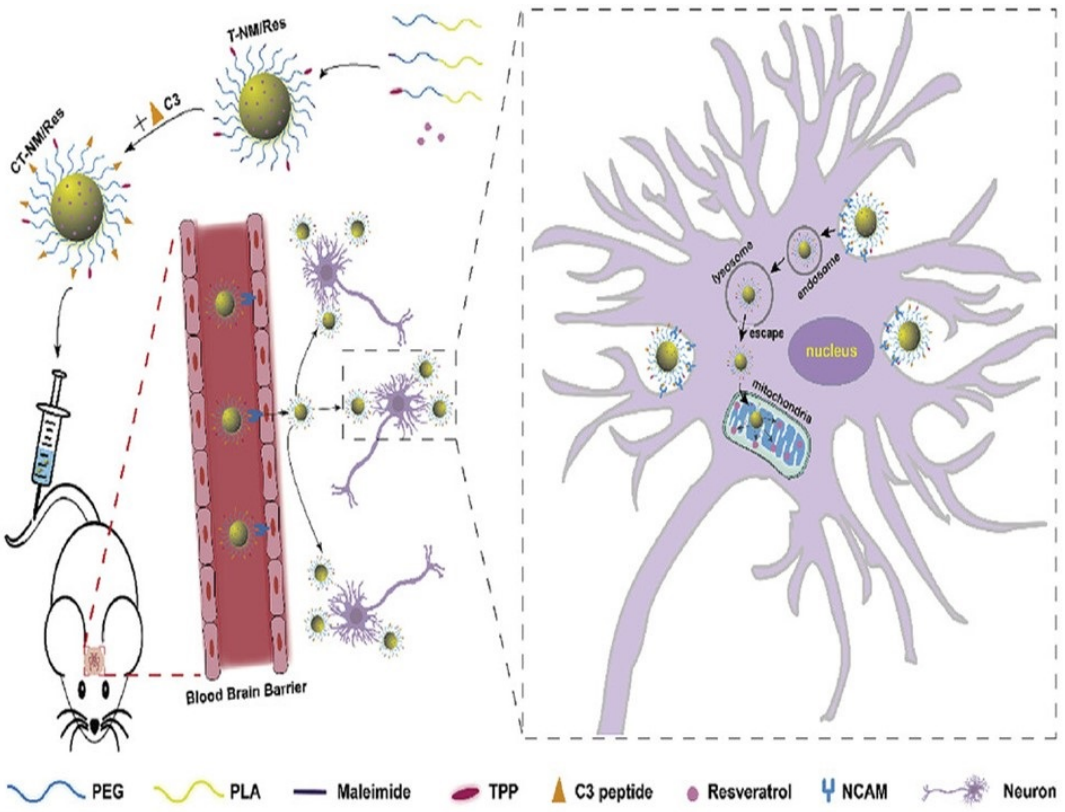

FIGURE 4.4
Schematics of Micelle delivery and mechanism of reaching Mitochondria (figure adopted from [47], published under Open access, Creative Commons (*CC*) License).

Although there are a number of cell organelles, that nanoparticles can interfere with and cause cellular damage, but these are major attachments ad interferences of nanoparticles inside a cell.

Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) represent highly reactive oxygen molecules generated during fundamental cellular metabolism. Cells have evolved universal intracellular defence mechanisms to counteract their undesired effects and prevent damage to essential biomolecules. Elevated levels of ROS, particularly under conditions of heightened stress, are believed to serve as a central mechanism through which NPs exert their inhibitory effects on bacterial growth [59, 60].

ROS formation occurs when oxygen undergoes undesired reduction states, transforming into free radicals, super-oxides, and peroxides instead of forming water. Various stressors, including UV light, DNA damage, and NPs, can amplify ROS production to levels that become toxic, leading to cell damage or death [61]. Notably, studies have demonstrated that NPs generate free radicals, with an observed correlation between increasing NP concentration and elevated ROS levels [62-64]. Even bacteria like *C. metallidurans*, which are adapted to heavy metal stress, experience an augmentation of ROS levels during exposure to NPs [65].

The escalating presence of NPs in the environment may disrupt native bacterial populations, such as nitrifying bacteria essential for ammonia transformation to nitrates in municipal sewage treatment. Investigations have indicated that, while ROS production occurs in nitrifying bacteria exposed to Ag^{-} NP and AgCl colloids, the inhibition of bacterial growth is primarily attributed to Ag^{+} ions, potentially due to the Ag concentration [66]. Exposure to sublethal concentrations of Ag-NPs upregulates nitrifying genes in bacteria. However, at higher concentrations, this upregulation diminishes, possibly due to interference with ROS generation caused by the loss of cellular integrity at elevated NP concentrations.

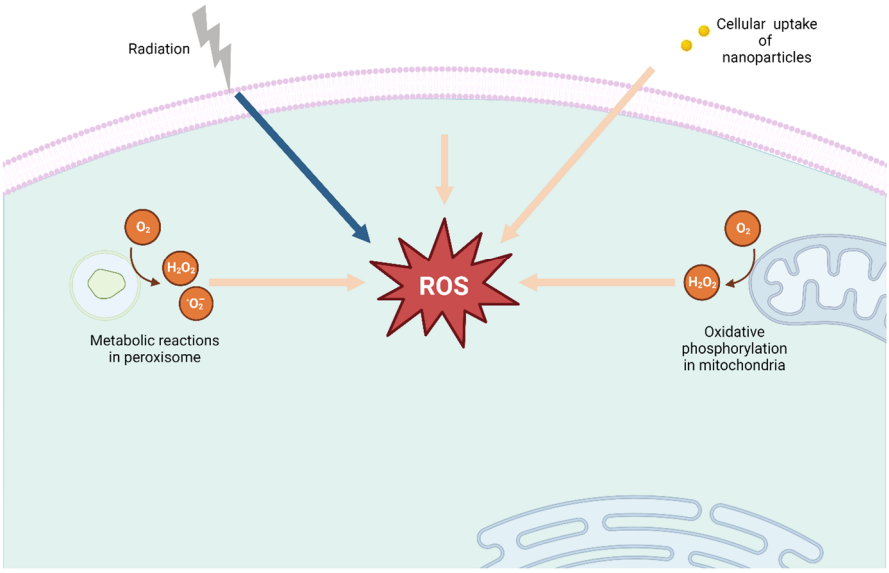

FIGURE 4.5
Schematics of Reactive Oxygen Species (ROS) generation by nanoparticles inside a cell and possible mechanism of cellular organelles damage.

The oxidation state of the metal in NPs could contribute to their bactericidal effect. For example, Cu_2O-NPs exhibit greater antibacterial activity than CuO-NPs, indicating that oxidation may play a role in toxicity [67]. The reaction of O_2 with Cu_2O to form Cu^{2+} can lead to sustained oxidative stress. Superoxide molecules reduce Cu^{2+} to Cu^{+}, generating H_2O_2. This H_2O_2 can further react with Cu, producing OH^{-}. Cells exposed to CuO-NPs exhibit higher concentrations of OH− compared to Cu_2O-NPs, suggesting that intracellular proteins interact more with Cu_2O than CuO [68]. Figure 4.5: Schematics of ROS generation.

Furthermore, the ROS interact with various parts of a cell, for instance, both intracellular and extracellular ROS have the potential to disturb cell membranes [61]. One way to modify the cell membrane involves lipid oxidation, a process easily triggered by free radicals [69]. Interestingly, in the context of *S. aureus*, lipids did not experience the anticipated level of impact, likely due to the thicker cell wall structure characteristic of Gram-positive bacteria. Certain ROS, such as OH radicals, carry a negative charge, making it challenging for them to easily breach the negatively charged cell membrane, irrespective of Gram classification [70]. However, H_2O_2, a commonly generated ROS, possesses the ability to penetrate the cell membrane and exert bactericidal effects [71]. Furthermore, the presence of oxidative stress can result in an amplified depletion of glutathione (GSH)[72]. Within Gram-negative bacteria, the generation of intracellular ROS can be gauged by assessing the ratio of GSH to oxidized glutathione (GSSG). GSH, a tripeptide thiol, functions by diminishing disulfide bonds to cysteines while concurrently undergoing oxidation to form GSSG. This biochemical process acts as a defense mechanism for the cell, guarding against potentially detrimental redox reactions through the scavenging of ROS molecules [61, 62]. Notably, when bacteria were exposed to Ag-NPs, there was a discernible reduction in GSH levels, coupled with an escalation in the production of GSSG [73]. Comparable outcomes were observed when E. coli encountered ZnO- and TiO2-NPs [74].

An increase in ROS levels within a bacterial cell may trigger the activation of genes responsible for shielding the cell against ROS. However, not every type of NPs is capable of instigating an antioxidant response, as exemplified by *E. coli* exposure to MgO-NP [14].

Treatment of *Pseudomonas sp*. cells with Ag-NPs resulted in the heightened expression of specific proteins, including translational ribosomal proteins S2 and L9, ketohydroxyglutarate aldolase (KHGA), AhpC (alkyl hydroperoxide reductase), and TSA (thiol-specific antioxidant) [75]. Both TSA and AhpC belong to the peroxiredoxin antioxidant enzyme family, serving to shield the cell from peroxide-induced damage and expressed during instances of oxidative stress [76]. This increased expression aligns with the notion that Ag-NPs induce oxidative stress in cells, evident from the elevated production of these enzymes to counteract escalating ROS levels. KHGA, associated with sugar metabolism, engages in the conversion of sugar acids, hexonates, and hexuronates into pyruvate and glyceraldehyde-3-phosphate [70]. It also regulates glyoxylate levels and prevents toxin accumulation. The expression of KHGA may be a consequence of Ag-NP-induced alterations in metabolism. Translational ribosomal proteins S2 and L9 play roles in translational regulation and contribute to structural and stress regulation [77].

Conclusion

The nanomaterials are known to cause cellular death by following various routes. The most followed method is the rapture of DNA. It has been well established the nanoparticles cause damage to the

Helical structure of the DNA. Although these methods look fascinating that nanoparticles can successfully kill the cancer cells, but there is another angel to look at it. That is that this could be cellular toxicity also. Therefore, some studies are needed to determine which could be the limitations and what doses could be recommended.

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