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Review Article

Mechanism, of Biophysicochemical Interactions and Cellular Uptake at the Nano-Bio Interface: A Review

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Abstract: Although numerous studies have investigated the interaction between nanoparticles and biological systems (proteins, cells, tissues, membrane etc.), and the growing interests of nanotoxicity of these engineered nanoparticles, much remains to be investigated. First, there are various factors to be explored, such as the physical or chemical properties of materials, different cell lines, and the systematic study of specific materials. Secondly, architectural structure (shape) conditions of NPs have not been well investigated and undestood. Third, the variations in cell line result in different cell uptake, toxicity, or transportation in the same materials, but systematic studies of this phenomenon are scanty. Fourth, the nanotoxicity issue and the accumulation of non-degradable materials relating to biosafety are yet to be understood. Fifth, the transformation of NMs' surface chemistry in living creatures is too complicated to investigate. In this article, we review the biophysicochemical mechanisms of the various interactions between nanomaterials and biological systems (proteins, cells, membrane). With the rapid increase in studies related to nanotechnology, investigations on nanomaterials can be more beneficial than others because of their size. A comprehensive understanding of nano-bio interactions can serve as a foundation for future biomedical applications.

Keywords: Nanoparticles, Cellular Uptake, Toxicology, Polymer

1. Introduction

In recent years, the interaction of nanoparticles (NPs) with biological systems has become an interesting area of fundamental and applied research at the interface between physical and life sciences [1]. As NPs are similar to typical cellular components and proteins, they may evade the natural defenses of biological organisms, utilize the endocytosis machinery for intruding cells and, thereby, lead to permanent cell damage [2]. While many studies have been performed using cell biology or toxicology approaches, recent work has shed light on the molecular aspects of the biological action of NPs. The application of sophisticated techniques such as molecular biophysics may facilitate the elucidation of the biomolecular interactions involved and reveal the fundamental factors governing the biological effects of NPs.

Whenever NPs come in contact with biological systems, physical and chemical interactions take place between the surfaces of the NPs and these biological components (e.g., proteins, membranes, phospholipids, endocytic vesicles,

organelles, DNA, etc.) - the so-called "nano-bio interface". It is well established that, upon the exposure of an organism to NPs, proteins from body fluids bind to NP surfaces [3]. The exposure leads to an interaction between living systems and protein-coated NPs [3]. The "protein corona" that forms around the NPs [4] largely defines the biological identity of the NP, and the efficiency of this interaction can be a decisive factor driving the biological response of an organism to NP exposure [5]. Nel and co-authors [6] have presented an in-depth discussion of the basic physical interactions happening at the nano-bio interface.

2. Nanoparticle–protein Interactions

NPs are composed of a core material and a surface modifier that can be used to change the physicochemical properties of the core material. These physicochemical properties include charge, hydrophobicity, or surface chemical properties that can be altered by attaching specific chemical compounds. Because these physicochemical characteristics influence NP efficacy in any application, both properties of the NPs determine their biocompatibility and biodegradation [7]. NP charge is a significant factor that affects the interactions between NPs and proteins to form NP–protein corona. The charge dictates the biodistribution of NPs *in vivo* [8]. Aubin-Tam and Hamad-Schifferli studied the effect of NP surface charge by using AuNPs functionalized with positive (aminoethanethiol), negative [bis (p-sulfonatophenyl) phenylphosphine], or neutral [poly (ethylene glycol) thiol] ligands attached to specific protein sites, such as cysteine 102 (C102) of Saccharomyces cerevisiae cyt C [9]. The study showed that changing ligand charge could influence the denaturation of the attached yeast cytochrome; protein denaturation is determined by the interaction between the NP ligands and amino acids localized around C102 affecting protein stability. These studies demonstrated that the NP surface charge, defined by the functionalized ligands, could have a significant impact on the structure of proteins on the NP surface.

Decuzzi and coworkers (2010) had demonstrated the effect of NP surface charge on NP–protein interactions [10]. Three major human hard corona proteins were used: human serum albumin (HSA), fibrinogen (Fg), and immunoglobulin G (IgG) in combination with 40 nm AuNPs and silver nanoparticles (AgNP) coated with citrate and lipoic acid to study time-dependent adsorption kinetics, individual protein corona formation on the NPs, and the effect of corona formation on NP dispersion and stability of saline (PBS), NP–HSA, and NP–IgG coronas. In the case of NP– HSA, all coronas exhibited a negative charge; however, in both lipoic acid–NPs and citrate–NPs, the net charge of the NP surface changed from negative to positive following coincubation with IgG. The zeta-potential value of lipoic acid–NP–IgG was higher than that of citrate–NP–IgG, indicating a higher stability of lipoic acid–NP–IgG coronas. This result explains the higher binding affinity of IgG for NP surfaces compared with citrate. However, the Fg corona induced agglomeration of AuNPs and AgNPs. This phenomenon could be due to the elongated rod-like conformation and unique molecular dimensions of Fg [11]. Despite the negatively charged surfaces of both lipoic acid–NPs and citrate–NPs, the binding affinity and corona formation tendency on the AuNP and AgNP surfaces was dependent on the characteristics of the individual proteins. This suggests that the nature of the interaction of each protein is different and could potentially affect the biological response of the NPs.

The interactions of the protein with the surface of NPs (e.g. Au NPs) largely depend on some factors such as the method of preparation, surfaces, and composition. Unfortunately, only a handful of efforts have been made previously to divulge the underlying mechanism that controls the adsorption capacities, interaction and binding processes of different blood proteins when they competitively and selectively bind to CNT surfaces. Exposure of our body to or inhalation of CNTs materials will most likely lead to interaction with internal tissues/organs exposed to these CNTs or CNT-based nanomaterials. Therefore, understanding the mechanisms underlying the interactions between CNTs and serum proteins is very critical in clarifying the potential hazard of CNTs, including the associated cellular trafficking and systemic translocation. Ge and co-workers [12] employed both experimental [fluorescence spectroscopy, circular dichroism, atomic force microscopy (AFM), and NMR spectroscopy] and theoretical methods (molecular dynamics simulations) to investigate the single-wall carbon nanotube (SWCNT)-protein interactions. They observed an amazingly competitive binding of different serum proteins onto the surface of SWCNTs, with different adsorption capacities and packing modes. The observation indicates that these competitive binding behaviors of serum proteins on the SWCNT surfaces are guided by each protein's unique chemical structure and the quantity of amino acid hydrophobic residues that each protein contains. These separate protein-coated SWCNTs often possess different cytotoxicity by swaying the next cellular responses. This investigation has shed light toward the design of safe carbon nanotube materials through a detailed understanding and accounting for their interactions with human blood proteins.

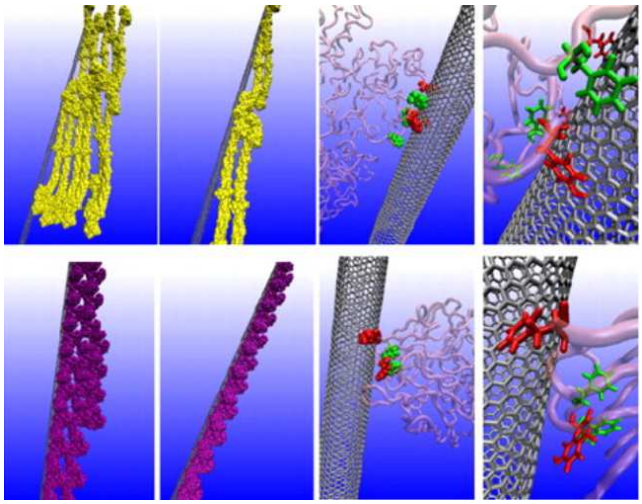

Figure 1. *Interactions between proteins and SWCNTs [12].*

3. Interaction of Polymers and Nanoparticles with Membranes

Hydrophobic/hydrophilic balance, as well as polymeric 3-D chemical structure, controls the interaction between polymers and lipid membranes. Adsorption, accompanied by reassembly of domains, will occur by introducing graft polymers [18]. Hydrophilic polymers such as polyethylene oxide can be inserted into membranes, giving rise to a ‘‘mushroom’’- or ‘‘brush’’-type assembly [19]. Polymers such as polyamides can bind effectively onto an oppositely charged lipid bilayer membrane [20], resulting to lateral phase separation via electrostatically induced flip/flops [21–25]. Finally, membrane alterations can produce a clear and visible increase in the curvature of the membranes if the bending modulus is smaller than the interacting forces [26, 27]. The presence of pores in polymers is another structural characteristic that has recently become debatable among membrane scientists [28] giving rise to different types of penetration, depending on the polymer’s 3D structure. Polycarboxylate [29] as well as di-, tri- [30-31] and starblock copolymers [32–35] composed of PEO–PS-diblock copolymers [36-39] have been studied and showed a strong impact on the molecular weight [40, 41] as well as displaying sealing effects [42, 43] or lipids membrane deterioration such as in cationic polymers [44, 45]. A large amount of separate deviation is observed when nanoparticles (up to 100 nm) interact either with polymersomal membranes or lipid, as stabilities of lipo- and polymersomes are not the same. Hence, hydrophilic nanoparticles are organized onto the inside or outside of lipid vesicles. Insertion of hydrophobic nanoparticles into lipid membranes poses a great challenge since the membranes are destroyed during the loading process [46, 47]. Macroscopically visible effects include curving of the lipid membrane, budding or fission effects [48, 49]. Negatively charged nanoparticles often result in clustering effects thus reducing charge movement/transfer and increasing the gel phase of the involved lipids [50]. However, lipid bilayer composed of polymers can largely and easily get assembled and decorated with nanoparticles by incorporation into the hydrophobic interior [51], or through outside attachment.

The interaction between graphene and cells has not been explored because of the high complexity of such interactions. Graphene - a two-dimensional single-atom-thick nanomaterial with unique structural, mechanical and electronic properties has potential biomedical applications [52-54]. Its high specific surface area allows high-density biofunctionalization for nanotechnology-based drug delivery [55-57]. Furthermore, its smooth, contiguous topography and bio-persistence play a unique role in foreign-body-induced carcinogenesis and tumor progression [58, 59]. Moreover, because graphene demonstrates ultra-high *in vivo* tumor uptake in mice, it is effective for photothermal ablation of the tumor [60]. Currently, recent studies have shown that graphene and graphene oxide have strong antibacterial activity [61, 62] towards Escherichia coli (E. coli). This cytotoxicity is due to physical damage resulting from direct interactions between the graphene and the bacteria cell membrane [63]. Graphene induced cytotoxicity is significantly reduced when the nanosheets are surrounded by proteins [64], such as serum proteins. Therefore, graphene is less a threat to humans or other mammalians but lethal to bacteria thus making it a novel antibiotic against bacteria. However, the detailed dynamical process and underlying molecular mechanism of graphene induced degradation of the bacterial cell membrane to remain unclear.

Studies by Tu and co-workers [65] have shown how graphene nanosheets can penetrate the cell membranes of E. coli while extracting phospholipids from the membranes. Experiments show that this process causes degradation of E. coli membranes and reducing bacteria viability. Their molecular dynamics simulations reveal atomic details on how graphene and graphene oxide interact with the inner and outer membranes of E. coli. These findings offer new insights for the design of graphene-based antibiotics for medical applications.

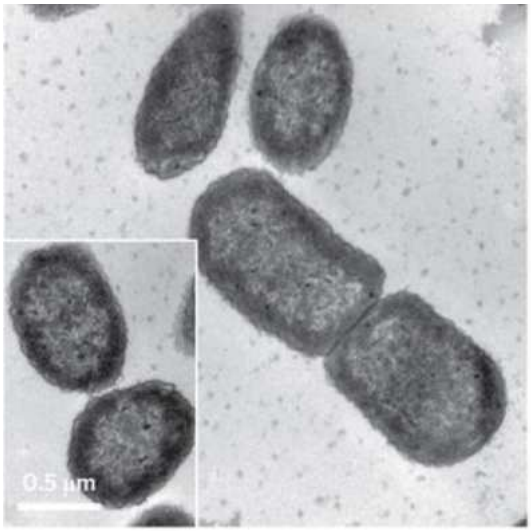

Figure 2. *Morphology of E. coli subjected to graphene oxide nanosheets. TEM images showing E. coli undergoing changes in morphology after incubation with 100 mg/ml [66] graphene oxide nanosheets at 37°C for 2.5 h. Three stages of destruction can be seen.*

4. Interaction of Nanomaterials (NMs) with Cell

The interactions between NMs and cells have been explored in recent studies since the physical and chemical properties of NMs have a considerable influence on the biochemical properties of cells that are in contact with each other. The physical properties include; size, shape, surface area, and surface compositions, while surface chemistry includes surface charge, surface functionalization, hydrophobicity or hydrophilicity. Physiological stability comprises of aggregation, agglomeration, biodegradability, and solubility. In nature, physicochemical properties are essential to the physiology of cells, including uptake properties (ratio amount, and mechanism), transportation

properties (accumulation location and transportation process), cytotoxicity (necrosis, apoptosis, and decreased cell viability), and exclusion. Due to the interaction between NMs and cells, these factors and behaviors should be considered before the application of NMs to any biological systems.

4.1. Effects of NM Size

The size of NMs strongly affects their optical properties. NM size is also crucial to physiological interaction. NMs have six size-dependent pathways for cell entry, with sizes ranging from over 1000 nm to less than 10 nm: phagocytosis, macro-pinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, clathrin/caveolin-independent endocytosis, and direct cell membrane penetration [67]. Each pathway possesses its limited size range and dynamics. The size range for phagocytosis is between 500 nm and 10 μm [68]. By contrast, most ligand-modified NMs, which are less than 500 nm, enter cells through endocytosis. To penetrate the cell membrane, the size of the material should be less than the thickness of the membrane bilayers, which is 4 to 10 nm. However, particles less than 5 nm are rapidly removed from the cell by renal clearance [69-71]. The perfect size for NMs used in bio-applications such as drug delivery or cancer therapy has been has been a debate. Chithrani et al. found that NMs with diameters <100 nm have strongly size-dependent intracellular uptakes [72]. NPs with diameters of 14, 50, and 74 nm exhibits size-dependent cell uptake numbers and uptake high life. NMs with 50 nm diameters exhibit higher cell uptake rates and numbers than the others because of the difference in wrapping time. The interaction between antibody (Herceptin)-Au NPs and SK-BR-3 breast cancer cell receptors is also size-dependent, and that NPs with sizes ranging from 25 to 50 nm in diameter exhibit the most efficient uptake [73]. The most efficient size, 50 nm, has been approximated by an experiment involving hydroxyapatite NPs (45 nm), Au NPs (50 nm), and polypyrrole NPs (60nm) [74-77]. The size of the NMs influences uptake properties during phagocytosis. The highest phagocytosis occurs when the diameter ranges from 2 μm to 3 μm [78]. In addition, size must be considered because of NMs' toxic properties. Pan et al. found that 1.4 nm Au NPs exhibit high toxicity, whereas 15 nm Au NPs exhibit non-toxicity at 100-fold concentrations of 1.4 nm experiments [79]. The difference of strong toxicity of Au clusters is because the cluster size can easily combine with DNA and their major groove dimension [80]. Park et al. demonstrated that Ag NPs with a diameter of 20 nm are more toxic than larger NPs (80 nm and 113 nm) and Ag ions [80].

4.2. Effects of Shape

The NM shape is another significant factor affecting the interaction between materials and cells. Tang group pointed out that mesoporous silica NPs (MSNs) with different aspect ratios have major effects on cellular functions, such as uptake rate, cytoskeleton formation, adhesion, migration, viability, and proliferation [81-83]. The longer nicotinamide riboside (NR) of MSNs (NLR450, aspect ratio = ~4) is more easily internalized by A347 human melanoma cells compared with shorter NR (NLR240, aspect ratio = ~2) and spherical (NS100, aspect ratio = ~1) MSNs. In addition, the cytotoxicity of the MSNs decreases as aspect ratio decreases. Gratton *et al*. obtained similar results using cylindrical PRINT particles with varying aspect ratios [84]. High aspect ratio (d = 150nm, h = 450 nm, aspect ratio = 3) rod-like particles are internalized more efficiently by HeLa cells than 200 nm symmetric cylindrical particles. Muro *et al*. investigated the targeted accumulation of various sizes (100 nm to 10 μm) and shapes (spherical versus elliptical disk-like particles) in endothelial cells, and discovered that the elliptical disks, which are micro-scale, had better targeting efficiency than any other spherical NPs [85]. However, the scale of NPs changes at around, or lower than 100 nm may present different results than those observed in previous discussions. Chan's group demonstrated that transferrin-coated spherical Au NPs (14 and 50 nm in diameter) showed higher uptake rates than transferrin-coated, rod-like Au NPs (aspect ratio =1.5 (20 nm × 30 nm), 3.5 (14 nm × 50 nm), and 6 (7 nm × 42 nm)) for HeLa cells, with uncoated Au NPs presenting the same result. Cell uptake efficiency also decreases as aspect ratio increases. Florez *et al*. showed that nonspherical polymeric NPs (191 nm × 84 nm, 279 nm × 70 nm, and 381 nm × 65 nm) exhibit lower uptake efficiency than their spherical counterpart for mesenchymal stem cells (MSC) and HeLa cells, and the uptake rate decreases as the aspect ratio increases [86]. Qiu *et al*. compared spheres (30 nm × 33 nm) and rod-like Au NPs with various aspect ratios (40 nm × 21 nm, 50 nm × 17 nm, and 55 nm × 14 nm), resulting in the higher-aspect-ratio rod-like NPs being more slowly internalized than the lower-aspect-ratio rod-like and spherical Au NPs in MCF-7 cells.

In the previous discussion, the interaction between shape and size is shown to have a powerful effect on the biophysical reaction of cells, and the volume of the particles determines the cell uptake efficiency. Moreover, the shape of NMs has also been proven by theoretical models and experimental studies to affect the internalization and vascular dynamics [87, 9, 10]. Nanostructured materials with controllable sizes and shapes, as well as their applications have also been demonstrated [11].

4.3. Effects of Surface Chemistry

The surface chemistry of NPs exerts various significant effects on the cells. The surface functional groups of NPs determine most of the physicochemical properties strongly related to the interaction between materials and cells. Among these physicochemical properties, the surface charge of NPs has the greatest effect on the interaction of NPs with cells. Cho *et al*. demonstrated that poly (vinyl alcohol) PVA-coated and citrated-coated Au NPs, which possess neutral and negative charges, respectively, absorb much less amounts on the negatively-charged cell membranes than positively-charged poly (allyamine hydrochloride) PAA-coated Au NPs, based on the I2/KI etchant method.

This method can selectively detect particles adhering to the cell surface [12]. The cellular uptake of positively charged NMs has resulted in higher uptake rates and efficiency in various cell types, as well as increased anionic particle adhesion to cell surfaces. These NMs include metal oxide [13, 14], metal QDs [16], polymeric NPs [17], mesoporous silica NPs *etc* [18]. This faster cellular uptake and higher uptake efficiency can improve cellular entry in several bio-applications, including drug delivery systems or therapeutic behaviors. Hydrophilicity or hydrophobicity is mostly determined by their surface ligands, surfactants, or stabilizers, which can be modified by chemical syntheses [19]. Hydrophobic NPs result in decreased dispersion in biological fluids and media [20]. However, hydrophobic property enhances the penetration ability of NPs into cell membranes and nuclear pores through hydrophobic interaction [21, 22]. As a result of attempts to balance dispersion property (hydrophilic) with high penetration ability (hydrophobic), amphiphilic NMs have been given much attention because of their excellent dispersion in aqueous and organic phases [23-25, 88].

5. Carbon Nanotubes (CNTs) and Cell

Given their remarkable optical, mechanical, and electrical properties, CNTs have been proposed for biomedical applications such as cell tracking and labeling, tissue engineering scaffolds, nanosensors, and vehicles for controlled release of drugs or delivery of bioactive agents. Detailed information about their biosafety is required for biomedical applications. Several *in vitro* and *in vivo* studies found significant cytotoxicity, DNA damage, micronucleus induction, or mutagenicity trends produced by carbon black or carbon-rich particles [89-91]. For example, MWCNTs can activate NF-κB, enhance phosphorylation of MAP kinase pathway components, and increase generation of proinflammatory cytokines in bronchial epithelial cells [90]. However, Zhong et al. showed that carbon black did not induce changes in DNA migration in V79 or Hel 299 cells [92]. Carboxylated MWCNTs did not have any significant effect on cellular morphology and viability of PC12 cells at lower concentrations. Moreover, short MWCNTs promoted neuronal differentiation of PC12 Cells [93]. In summary, surface chemistry, physical properties, and dose are important factors in determining the toxicity of CNTs. For example, CNTs are readily taken up by cells and are noncytotoxic with appropriate surface modification and certain limit concentration [94, 95].

The reported underlying mechanisms are also controversial. As shown in Figure 6, CNT-induced oxidative stress is regarded as the principal toxic mechanism [96-98]. Conversely, Fenoglioet demonstrated that MWCNTs exhibited a remarkable scavenging capacity against an external source of hydroxyl or superoxide radicals [99]. In addition, the iron impurity of CNTs is considered as another reason for CNTs' toxicity [100]. Complications arise when comparing these investigations as there are often considerable variations in the methodologies used including differences in exposure protocols and duration, and length and frequency of post-exposure sampling. More importantly, pristine CNTs are highly hydrophobic, whereas surface functionalization (carboxylated, aminated, or PEGylated) renders hydrophilicity and dispersibility in an aqueous phase, enabling varied interactions with biological systems [101, 102]. Further extensive in vitro and in vivo investigations are necessary to reach more definitive conclusions about the genotoxic properties of CNTs and the possible mechanisms involved in such toxicity.

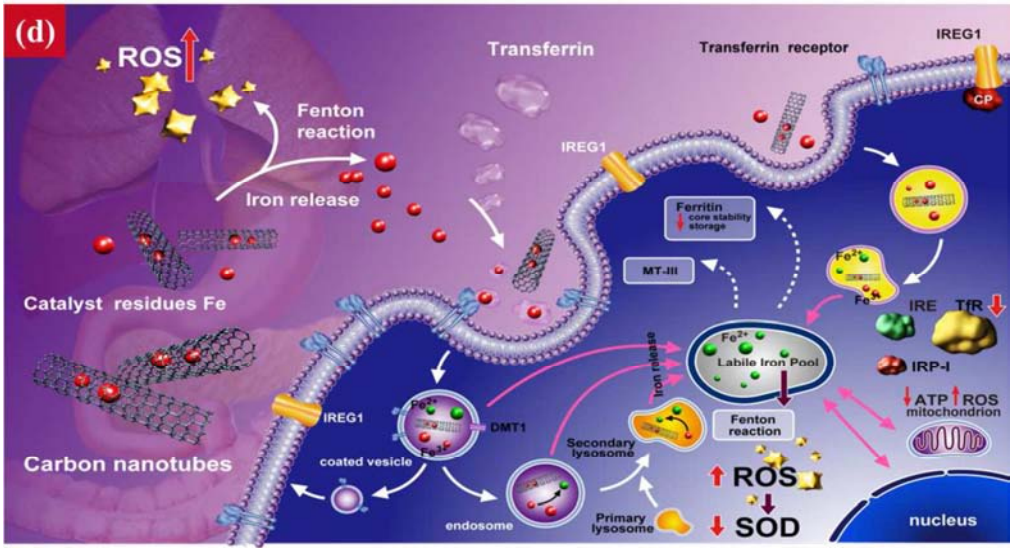

Figure 3. *The toxic effect of CNTs on cells and underlying mechanism. (a) Rapid transport of MWCNTs in PC12 cells. The cellular uptake and rapid removal of short MWCNTs at different time points were tested by HE staining during exposure to MWCNTs (30 μg/mL) for 2 days and the following culture in fresh medium in the absence of MWCNTs for 5 days. Cell images were taken at 1, 3, 5, and 7 days, respectively. (b) Hydroxyl radical formation from nanotubes having different metal content at different pHs. (c) The cell viability after treatment with CNT with different iron impurities. (d) Transport pathway, molecular, and cellular mechanisms of toxicity linked to cellular exposure to carbon nanotubes and leached metal. Reprinted from ref. [103] with permission. Copyright (2012) Nature.*

Table 1. *The Scientific Review Summary of the Toxicologically-Engineered Nanoparticles.*

NPs	Protein	Toxicity	Interaction mechanism	Reference
AuNP	human plasma (HP) or human serum albumin (HSA)	Immunotoxicity, steatosis and mitochondrial metabolism DNA damage and repair, apoptosis	AuNP were incubated in microcentrifuge tubes at physiological concentrations of HP and HAS	[104]
cationic gold nanorods	Dermis layer proteins	No	Protein adsorption	[26]
AuNP-allergen	IgE epitopes	Der p 1 upon binding to AuNPs reduced the integrity of a tight cell monolayer	AuNP were incubated with different concentrations of purified recombinant allergens in order to assemble their conjugates	[27]
ZnO, TiO_2, SiO_2 and Au nano particles	plasma protein, adsorbome	Most nanomaterials are modified with anti-fouling polymers to suppress protein adsorption altogether. This reduces off-target cell uptake, but also lowers targeting efficiency.	Adsorbs(diffusion, or by traveling down a potential energy gradient.) remains bound, or dissociates during biophysical interactions or translocation	[28]
Aluminum oxide nanoparticles	hydrophobic proteins SP-B and SP-C	No	Aggregation (mixing to make a colloidal system)	[29]
(Ag, Au, and Cu). NPs	Endothelial cell proteins	Induce oxidative stress and generate free radicals	Adsorption/Absorption	[30]
Cu NPs	Renal Proteins	Glomerulitis, degeneration, and necrobiosis of renal tubules	Absorption	[105]
Ag NPs	Cytokines	pro-inflammatory cytokines increased maturation and expression of co-stimulatory molecules on dendritic cell	In vitro construct	[31]
ZnO	Cytokine	pro-inflammatory cytokines and mitochondrial injury	Dissolution in cell culture medium	[106]

Nanoparticles can gain entry into the body through the following routes; ingestion, inhalation and skin (Figure 4). After entry into the human body they come in contact inevitably with a big variety of biomolecules such as sugar, protein and lipids which are soluble in the body fluid. The protein corona is formed by immediate coatage of biomolecules and NPs surface, [32, 33] and this is significant in determining the identity of NP biologically [34].

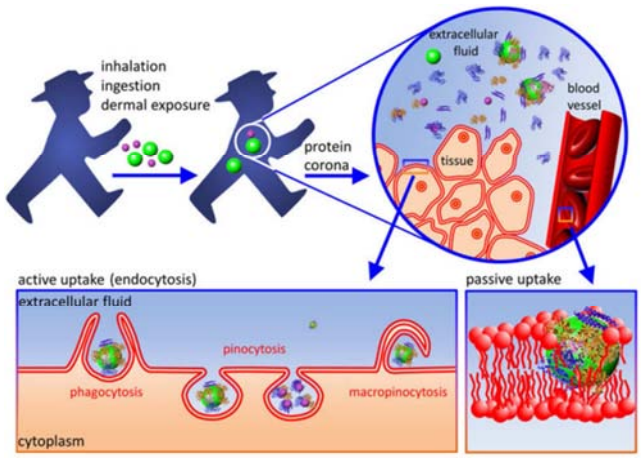

Figure 4. *The Uptake of Nanoparticles.*

The cell is an important functional unit and building block in all living organism especially in determining interaction of engineered nanomaterial with the living system. NPs has potential in passing through the plasma membrane, despite of it being selectively permeable to allow passage of ions and molecules in and out of the cell and maintaining osmortic pressure and electric potential of the cell. Transportation of NPs in and out of cells are by endocytosis and exocytosis and are facilitated by it being encapsulated in vesicle (Figure 4).

NPs can gain entry into the body by inhalation, ingestion or through the skin. The interaction of nanoparticles with the cell can be divided according to specific physical and chemical interaction at the nano-bio interface, and also based on organelle or molecular level. For nanoparticles to be considered useful in biological application it needs to have diverse physicochemical property and biocompatibility targeting biological environments, which are largely protein, nucleic acid and lipid. After integration and conformational change of biomolecules, the surface chemistry of NPs is modified by host enzyme system (Figures 5 and 6). Additionally, the flexibility and heterogeneous nature of non-uniform plasma membrane is subjected to changes that lead to exchange of energy from NP-lipid/protein interaction. The creation of a dynamic interface by a cell is as result of biological activities, in addition to dimension complexity of the interaction; protein, ion and biomolecules are transported by active transport; adenosine-5-triphosphate (ATP) dependent on endocytosis and exocytosis; polymerization of cell skeleton protein; active transport of NPs and the process after internalization of NPs (Figures 2-3) [35].

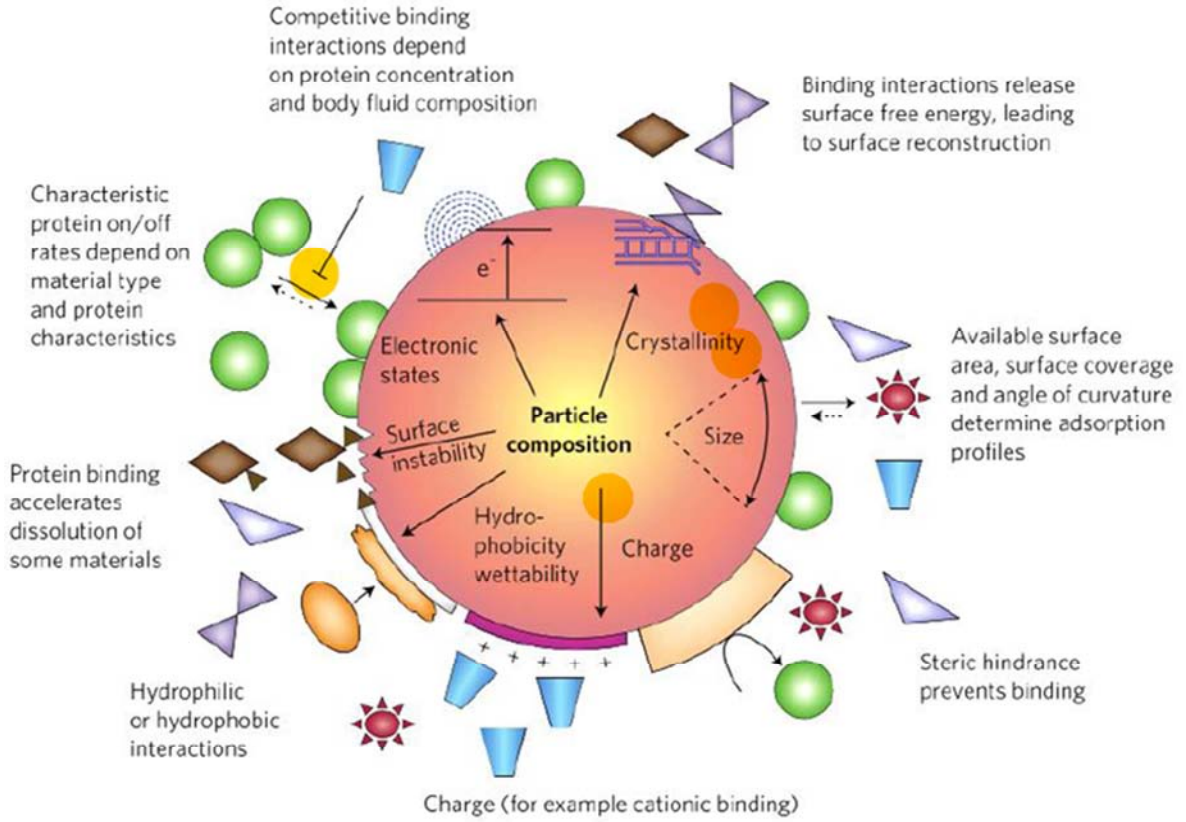

Figure 5. *Interaction of Biomolecules with the Surface of NP.*

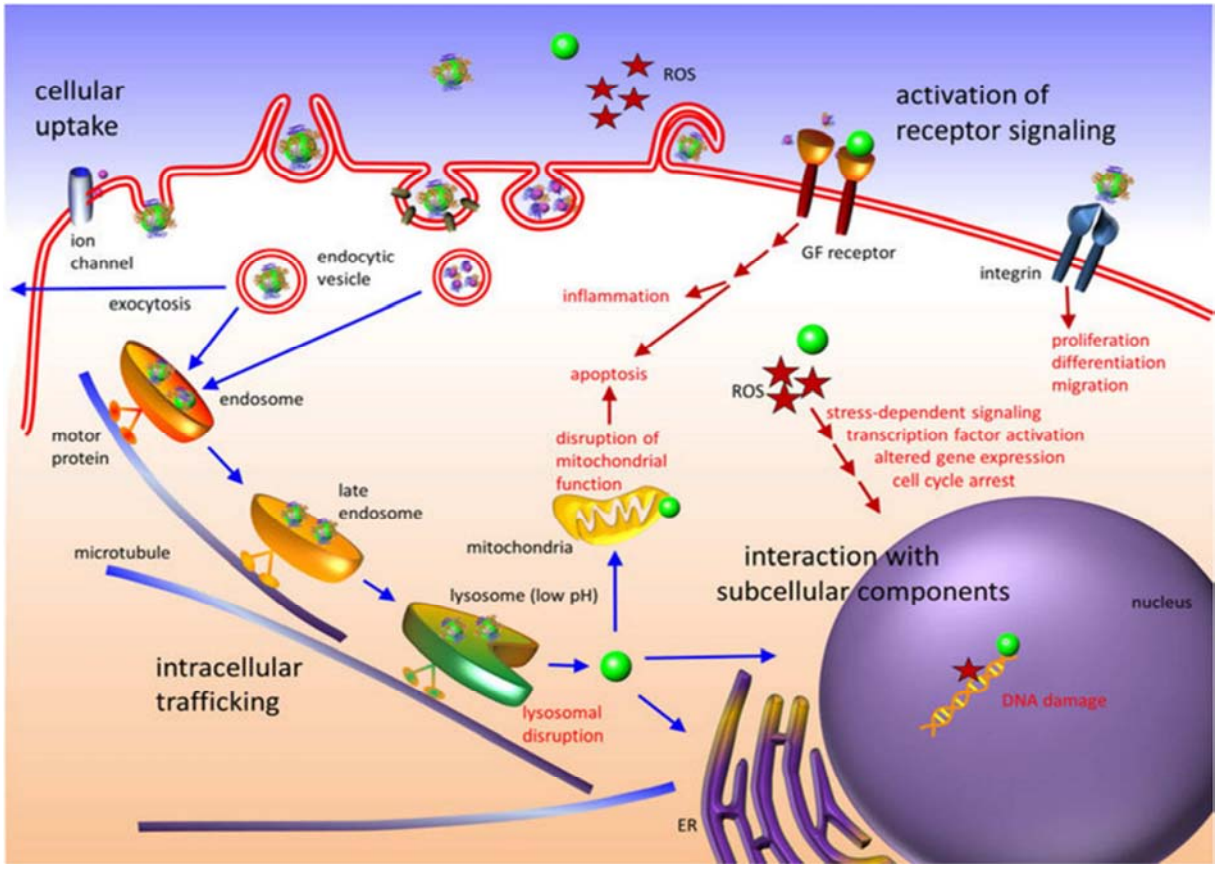

Figure 6. *The Effect of Cytotoxicity Caused by Nanoparticle.*

The integrity of plasma membrane is essential for normal cell function, and it consists of bilayer of phospholipid that has a tail of hydrophobic fatty acid which is shielded at the centre of the layers while the hydrophilic head is pointing outward to the aqueous environment. The dynamics of biological membrane is formed by assembly of hydrophobic forces in the environment that is aqueous; the presence of lipid movement is driven dynamically by thermal. The differences in physicochemical features is related to the head of lipid membrane, and this can be in terms of size of the head, charge and polarity, and also the length, configuration of isomerism and degree of saturation of the chain of the fatty acid, fluidity, and permeability of water. The dynamics of lipid membrane could change simultaneously and surface chemistry from such membrane could exert great effects on the dynamics of the membrane. Molecular dynamics (MD) studied by Nielsen et al, indicated lipid bilayer perturbation which is as a result of transmembrane presence of nanotube [36].

The physicochemical interaction between engineered nanoparticles and plasma membrane are of uttermost important in understanding the response in cellular function to the presence of NPs. For biological activities such as receptor recognition of the ligand on the surface of NPs, endocytosis and exocytosis are involved. As one of the key pathways in cellular trafficking, endocytosis facilitate movement of molecules or particles in the extracellular environment and it is present in all types of cells found in the body. The polar macromolecules: protein, growth factors and hormones that penetrate the amphilic plasma membrane are transported via membrane bound vesicle formed as a result of invagination and pinching off of the plasma membrane. The mechanism used by endocytosis are; phagocytosis and pinocytosis. Phagocytosis plays vital roles of immunological activity and inflammatory response against invasion of foreign pathogen. Another importance is that it triggers immunological response when exposed to NPs. It currently has great significance in understanding nanotoxicity mechanism by toxicologists and immunologists. Pinocytosis involves fluid uptake from extracellular space to the cell and this is located in all cell types and this are; macropinocytosis (phagocytosis), caveolae-mediated endocytosis, clathrin-mediated endocytosis, and clathrin-and caveolae independent endocytosis. The initiation of all these processes are by highly specific binding between receptors found in the endocytotic pit with ligand on the target, clathrin and caveolae processes which are mediated by endocytosis are used in carrying material needed by the cell which are LDL, transferrin, and antibodies growth factors [37].

The homogeneity of NPs with the unstructured surface was internalized primarily by energy-dependent endocytosis while the one with structured surface had the ability to penetrate the plasma membrane at reduced temperature when activities of the cell such as endocytosis are inhibited [38]. In biomedical application the NP used interact with fluid in the body such as blood [39-42], pulmonary lipid surfactant [43], or gastric mucus [44] before they are absorbed in the tissue or cell. Interaction of nanomaterials and biological macromolecules affects the normal function of the molecule; and can be by binding of protein to NPs which leads to changes in the secondary structure of protein and these impair with protein stability and folding; the binding of unfolded protein to receptor membrane result to immune response by the cell. The interaction of protein-NPs is termed as hydrophobic interaction and is driven by means of entropy with the effect originating from ability of water molecules to cage in the non-polar surface. Hydrophobic interaction among protein and NPs could also intergrade the NPs-to-protein core. Recently, a number of studies have been conducted regarding the toxicity of engineered nanoparticle (ENP) which shows that nanomaterials have been identified as the primary causative agent of toxicity in microorganism, invertebrate and vertebrate as summarized in Table 2 [56].

Table 2. *Toxicity and Interaction of Different Nanoparticles in Different Organism.*

NPs	Organism	Toxicity	Intereaction mechanism	Reference
AgNPs	Nitrifying bacteria (N. Europaea)	Inhibition of N. europaea, bacterial surfaces were adhered by AgNPs and appeared breakages and Holes, damage of cell wall, and could result in the leakage of intracellular contents	physicochemical reaction in the medium (for example precipitation and aggregation	[45]
TiO_2, SiO_2	Mytilus edulis (gill mucus)	No	Adsorption	[46]
Amino modified polystyrene NPs (PS-NH2)	Mytilus hemocytes, hemolymph serum (HS)	Cellular damage, disregulation of p38 MAPK signalling	Incubation	[47]
ZnO	*Azotobacter* and *Pseudomonas,* Bacteria	Generation of reactive oxygen species, disruptions in cell membrane and increased permeability	Electrostatic forces, Incubation	[48]
AgNPs	Fish	Dissolved silver, nanoparticle-specific effect	Cellular uptake via endocytic pathways	[49]
AgNPs	Algae	Dissolved silver	No cellular uptake, adsorbed on algal surface	[50]
GO-TiO_2	*E. coli, C. metallidurans*	Metabolic activity reduction, Growth inhibitation	van der Waals forces	[51]
oxidized CNTs	microorganism	Cytotoxicity	Adsorption	[52]

NPs	Organism	Toxicity	Intereaction mechanism	Reference
89 (O-CNTs) were well-dispersed in polyvinyl alcohol (PVOH)	*Pseudomonas aeruginosa*			
AuNPs	*Shewanella* or *Bacillus*	Toxicity reduction in the viability of bacteria	Surface binding, electrostatic interactions	[53]
Carboxyl-CdSe/ZnS core shell QDs	*Cupriavidus metallidurans*	Loss of membrane integrity	Simple Adsorption	[54]
Tungsten carbide	Rainbow trout Oncorhynchus mykiss	Toxicity	Simple Adsorption	[55]
Nano-CuO andnano-Fe_3O_4	extracellular polymeric substances (EPS) from algal aggregates	Growth inhibition	Simple Adsorption	[56]
CuO NPs	eukaryotic algae *Chlorella pyrenoidosa*	Membrane damage, Reactive oxygen species generation and mitochondrial depolarization	Surface attachment, Deposition, Direct contact	[57]
ZnO and TiO_2	*Picochlorum sp*	Negative effect on algal growth and chlorophyll a concentration,	Negative effect on algal growth and chlorophyll a concentration,	[58]
TiO_2	Algal Extracellular Polymeric Substances	Effect on cellular membranes, toxic for both marine and fresh water organisms	Electrostatic interactions and chemical bonding (bridge-coordination	[59]
Fe_3O_4 NPs	Gram positive bacterium *Staphylococcus aureus* and gram negative bacterium *Escherichia coli*	No toxic effects observed except the Fe nanoparticles with other microorganisms cause inhibition and Toxicity and also damage to the cell membrane integrity	Adsorption due to Electrostatic interactions (Coordinate bonding)	[60]
Au@Ag NPs	*S. aureus*, a pathogen	Toxic to mammalian cells, dermal fibroblasts cell viability decreased	Electrostatic interactions	[61]
Zero-valent iron nanoparticles	bacterium*, P. putida* G7	High redox activity, which may cause the reductive decomposition of functional groups from proteins and lipopolysaccharides of the outer membranes	Physical interactions	[62]
TiO_2, microporous and spherical SiO_2, and Al_2O_3	algal cells (*Chlorella pyrenoidosa)*	Toxicity is present	Derjaguin–Landau–Verwey–Overbeek (DLVO) colloidal interactions and electrostatic and hydrophobic interactions	[63]

6. Conclusion

This review summarizes the NMs most commonly used in biomedical applications in the past decade. Cellular uptake behaviors are strongly dependent on size, shape, surface charge, and chemistry. NPs with diameters <100 nm have strongly size-dependent intracellular uptakes, elliptical disks had better targeting efficiency than any other spherical NPs and positively charged NPs have resulted in higher uptake rates, efficiency and increased anionic particle adhesion to cell surfaces.

Owing to the different compositions, physical properties, and surface properties of these NMs, their use in the treatment of some chronic diseases has resulted in various toxic effects. The cytotoxicity of these materials is also related to several factors, including material compositions, size, and surface ligands. The physicochemical properties of NMs should be traced from their synthesized procedures, intrinsic properties, and surface chemistry. Each material offers unique properties for use in different applications, but also has their particular limitations.

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