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REVIEW

Methods to evaluate the toxicity of engineered nanomaterials for biomedical applications: a review

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Abstract

Nanomaterials can be engineered with distinctive properties for their use in agriculture, environment, medicine, cosmetics and household commodities. Nonetheless, knowledge on the toxicity of engineered nanomaterials is actually limited, and their potential adverse effects should not be overlooked. In particular, it is important to understand the dynamics and mechanism of nanotoxicity. Toxicity of engineered nanomaterials arises mainly from their ability to produce reactive oxygen species, their ease of absorption and distribution into various tissues, and their kinetics of elimination from the human body. Therefore, toxicity mechanisms should be tested in model biological systems, with focus on properties such as size, shape, surface modification, composition, and aggregation. Here we review the fundamentals of nanotoxicity, methods to assess the toxicity of engineered nanomaterials, approaches to reduce toxicity during synthesis, and prospects of engineered nanomaterials in nanomedicine.

Keywords Engineered nanomaterials · Physicochemical properties · Biology · Nanomedicine · Nanotoxicity

Abbreviations

AMPK	Adenosine monophosphate-activated protein kinase
AP-1	Activator protein-1
ATP	Adenosine triphosphate
BBB	Blood–brain-barrier
CTAB	Cetyltrimethylammonium bromide
DCFH-DA	Dichloro-dihydro-fluorescein diacetate
EPR	Enhanced permeability and retention
ENMs	Engineered nanomaterials
GI tract	Gastrointestinal tract
GSH-Px	Glutathione peroxidase
GO	Graphene oxides
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinase
MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWCNTs	Multi-walled carbon nanotubes
NAD	Nicotinamide adenine dinucleotide
nano-SAR	Nano-structure activity relationship
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
mTOR	Mammalian target of rapamycin
ROS	Reactive oxygen species
QSARs	Quantitative structure–activity relationships
QSTR	Quantitative structure–toxicity relationship
QTTR	Quantitative toxicity–toxicity relationship
SWCNTs	Single-walled carbon nanotubes
TiO_2	Titanium dioxide
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
UV	Ultraviolet
WST-1	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium
XTT	2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
ZnO	Zinc oxide

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Introduction

Nanotechnology is an evolving field that was quietly initiated in 1959 at the California institute of technology, USA, through a talk by Richard Feynman (Feynman 1960). In "There is plenty of room at the bottom," Feynman predicted that matter could be operated and governed at the atomic or molecular level to produce novel materials and technologies. Unique opportunities originate from nanomaterials through their high surface-to-volume ratio and nanoscale size. These properties give rise to quantum confinement, leading to increased chemical activity, altered electronic and optical properties (Nasrollahzadeh et al. 2019).

Engineered nanomaterials (ENMs) of different types are being produced through physical, chemical, and biological methods for a wide range of applications, including bioimaging, drug delivery, biosensors, antimicrobial agents, and photodynamic therapy against cancer (Ghosh et al. 2008; Moyano and Rotello 2011; Daima et al. 2013; Daima and Bansal 2015; Navya et al. 2019a, b; Umapathi et al. 2019, 2020; Yata 2019; Madhyastha et al. 2020; Matur et al. 2020; Makvandi et al. 2021). Their absorption and distribution into the human body results in their interaction with cell membranes, proteins, deoxyribonucleic acid (DNA), and cellular organelles. The extent and duration of the interactions depend on the physicochemical properties of engineered nanomaterials, and therefore the intensity of biological effects. For instance, engineered nanomaterials dispersed in biofluids develop protein corona that create an initial nano-bio interface facilitating their entry into cells (Nel et al. 2009; Kaphle et al. 2018; Umapathi et al. 2018).

Similarly, cationic nanoparticles cause cell membrane disruption, leading to an electrolyte imbalance and cell death (Chen et al. 2009). It is reported that nanoparticles of size ~ 10 nm induce epigenetic effects by adhering to DNA grooves (Soenen et al. 2011) altering gene expression and impacting DNA repair genes (Tang et al. 2015). Gold (Au) nanoparticles do not provoke toxicity, but removal of adhering cetyltrimethylammonium bromide (CTAB) results in toxicity, highlighting that reducing or functionalizing molecules used for the design of nanomaterials have a propensity to induce toxicity (Alkilany et al. 2009). From these discussions, it is apparent that mechanisms of toxicity must be evaluated to enable safe use of engineered nanomaterials (Uddin et al. 2020).

Here, different aspects of nanotoxicity have been elaborated. In the beginning, information about the fundamentals of toxicity and its origin, followed by the essential methods for assessment of toxicity in vitro and in vivo, and subsequently the strategies for controlling nanotoxicity and perspective on the next-generation nanomedicine is provided.

Emergence of nanotoxicity

The nanotoxicity of engineered nanomaterials can be acute, chronic, or it may affect the developmental stages of an organism. While their small size, high surface-to-volume ratio, ions released under different conditions from the nanoparticles, and surface structure or composition make them dissimilar from their bulk phase and thereby favorable for their use in certain applications, the same properties also lead to increased toxicity (Navya and Daima 2016; Kaphle 2017a, b; Kaphle et al. 2018; Sardoiwala et al. 2018; Madhyastha et al. 2019; Mulenos et al. 2020). In fact, toxicity and particle size are interconnected, and higher the surface-to-volume ratio, greater the bioactivity to create significant cytotoxic effects to living cells (Navya and Daima 2016). However, toxicity is dependent on the potential for absorption by inhalation or direct oral ingestion (Umapathi et al. 2018).

In addition to engineered nanomaterials, nanoparticles are produced by natural sources. Thus, volcanic eruptions, forest fires, and soil erosion produce nanoparticles. Volcanic eruptions that are suspended in the atmosphere are toxic to humans and consists of heavy metals nanoparticles. A typical volcanic eruption can produce up to 30 million tons of ash, and it can spread worldwide, and compromise the earth's atmosphere for years (Taylor 2002; Buzea et al. 2007). The short-term effect of volcanic ash includes respiratory effects, eye, and skin irritation, while in long-term they cause skin diseases including podoconiosis, in which nano-micro-particulate matter absorbed through the skin of the feet (podos) from the soil (konia) leads to failure of the lymphatic system (Fuller 2005). On the contrary, engineered nanomaterials constitute anthropogenic nanomaterials that are produced deliberately or incidental in industrial activities. For instance, the incidental nanoparticles could be a product of combustion processes. These may be toxic and characterized by irregular sizes, shape and composition and may contain significant amounts of polynuclear aromatic hydrocarbons, including carcinogenic material such as benzo-a-pyrene (e.g., diesel and automobile exhaust) (Penn et al. 2005).

Several household activities such as cooking, cleaning, and combustion in fireplaces or candles also generate a significant amount of nano-micro particles, and they have the potential to cause adverse effects because of long-time exposure in indoor environments. Other sources of incidental nanoparticles include cigarette smoke, building demolition, welding fumes, and industrial processes. All these are highly deleterious to human health. Unlike the anthropogenic nanomaterials, engineered nanomaterials could be precisely controlled in terms of size distribution, shape, and composition. Thus, engineered nanomaterials can be commercialized in

products as cosmetics, sunscreens, toothpaste, strengthening materials, sporting goods, and clothing without many adverse/side effects. Table 1 shows some of the applications of nanomaterials, their benefits, and accompanying risks (Klaine et al. 2012).

Dynamics of exposure, uptake, and distribution of nanomaterials in the body

The National Institute for Occupational Safety and Health (NIOSH) states that the "workers within nanotechnology-related industries have the potential to be exposed to uniquely engineered materials with novel sizes, shapes, and physical and chemical properties. Occupational health risks associated with manufacturing and using nanomaterials are not yet clearly understood" (https://www.cdc.gov/niosh/topics/nanotech/). The potential for toxicity is dependent on stability and durability of nanomaterials, which are in turn determined by their size, shape, surface charge, or coatings. Further, the toxicity depends on the routes of uptake into the body, including skin, gastrointestinal tract, lung, nasal cavity, or eyes as shown in Fig. 1 (Yokel and MacPhail 2011; Daima 2013; Daima and Bansal 2015; Navya and Daima 2016; Umapathi et al. 2018; Sengul and Asmatulu 2020). The uptake mechanism is also dependent on the physicochemical properties of nanoparticles. The surface properties of the nanoparticles may make them suitable for selective uptake and distribution in the body. Moreover, various medical equipment's like surgical instrument, catheters, bone, and dental implants may be coated with nanoparticles such as silver nanoparticles which can easily penetrate inside human body (Pandiarajan and Krishnan 2017).

Exposure and uptake in the lungs

Nanoparticles are frequently taken up by inhalation and get deposited throughout the respiratory tract up to the lungs. Nanoparticles in the range of 1–5 nm cannot reach the alveoli but accumulate along the mucus-lined upper airways or tracheobronchial region (Smith 1994). Spherical nanoparticles show a greater accumulation on lungs surface compared to other shapes, and particles with larger diameter or higher aspect ratio fibers are likely to be found mainly in the upper respiratory tract (Lippmann 1990).

Dermal exposure and uptake

Skin, the largest organ of the body, is composed of three layers. It offers formidable resistance to penetration of nanoparticles. However, their infiltration can occur through hair follicles and deformed or broken skin. Spherical nanoparticles penetrate the skin up to a depth of 2400 µm along hair follicles (Toll et al. 2004). Broken skin permits facile entry of larger size particle in the range of 500 nm to 7 µm (Oberdorster et al. 2005). Nanoparticulate titanium dioxide (TiO_2), which is being commercialized in sunscreens as an ultraviolet (UV) protector, penetrates the stratum corneum layer of the skin and they can interact with the immune system (Lademann et al. 1999). Several in vitro studies have reported the penetration abilities of nanoparticles across the skin and their subsequent transport to various organs (Lademann et al. 1999; Kreilgaard 2002).

Oral exposure and uptake

Gastrointestinal (GI) tract is exposed to two sources of nanoparticles: exogenous sources—such as food, water, cosmetics and inhaled nanoparticles, and endogenous sources—such as intestinal calcium and phosphate secretion (Lomer et al. 2007). In developed countries, an average person takes up to 10^{12} micro-nano particles in a day (Oberdörster et al. 2004), whereas the use of toothpaste, sunscreen, or lotions that contain a significant amount of titanium dioxide nanoparticles as a whitening agent can increase the ingestion multiple times. The uptake of nanoparticles would be affected by the physicochemical properties including size, shape, and surface charge. A study on polystyrene particles showed that 50 nm, 100 nm, 1 µm, 3 µm particles are up transported across the epithelium at 6.6%, 5.8%, 0.8%, and 0%, respectively (Jani et al. 1990). Disease such as diabetes may increase the uptake of particles through the gastrointestinal tract (Buzea et al. 2007).

Other exposure sites

Neuronal uptakes of particles via olfactory nerves and the blood–brain-barrier (BBB) have also been reported. The presence of nanoparticles in the olfactory bulb is reported to be via olfactory nerves (Oberdörster et al. 2004). For instance, studies of rat inhalation with magnesium dioxide (MgO_2) nanoparticles (30 nm) suggested the accumulation of particles in the olfactory bulb (Elder et al. 2006). These phenomena have raised concerns about their toxicological effects of nanoparticles. Another important site of exposure and uptake is the ocular surface, which is exposed to airborne nanoparticles and those in cosmetics (Yokel and MacPhail 2011).

The role of physicochemical characteristics on toxicity

As illustrated in Fig. 2a, various physicochemical properties of nanomaterials make them promising materials for a range of diverse fields. However, the same properties also lead to adverse effects by enhancing their uptake as well as invasion into biological systems (Fig. 2b). Consequently, it

Table 1 Nanomaterials applications, benefits, and associated risks

Application	Risk	Benefit
Nanocrystals harvest light in photovoltaic devices	Light pollution in rural areas, opportunity to fossil fuel economies	Green, renewable energy, new self-lighting displays for electronic devices
Antimicrobial wound dressings contain nanocrystalline silver	Release of antimicrobials into the environment, hazard to natural microbial systems	Improved healing in wounds and reduced risk of infection
Sunscreens containing titanium dioxide (TiO_2) nanomaterials are extremely effective at absorbing UV light	Titanium hazard to intertidal organisms and sandy shore ecosystems	Consumer preference for transparent but effective sun creams, decrease in skin cancer due to increased sunscreen use
Metal nanomaterial supplements to increase the burn efficiency of fuels	Respiratory exposures to nanomaterials in fuel exhausts. Long range of transport of particles in the atmosphere	Less soot from diesel vehicles and urban air pollution. Burn efficient aviation fuels. Reduced green-house gases
Medical application of hydroxyapatite and nano-silica in bone reconstruction	Durability-particles eroded from the surface may cause pathology in other internal organs in long term	Structural repairs to teeth and bone using a natural material already in the body (no adverse immune responses)
Nanomaterials in food packaging	Unintended transfer of nanomaterial from the packaging to the food. Uncertain lifetime oral exposure risk	Stronger lighter packaging to protect soft foods, antibacterial packing to improve shelf life. Increased food safety
Use of carbon nanotube to improve strength and flexibility of sports equipment	Life cycle analysis, what happens to the material in landfill at the end of their use?	Better product that lasts longer for the consumer. Reduced sports related injuries
Use of nanomaterials as catalyst in industrial processes such as coal liquefaction and producing gas	Inadvertent incorporation of toxic catalysts in consumer products, waste disposal of catalytic converters to landfill	Improved efficiency and economy of industrial processes. Less industrial wastes/ton of pollution
Usage of nanomaterials in water filtration and purification	Unintended waterborne exposure to wildlife of engineered nanomaterials	New source of portable, safe drinking water in poor regions of Africa/Asia. More efficient purification systems for the water. Reduced exposure to waterborne pathogenic organisms and toxins

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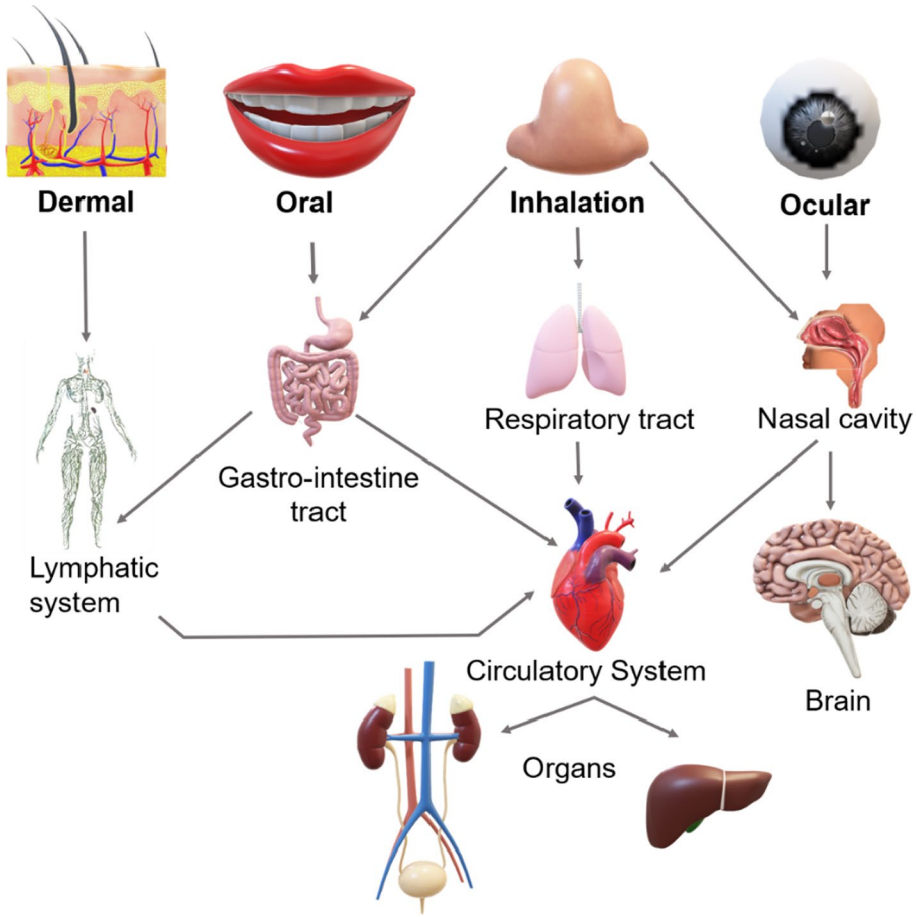


Fig. 1 Possible routes of engineered nanomaterials exposure and uptake, and organ systems for translocation of engineered nanomaterials. Reprinted and modified with permission from (Yokel and MacPhail 2011). *The Creative Commons Attribution License (*http://creativecommons.org/licenses/by/2.0*)*

is necessary to assess the physical and chemical properties of engineered nanomaterials vis-à-vis their use in nanomedicine (Fig. 2c).

Size, shape, and composition

Particle size is one of the main physicochemical properties of nanomaterials. Particles having a size below 1 µm can easily infiltrate into cells, whereas greater size particles cannot cross the outermost layer of the skin in most of the cases. Numerous studies have been performed to recognize the size-dependent biological effects and biodistribution using a variety of nanomaterials. A study regarding the assessment of cellular toxicity of size-dependent silver (Ag) nanoparticles (10 nm, 50 nm, and 100 nm) against a series of cell lines has revealed their biological responses. The study confirmed that the smallest size particles produce toxicity and induce reactive oxygen species (ROS) production, lactate dehydrogenase (LDH) release, and apoptosis. This is attributed to their high surface-to-volume ratio that occurs with reduction in size (Kim et al. 2012). Likewise, gold nanoparticles of 2–15 nm size have been used in tissue engineering, 20–60 nm are employed for drug delivery and DNA testing, and 80–250 nm have been utilized for medical, electrical and X-ray optics (Gu 2021). In another study, three varying sizes (30 nm, 150 nm, and 300 nm) of ferromagnetic nanoparticles exhibited size-dependent peroxidase-like activity and depicted that the smaller particles work as more powerful catalysts.

The shape is also crucial since catalytic properties of the nanoparticles are affected by the shape of the particle; moreover, it affects interaction of the nanoparticles at the nano-bio-interface and facilitates their cellular uptake (Navya and Daima 2016). Furthermore, the surface area differs depending upon the shape of the material. For example, an octagonal-shaped particle possesses different surface area when compared to a spherical particle irrespective of their sizes. Thus, the efficacy of the material changes with varying sizes and shapes. A study confirmed that the spherical nanoparticles show greater uptake over nanorods (Mahmoudi et al. 2011), whereas antimicrobial activity against *Escherichia coli* is much higher for triangular nano-plates when compared to spherical and rod-shaped Ag nanoparticles (Pal et al. 2007).

In addition to the size and shape of nanomaterials, the type of material or the composition of nanomaterials also have considerable influence on their toxicity (Fig. 2). For instance, polymeric nanoparticles will have different

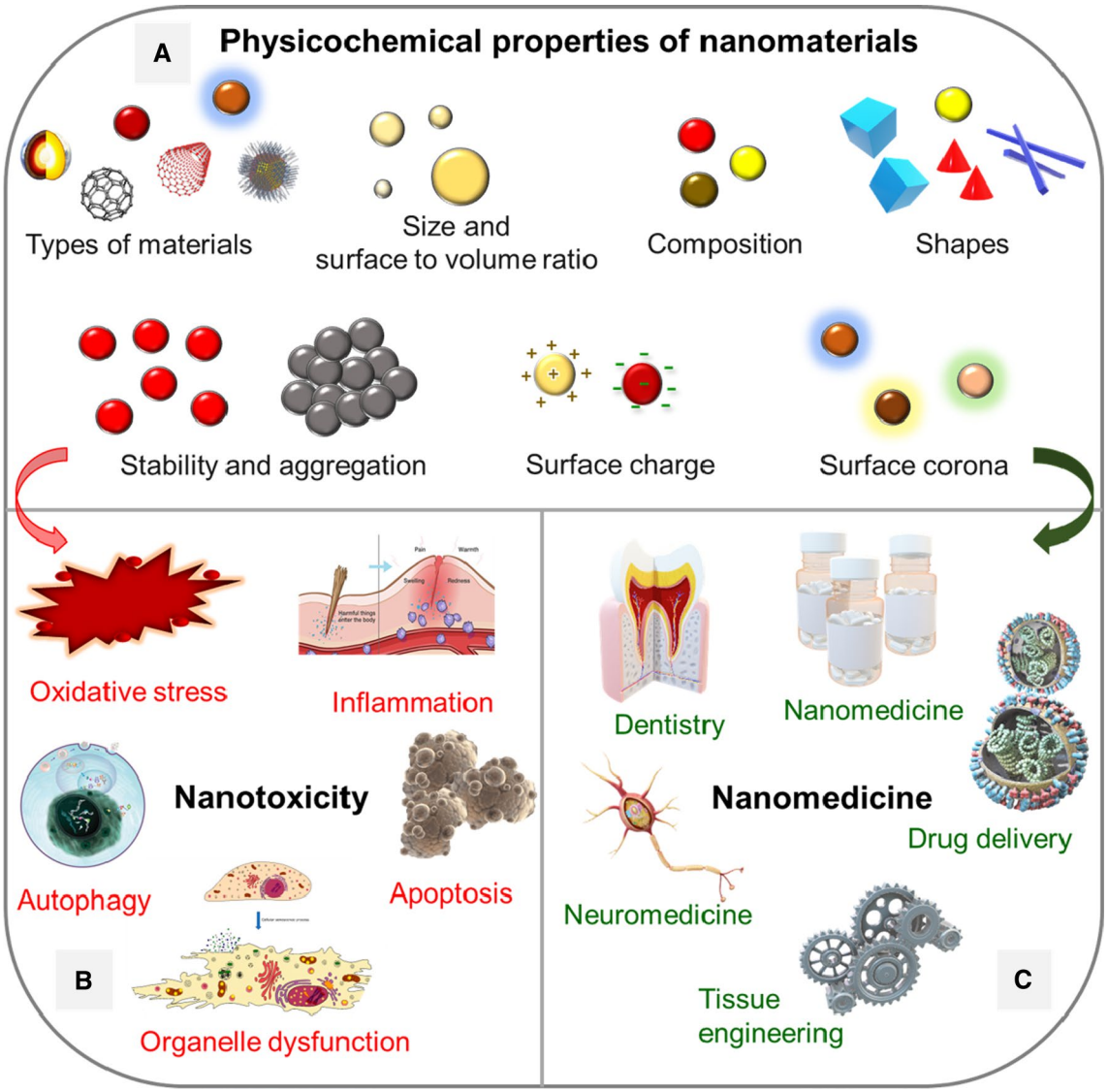


Fig. 2 Physicochemical properties of nanomaterials, which are essential for their biological or toxicological performance (a). The important processes by which nanomaterials may generate toxicity (b), and some of the applications of nanomaterials in medicine to realize full potential of nanomaterials (c)

behavior than metallic or ceramic nanoparticles. Several studies have reported the antimicrobial profile of different nanomaterials, and the potential of antibacterial activity has been found to be significantly impacted by the composition of nanomaterials. The toxicity is affected by the crystal structure as well, as shown for titanium dioxide nanocrystals, wherein the titanium dioxide nanoparticles could cause DNA damage, lipid peroxidation, and micronuclei formation (Shukla et al. 2013).

Surface-to-volume ratio and crystal defects

Another important attribute of nanomaterials is the surface-to-volume ratio, which improves as the size of material decreases. At the nanoscale level, the number of surface molecules increases exponentially with the decreased size, and thus making them more reactive, and consequently decide their toxicological profile. For instance, nanoparticles having a size of 30 nm will have 10% of its molecules exposed on the surface, whereas particles with a size of 10 nm and 3 nm will have 20% and 50% molecules on surface, respectively. Therefore, the surface-to-volume ratio or, more specifically, surface molecules will be of great interest to define the behavior of nanomaterials (Nel et al. 2006).

Additionally, the reduction of the size of the material may influence the defects in crystal planes by disrupting the electronic configuration of materials. Certain changes may give rise to some unknown functions of the material. These can

cooperatively work as reactive sites such as hydrophilic or hydrophobic, and catalytic activity (Oberdorster et al. 2005; Nel et al. 2006, 2009). Hence, while engineering nanomaterials for biomedical applications, the surface-to-volume ratio, and crystal planes should be thoroughly determined since these are predominantly responsible toward modified functionality of the materials. Here, it is essential to indicate that sometimes these parameters are underestimated when compared to other physicochemical properties.

Mechanisms of nanotoxicity

Nanoparticles can interact with cells or biological entities in several ways, and they can promote adverse effects due to oxidative stress, inflammation, autophagy, apoptosis, or organelle dysfunction as shown in Fig. 3.

Oxidative stress

Nanoparticles promote generation of several reactive oxygen species, including hydrogen peroxide, superoxide, and hydroxyl radical. However, their adverse impact is prevented by antioxidants such as superoxide dismutase (SOD), catalase (CAT), or glutathione peroxidase (GSH-Px). Thus, excessive exposure to nanoparticles could overwhelm the capacity of antioxidants, leading to toxic oxidative stress (Armstrong et al. 1999; Okuda et al. 2002; Jaiswal 2004; Starkov 2008). The oxidative stress created by nanoparticles may cause cellular or mitochondrial dysfunction (Fig. 3), leading to a disturbance in reactive oxygen species signaling. Thus, nanoparticles could precipitate activation of macrophages and neutrophils, which further exacerbate the situation with enhanced generation of reactive oxygen species causing inflammation and DNA damages. For instance, two different sizes of Ag nanoparticles (4.7 nm and 42 nm) with cell lines HepG2 (liver) and HL-60 (leukemic) revealed that the smaller size particles induced ROS at higher level compared to the larger sized nanoparticles (Avalos et al. 2014).

Besides size, it has also been reported that surface properties of nanoparticles can also affect ROS production. For example, gold nanorods coated with cetyltrimethylammonium bromide produce reactive oxygen species in HCT116 (human cancer) through damage of mitochondria, leading to the onset of autophagy. In the same way, (Manke et al. 2013) the toxic profile of zinc oxide (ZnO) and silicon dioxide (SiO_2) nanoparticles having different shape and size showed the ability of nanoparticles to generate oxidative stress as an effect of surface properties rather than size and shape. ROS can also be produced, when there is an interaction of nanoparticles with mitochondria; for example, oxidative stress was induced by multi-walled carbon nanotubes (MWCNTs), causing damage to mitochondria. Many studies have shown that reactive oxygen species generated by nanoparticles can induce inflammation. Single-walled carbon nanotubes (SWCNTs) exposed to human mesothelial cells led to reactive oxygen species production, and consequent activation of pro-inflammatory transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and AP-1(activator protein-1) (Pacurari et al. 2008).

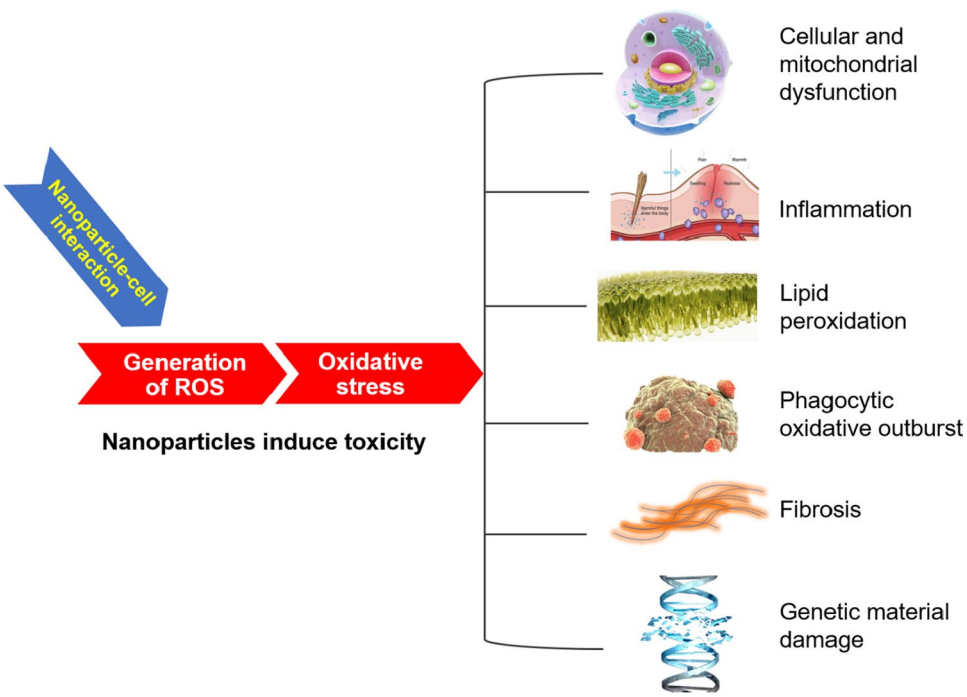


Fig. 3 Nanoparticle–cell interaction, which may induce nanotoxicity through reactive oxygen species (ROS)-mediated oxidative stress. The oxidative stress can lead to cellular or mitochondrial dysfunction, inflammation to the cells, lipid peroxidation, phagocytic oxidative outburst, fibrosis, and DNA damage, respectively

Inflammation

Nanoparticles are known to trigger an inflammatory response in many cells and tissues (Padmanabhan and Kyriakides 2015). For instance, single-walled carbon nanotube-treated A549 cell lines showed increased expression of interleukin 8 (Baktur et al. 2011). Vascular endothelial cells in the brain showed increased expression of cytokines such as interleukin-1β, tumor necrosis factor-α (TNF-α), and prostaglandin E2 when Sprague–Dawley rats were exposed to metal nanoparticle such as Ag. This study also implies that there is a breakdown of the blood brain barrier via nanoparticles, leading to neurodegeneration, neuronal disorders, and neuroinflammatory response (Trickler et al. 2010).

Two inflammatory pathways, namely the NF-κB pathway and mitogen-activated protein kinase (MAPK) pathway, regulates the cytokine production, DNA transcription, and survival of the cell (Arthur and Ley 2013). When RAW264.7 macrophages were exposed to a low dosage of nanoparticles such as silver, titanium dioxide, and zinc oxide nanoparticles led to activation of NF-κB and pro-inflammatory genes, including interleukin-6, interleukin-1β, and tumor necrosis factor-α. Similarly, ICR mice showed neuroinflammation through an upregulation of NF-κB pathway, toll-like receptors (TLRs), and tumor necrosis factor-α, when exposed to titanium dioxide nanoparticles (Ze et al. 2014). Another study exposed increased inflammation, mitogen-activated protein kinase activation, and cytokine levels when Balb/c mice were exposed to copper oxide (CuO) nanoparticles (Park et al. 2016). In Sprague–Dawley rats, exposure to cerium oxide (CeO_2) nanoparticles led to elevated p38 mitogen-activated protein kinase phosphorylation in the lungs (Rice et al. 2015).

Autophagy

Autophagy is induced when nanoparticles are exposed to cells. Thus, autophagy flux was amplified in human colon adenocarcinoma (HT29) cells when exposed to titanium dioxide nanoparticles (Yu and Li 2011). Gold (Au) nanoparticles provoke accumulation of autophagosome in human fetal lung fibroblast MRC-5 cells. Manganese (Mn) nanoparticles induced autophagosomes in dopaminergic neurons as noted by an increase in LC3 and Atg7 expression along with cleavage of Beclin-1 (Ngwa et al. 2011). Activation of autophagy in response to nanoparticles may involve mammalian target of rapamycin (mTOR). For example, silica nanoparticles induce autophagy in A549 cells by P13-K/Akt/mTOR pathway. Beside mammalian target of rapamycin, cellular energy sensor 5'-AMP-activated protein kinase is also implicated. When mouse podocytes were exposed to titanium dioxide nanoparticles, both adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin pathway were shown to be associated with elevated LC3-II/LC3-I level ratio, p62 and Beclin 2 (Zhang et al. 2016).

Apoptosis

Several reports have demonstrated that silver nanoparticles with the aid of caspases-8 and caspases-9 prompt apoptosis in PC12 cells, and when exposed to zebrafish, lead to the generation of oxidative stress with an enhanced caspase-3, caspase-9, Bax and Nova expression levels (Ahamed et al. 2010; Choi et al. 2010; Hadrup et al. 2012). Likewise, the intrinsic pathways can also initiate apoptosis, which depends on several incentives like oxidative stress, the inadequacy of growth factors, and the loss of apoptotic suppression. As mitochondria are involved in intrinsic pathways that play a central role, intrinsic pathways are also often regarded as the mitochondrial pathway. It has been reported that zinc oxide nanoparticles enhance the caspases-3 and caspases-9 in zebrafish embryos, with the production of oxidative stress and the Bax/Bcl-2 ratio is simultaneously also found to raise in the embryos. All these preceded the apoptosis pathway mediated via mitochondria and caspases (Zhao et al. 2016). Carbon black—a nonmetal nanoparticle also induces apoptosis, and studies have displayed that exposure of carbon black to 16HBE14o and normal bronchial epithelial cells steer to falling off in membrane potential of mitochondria, thereafter release of cytochrome c inside cytosol and trigger of Bax (Hussain et al. 2010).

Organelle dysfunction

Nanoparticles have been demonstrated to penetrate the cell membrane and ultimately ingress the nuclei with the assist of diffusion or transportation via nuclear pores and induce genotoxicity. For example, silver nanoparticles resulted in DNA damage when exposed to human mesenchymal stem cells. Similarly, zinc oxide nanoparticles lead to fragmentation of DNA in human nasal mucosa cells. Nanoparticles caused disturbance to cell cycle when titanium dioxide nanoparticles were exposed to NIH 3T3 (cell lines of mouse embryonic fibroblasts) and human fibroblasts. The nanoparticles affected anaphase and telophase during mitosis through multipolar spindles and chromosome dissociation (Huang et al. 2009).

Nanoparticles can also cause mitochondrial dysfunction, which can reduce adenosine triphosphate (ATP) production and enhance the production of reactive oxygen species (Valko et al. 2007). For example, adenosine triphosphate content in IMR-90 and U251 cells decreased because of mitochondrial damage in response to silver nanoparticles (Nair et al. 2009). When titanium dioxide is administered to rat cortical astrocytes, the mitochondrial

membrane potential was depolarized and simultaneously led to increased oxidative stress (Wilson et al. 2015). Iron oxide (Fe_2O_3) nanoparticles have also been shown to alter mitochondrial membrane potential in A549 cells in a dose-dependent manner (Khan et al. 2012).

The plasma membrane, which harbors many proteins with ion transporters and receptors essential for cell signaling and cell–cell recognition (Dowhan 1997), is susceptible to lipid peroxidation in response to nanoparticles via production of free radicals. For instance, when cadmium telluride (CdTe) quantum dots (QDs) elevated malondialdehyde (MDA) and reactive oxygen species level (Zhang et al. 2015) in murine hepatoma alpha mouse liver cells (AML12) and liver of ICR mice along with escalated lipid peroxidation. Cadmium telluride quantum dots exposed to human neuroblastoma (SH-SY5Y) cells also led to lipid peroxidation (Choi et al. 2007). Lysosomes having an acidic environment aid the cell in the degradation of foreign and toxic particles. Studies have reported that nanoparticles are also responsible for the dysfunction of lysosomal, which triggers apoptosis (Stern et al. 2012). Gold (Au) nanoparticles in one of the studies, alkalinize lysosomes and fallout as inadequate autophagy, suggest as evidence of autophagosome accumulation in the cells (Ma et al. 2011). In another study, single-walled carbon nanotubes (SWCNTs), gold nanoparticles, and graphene oxides (GO) destabilized the lysosomal membrane and led to the accumulation of autophagosome in murine peritoneal macrophages and normal rat kidney cells, respectively (Ma et al. 2011; Wan et al. 2013).

Assessment of nanotoxicity

As shown in Fig. 4, the in vitro, in vivo, and in silico methods are frequently employed for the assessment of nanotoxicity. All these methods can provide very specific information on the toxicity potential of nanomaterials, and the approaches can lead toward safe design of nanomaterials for nanomedicine applications.

In vitro toxicity assessment of nanomaterials

Industries and governing bodies consistently exploit the in vitro models for toxicity assessment and risk evaluation. This kind of assays can be useful to generate first information about the toxicity of nanomaterials. Therefore, in the following section, we discuss different methods employed for evaluating nanotoxicity in vitro along with the fundamental concepts of each method.

Trypan blue Trypan blue exclusion assay is the most frequently used method employed to assess cell viability. Living cells possess intact cell membranes, which excludes trypan blue dye. Typically, a cell suspension is exposed to the dye along with the nanoparticles in question. The cells showing clear cytoplasm and blue cytoplasm are grouped as viable cells or dead cells, respectively. With the help of light microscopy, the count of cell viability and cell death can be determined as a ratio of unstained control cells. This assessment of toxicity in vitro is simplistic and highlights damage to membrane integrity.

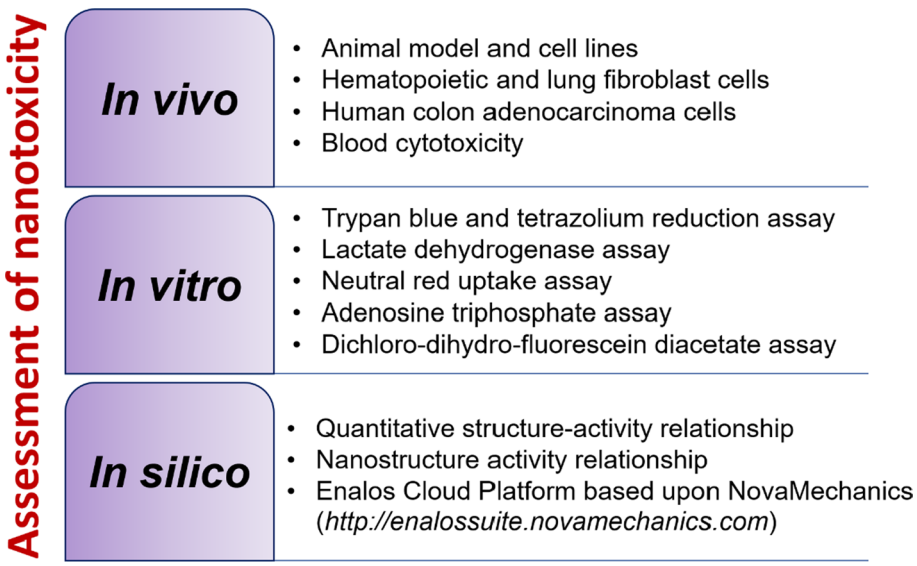


Fig. 4 Assessment of nanotoxicity by in vitro, in vivo, and in silico methods or models

Tetrazolium reduction assay The different cellular phenomena, like metabolic function, cell proliferation, and DNA synthesis, can be exploited to evaluate cellular viability. To detect this viability, several tetrazolium compounds have been used namely, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]; XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide]; and WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium]. These are differentiated into two types: (a) positively charged, such as MTT that readily penetrate the viable cell, and (b) negatively charged, such as MTS, XTT, WST-1 that do not penetrate the viable cells. When cells are treated with soluble MTT, the viable cells with active mitochondrial activity convert MTT into purple color formazan through succinate dehydrogenases. The mechanism involves reaction with reduced nicotinamide adenine dinucleotide (NADH) or similar reducing molecules that transfer electrons to MTT. The formazan that is produced must be solubilized, which can be accomplished by acidified isopropanol, dimethyl sulfoxide (DMSO), dimethylformamide, sodium dodecyl sulfate (SDS), or combinations of detergent and organic solvent (Mosmann 1983; Hansen et al. 1989). For the negatively charged MTS, XTT, or WST-1, different sets of salts have been used, which can combine with intermediate electron acceptors such as phenazine methyl sulfate (PMS) or phenazine ethyl sulfate (PES). These formed entities, then can easily enter into the viable cells, reduced in the cytoplasm or on the cell surfaces, which can further convert tetrazolium into soluble formazan product (Berridge et al. 2005).

Lactate dehydrogenase assay This is a colorimetric assay for evaluating cellular cytotoxicity. Lactate dehydrogenase, which is expressed in all cells, is released upon cell lysis from the cytoplasm. Hence, this assay quantitatively measures the enzyme lactate dehydrogenase, which is released into extracellular from a damaged cell. This can be done with the help of coupled enzymatic reactions that convert tetrazolium salt iodonitrotetrazolium (INT) into a red color formazan by diaphorase. During the initial stage, there is a conversion of lactate to pyruvate, which is catalyzed by lactate dehydrogenase, and thus nicotinamide adenine dinucleotide (NAD) is reduced to $NADH/H^+$. In the next procedure, diaphorase transfers H/H^+ from $NADH/H^+$ to 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, which on reduction shows red color formazan. The red formazan shows maximum absorbance at 492 nm and can be measured quantitatively. To determine the maximum release of lactate dehydrogenase, a detergent Triton X-100 is used as a positive control (Kumar et al. 2018).

Neutral red uptake assay This is a colorimetric cytotoxicity assay and measures the ability of a compound to inhibit the uptake of the dye. Neutral red uptake depends upon pH gradient maintained by cells, the dye has net zero charge at physiological pH, and therefore, enables the dye to penetrate the cell membrane. The dye gets charged and remains in the lysosome as it has a proton gradient to sustain pH lower than the cytoplasm. The dye is not retained when cells are dead. The uptake of neutral red dye by the lysosome is a very sensitive marker of cell viability and a good marker for lysosomal damage. The absorbance of dye is measured at 540 nm in a multi-well plate spectrophotometer.

Adenosine triphosphate (ATP) assay Adenosine triphosphate level decreases when cells are damaged and lose membrane integrity. Adenosine triphosphate provides a luminescent signal when the enzyme luciferase catalyzes the conversion of luciferin to oxyluciferin in the presence of Mg^{2+} ions. Adenosine triphosphate is released from the cells with the help of detergent that lyse the cell; luciferin is used as a substrate, and the stable form of luciferase to catalyze the reaction that produces light. The adenosine triphosphate assay can recognize less than 10 cells per well, and consequently, it has been broadly used 1536-well plate setup.

Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay This is a fluorogenic dye that measures hydroxyl, peroxyl, and other reactive oxygen species activity. Upon entry into cells, the dye is deacetylated by esterases (hydrolase enzyme that splits esters into an acid and an alcohol) to a non-fluorescent compound. The later oxidized by reactive oxygen species into dichloro-dihydro-fluorescein, which is fluorescent and can be detected by fluorometer, flow cytometer, or fluorescence microscope with excitation at 485 nm and emission spectra at 535 nm.

In vivo toxicity assessment of nanomaterials

The toxicity of nanomaterials has been reported in a wide range of animal models. The acute toxicity tests of nanomaterials entail a short duration of exposure, usually up to 24 h, administered in a single dose or as multiple doses; whereas the long-term studies include sub-acute, sub-chronic, and chronic studies, also referred to as multi-dose studies of toxicity potential of nanomaterials. A relatively long-time-interval is adopted to summarize the toxicity effects of nanomaterials with the repetition of short doses and, at the same time, with numerous functional, biochemical, physiological variations. The time duration for the sub-acute toxicity study is up to one month, whereas sub-chronic studies are conducted up to three

months of period. Furthermore, chronic studies could take up to a year, depending upon the endpoints to be understood.

These studies comprise a larger number of animals such that the yielding becomes statistically significant. In general, 1–5 animals/sex/group are selected for range-determination studies. Sub-acute studies incorporate 2–3 dogs/sex/group also at the same time, 10–20 sex/group/rodents are needed for sub-chronic studies. The doses to be distributed among the group of animals are inferred from outcomes of acute toxicity studies (single dose), which determines the stretch of minimal effective dose to the maximum tolerated dose. Conclusions must be drawn for specialties like illness, morbidity, or mortality. Moreover, observation is necessary for skin, eyes, mucosa, posture, circulatory, respiratory, and nervous system abnormalities, and general actions. Not only doses but also variations in food and water consumption can also be planned as a parameter to obtain data about changes in body weight. Hematopoietic and blood biochemistry investigations are also suggested for evaluating the toxicity related to any specific organ along with general health effects. In the case of long-term studies, the routes for administration may differ from acute toxicity studies based on exposure and anticipated routes in human as well as reasonable and valid justifications of the conclusions of other multi-dose studies.

One such example of evaluation of the toxicological features concerning therapeutic nanoparticles has been provided, wherein toxicity potential of curcumin-loaded polymeric nanoparticles to treat ulcerative colitis is evaluated. The unfunctionalized nanoparticles did not reveal any significant toxicity against HT-29 (human colon adenocarcinoma) cells in the MTT assay for 72 h of the test period. So, the curcumin-functionalized nanoparticles were then examined for their cytotoxicity effects on rats for both single-dose (acute toxicity study) as well as multi-dose (28 days sub-acute study) administrations. Throughout the study, no evidence of health issues, and no abnormalities were found to the group of animals. Rather they were observed being healthy in the hematological and blood biochemistry parameters as well as other endpoints tests. This indicated the safety of engineered nanoparticles for both short-term and extended periods (Dandekar et al. 2010).

Nanoinformatics for in silico toxicity prediction

Toxicity methods are relatively expensive, challenging, and inflexible. To overcome these limitations, a new computer-based approach has been developed known as in silico modeling. Applying the computer-based techniques, toxicological effects can be predicted concerning nanomaterials using databases maintained with training data sets. These databases are generated with information regarding the structure of nanomaterials and their physicochemical properties. One such important algorithm is quantitative structure–activity relationships (QSARs) modeling. The organization for economic cooperation and development (OECD) defined guidelines must be followed for validating the model in the third phase of model construction.

In this context, a research group has developed a nanostructure activity relationship (nano-SAR) for the classification of cytotoxicity with high throughput for the bronchial epithelial (BEAS-2B) cell covering exposure to nine nanoparticles of metal oxide. The model works on a logistic regression scheme, and further, it was assessed with the guided quantitative structure–activity relationships validation protocols. With the help of high throughput screening dataset, the identification of toxic and non-toxic events followed by labeling with respect to cells that are not exposed. Accurate prediction of this model consisted of atomization energy of the metal oxide (EMeO), the primary size (d), and the period of the metal (PMe), the relation between all three is shown in the following equation:

$$\ln \frac{P(NP \in T)}{P(NP \in N)} = 3600.3 + 103.5 \times d + 9.5 \times \theta_v + 97.6 \times P_{Me} - 58.5 \times E_{MeO}$$

where P(NP∈T) and P(NP∈N) indicate the probability of a nanoparticle as toxic or non-toxic, respectively, θv is the volume fraction of nanoparticle (Georgios Leonis 2018). The purpose of this model is to operate a strategy based on high throughput screening data to predict the toxic profile ranking of metal oxide nanoparticles.

The dataset of 17 metal oxide (Puzyn et al. 2011) has been used to build the quantitative structure–toxicity relationship (QSTR) model to develop the cytotoxic correlation between prokaryotic (*Escherichia coli*) and eukaryotic system (human keratinocyte cell line, HaCaT) (Kar et al. 2014). A new method nano-quantitative toxicity–toxicity relationship (QTTR) was constructed to predict the HaCaT and *Escherichia coli* cytotoxicities, in which specific features like electronic chemical potential (μ), the standard enthalpy of metal oxide nanocluster (ΔHfc) formation, and experimental cytotoxicity evaluation of *Escherichia coli* were displayed to be the most fitting information for HaCaT cytotoxicity predictions. Similarly, with the other parameters such as enthalpy of a gaseous cation formation (ΔHMe^{+}), the charge of cation analogous to a given oxide (χox), and the test cytotoxicity of human cell lines shows relevant erudition for *Escherichia coli* cytotoxicity prophecy. Hence, when the data for metal nanoparticle cytotoxicity are provided for HaCaT or *Escherichia coli*, the nano-quantitative toxicity–toxicity relationship method enables for deducing cytotoxicity of other species. The expression below gives information on the correlation of cytotoxicities between the two species (Georgios Leonis 2018);

Escherichia coli cytotoxicity = f (human cytotoxicity, H_{Me+}, χ_{ox}).

pEC_{50} *Escherichia coli* = 3.494 − (0.0006 × ΔH_{Me+}) − (0.308 × χ_{ox}) + (0.345 × pEC_{50} HaCaT).

Human cytotoxicity = g (*Escherichia coli* cytotoxicity, ΔH_f^c, μ).

pEC_{50} HaCaT = 0.968 − (0.222 × μ) + (0.0003 × ΔH_f^c) + (0.166 × pEC_{50} *E. coli*).

Furthermore, the correlation between the charge of the nanoparticle and the *Escherichia coli* cytotoxicity was witnessed, and it was explicated that the cytotoxicity to *Escherichia coli* was heightened by nanoparticles with a lower positive charge because of a trouble-free detachment of electrons from the surface of nanoparticle in contrast to the nanoparticles having a higher charge on their surface (Gajewicz et al. 2015).

A study has been conducted on 24 metal oxide nanoparticles to decipher the toxicity in relation to conduction band energies through which it was perceived that when conduction band energy overlay with cellular redox potential, nanoparticles such as cobalt tetra oxide (Co_3O_4), chromium oxide $(Cr_2O)_3$, nickel oxide (Ni_2O_3), and manganese oxide (Mn_2O_3) are prone to create reactive oxygen species and oxidative stress. Also, these nanoparticles were capable of oxidizing cytochrome c, consequently staying involved in redox homeostasis. Inductively coupled plasma mass spectrometry (ICPMS) investigation revealed that the harmful biological effects of these metal oxide nanoparticles could be supported by their solubility (Zhang et al. 2012). Thus, this outcome rendered a new platform for modeling the structure–activity relationship (SAR) based upon conduction band energy levels and dissolution of nanoparticles. At last, it was pointed out that in silico ranking scheme, including relevant statistics can be consolidated to predict toxicological model, through which in vivo toxicological profile may be cautiously predicted with the in vitro toxicological ranking. An online tool is available through the Enalos Cloud Platform (http://enalossuite.novamechanics.com) based upon NovaMechanics, and it is open software. This web service supports the computer-aided architect to predict the activity of nanoparticles. The motive of the Enalos Cloud Platform is to build an application for researchers approaching safe-by-design nanomaterials.

Nanomedicine: biomedical applications of nanotechnology

Nanomedicine is application of nanotechnology for diagnostics, drug/gene delivery, imaging, scaffolds for tissues, and as biosensors for disease detection (Daima et al. 2018, 2021; Chamundeeswari et al. 2019; Navya et al. 2019a, b; Shruthi et al. 2019; Chaudhari et al. 2020). In the following section, tailoring the physicochemical properties of nanomaterials with its application as nanomedicine in various aspects has been discussed.

Rational engineering of nanomaterials for medical applications

The physicochemical parameters, including size, surface-to-volume ratio, shape, surface modification or functionalization influence uptake, biocompatibility, and toxicity of engineered nanomaterials (Fig. 2). With decrease in size, uptake of the nanomaterials into cells is elevated. The shape also alters the uptake rate by the biological systems. Spherical nanoparticles are engulfed more rapidly than nanorods. The surface-to-volume ratio is remarkably a prime factor, given the fact that surface molecules/atoms boom up when the size of materials reduced below 100 nm. The number of atoms/molecules on the surface of nanomaterials is the decisive factor for materials reactivity, which will direct the chemical and biological characteristics along with stability of these nanomaterials. The answer to this question can be supported by the pH of the medium, concentration of electrolyte in the solvent, and shielding or capping agents, which adhere to the surface of nanoparticles and prevent agglomeration or aggregation. Aggregation property can be exploited for multiple applications like immunoassays, diagnosis, biosensing, and many more. For the stability and desired application of nanomaterials, functionalization is an attractive possibility to construct the smart nano-agents for nanomedical operations.

Another important facet of physicochemical properties is protein corona, which determines the fate of nanoparticles inside the living system. When nanoparticles enter the biological system, various proteins and moieties bind to the surface of nanoparticles leading to the surface covering nanomaterials. This coating is termed as protein corona. The biomedical application is fully ruled by nanomaterials-surface corona and functionalization. Nanomaterials can passively enter the cellular medium with the help of its surface charge, which plays the primary role in electrostatic interaction at the interface of nano-bio. As the cell membrane possesses an anionic charge, nanomaterials with the cationic charge internalize more leisurely as compared to negative surface charge. Hence, all these studies regarding size, surface charge, stability, functionalization, and corona are demanded to support the engineered nanomaterials for specific cellular targets for nanomedical applications (Navya and Daima 2016).

Nanomedicine for drug delivery

Drug delivery must bypass a series of barriers, viz., epithelial barriers, diffusional resistance in the connective tissues, opsonization and subsequent sequestration by mononuclear phagocyte system, cellular internalization, and nonspecific distribution. Nanoparticles can be engineered to overcome some of these barriers and permit release of therapeutic agents at a target site.

Chitosan shows mucoadhesive attributes and also break open tight junctions of the epithelia. Nanomaterials of chitosan are very much used for drug delivery to/across the buccal, nasal, eye, and intestinal epithelia. For example, carboxymethyl chitosan nanoparticles have been prepared to release intranasal carbamazepine to overcome the blood brain barrier, hence raising the medication in the brain at reduced systemic drug exposure. Chitosan nanoparticles delivered antigen in a controlled manner and displayed initiation immunization through anti-HBsAg IgG levels (up to 5500 mLU/mL) (Prego et al. 2010).

Alginate nanoparticles have also been utilized for drug delivery, because it is biopolymeric material grouped as an anionic mucoadhesive polymer. They have been synthesized to release venlafaxine through intranasal for treating depression. These nano-formulation are reported to straight away transporting the drug to the brain (Haque et al. 2014).

Liposomes are commonly used in the pharmaceutical and cosmetics industry. Four different liposomes have been prepared: (a) conventional types of liposomes, which are having cell membrane-like structure, (b) PEgylated liposomes with polyethylene glycol (PEG) at the surface for steric equilibrium, (c) ligand-targeted type, and (d) theranostic liposomes (Sercombe et al. 2015). A drug of interest can be loaded into liposomes at the time of liposome formation or after the liposome formation.

Micelles nanostructures are amphiphilic with core–shell structure in aqueous solution. Hydrophobic drugs can be conjugated with a hydrophobic core, and their hydrophilic shell make the miscible in water. Drugs can be loaded into micelles by solvent evaporation, dialysis, or direct dissolution process. Polymeric micelles have been employed for ocular (Mandal et al. 2017) and cancer drug delivery (Zhang et al. 2014).

Dendrimers are monodispersed and globular-shaped nanostructures. Dendrimers can be readily functionalized for drug delivery, and drug release can be controlled based on physical parameters like pH and temperature. A drug carrier dendrimer has been developed for cancer inhibition having target specificity, antiangiogenic, pH-dependent drug release, which display increased in the concentration of doxorubicin in the tumor by 121.5-fold after a day contrasted with free doxorubicin (Jain et al. 2014).

Inorganic nanoparticles as a drug vehicle have been studied, but their mechanism of toxicity is not yet well understood. Au nanoparticles can be exploited by the conjugation of the drug through the ionic/covalent bond, which further delivers or control the release of drugs via external stimuli. Iron oxide nanoparticles were capped with violamycine B1 and anthracycline antibiotics synthesized via laser pyrolysis and were examined against the MCF-7 cells for its anti-proliferation properties in comparison with iron oxide nanoparticles that are available in the market (Marcu et al. 2013).

Nanocrystals are nano-formulations of a solid drug without any covering. They could be stabilized by polymeric stabilizers or surfactants. For example, chitosan nanoparticles embedded with cinaciguat nanocrystals were utilized for pulmonary drug delivery. Quantum dots are another type of nanocrystals with size ranging from 2–10 nm. They are semiconductor materials having size-dependent properties such as optical properties, which has captivated much toward nanomedicine. In one of the recent studies, quantum dots-antibodies conjugates are prepared, and they were coated with norbornene-displaying polyimidazole ligands. The coated fluorophore was employed to label in vivo bone marrow cells. Moreover, it was found that the fluorophore conjugate was capable of disseminating into the entire bone marrow, and even it could tag rare populations of cells, including progenitor and hematopoietic stem cells (Han et al. 2015). Also, quantum dots with the anticancer drug designed in lipid carriers for theranostic application have been shown to detect H22 tumor cells with specificity (Olerile et al. 2016).

Nanomedicine for tissue engineering

Customized nanoparticles can mimic the natural properties of tissues (Hasan et al. 2014a, b; Hasan et al. 2014a, b); therefore, nanoparticles have been utilized for various functions within tissue engineering. Nanomaterials can remarkably elevate the electrical, biological, and mechanical features of scaffolds (Memic et al. 2015). For instance, a biodegradable patch enclosed with titanium dioxide displayed greater tensile strength in augmenting the scar after myocardial infarction (tissue death or necrosis due to poor blood supply) (Jawad et al. 2011). Whereas carbon nanotubes have shown an increase in mechanical strength for tissue engineering. For example, incorporating carbon nanotubes in polycaprolactone enhanced the mechanical properties by several folds. This strategy has been employed to increase the proliferation of rat bone marrow stem cells (Pan et al. 2012). Gold nanoparticles inside fibrous decellularized matrices improved electrical properties of cardiac cells (Shevach et al. 2014).

Scaffolds loaded with silver nanoparticles and alginate and hydroxyapatite (HA) scaffolds acquired antimicrobial properties, which are of great benefit in development of prosthesis (Marsich et al. 2013). The 3D tissue-engineered scaffolds have been fabricated with the assist of nanomaterials for bone, skin, and other tissues, (Xu et al. 2004; Li et al. 2005) also nanostructure exhibited to regulate the activities of primary stem cells like growth, adhesion, and differentiation (Namgung et al. 2011; Teo et al. 2013).

Neuronanomedicine

Neuronanomedicine is promising for theranostic strategies of neurological disorders. Alzheimer's and Parkinson's diseases are highly prevalent neurodegenerative disorders (Gitler et al. 2017). Nanocarriers can be employed for the treatment of various neurodegenerative diseases, for example, polyethylene glycol biodegradable polymer nanoparticles conjugated with particular antibodies exploited for the abrogation of amyloid fibrils in Alzheimer's disease (Carradori et al. 2018). In vitro studies revealed that utilizing polymeric nanocarrier enhanced the delivery of curcumin with reduced oxidative stress and inflammation (Barbara et al. 2017). Another study showed the accumulation of polymer-lipid nanoparticles containing docetaxel (DTX-NP), heightening the inhibition of tumor growth and increased survival as compared to the clinical dose of docetaxel as shown in Fig. 5 (He et al. 2017).

Stroke appears to be a medical emergency that may fallout into death or disability. Therapy for strokes has been scrutinized for poly lactic-co-glycolic acid nanoparticles with surface functionalized chlorotoxin for the co-conveyance of Lexiscan and Nogo-66, toward raising permeability of blood brain barrier along with the capable targeting ligand at the

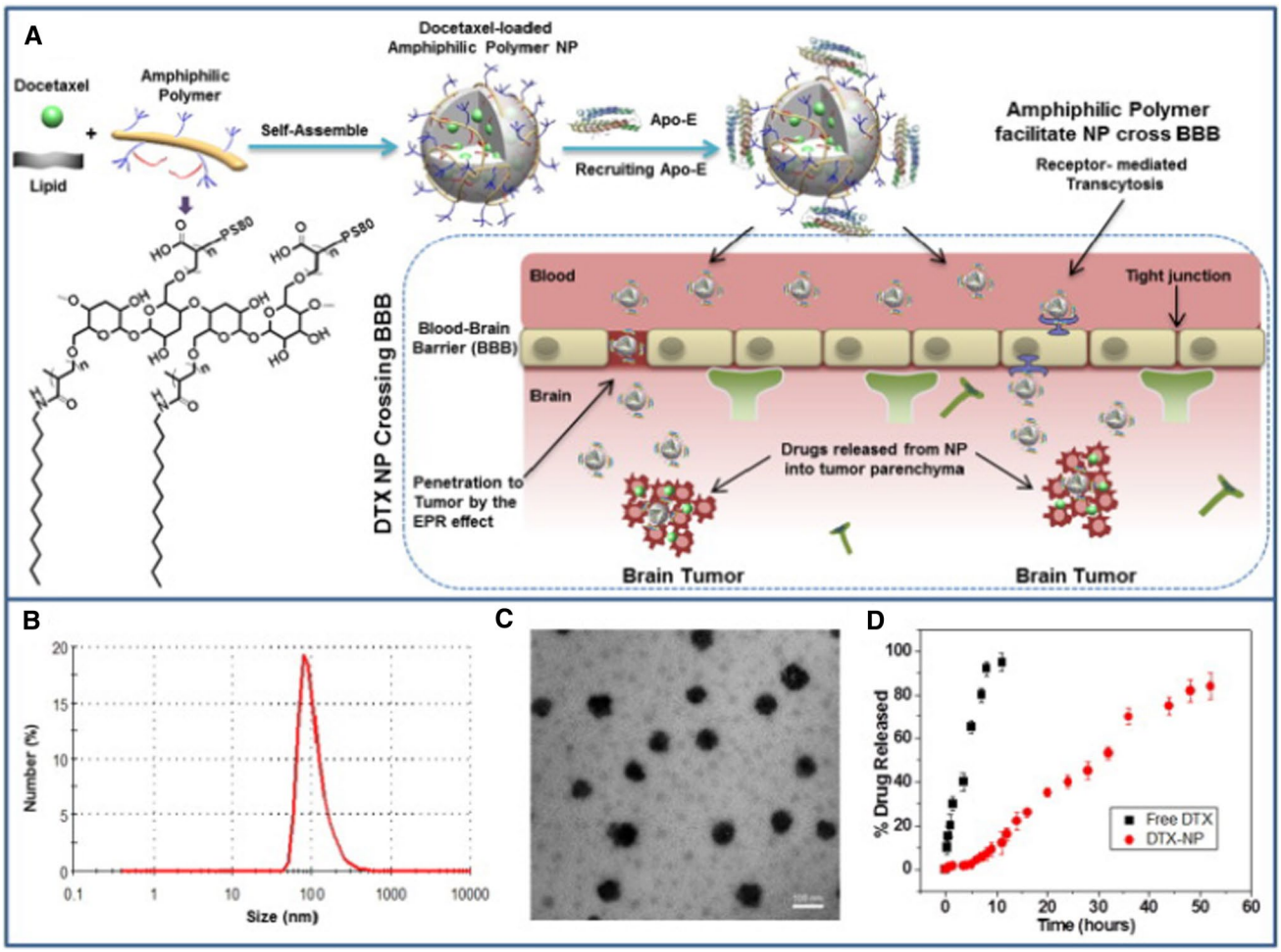


Fig. 5 Docetaxel-loaded polymer-lipid nanoparticles (DTX-NP) formation from the amphiphilic polymer, and use of apolipoprotein-E (Apo-E) on the surface. The DTX-NP conjugate shows enhanced permeability and retention (EPR) effect, and receptor-mediated transcytosis mechanism for surmounting the blood–brain-barrier (a). The particle size distribution (b), transmission electron microscopy image (c), and *in-vitro* release of pristine DTX and DTX from the loaded-NPs (d) are also shown. Here, scale bar = 100 nm (c) and data presented as means SD, $n = 3$ (d). Reprinted with permission from (He et al. 2017)

stroke location, which confirmed for an increase strokes survival (Han et al. 2016). Transcend-peptide (xB3) a patented stage developed by 'Bioasis scientists' for the purpose of neuro-medicine which consists of human transport protein wandering in blood, which put on display as efficient delivering molecules beyond blood brain barrier via transcytosis through receptor-mediated, with which preclinical studies showed the capability of xB3 to deliver antibodies, enzymes, and gene therapies thus holding a potential to treat brain cancers and neurodegenerative diseases.

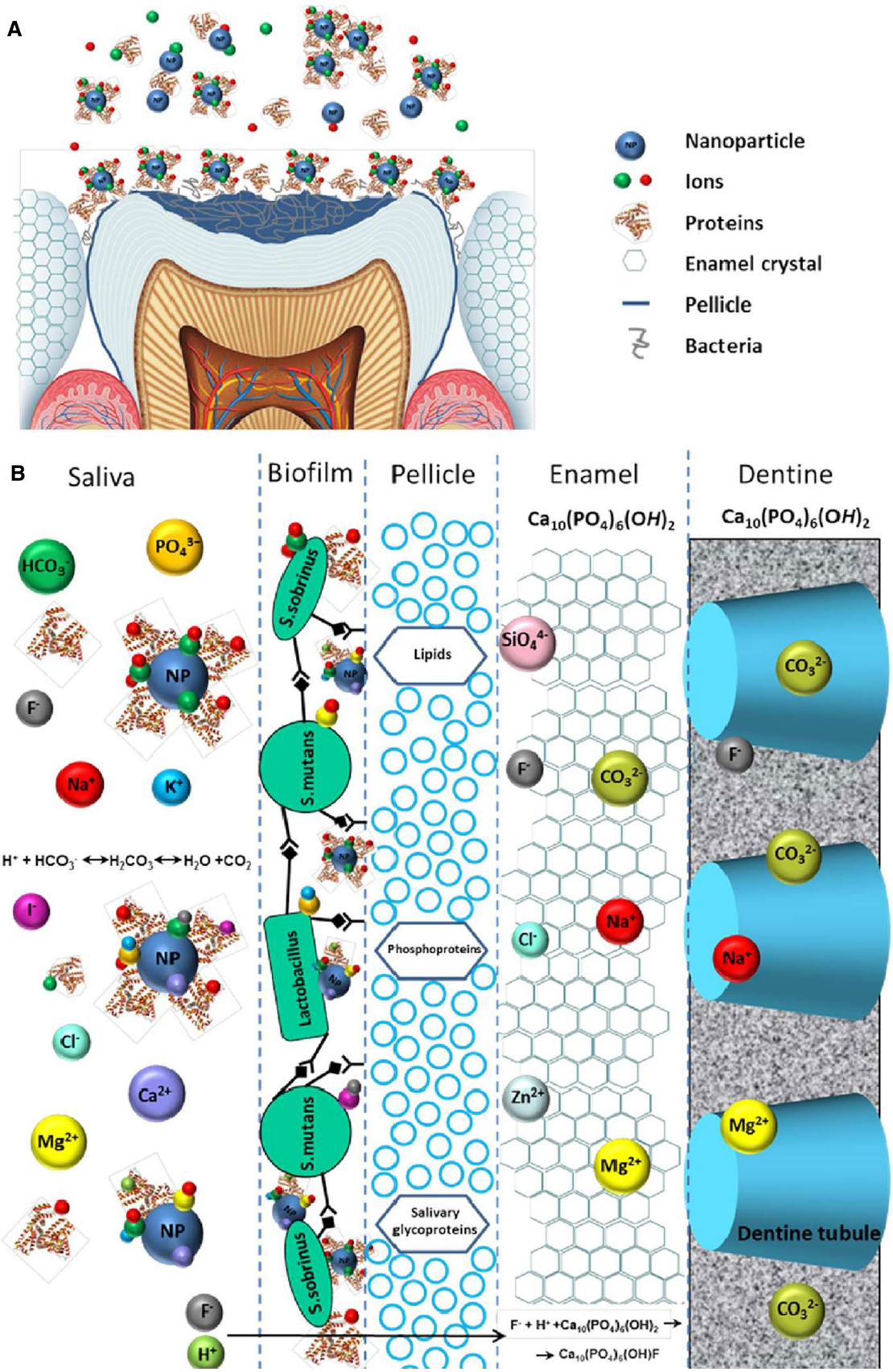
Nanomedicine for dentistry

Nanodentistry is an emerging field with great potential to generate advanced biological materials for orthodontics, periodontics, prosthodontics, or restorative dental science. Enhanced mechanical, aesthetic, optical properties, and lower shrinkage are the significant assets for utilizing nanoscale biomaterial (Kim et al. 2005) in the field of oral surgery, prosthodontics, endodontics, diagnostics of saliva, and dental implants.

In one of the recent reviews, evidence-based applications of engineered nanomaterials in dentistry have been discussed, wherein, it was indicated that the complexes of nanoparticle-ion-protein can selectively adhere to the pellicle layer or the growing biofilm as shown in Fig. 6a (Besinis et al. 2015). Figure 6b presents the distribution of nanoparticles and various ions within the oral-environment, -biofilm and mineralised dental tissues. Normally, different ions and proteins are present in the natural form of saliva. In the presence of nanoparticles, these ions and proteins form the complexes with the nanoparticles, wherein the conditions of oral cavity may accelerate the sedimentation and agglomeration of nanoparticles on the dentinal surfaces. Interestingly, the pellicle due to its globular structure can support the oral biofilm formation due to the presence of a proteinaceous layer, that can easily provide surface for the colonizing species. This formed biofilm prevents the diffusion/permeation of nanoparticles, thus acting as a barrier for the enamel-pellicle interface. As discussed, various types of ions are present at the different surfaces of teeth. The ions like F^-, Cl^-, SiO_4^{4-}, Zn^{2+} are abundantly found near the external surface of enamel, whereas the dentino-enamel junction has higher concentration of Na^+, Mg^+, Co_3^{2-}, respectively (Besinis et al. 2015).

Nanostructures such as nanorods, nanoparticles, nanotube, nanofibers, liposomes, dendrimers, and many others are exploited as a nanocarriers and theranostic agents for dental diseases. The most well-liked restorative materials for filling are dental composite, which comprises a resin polymer matrix, inorganic filler, and coupling agent. Metals and their oxides such as silver, zinc, titanium dioxide nanomaterials are assimilated for dental applications with antimicrobial properties as dental composite replacing traditional metal granulate (Besinis et al. 2015). The most critical step in revascularization procedures in clinical is root canal disinfection. Bioactive nanofibrous scaffolds were fabricated, which can be employed as a drug delivery system before regenerative endodontics for root canal decontamination (Bottino et al. 2015).

An alternate of restoring teeth with the help of crowns and bridges is dental implants, which is widely used in the present world. An in vitro study was conducted to compare the fallout of different implants and nanotopographies on the production of cytokine via gingival fibroblasts of human, which showed enhanced secretion of interleukins (Stavroullakis et al. 2015). Bioactive glass nanomaterials are captivating as a consequence of their excellent activity in composite to act as scaffolds for periodontal than its conventional micron-scale materials (Hong et al. 2010; Qasim et al. 2015), because of its distinctive attributes, when comes in contact with the body a covering of carbonated apatite is formed which communicate with tissue to produce healthy bone. Another in vivo study was performed with triclosan-loaded nanoparticles in dogs only with gingival index, and bleeding on probing was examined, which showed that these nanoparticles were capable of reduction of inflammation


A
Nanoparticle
Ions
Proteins
Enamel crystal
Pellicle
Bacteria
B
Saliva
Biofilm
Pellicle
Enamel
Dentine
Ca10(PO4)6(OH)2
Ca10(PO4)6(OH)2
HCO3-
PO4 3-
NP
F-
Na+
K+
H+ + HCO3- ↔H2CO3↔H2O +CO2
I-
Cl-
Ca2+
Mg2+
S.sobrinus
S.mutans
Lactobacillus
S.mutans
S.sobrinus
Lipids
Phosphoproteins
Salivary glycoproteins
SiO4 4-
CO3 2-
Zn2+
F- + H+ +Ca10(PO4)6(OH)2 →
→ Ca10(PO4)6(OH)F
Dentine tubule

◂**Fig. 6** Dentinal tissues, and nanoparticles (NPs) presence in saliva and dentinal tissues. The complexes of nanoparticles, ion, and protein cannot stick to the surfaces of the tooth; however, the complexes can adhere to the pellicle layer or the growing biofilm (a). Schematic representation of the distribution of nanoparticles and ions in the oral environment, biofilm, and dentinal tissues (b). Reprinted with permission from (Besinis et al. 2015)

(Piñón-Segundo et al. 2005). Hence, to inflate the nanotechnology commodities in dentistry needs a comprehensive analysis with the full judgment before consolidating it to clinicians. Nanotechnology will make a pathway for the addition of various techniques that permits early diagnosis and maintaining oral conditions.

Challenges and translation to commercial products

Engineered nanomaterials are sought in nanomedicine to exploit their therapeutic advantages, which include bioavailability, reduced toxicity, target site delivery, and theranostic in one system. For the successful commercialization of engineered nanomaterials, the high therapeutic effect must be combined with safety. Another challenge for engineered nanomaterials is associated with their formulation, which requires sonication, centrifugation, size reduction, sterilization, and solvent evaporation, many of which at large scale impose a challenging task for the manufacturing process. The retention of the physicochemical properties is mandatory for nanomedicine throughout the mass production, and also during their life cycle, if there is variation at the time of manufacturing, then it may alter the physicochemical properties, in the end, twisting the therapeutic outcomes. Nanomedicine characterization is another crucial step, which appends cost to the manufacturing, and testing of it; hence, it demands an expertise team for data analysis and interpretation. In this context, Table 2 depicts the major challenges associated with the delivery of cancer therapeutics (Navya et al. 2019a, b).

Clinical trials of nanomedicines are enduring from the shortfall of reliable screening that can appraise the efficacy and toxicity with the best in vivo correlation. In vitro assessment consists of drug release, cellular uptake, and toxicity, which are performed using cell cultures in monolayers (2D). Nevertheless, the uptake of nano-formulation is affected by the interaction surrounded by its physicochemical parameters, and thus 3D cell system may come up with preferable fallouts. Nanomedicine in healthcare reshapes the concurrent diagnosis and therapy, regardless of the impact of nanomedicine, only a few products extended to the market. Translation of nanomedicine refurbishes the infrastructure of the industry with excellent manufacturing amenities and characterization of nanomedicine, which include quality control at every place to perpetuate the quality and efficacy.

Nanomedicine characterization is executed under the condition with novel methods like microfluidics for mimicking the in vivo environment and selection of the propitious model. There is a significant headway in providing better health by nanomaterials. As stated by Grand View Research, Inc., the worldwide market of nanomedicine is to be expected to set foot at $350.8 billion by 2025. Innovation in healthcare is anticipated to introduce more nanomedicine in the market. Hence, it is indispensable to evaluate the methods that can assess the delivery, efficacy, and safety of nanomedicine.

Table 2 Challenges associated with the delivery of cancer nanotherapeutics, showing the vital physicochemical properties of nanomaterials which are essential to be controlled and measured; large-scale production of nanomaterials and allied concerns; and the toxicity-regulatory issues

Challenges in nanodrug delivery		
Physicochemical properties	Large-scale production	Toxicity and regulation
Size and shape	Good manufacturing practices	In vitro and in vivo correlation
Storage and stability	Planning and designing	Nano-bio-interface interaction and protein corona
Surface area and composition	Batch-to-batch variation	Environmental concern
Surface chemistry and surface charge	Multi-stage production	Time consumption and quality control
Crystal plane and aggregation	Polydispersity and high cost	Complementary toxicity assays
Drug loading and porosity	Reproducibility	Food and drug administration (FDA), European medicines agency (EMA) guidelines
Release profile	Sterility issues	Regulatory approval

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Declarations

Conflict of interests The authors of the manuscript do not have competing interests.

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