



Published in final edited form as:

Biomed Mater. ; 16(4): . doi:10.1088/1748-605X/abe5fa.

Biocompatibility of nanomaterials and their immunological properties

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Abstract

Nanomaterials (NMs) have revolutionized multiple aspects of medicine by enabling novel sensing, diagnostic, and therapeutic approaches. Advancements in processing and fabrication have also allowed significant expansion in the applications of the major classes of NMs based on polymer, metal/metal oxide, carbon, liposome, or multi-scale macro-nano bulk materials. Concomitantly, concerns regarding the nanotoxicity and overall biocompatibility of NMs have been raised. These involve putative negative effects on both patients and those subjected to occupational exposure during manufacturing. In this review, we describe the current state of testing of NMs including those that are in clinical use, in clinical trials, or under development. We also discuss the cellular and molecular interactions that dictate their toxicity and biocompatibility. Specifically, we focus on the reciprocal interactions between NMs and host proteins, lipids, and sugars and how these induce responses in immune and other cell types leading to topical and/or systemic effects.

Keywords

nanomaterials; nanoparticles; biocompatibility

1. Introduction

Advances in nanotechnology and nanofabrication have allowed the development of a wide range of material classes, geometry, and sizes [1, 2]. As per ISO/TS 80004, the term NM is defined as 'a material with any external dimension in the nanoscale or having internal structure or surface structure in the nano-scale.' Many material features are unique at this

scale due to size-dependent properties like high surface to volume ratio, high reactivity, pronounced quantum effects, etc [3]. In addition, NMs or materials with nano features can be engineered and optimized for optimal performance due to their ability to be processed at the nanoscale [4, 5]. Biomedical applications are one area where NMs have been utilized and their versatility in creating infinite geometries and hierarchical designs has allowed rapid and remarkable development [6–10]. Naturally, exposure of living systems to NMs, intentional or otherwise, leads to complex interactions including the FBR [11–13]. These interactions can vary significantly and depend on the type of NMs and the tissues and cells involved [14]. For example, some NMs display desirable interactions with the immune system and are tailored for therapeutic purposes [7, 15, 16]. Others elicit undesirable responses that might lead to health complications [17, 18]. Moreover, due to their novel scale, NMs might pose a threat to those involved in their manufacturing [19].

2. Nanomaterial compositions and applications

Despite the major breakthrough in engineering NMs for biological applications, the NM design parameter, and the tissue- and cell-specific responses they can potentially elicit, continue to pose unique challenges [10, 14]. For example, the biocompatibility of a given NM is largely dependent on its surface properties [20]. In addition to the desired properties such as drug release, biointegration, etc, acute or chronic inflammation is almost always present as a result of the FBR [11]. Inflammation and encapsulation by themselves are not necessarily undesirable, in fact, in some cases, it can also be beneficial such as for intrauterine contraceptives [21]. Given its ubiquity, modulation of the inflammatory response is perhaps a more suitable goal for bioengineers. Suggestions for integrating biological regulators such as immunomodulatory compounds with biomaterials have been made in this direction [22].

Here, we discuss five common classes of NMs that have been utilized in or suggested for therapeutic purposes: polymeric, metal, carbon-based, liposomes, and surfaces with nanofeatures (discussed in sections 2.1 to 2.5). The macroscopic surfaces with nanofeatures include macroscale materials like implants whose surfaces have engineered nanofeatures like nanorods or nanopores [23]. Table 1 summarizes the major NM classes and their most common features and applications. It also features diagrams showing the geometry of various NMs.

2.1. Polymer-based nanomaterials

Polymers, long chains of repeating monomers, are widely used in nanomedicine due to their biocompatible and biodegradable properties. Polymeric NMs are primarily composed of carbon, hydrogen, oxygen, and nitrogen and are sourced from either synthetic or biological sources. Biodegradable polymeric NPs have been identified as great candidates for controlled drug delivery, as they are capable of selective targeting, controlled drug release, protection of the encapsulated payload, and prolonged circulation time [24]. Polymers can be used to form various types of NMs such as drug/protein conjugates, polymeric micelles, and NPs. Micelles are composed of a hydrophilic outer shell and a hydrophobic core, a design that increases drug stability [24]. Polymer conjugation occurs

when a polymer chain is attached to a protein or drug, altering its delivery properties. For example, PEGylation, also known as PEG conjugation, is often used for drug delivery due to its hydrophilic nature, resulting in the body's inability to recognize it as foreign [68]. Liposomes, metals, drugs, and other proteins have also been modified by PEGylation to avoid immune detection.

Polymeric NM synthesis can occur through various methods. The polymer used and the molecule or drug encapsulated will determine the optimal synthetic method. When beginning with a monomer, the first step is polymerization. The polymer is processed at the nanoscale through an emulsion technique, where a mixed water-surfactant system is used to generate nanodroplets, which are then precipitated to form NPs [24]. Other methods of synthesis include interfacial polymerization and controlled living radical polymerization, which includes nitroxide-mediated polymerization and reversible addition-fragmentation chain transfer [24]. When beginning with a preformed polymer, the first step is to disperse the polymer. The NPs are formed through solvent evaporation, nanoprecipitation, salting-out, dialysis, or supercritical fluid technology [24].

Solvent evaporation, where the polymer is first emulsified into an aqueous phase followed by solvent evaporation resulting in precipitation into NPs, is a common synthesis process. NP size can be altered by adjusting the temperature or the stir rate during evaporation [25]. Additionally, conjugation on the surface of polymeric NMs allows for potential targeted delivery. Many characteristics of polymeric NPs can be modified such as the surface charge, surface receptors for targeting, size, and morphology [24]. These characteristics can alter NP behavior by altering the cellular uptake, biocompatibility, or specificity. Most polymer NMs range from 10 nm to 200 nm as materials at those sizes have a high potential for prolonged circulation [24].

Polymeric NPs can be categorized as natural or synthetic. Synthetic NPs can be further categorized as non-degradable or biodegradable. Historically, synthetic polymers used for NPs were non-biodegradable such as poly(methyl methacrylate), polyacrylamide, and polystyrene [24]. Due to their toxicity and inflammatory effects, the focus shifted to biodegradable polymers such as PLA, PLGA, and PEG [24]. Natural polymers such as starch, cellulose, and chitin have also been investigated as materials for nanomedicine. Starches have been used to produce films with nano features and nanocomposites for use in biomedical applications [69]. CNF, CNC, and bacterial nanocellulose represent NMs derived from cellulose, a highly abundant, and therefore considered a renewable, source of biopolymers [70]. Cellulose can be processed into nanofibers, nanowhiskers, or NPs. Chitosan, a derivative of chitin, is biocompatible but displays varying degrees of toxicity under different chemical modifications, such as different levels of deacetylation [69]. The adhesive nature of chitosan allows adherence with tissues for many applications including dentistry, wound healing, and ophthalmology. Furthermore, chitosan possesses a variety of biological properties such as mucoadhesion and enhanced permeation [71].

When used in cancer treatments, polymeric NPs were found to decrease systemic drug toxicity while providing targeted treatment to the cancerous site [24]. Due to the ease of functionalization, they have also been engineered to penetrate the BBB for the potential

treatment of neurodegenerative diseases [24]. A newer innovation in polymeric NPs is the creation of stimuli-responsive NPs. These NPs experience a conformational change due to a stimulus, such as temperature or pH, which triggers controlled release [24]. For example, cancerous tissues exhibit hyperthermia and temperature triggered NPs have been designed as a treatment method [24]. pH-triggered NPs can be used for treatment because many tumors create an acidic extracellular space [24]. Polymeric NPs are also being investigated for nucleic acid delivery since they have been found to protect them from degradation and increase cellular uptake [24]. It should be noted that most of these applications are still in the experimental phase. Polymeric NMs on the market are restricted to PEGylated nanopharmaceuticals, such as Cimzia or Adagen [27]. There are also a number of polymeric NPs and micelles in clinical trials for cancer therapy [24].

2.2. Metals and metal oxide-based nanomaterials

Metal NMs have been used for various applications, mostly in the form of NPs on the scale of 1–200 nm [20]. Due to their optical properties and propensity for scattering light, metals are optimal materials for diagnostics and have been used in imaging as contrast agents, for cancer detection, or disease therapy [72]. Metal NPs are synthesized through various chemical or physical methods. In chemical synthesis, metal salts undergo a redox reaction by either electrochemical synthesis, which involves conduction of current through an electrolytic solution, sonochemical synthesis, where a high energy pulse is used to generate radical species, or chemical reduction, which utilizes metal precursors or reducing agents to form NPs [26]. Physical methods of synthesis include thermal decomposition, which uses high temperatures to create NMs, and laser ablation, where the metal is removed by a laser to create highly controlled NMs [72].

All metal NMs induce an inflammatory response that is dependent on composition, size, and shape. Studies have shown that immune responses can be triggered by inhalation or direct contact, impacting not only patients but those who manufacture them [20]. Generally, as the size of metal NPs decrease, they become more toxic [73]. Other properties that can influence the toxicity of metal NPs include elemental composition, charge, shape, crystallinity, and NP solubility [73].

Gold and silver are two noble metals used often in nanomedicine. Silver NPs contain unique optical, anti-microbial, and electronic properties, while gold NPs have a relatively low toxicity, are easy to prepare and are often used in diagnostics due to their surface plasmon resonance effect [72]. Similarly, surface-enhanced Raman spectroscopy properties of silver NPs have been utilized to develop a blood test for esophageal cancer detection [74]. Reactive metals are also used for NPs and include copper and aluminum. Copper NPs are inexpensive, have good physical properties, and can be used in a wide range of applications [72]. Copper selenide nanocrystals have been created to produce photo-thermal heating, which can destroy human colorectal cancer cells upon irradiation [75]. Aluminum NPs are used to create vaccine adjuvants [29]. Magnetic metals, such as iron, nickel, cobalt, and manganese are useful for MRI imaging [72]. Gold, silver, titanium, and zinc are known to be anti-microbial which, when formed into NPs, can be used for antibacterial therapies [72]. Although many metal NMs are being investigated in an experimental setting, due to their

aforementioned relative toxicity, only a few metal NMs have been approved by the FDA. Currently, super-paramagnetic iron oxide is approved as an MRI contrast agent while other iron NPs have been approved for treating iron deficiencies [28]. Many vaccines containing aluminum adjuvants are approved by the FDA [29].

2.3. Carbon-based nanomaterial compositions and applications

Carbon nanoforms are one of the most widely studied categories of NMs, with sizes ranging from several nm to a few 100 nm. Carbon atoms can bond together in various ways depending on their hybridization state, giving rise to a vast array of NMs [76]. These NMs are found in various kinds of geometry and size ranges. For example, graphite is one atomic monolayer thick sheet made of hexagonally arranged carbon atoms. Often, many carbon NMs are referred to as graphitic NMs, because they have carbon atoms arranged primarily in hexagonal rings, like those in graphite. These may be cylindrical CNTs, spherical fullerenes, nanocones, helical tubes, and can even combine to form multiwalled CNTs, nano-onions, and nano-peas (fullerenes in tubes) [37–39, 77–79]. Other structures include nanodiamonds with tetra-hedrally arranged carbon atoms and amorphous carbon black particles. Sometimes, CQDs are referred to as zero-dimensional materials as they are extremely small even amongst NMs. They may be crystalline or amorphous and can be generated by breaking down other NMs like graphite, nanodiamonds, CNTs, etc into sizes less than 10 nm [80].

Some common methods for generating graphitic NMs include graphite vaporization using a discharge arc or laser, CVD using hydrocarbons, HiPco, and hydrocarbon combustion (see table 1 for details) [30]. Size and geometry selectivity for the resultant NMs can be achieved using catalysts. Alternatively, processes like gel chromatography (used for CNTs) may be used for extracting required NM after a nonselective generation process [81]. High pressure is needed in addition to high temperature for the production of nanodiamonds, mimicking the natural process of diamond formation [43].

The physical and chemical properties of many of these nanoforms are dependent on their size, geometry, and surface chemistry, and so are their biological interactions [18, 45, 82]. Functionalization of these NMs by adding certain functional groups/surfactants/polymers on their outer surface is often done to make them more water dispersible, less cytotoxic, and more biocompatible [46]. Specific processes like Hummer's or Brodie's method are used for oxidizing graphene sheets [32]. Other materials like CNTs can be functionalized with –COOH through ultrasonic treatment in a mixture of acids [83]. They can also be modified with therapeutic loads for delivery applications.

NMs like nanotubes and fullerenes stand out as therapeutic delivery systems. They can encapsulate therapeutic loads and bind with other molecules covalently on their external surface. In this manner, they can be conjugated with anticancer drugs, bioactive peptides, proteins, nucleic acids, DNA strands, etc [31]. CNTs can be used as photothermal agents owing to significant near-infrared absorption [84]. They have been shown to be promising candidates as diagnostic and imaging tools, tissue engineering scaffolds and are used in neuron prosthesis applications [76]. Fluorescent properties of CQDs are extensively studied

and, owing to their extremely small sizes and passivated surface, seem to have good potential for passing the BBB [80].

Similar nanoforms can be produced unintentionally during the burning of fossil fuels, and other incineration processes [85]. They can easily get introduced through pulmonary routes and result in adverse responses. Hence, studies have focused on resulting conditions such as fibrosis caused by exposure to CNTs [86, 87]. A study indicated that SWCNTs may be perceived as pathogens, and the conditioned medium from SWCNT-exposed cells acted as a chemoattractant for DCs [88]. Since these materials are manufactured for various laboratory and industrial applications, there is a growing concern for the safety of personnel working at mass manufacturing sites owing to frequent, long-term exposure. A recent study discussed functional immune response in workers from 12 different U.S. facilities exhibiting generally low levels of exposure to CNTs or nanofibers [19]. However, recent studies with macrophages and fibroblasts exposed to graphene and carbon-based nano-onions, respectively, showed very low ROS generation and cytotoxicity [89, 90]. These observations prompted investigators to suggest that cytotoxicity results may be influenced by additional contaminating factors, such as bacterial endotoxin, introduced during manufacturing [89].

Carbon-based NMs have also been shown to activate the inflammasome complex in macrophages, followed by IL-1 β secretion. It has also been suggested that inflammation (in many cases unavoidable for carbon-based NMs) is not always a detrimental response, and strategies should not seek to prevent acute inflammation at every cost, but rather focus on chronic effects [91].

2.4. Liposomes and other self-assembled material compositions and applications

Liposomes are self-assembled lipid bilayer vesicles often but not always composed of phospholipids, one of the most common examples being phosphatidylcholine [48, 92]. Liposomes may be mono- or multi-layered and have sizes ranging from 30 nm to several micrometers [48]. They are suitable candidates for delivering therapeutic payloads owing to their biocompatibility and ability to carry both hydrophilic and hydrophobic loads [48, 92, 93].

Sonication of a suspension containing lipid membranes to disrupt the layers and allow for self-assembly into smaller vesicles is one of the most popular synthesis methods. Multilayered liposomal suspension can also be extruded through a polycarbonate filter to yield particles with a diameter near the pore size of the filter [48]. A heating method developed by Mozafari *et al* is also popular as it does not involve any toxic components [94]. They are often PEGylated, which reduces surface protein adsorption and uptake by macrophages and results in an increase in their residence time [51, 92]. PEGylation can be done on the lipid bilayer before or after vesicle formation (pre- or post-insertion), resulting in PEG chain(s) on both or only the external surface of the liposome [50]. Water-soluble drugs may be dissolved in an aqueous suspension used to disperse the bilayer and consequently, drug molecules get trapped in the aqueous liposome core. For loading hydrophobic loads, they can be trapped in the liposomes' nonpolar bilayer compartment [51]. Both the main components of these carriers, lipid bilayer and PEG polymer, are generally considered to be biocompatible, and hence such liposomes are used extensively

for drug carrier applications [48, 92]. Consequently, they form the single largest combined category of NMs utilized in FDA approved and investigational drugs [28, 95]. Even so, there have been some unanticipated immune responses against these carriers including accelerated blood clearance, CARPA, and some lipid related allergies, e.g. immediate hyper allergy on the first administration of Doxil® [92].

Non-PEGylated liposomes are preferred for specific cases. For example, conventional liposomes perform better for the delivery of high membrane permeability drugs like vincristine, as PEGylation may make the liposome walls less rigid by hindering hydrogen bonding in the bilayer [53]. Other self-assembled materials, such as nucleic acids, polypeptide nanofibers, etc are being evaluated in preclinical studies for tunable drug delivery and immunological response properties [93]. These may be synthesized using various wet synthesis methods such as emulsification, desolvation, or complex coacervation [54].

2.5. Nanopatterned surface compositions and applications

Apart from surface chemistry, which may be altered through functionalization, topology at micro- and nano-scales can also be utilized to modulate cell response, and consequently immune response in more complex biological systems [96–99]. Patterning may directly affect properties like hydrophobicity and alter more complex cell response pathways. Such patterning may also mechanically force the cells to grow along a certain direction in specific morphology and/or may alter cell response by locally altering the stiffness of the substrate [100, 101]. A surface may be nanopatterned using laser sculpting, ion/electron beam drilling, chemical etching, electrochemical anodization, EBL-assisted RIE/imprinting, or thermo-mechanical nano molding [58, 61, 102].

It has been shown that nanopatterned surfaces may have desirable interactions with certain cells involved in the FBR [57]. Nanopatterning may include creating protrusions, such as nanorods on the surface or pores of certain size distribution. These features can be used to engineer cellular responses such as improved inflammatory and re-endothelialization to increase stent performance [103]. Another example involves improving titanium biocompatibility by surface nanostructuring [104]. Similarly, modulation of macrophage responses has been achieved by BMG nanopatterns [57].

2.6. Nanoceramic composition and applications

Ceramics are a broad class of materials defined as inorganic compounds of metal or metalloid and non-metal with ionic or covalent bonds. They have high mechanical strength and are pH and temperature resistant but have low biodegradability [67]. Synthesis of ceramic NMs can occur through methods such as microemulsion precipitation or hydrothermal synthesis [67]. Ceramic NMs are often used for coatings due to their heat resistance and chemical inertness and their applications range from drug delivery and tissue engineering to diagnostics and biosensors [67].

There are certain materials that stand out in their category for their prevalent use in biomedical applications, e.g. HA. Other materials used for ceramic NMs are based on calcium phosphates, silicon, titanium, and zirconium. Ceramic NMs can be synthesized

into a variety of shapes. Ceramic NPs consist of an inorganic core that can encapsulate and protect a drug [67]. Ceramic nanoscaffolds contain pores that allow for high porosity, high surface area, and long degradation times for controlled drug release [67]. Nano-clay is composed of thin nanosized layers of ceramic material known for their high adsorption ability, surface area, and chemical inertness [67]. Some HA based drugs and formulations, such as nanocrystal-line HA-paste based Ostim®, have been approved by the FDA for bone growth stimulation.

3. Nanomaterial toxicity and biocompatibility

NMs designed for biomedical applications must be assessed in terms of cytotoxicity and overall biocompatibility [20, 105]. Cytotoxicity refers to their effects on cell functions and viability. Biocompatibility, on the other hand, involves a comprehensive evaluation of NMs *in vitro* and *in vivo*. To ensure their biosafety, they must get tested following standard protocols. For example, under the guidance on NMs by ISO, ISO/TR 10993-22:2017 part 22, it is required that NMs must be tested on (a) cytotoxicity, (b) genotoxicity, carcinogenicity, and reproductive toxicity, (c) immunotoxicity, irritation, and sensitization, (d) hemocompatibility, (e) systemic toxicity, (f) pyrogenicity, and (g) implantation [106]. These tests are crucial to ensure the safety of patients that receive NM-based treatments, as well as the manufacturing workers [107].

Although toxicology tests are crucial to prevent adverse effects, fundamentally understanding the associations between NM physicochemical features and their specific bio-effects is important to broaden their applications. For example, research that focuses on the therapeutic applications of NMs has shown their ability to control the scarring process, attenuate fibrosis, and suppress cancer growth [108]. Wide ranges of material types, biological systems, and analytical metrics that quantify their interactions, however, complicate such assessment. A previous review has listed a number of challenges in understanding the bio-interactions of NPs [109]. Here we expand the list to NMs. It is challenging to understand the association between physicochemical features of NMs and their effects on biological systems because of (a) a broad scale of different types of NMs, varying in chemical composition, size, shape, and surface coating [109, 110] (b) a large number of different model systems that can be used for testing that often interact with different NMs in varying manners (such as changes specific to different cell types) [111], (c) the lack of standardized protocols (e.g. incubation times and NP concentrations used in cell exposure [112, 113], and (d) increased complexity related to *in vivo* tests [114]. Overall, these challenges limit the number of materials that can be tested and prevents our complete understanding of the bio-effects of NMs.

Here, we opted to cover the most relevant tests and evaluate them based on the specific application requirements. We focused on the effects of NMs on cytotoxicity, immunomodulation, fibrosis, and genotoxicity, which are most commonly described in the literature and also relate to occupational and manufacturing exposures [107]. Figure 1 highlights the most common NM features that influence these processes.

3.1. Nanomaterial cytotoxicity and genotoxicity

Multiple parameters such as composition, molecular structure, and size can influence NM cytotoxicity, and their effects are mediated by engaging pathways that cause direct necrosis, induced apoptosis, or antibody-triggered immune clearance [115]. Common cytotoxicity tests are based on assessing the following criteria associated with injured/dead cells: (a) damaged cell membrane, (b) production of cell-death markers, and (c) impaired metabolism. Figure 2(a) provides an example of a cytotoxicity assay for ZnO NPs.

Some specific examples include the LDH test that detects the release of LDH, which usually resides inside intact cells. Caspase-3/7 assay assesses the production of caspases, which are mediators of apoptosis. Other tests, such as the MTT assay, measure changes in metabolic activity to evaluate cell viability. Specifically, the MTT assay measures the reduction of tetrazolium salt using redox indicators that correlate with cell viability. Alternatively, the reduction of resazurin can be assayed to evaluate cell viability in a similar manner [119]. Interestingly, other *in vitro* assays utilize cell-membrane mimic lipid models to study specific damage by the interactions with NMs [120]. In such studies, the mechanism behind those interactions includes membrane disruption, which leads to cell death [120].

NMs can also reduce cell viability by damaging DNA and impairing metabolic activities based on their composition or geometry. For example, CuO, which is a semiconductor and also a popular catalyst used in chemical reactions, has been reported to be cytotoxic [115]. Specifically, it was shown that when compared to Fe₂O₃, Fe₃O₄, and TiO₂, CuO NPs caused more damage to DNA and mitochondria leading to cell death [89]. In addition, SiO₂ NPs and CNTs were reported to be more cytotoxic to bronchiolar epithelial cells than TiO₂ [115]. NM geometry was also shown to influence cytotoxicity via ROS generation [121]. For example, cell viability was reported to increase with a decrease in the size of a silver NP (5, 25, 50, and 110 nm silver NPs in diameter were tested) [121]. In comparison on a larger scale, nanometer-sized particles of CuO were shown to be more toxic than the micrometer particles [115].

When a material does not affect cell mortality, it may still induce sub-lethal effects on the genome and epigenome. These changes can happen especially at low doses. The genotoxicity of NMs has been previously reviewed [122, 123]. Standard methods to test for such effects include the Ames test, comet test, micronuclei test, DNA laddering test, and chromosome aberration test [122, 123]. More recently, next-generation sequencing has also been used in chemical mutagenicity evaluation [124]. It should be noted that NMs can not only cause genomic alterations by directly entering the cell through passive diffusion and endocytosis but can also catalyze the intracellular production of ⁻OH radicals, which can lead to elevation of ROS and DNA damage [122]. For example, NMs that have been shown to raise ROS level include Ag [125–130], TiO₂ [131, 132], silica [133], quantum dots [134], and CNTs [122, 135, 136]. Besides material composition, studies have also shown that surface charges affect the genotoxicity of liposomes and that positively charged nanocarriers were less genotoxicity [81]. Figure 2(b) shows an example of chromosomal aberrations induced by Ag NPs.

3.2. Nanomaterial immunomodulation

NMs, by design or otherwise, can lead to direct and indirect immunomodulation, which includes immunosuppression and immunostimulation [137]. The role of NMs in immunomodulation is of particular interest in drug delivery applications, in which NPs serve as vehicles for vaccines or drugs. Here, however, we focus on the immune reaction caused by the NMs themselves, which involves the innate and adaptive immune responses [138]. Innate immunity refers to the interactions between NMs with non-specific immune cells such as macrophages and natural killer cells. Whereas adaptive immune response activates T cells and B cells that elicit antigen-specific effects. For example, zinc oxide and silver NMs can significantly increase IL-6 and IL-8 production in kidney cells, implying an increase in both the innate and adaptive responses [139].

Immunomodulation by NMs is a complex process influenced by many parameters, including chemical composition, molecular structure, surface chemistry, and size [20]. Previous reviews have addressed this process but are limited to a subset of parameters. For example, some focus on immune adjuvants and their therapeutic effects [137, 140]. Other reviews have more extensively elaborated on the effects of various NM categories and compositions on systemic immunomodulation [105, 141, 142]. In addition, the role of biophysicochemical features at the nano-bio interface such as protein adsorption and corona formation in immunomodulation has been addressed [20, 143, 144].

NP size, as mentioned above, has also been shown to be relevant in this process. For example, nano-size (193 nm) particles induced a stronger immune response than micro-size ones (1530 nm) [145]. Interestingly, studies have also shown that larger size may elicit stronger serum immunoglobulin response [137]. In addition, bulk material with spiky nanostructures was shown to modulate the immune response. Specifically, *in vitro* studies showed an increased expression level of CD 40 in DCs after 12 h incubation with spiky particles [146]. Usually, the expression of CD 40 indicates a relevant higher level of the immune response of APCs. The nanostructures in a bulk material such as nanospikes might exert mechanical stress on the cells, which results in potassium efflux and inflammasome activation in DCs [146]. Due to the diverse immunomodulatory responses elicited by different NMs, coupled with the somewhat inconsistent methods used to assess them, it is difficult to associate them with specific features. In addition, multiple parameters have been shown to have synergistic effects on this process, which complicates interpretation [144].

3.3. Nanomaterial-induced fibrosis

Fibrosis involves the excessive deposition and remodeling of dense ECM and it can occur in response to NMs [147–151]. As a process, it is not entirely understood because it can occur in the absence of an acute response and the lack of standardized predictive tests. *In vitro* tests for fibrotic responses include analyzing cells for the expression of fibrosis-associated proteins such as α -SMA, TGF- β , and ECM components such as fibronectin, laminin, COL I, and hydroxyproline (modified amino acid common in collagens). However, cells are most often cultured on a stiff substrate (2D culture) and this can lead to alteration of gene

expression due to the nucleus pore opening caused by mechanical forces, which might require *in vivo* studies to be performed [152, 153].

NPs, due to their small size, can gain easy access to the lung alveoli. Upon contacting lung cells, NPs can affect them in multiple ways leading to fibrosis. For example, NPs can induce the production of ROS and increase TGF- β production [154]. But such effects may not cause near-term discomfort in the subject, therefore making it difficult to associate NPs with these adverse events. Moreover, NMs engage in complex mechanisms to induce fibrosis. For example, CNTs have been reported to activate type 2 immune mechanisms and induce fibrosis and cancer through the IL-1—IL-17—TGF- β axis [86, 155–157]. In contrast, metal oxides, such as those of copper [158], titanium [159], cerium [160], have been shown to induce fibrosis via ROS elevation, inflammation, and increased TGF- β expression. Figure 2(c) shows an example of silica NPs causing lung fibrosis in mice.

3.4. Therapeutic use of nanomaterials

Because of the exceptional ability of NMs to transverse biological membranes, therapies involving drug delivery and photodynamic therapy have been developed [161]. More interestingly, the cytotoxic attributes of NPs have been co-opted to treat illnesses associated with excessive cell growth [162, 163]. For example, studies have used NPs to treat hypertrophic scarring [163]. Moreover, some NPs could be used to treat solid cancer too. For instance, it was found that a biosynthesized gold NP was cytotoxic to a breast cancer cell [164]. In addition, NMs such as Au, quantum dots, and fullerenes can quench ROS and have been used to alleviate genome damage [122]. However, precise targeting in these therapies has proven to be challenging, and topical and systemic responses of these therapies need to be evaluated.

4. Topical vs systemic interactions of nanomaterials

NMs present both opportunities and challenges because they can overcome size limitations and achieve unique distributions in physiological systems. In this section, we review applications pertaining to tissue regeneration and drug delivery for immunotherapies while considering their intended topical and systemic effects. *Systemic* refers to those processes affecting the whole body or at least multiple organ systems whereas *topical* describes a response that affects the immediate area of contact with the NM, whether it be a single organ or tissue type. The term *topical* in this section, is not used in its common definition of being physically superficial, but rather affecting a localized area of the body. Particularly, topical and systemic effects are observed as NMs have enhanced routes of delivery, including oral, implantable, and injectable administrations. In addition, targeting of specific organs or tissues is attempted through surface modifications, which aim to evade innate immune responses and oxidizing inflammatory mechanisms. For example, CNTs and graphene oxide, while designed for effective drug delivery, face enzymatic degradation, and other immune regulatory challenges [165]. Finally, NMs used for topical reparative and regenerative processes can be subjected to systemic immune responses. For example, biopolymeric fillers, carbon-based NMs, and porous metals cannot achieve full restoration of damaged musculoskeletal tissues due to the innate immune response they elicit [166]. It

is therefore important to consider the reciprocal impact between NMs and immunity and examine it in the proper context in terms of intended and unintended topical vs systemic effects. Figure 3 exemplifies possible unintended systemic and topical effects that can be anticipated from NMs depending on their route of administration.

4.1. Polymer

Polymeric NMs are widely used in drug delivery and drug targeting systems as their particle sizes are conducive to prolonged circulation in the blood, increasing payload half-life [70]. Atrisorb® (Tolmar Inc., Fort Collins, CO, USA), a PDLLA polymer, is a biodegradable material made from the ring-opening polymerization of lactide monomers. Membranes functionalized with antibiotics and growth factor-coated PDLLA nanofibers have been shown to facilitate healing while reducing the risk of bacterial infections [169]. While this nanopolymer offers both great surface area to volume ratio and many possible chemical modifications, its efficacy is hampered by host interactions. For example, PDLLA may be eliminated following application to a focal periodontal defect via phagocytosis. Moreover, if the PDLLA nanofibers gain access into the GI route, they can either be endocytosed by intestinal epithelial cells, remain in the submucosa, or enter the bloodstream and accumulate in the liver and spleen [170]. Accumulation of polymer NPs, due to slow biodegradation, could potentially result in hepatocellular injury through protein synthesis inhibition while accumulation in the spleen may affect immunopathology [71].

CNCs, typically on the order of a few hundred nanometers in length and a few nanometers in width, are rod-like acicular structures extracted from ligno-cellulose fiber by hydrolysis with a strong acid [171]. CNCs have been investigated for topical treatments. For example, a nanobiocomposite hydrogel consisting of a mixture of CNCs and silver NPs mixed in a Vaseline® base was used to treat wounds in a diabetic mouse model [172]. Semi-quantitative analysis of H&E images showed reduced inflammation as Ag is thought to decrease the time needed for granulation tissue formation, fibroblast invasion, and possess anti-inflammatory properties. These nanocomposites significantly enhanced the expression of vascular endothelial growth factor and fibroblast growth factor allowing for increased angiogenesis, permeability, and recruitment of inflammatory cells to the injured site, aiding the proliferation and migration of endothelial cells [172]. Although recent studies have not provided evidence of permeation of the dermal barrier by CNCs, it is possible that skin sensitization can occur at high concentrations. In this scenario, nanocellulose could penetrate the stratum corneum and form a stable association with proteins, triggering DCs to migrate to the lymph nodes and consequently activate T lymphocytes [173]. Therefore, it is possible for topically applied NMs to induce a systemic immune response.

Chitosan, another naturally derived polymeric NM, is cationic in nature and its electrostatic interactions with nucleic acids allow it to act as a suitable carrier for strong immune adjuvants for cancer vaccines [71]. In a study evaluating its potential to target bladder cancer, peptide-carrying glycol chitosan NPs demonstrated long circulation times and tumor accumulation for at least 48 h [71]. Despite such enhanced and targeted penetrations, the chitosan NPs also accumulated in the liver and kidneys. In this circumstance, an intended

systemic targeted delivery system resulted in non-targeted accumulation in other tissues, which is fairly common with NMs.

4.2. Carbon

Carbon-based NMs can achieve nanofibrous architectures and have tunable physical properties that make them suitable for the fabrication of scaffolds [174]. In nerve regeneration studies, for example, CNTs are often employed as scaffolds since they display morphological features analogous to neurites such as their anisotropic conductivity and elongated structure. Furthermore, bundles of CNTs mimic dimensions similar to that of dendrites, and the tunable physical and chemical properties of CNTs, such as length, diameter, chirality, surface functionalization, and band gaps are compatible with neural activity [175]. While CNTs have demonstrated biocompatibility, there are studies showing accumulation-related side effects. Figure 3 provides an example of this phenomenon where in the majority of non-functionalized CNTs, inhalation can lead to pulmonary inflammation where CNTs engulfed by macrophages can be encapsulated by granulomas. In both acute and chronic inflammatory settings, phagocytic and necrotic tissues contain cytotoxic factors such as ROS, digestive enzymes, and cytokines that can enhance progression to fibrosis and tumorigenesis of the local tissue [176]. Furthermore, distant from the local site of administration, CNTs can induce inflammatory responses to pleural and mesothelial tissues, as well as the liver upon unintended systemic exposures [156].

Carbon NMs are also used in designing innovative and biocompatible targeted drug delivery strategies. For example, fullerenes/buckyballs possess a strong apolar character and their specific geometry, size, and surface characteristics make them appropriate for crossing cell membranes [39]. While intravenous injection and oral delivery are the most common routes of administration, pulmonary, transdermal, transmucosal, and ocular introductions have also been demonstrated [177]. In a study by Misra *et al*, the FDA-approved anticancer drug DTX was tested for its effectiveness using C60 fullerenes conjugated with APA. C60-OH-APA-DTX fullerenols administered intravenously demonstrated hemocompatibility and pharmacokinetic neutrality, allowing them to bypass the mononuclear phagocytic system and achieve prolonged circulation time [178]. It should be noted that local and systemic biocompatibility of fullerenes may have a dosage threshold. For example, in a study by Prylutska *et al*, at C60 fullerene concentrations of 300 mg kg⁻¹ or higher, behavior disturbances, hematotoxicity, and spleen, kidney, and liver pathologies were observed [179].

4.3. Metal

4.3.1. Noble metals—Many studies have demonstrated that gold is an efficient nanocarrier for systemic delivery. AuroShell®, for example, is an FDA approved gold nanoshell for treating head and neck cancers using photothermal therapy [180]. After intravenous administration, these nanoshells accumulate in the tumor through EPR effects. A near-infrared laser is then applied to the tumor, allowing the particles to selectively absorb the laser energy and convert the light into heat. As a result, the tumor and its blood vessels are destroyed [180]. However, there are also unintended findings that suggest the toxicity of gold NPs. For example, after intravenous injection of polyelectrolyte multilayer coated

gold NPs, which are used for treating prion disease, the particles can accumulate in the hippocampus, thalamus, hypothalamus, and the cerebral cortex [181].

4.3.2. Reactive metals—Although copper (Cu) is essential for normal physiological functions including hemoglobin formation and catecholamine biosynthesis, concentrations in excess of the range of biological tolerance may cause unintended effects such as hemolysis, GI distress, liver, and kidney damage [182]. In a comparative study investigating the effects of single oral administrations of Cu NPs, Cu was reported to accumulate at high concentrations and low solubilities in the liver, kidney, heart, and spleen [183]. Histopathological analysis of these tissues showed unintended local effects such as dilated liver sinusoids, atrophy of glomeruli in the kidneys, and multinucleated cells in the spleen [183].

Aluminum oxide NPs (Al_2O_3 NPs), despite being commonly employed, have been shown to contribute to pulmonary fibrosis due to occupational exposure in the workplace [184]. Using a nose-only inhalation system in Sprague Dawley rats, researchers found that despite a lack of adverse systemic effects, aluminum accumulation in the kidneys and increases in LDH, IL-6, and TNF- α in the lung were due to Al_2O_3 NPs inhalation exposure and were dependent on particle size [185]. In studies looking at aluminum oxide NPs after oral administration, mice were observed to have significant Al_2O_3 NPs accumulation in the liver, lung, and kidney [186].

4.3.3. Magnetic metals—Feraheme®, an FDA-approved iron oxide NP for treating iron deficiency, offers another example of desirable systemic effects. In addition to treating anemia, Feraheme® has been shown to kill acute myeloid leukemia cells, as well as the stem cells that give rise to them, in patient samples and mouse models [187]. It does so by providing a source for induction of ROS, which in turn leads to an increase in oxidative stress, resulting in cell death. However, one major unintended effect is interference with MRI, which could last up to three months after injection [187].

As an example of a locally intended approach, cobalt ions have been frequently investigated in alveolar bone regenerative studies for clinical dentistry applications. Cobalt is of interest in regenerative approaches since it has anoxic-mimicking capabilities that promote tissue angiogenesis and osteogenesis [188]. On the other hand, cobalt chloride-based cobalt-substituted HA (COHAC) NPs, despite demonstrating anti-inflammatory and antibacterial effects and enhanced differentiation of bone cells due to their high degradation rate, caused a decrease cell viability in concentrations higher than 10 ppm [189].

4.4. Self-assembled materials (PEG, non-PEG, protein-based)

4.4.1. PEGylated liposomes—Liposomes possess several characteristics that hold a great promise in drug delivery with relatively low levels of negative effects, including solubility, large payloads, protected carrier, and reduced toxicity [190]. After intravenous infusion, PEGylated liposomes can allow a systemic response with prolonged circulation time for delivering drugs to target sites such as infected tissues and tumor areas [191].

Doxil®, for example, is an FDA approved chemotherapy drug used to treat ovarian cancer, AIDS-related Kaposi's sarcoma, and multiple myeloma through intravenous infusion. However, there are lipid-related side effects associated with Doxil® via interactions with the complement system. HSRs have been reported immediately after the first intravenous injection in up to 45% of patients [92, 192]. The reactions are mainly due to CARPA in which an adverse immune response is triggered through the activation of the complement system upon infusion. Particularly, EPR effects of Doxil® also contribute to CARPA [92, 193]. The hypothesized process is that anaphylatoxins C5a and C3a are released after complement activation and stimulation of mast cells and basophils, followed by secretion of inflammatory mediators that activate endothelial and smooth muscle cells, resulting in CARPA. Symptoms of CARPA observed in patients include flushing, shortness of breath, and chills, which usually decrease or disappear after subsequent infusion.

4.4.2. Non-PEGylated liposomes—Non-PEGylated liposomes are delivered in the same way as PEGylated ones. Upon intravenous administration, they circulate systemically in the bloodstream for delivering drugs and interact with circulating proteins or target sites, resulting in local responses [191]. AmBisome®, for example, is a liposome that encapsulates fungal pathogen amphotericin B used for treating a wide range of fungal infections, including cryptococcal meningitis in HIV patients, visceral leishmaniasis, and those caused by *Aspergillus* species. After administering intravenously, AmBisome® circulates in the blood and binds to fungal cell walls at local sites, followed by the disruption of liposome, and the release of amphotericin B [194]. Unintended effects of AmBisome® include HSRs and CARPA due to local cell–cell interactions from the activation of the complement system. Similarly, several other non-PEGylated liposomal drugs such as DaunoXome® and Visudyne® also induce CARPA [92].

4.4.3. Protein-based liposomes—Protein-based liposomes are administered intravenously as well, leading to systemic response and topical effects at target sites. The effects of protein-based liposomes depend largely on the protein themselves due to their specific binding. For example, Abraxane®, an FDA-approved NP formed by albumin with conjugated paclitaxel, dissolves into soluble complexes after intravenous injection [27]. The particles can then accumulate in tumors due to the EPR effect and can be transported across endothelial by binding to membrane protein gp60 [90]. Albumin serves as a delivery vehicle and allows Abraxane® to achieve a high response rate for treating breast and pancreatic cancer. As described in figure 3, Abraxane® also has several unintended side effects such as low blood cell counts, hair loss, peripheral neuropathy, and abnormal electrocardiogram.

Ontak®, consisting of recombinant diphtheria toxin protein conjugated to IL-2, selectively binds to IL-2 receptors upon intravenous injection and delivers diphtheria toxin to target cells [27]. This allows T-cell stimulation for the treatment of leukemia and lymphoma. However, it was discontinued in 2014 due to severe HSRs and loss of vision effects.

5. Nanomaterials and molecular interactions

NM cytotoxicity and biocompatibility are dictated by complex interactions that are not fully elucidated. Physicochemical properties, such as morphology, size, defects, and chemical

stability of NMs, trigger biological responses that cause varying levels of toxicity and must be studied intensely prior to the application in humans [195, 196]. Protein corona, the coating created when the NM acquires proteins on its surface upon entering biological environments, plays a critical role in triggering immune responses and giving NMs new functional identities [197]. Other than the characterization of the biological identity of NMs, the physiological response triggered within the body is also an important area of concern in NM design and production. For most applications, NMs must evade innate immune cell activity and the body's adaptive immune response.

5.1. Protein corona

NMs adsorb proteins that form a layer, also known as the 'protein corona,' on their surfaces that then mediate interactions with the cells and tissues [198]. Protein corona, with its efficiency and efficacy, has proven useful in the diagnosis and treatment of many diseases, interacting and interfering with distinct biological microenvironments [199]. In NPs, it also plays a role in the alteration of immune responses, governing cell uptake, accumulation, biodegradation, and clearance [200]. Existing in two forms on the surface of NPs, protein corona can be either 'soft' or 'hard,' consisting of loosely bound proteins with a short lifespan in the former or of tightly bound proteins with a long lifespan in the latter [199]. Its formation affects the biological identity of NMs and their ability to trigger immune responses and is modified by a variety of factors, such as physicochemical properties of the NM, denaturation of proteins bound to the NM, protein source, environmental factors, and route of administration. A better understanding of protein corona modifications and interactions will allow for improved biocompatibility and further the development of drug delivery and other applications. Figure 4 shows how different kinds of protein corona can be formed on various NMs.

5.2. Molecular interactions and nanomaterial biological identity

As NMs enter biological environments, biomolecules adhere to their surfaces. As mentioned above, the nature of the protein corona is affected by many factors, including the composition of the NM. Characterization of corona formation on polystyrene NPs showed that equilibrium in the protein corona was reached a few minutes after dilution in plasma and over time continued to change in terms of the amount of protein-bound but not composition [201]. Furthermore, it was also shown that the newly formed corona influenced how NMs interacted with platelets and blood cells to affect hemolysis, thrombocyte activation, NP uptake, and endothelial cell death at an early exposure time. Recently, the protein affinity of three core-crosslinked polymeric NPs with long circulation times (poly(N-(2-hydroxypropyl) methacrylamide, polysarcosine, and PEG) was investigated and it was found that neither of these three polymers formed a substantial protein corona that could affect their *in vivo* interactions [202]. Another experiment used disulfide-stabilized poly(methacrylic acid) nanoporous polymer NPs to show that their internalization in monocytes and macrophages was differentially affected by the presence of protein corona [203]. It was observed that while a protein corona decreased the internalization efficiency in human monocytic cells, it induced a class A scavenger receptor-mediated phagocytosis in macrophage-like-cells without affecting the overall internalization efficiency. In addition to polymeric NPs, metallic NPs have also been extensively investigated. Recently, it was

shown that DNA can be hybridized onto gold NPs with a bovine serum albumin-induced protein corona and that the conjugate had greater colloidal stability in different solutions of salt, acid, and alkali [204]. PEG-grafted gold NPs were also used to investigate the role of size and surface chemistry in mediating serum protein adsorption and their subsequent uptake by macrophages [205]. Specifically, this study found that variations in serum protein adsorption correlated with differences in the mechanism and efficiency of NP uptake by a macrophage cell line. The differential effect of a protein corona was also corroborated in another study where ultra-small gold NPs were used to investigate the effect of the human serum albumin protein corona and it was found that the conformation-transited protein corona-AuNP complex could induce cell apoptosis [206].

Corona formation also affects the dissolution rates of NPs. Cobalt NPs were found to not dissolve in the presence of amino acids but the dissolution rate was higher in the presence of bigger proteins that adsorbed onto the NPs [207]. Aside from metallic NPs, liposomal NPs have also been investigated. A library of ten liposomal formulations was created and these formulations exhibited different targetability and levels of uptake by PANC-1 (human pancreatic cancer cell line) and INS-1 (rat insulinoma cell line) cells when exposed to human plasma [208]. Similarly, dioleoyl-3-trimethylammonium propane based lipoplex formulations were used to show that different lipid compositions resulted in different degrees of total protein adsorption as well as different compositions of individual proteins from fetal calf serum [209]. The same study also showed that PEGylation increases protein adsorption but attempts to identify individual proteins that alter protein adsorption and cellular uptake of NPs was unsuccessful.

In the past 20 years, carbon-based NMs have revealed many chemical and physical properties that are both academically fascinating and commercially appealing. Recently, it has been shown that human serum albumin corona formation on CQDs reduced their cytotoxicity and changed the mode of energy metabolism in mouse cells. In addition, the impact of the size of carbon NMs on protein binding has been confirmed and is consistent with previous studies showing that binding was more efficient with larger CNTs and NPs [210].

One of the major issues with NM-mediated drug delivery is the inevitable denaturation of proteins bound to the NM during interaction with immune cells. As a potential way to evade this phenomenon, it has been shown that corona formation on silica NPs provided a stealth effect on macrophage recognition [211, 212]. Efforts to exploit the properties of the protein corona were made by pre-coating polystyrene NPs stabilized with the PEG-based surfactant lutensol AT50 with immunoglobulin-depleted plasma, which showed a marked reduction in cellular uptake by immune cells [213]. Another example of a stealth effect was shown in a study where the protein corona of liposomes played a major role in their sequestration from peripheral blood mononuclear cells [214]. Capture of NMs by immune cells continues to be one of the most significant barriers to *in vivo* anti-tumor drug delivery. To overcome that, designer-artificial coronas were fashioned that were able to reduce capture by distinct populations of circulating leukocytes [133]. Another example of a bio-inspired NP surface capable of evading components of the immune system was described recently where CD47 was added to NPs and prevented phagocytic clearance [215].

Engineering artificially designed protein coats and developing strategies to avoid capture by the immune system requires a thorough understanding of the protein interactions at play. Investigations of the biological identity of different liposomal formulations after incubation in human and mouse plasma revealed that in the latter, liposomes were more negatively charged, less enriched in opsonins, and appreciably more enriched in apolipoproteins [211]. In addition to protein sources, there are other environmental factors that affect the biological identity of NMs such as protein concentration, temperature, incubation time, shear stress, etc. Researchers were able to show that increasing serum protein concentrations affected corona formation and led to desorption of abundant serum proteins (such as albumin, immunoglobulins, transferrin, etc) and adsorption of lower abundance proteins with higher affinity (apolipoproteins, lipoproteins, and complement factors) [216]. Another study with silver NPs showed that by varying the pH and temperature, the different conformational spaces and charge localization of the plasma proteins could be assessed, and this was correlated with the binding affinities of the proteome [217].

Corona formation is also affected by the flow. Specifically, protein-NPs complexes formed from fetal bovine serum after flow had decreased cellular binding, as measured with flow cytometry [218]. Surface charge is another important factor for corona formation. It has been shown that compared to polyanionic NPs, distinctly lower amounts of proteins were adsorbed onto polyzwitterionic hybrid NPs and the corona composition showed elevated relative ratios of medium molecular weight proteins [219]. Finally, the route of administration: systemic or topical also plays an important role in determining the biological identity of a NM. Orally delivered NMs need to cross: (a) the harsh GI environment (i.e. low pH and digestive enzymes); (b) the mucus barrier, and then (c) the intestinal enterocyte lining before reaching the bloodstream. In protein-rich GI fluids, a protein corona is likely to form and affect the surface characteristics [220]. NMs may also be inhaled, and research has revealed that when they come into contact with porcine lung surfactant, the protein coronas that formed were different from the ones formed in plasma [221]. Specifically, there was a striking prevalence of molecules with high lipid and surface binding in the protein corona that forms with the lung surfactant, which is markedly different from coronas formed with plasma proteins.

With regards to protein corona formation, an additional consideration is that it leads to loss of targetability and undesired off-target effects. For example, it was previously shown that silicon NPs functionalized with bicyclononyne and exposed to serum proteins exhibited a significant loss in targeting efficiency as compared to NPs with pristine surfaces [222]. Such observations prompted the development of strategies to exploit protein corona as a targeting ligand by synthesizing NMs that are coated by plasma proteins that are naturally shuttled to cells. Two proteins that have been correlated with the cellular association are apolipoproteins and vitronectin. One research group has developed predictive-validation modeling that provides a means of assessing the relative significance of the identified corona proteins [223]. They found that just eight proteins including vitronectin and some apolipoproteins were the main promoters of liposome-HeLa cell association. More recently, the targeting ability of the protein corona was studied using four temozolomide-loaded liposomes and a few typical proteins (vitronectin, apoA1, apoA2, apoB, apoC2) were identified, which bind

to receptors overexpressed at the BBB (e.g. scavenger receptor class B, type I and LDLR) [224].

5.3. Molecular interactions and nanomaterial immunological identity

Physicochemical characterization of NM biological identity can also provide information about their equally important immunological identity. Understanding the interactions of NMs with the immune system, which is designed for the efficient detection and elimination of foreign substances, is critical to either 'avoid immune recognition or to specifically interact with the immune cells' [225]. Therefore, it is important to consider both the material-intrinsic properties of the NM and the context-dependent identity formed through pattern recognition of the immune system. Through the introduction of non-stealth NMs, the initial response is dominated by components of innate immunity [226]. This response triggers the release of pro-inflammatory cytokines, activating key pathways in the immune and other cells [142]. Because of such effects, cytotoxicity assays are routinely employed to assess the safety of NMs. However, NM features including high adsorption capacity, hydrophobicity, surface charge, optical, and magnetic properties, or catalytic activity have been shown to interfere with such assays [227]. In addition, protein corona has also been shown to affect the nutrition balance of cell culture media, which interfered further with the accuracy of cytotoxicity assays [228].

It is appreciated that different NM types trigger the release of unique cytokine profiles, causing distinct symptoms and responses. A variety of assays are employed to characterize these responses including bead-based immunoassays and other cytotoxicity assays to measure the levels of released cytokines such as IL-6, IL-1, IL-8, IFN- γ , TNF- α , and CCL2 [139]. During inflammation and observation of high cytotoxicity induced by zinc oxide and silver NMs, IL-6, and IL-8 production increased significantly [139]. Increased NF- κ B activation, a hallmark of inflammatory responses, was also observed when a conformational change in serum albumin bound to NPs switched cellular uptake from the albumin receptor to scavenger receptor-mediated internalization [229].

Many different types of toxicological assays exist, yet the focus has shifted to *in vitro* testing due to ethical issues and high cost of *in vivo* testing. Cell culture assays test for cytotoxicity (altered metabolism, decreased growth, lytic, or apoptotic cell death), proliferation, genotoxicity, and altered gene expression [230]. In addition to increased cytokine production, interactions between NM corona and macrophage receptors led to a generation of ROS and permeability of lysosomes [142]. The release of proinflammatory cytokines also can stimulate the activation of phagocytes. Compounding these effects, another study has shown that the NM-specific release of cytokines could also stimulate the activation of phagocytes [231]. Specifically, it was shown that cobalt and nickel NPs and HA crystals have inflammogenic effects, stimulating macrophage release of TNF- α , and activating phagocytes [231]. The presence of corona also influenced the internalization of poly(methacrylic acid) nanoporous polymer NPs by monocytes and [203]. Consistent with this observation, the uptake efficiency of NM-corona complexes by macrophages was higher than that of pristine material, resulting in a remarkable increase of TNF- α and nitric oxide production [232]. It was also discovered that protein-coated systems enhanced the

expression of the Fc γ RIIB inhibitory receptor, a key negative regulator of B cell activation, leading to the impairment of cellular uptake and activation of cell apoptosis via the AKT/Caspase 3 signaling pathway [233].

While the innate immune system relies on the complement system and phagocytic cells, the adaptive immune system functions through T and B lymphocytes [234]. With cells and tissues that have already been compromised with ongoing innate immune reactions, the adaptive immune response occurs and takes over, enhancing the existing inflammatory process [200]. The response begins with the macrophage cytokine release and then progresses to the involvement of T and B lymphocytes, further attacking the NMs. For example, in a study of multiwalled CNTs, the mRNA expression of proinflammatory macrophage-produced cytokines significantly increased upon injection, leading to the stimulation of T lymphocytes [142]. It is also appreciated that NMs are recognized by the adaptive immune system due to their shared biochemical properties with viruses [200]. In addition, if only a soft corona is formed on the NP, or if the NP is not sterile, the adaptive immune response treats the NP as a foreign 'danger' signal [200]. Secondary and tertiary conformational changes of the absorbed proteins can also lead to adaptive immune response, with the tertiary structure change from the NP-protein corona formation leading to protein aggregation and the induction of immunogenic potential [200].

6. Cellular interactions

Before delving into specific cellular interactions with NMs, we briefly discuss the general mechanism of NM uptake by cells. It should be noted that this is only a short overview and that more in-depth coverage of these topics can be found in recent reviews [235, 236]. Cell–NM interactions can be broadly divided into endocytic uptake and receptor-mediated recognition. Endocytic uptake can then be divided into clathrin-dependent endocytosis, caveolae-dependent endocytosis, macropinocytosis, and phagocytosis. For larger NMs (>200 nm), phagocytosis and micropinocytosis are the dominant uptake methods. Phagocytosis is carried out by professional phagocytes (neutrophils and macrophages) and is initiated by NMs being recognized by specific receptors [235]. These receptors then mediate actin cytoskeletal activity that allows the plasma membrane to enclose the NM in a cup-shaped protrusion and subsequently pinch off inwardly to become a phagosome. In contrast, macropinocytosis does not rely on receptor-mediated NM recognition, and it non-specifically engulfs NMs into the cell. For smaller NMs (50–80 nm), cells will rely on either clathrin- or caveolae-dependent endocytosis. Both clathrin and caveolin are proteins that facilitate plasma membrane invaginations of NMs into the cell [236]. These vesicles are then trafficked to lysosomes for subsequent degradation. However, some NMs may take advantage of the acidic environment of lysosomes to escape degradation and deliver drugs or proteins to the cell [237].

While cellular uptake of these NMs can lead to therapeutic effects via internalization, NMs may also interact with cells via specific membrane receptor-mediated pathways. NMs can interact with cells by activating certain signaling cascades via specific receptors and may not require uptake into the cell. Some NMs can inherently activate certain signaling pathways while other NMs can be coated with antibodies or other ligands for specific receptors to

stimulate pathways. In the context of the immune response, specific receptors include but are not limited to TLRs, Fc receptors, scavenger receptors, and integrins [238]. Table 2 outlines the most common cellular interactions of different NMs and the subsequent cell-specific responses they elicit.

6.1. Cellular interactions of polymer-based nanomaterials

Polymer-based NMs represent a popular method for immune modulation due to their loading capability, shape, and size tunability, and biodegradability, which allow for precise NM optimization specific to individual cell types. Polymer NMs are usually loaded with cytokines to stimulate the immune response or are coated with antibodies specific to immune response receptors [26]. Polymer NPs have been tested on a wide variety of immune cells with their cellular effects being well-documented. Common polymer NM bases are PLGA, PBAE, and polystyrene with special attention given to PLGA NPs. PBAE NPs have mainly been tested on immune cells in cancer mouse models. For example, PBAE NPs loaded with IL-12 were delivered into a melanoma mouse model, causing M2 macrophages to repolarize into M1 macrophages and thus limiting subsequent tumor progression [246]. More recently, PBAE NPs loaded with cyclic dinucleotides, potential inhibitors of tumor growth, were used to target monocytes and improve outcomes in the same mouse model [260]. Numerous studies have used PLGA based NPs conjugated to antibodies specific APCs for immune tolerance induction. For example, PLGA NPs coated with the DC specific receptor DEC205 was shown to increase the levels of IL-10 produced by DCs and T cells *in vitro* [261]. In more recent studies, PLGA NPs were combined with the delivery of nucleic acid-based approaches to induce changes in the immune system. Specifically, PLGA NPs carrying siRNA against PD-1 and PDL1 were used to target tumor lymphocytes and suppress growth in a colon cancer mouse model [262]. In addition, PLGA NPs were loaded with an eCRISPR-Cas9 system to target and genetically edit murine-derived macrophages [263]. Collectively, these studies highlight the potential of NPs to be used for *in vivo* gene modulation.

6.2. Cellular interactions of carbon nanomaterials

The carbon NM family consists of a number of formations such as carbon black NPs, fullerene, carbon nanohorns, nanographite, and CNTs. The cellular interactions with each of these materials have been reviewed recently [142]. Here, we emphasize the cellular interactions with CNT's due to their prevalence in the field. We also paid special attention to their interactions with immune cells and more specifically with macrophages. It has been shown that stimulation of macrophages via CNT uptake is modulated by molecular recognition receptors such as TLRs, scavenger receptors, complement receptors, integrins, and lectin-like receptors [264]. The method by which CNT's are sensed ultimately affects the downstream signals that are activated. It has been suggested that complement opsonization on CNT's leads to reduced expression of proinflammatory cytokines and upregulation of anti-inflammatory cytokines [248]. While PEG-coating is commonly used to bypass these interactions and reduce cytotoxicity, it has been shown that this may also reduce the level of uptake by macrophages. Furthermore, the shape of CNT's affects macrophage activity. Specifically, Palomaki *et al* showed that while most CNT's can induce IL-1 β expression, only long needle-like CNT's can induce IL-1 α expression in LPS-primed

macrophages [265]. Many recent studies have focused on CNT's as potential vaccines against viruses, though these studies have been performed in nonmammalian organisms and lower order mammals, such as in fish and in hamsters [266–268].

A common concern with CNT's is their stability and susceptibility to degradation by the immune system during therapeutic use. It is thought that macrophages can uptake CNT's and activate NADPH oxidase which stimulates ROS that contributes to their degradation. However, the rate at which this happens varies in different organ systems. *In vivo* studies on mice found multi-walled CNTs injected into the cortex of the brain can be degraded as early as 2 d post injection while those injected into lungs could last for as long as 6 months [269]. In addition, a recent study showed that the cytotoxicity effect of CNT's on lung cells increased susceptibility to H1N1 infection [19].

6.3. Cellular interactions of metal/metal oxide nanoparticle

The interactions between macrophages and metal oxide NPs as well as the downstream responses have been reviewed previously and therefore will not be addressed in detail here [249]. In brief, besides being directly endocytosed, binding of metal oxide NPs to integrins, carbohydrate receptors, TLRs, nucleotide oligomerization domain-like receptors, Fc receptors, or scavenger receptors can induce macrophage activation [249]. The downstream effects are complex, including pro-inflammatory/anti-inflammatory cytokine production, toxic compound production, or pre-apoptosis membrane component translocation. These downstream processes are dependent on the intrinsic properties of NPs and the activation states of macrophages [249]. Commercial silver NPs have been reported to induce inflammation biomarker expression in murine RAW 264.7 macrophages and anti-inflammation biomarker expression in human whole blood cell cultures [251]. Gold NP-loaded mesoporous silica has been demonstrated to induce M2 polarization and promoted the secretion of anti-inflammatory factors of macrophages [252]. Cobalt NPs, mimicking the debris coming from failed CoCr alloy hip prostheses, have been shown to alter the migration, microtubule acetylation, spreading, and podosome formation of U937 macrophages owing to ROS production and downregulation of RhoA [253]. Neutrophils have been reported to interact with gold and silver NPs with the induction of neutrophil extracellular traps. Detailed mechanisms and downstream effects can be found in a review by Yang *et al* [270].

A recent study described the development of an antibody targeted amphiphilic organic ligand-protected gold NP vehicle capable of delivering small molecular weight drugs to lymphocytes [254]. The untargeted NP vehicle's gold core was surrounded by a mixed monolayer of alkanethiols terminated by hydrophobic methyl and water-solubilizing sulfate groups. This NP vehicle was capable of entering cells through lipid membrane penetration [235]. When conjugated with anti-CD8 antibody and intravenously delivered in mice *in vivo*, compared to the untargeted group, the uptake of anti-CD8 NP vehicles by CD8⁺ T cells increased 35-fold [254]. In addition, radionuclide-embedded gold NPs were demonstrated to be able to induce DC maturation and antitumor immunity towards cervical cancer [255].

6.4. Cellular interactions of nanoliposome

Nanoliposomes are widely used as antigen delivery vehicles and immune adjuvants in anti-cancer drugs, anti-infection drugs, and vaccines. The liposomal membrane can be prepared with natural or synthetic phospholipids and cholesterol similar to the lipids that are naturally present in cell membranes [271]. Nanoliposomes can be taken up by cells through phagocytosis or fusion. Immunological effects and factors influencing the outcome of the process of nanoliposome administration as well as specific examples of nanoliposome delivery systems used in cancer immunotherapy were reviewed in detail by Zamani *et al* [271].

To summarize, when employed as adjuvants, the physicochemical properties of nanoliposomes, such as membrane fluidity, surface charge, and size all influence the degree of immune responses [271]. Nanoliposomes encapsulating antigens designed to be phagocytosed by APCs are preferred to possess higher membrane fluidity in order to elicit humoral immune responses whereas rigid membranes are preferred for nanoliposomes designed to present antigens on the membrane [256, 271]. In general, liposomes with antigens encapsulated by positively charged lipids exhibit higher APC uptake efficiency than that of those composed of negatively charged or neutral lipids [271, 272]. Larger nanoliposomes induce more efficient immune responses owing to their better retention at the site of injection and more effective phagocytic uptake in the lymph nodes [271]. A previous study reported Ag formulated in liposomes with vesicle size ≥ 225 nm preferentially induced Th1 immune response in BAL-B/c mice whereas vesicles of the same type with the size ≤ 155 nm preferentially induced Th2 immune response [273].

6.5. Nanotopology

Nanopatterning and other techniques allow the processing of bulk biomaterials at the nanoscale and the creation of nanofeatures. Several studies have reported the ability of nanopatterned implants to modulate the behavior of cells involved in FBR. For example, Padmanabhan *et al* reported cell-type-specific remodeling of the actin cytoskeleton based on nanorod size [23]. Specifically, fibroblasts exhibited increasingly smaller size and circularity in response to the increasing size of nanorods (50–200 nm) on Pt-BMG. In contrast, macrophages were only able to detect nanofeatures greater than 200 nm. Finally, endothelial cells were able to detect BMG nano-features greater than 55 nm and exhibited greater area, perimeter, and elongation. Importantly, intracellular levels of Rho-A, which regulates actin remodeling and type I collagen production, were reduced in fibroblasts cultured on the nanopatterned BMG. These results suggested that fibroblast behavior in FBR could be modulated by specific nanopatterns. In a separate study with the same material, it was reported that nanotopography could modulate macrophage polarization [57]. Specifically, IFN/lipopolysaccharide-induced polarization was reduced in macrophages cultured on Pt-BMG with 55 nm diameter nanorods. In addition, implantation of the nanopatterned Pt-BMG *in vivo* induced reduced foreign body giant cell formation and fibrous capsule thickness and increased blood vessel formation when compared to that of the flat BMG group. Therefore, processing of bulk materials at nanoscale has the potential to modulate complex biological processes.

7. Conclusions and future perspectives

The relationship between NM biological identity and physiological responses is central to both their safe application and successful targeting. For example, lipid-based NPs have enabled the successful development of COVID-19 mRNA vaccines [274]. However, in order to improve the efficiency and effectiveness of NM-based treatments, undesired off-target effects must be minimized, the shuttling of NMs to desired cells must be optimized, and further studies regarding protein corona composition and cellular association should be pursued. A recent review identified key remaining challenges including the difficulty in quantifying and characterizing the vast number of proteins on NPs, the lack of reliable theoretical models, the difficulty in correlating theoretical and experimental models, the variation in serum proteins from species to species, discrepancies between *in vitro* and *in vivo* studies, and the subsequent issues in translating novel NMs into clinically relevant therapies [275]. It is expected that once the molecular mechanisms of these interactions are clarified, more effective NMs can be produced to fully benefit the field of nanomedicine. Moreover, issues involving scale-up, correlation between preclinical data and clinical outcomes, and difficulties of incorporating NMs in a final compatible form have been raised [276]. Despite these many challenges, the field is poised to continue improving existing NMs and discovering new ones that can interface with biological systems with greater efficacy. For example, the emergence of green NMs represents a new avenue to explore alternatives to chemical and physical methods for nanosynthesis [277]. In terms of new applications, exciting opportunities include the delivery of machinery for genome editing (CRISPR/Cas9), engineering of chimeric antigen receptor T cells for immunotherapy, and biofunctionalization of bulk materials via nanoprocessing. However, potential health hazards still exist both for those exposed during manufacturing as well as patients receiving treatments. Therefore, attempts to develop NM-based therapeutic and diagnostic strategies should also include more extensive and targeted studies to evaluate their potential cytotoxicity and overall biocompatibility. It is our belief that better understanding of these processes will contribute to enhanced designs with greater therapeutic potential.

Acknowledgments

This work was supported by NIH grant DK115969 (T R K) and T T was supported by NIH T32 GM007324 (T T). Figures 1, 3, and 4, and the icons in table 1 are created with the help of BioRender software.

Abbreviations

APA	aspartic acid
APC(s)	antigen-presenting cell(s)
BBB	blood brain barrier
BMG	bulk metallic glass
CARPA	complement activation-related pseudoallergy
CCL2	chemokine ligand 2, also known as monocyte chemoattractant protein 1

CD	cluster of differentiation
CNC	cellulose nanocrystal
CNF	cellulose nanofiber
CNT	carbon nanotube
COL I	collagen type I
CQD(s)	carbon quantum dot(s)
CRISPR-Cas9	clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9
CVD	chemical vapor deposition
DC(s)	dendritic cell(s)
DTX	docetaxel
EBL	electron-beam lithography
ECM	extracellular matrix
EPR	enhanced permeability and retention
FBR	foreign body response
FDA	Food and Drug Administration
GI	gastrointestinal
H&E	haematoxylin and eosin
HA	hydroxyapatite
HiPco	high pressure conversion (reduction) of carbon monoxide
HIV	human immunodeficiency virus
HSR	hypersensitivity reaction
IFN	interferon
IL	interleukin
ISO	International Organization for Standardization
LDH	lactate dehydrogenase
MRI	magnetic resonance imaging
MTT	3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide

NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NM	nanomaterials
NP(s)	nanoparticle(s)
PBAE	poly(B-amino ester)
PDA	pancreatic ductal adenocarcinoma
PDLLA	poly-DL-lactic acid
PEG	polyethylene glycol
PLA	poly(lactide)
PLGA	poly(lactide-co-glycolide)
Pt-BMG	platinum-based BMG
RIE	reactive ion etching
ROS	reactive oxygen species
SMA	smooth muscle actin
SWCNT	single-walled CNTs
TGF-β	transforming growth factor beta
TLR	toll-like receptor
TNF	tumor necrosis factor

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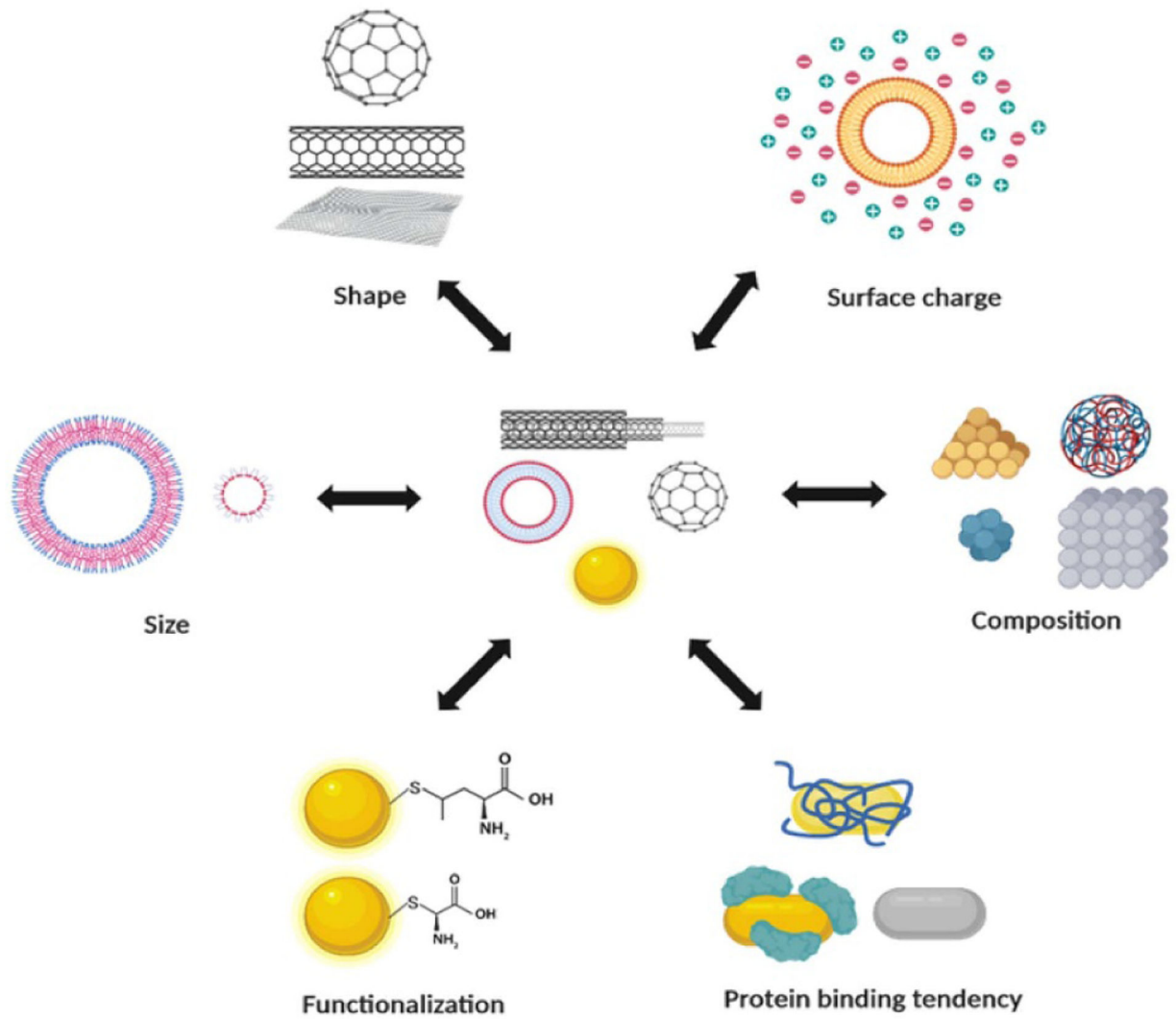


Figure 1. Examples of NM features that are reported to affect biocompatibility and cytotoxicity.

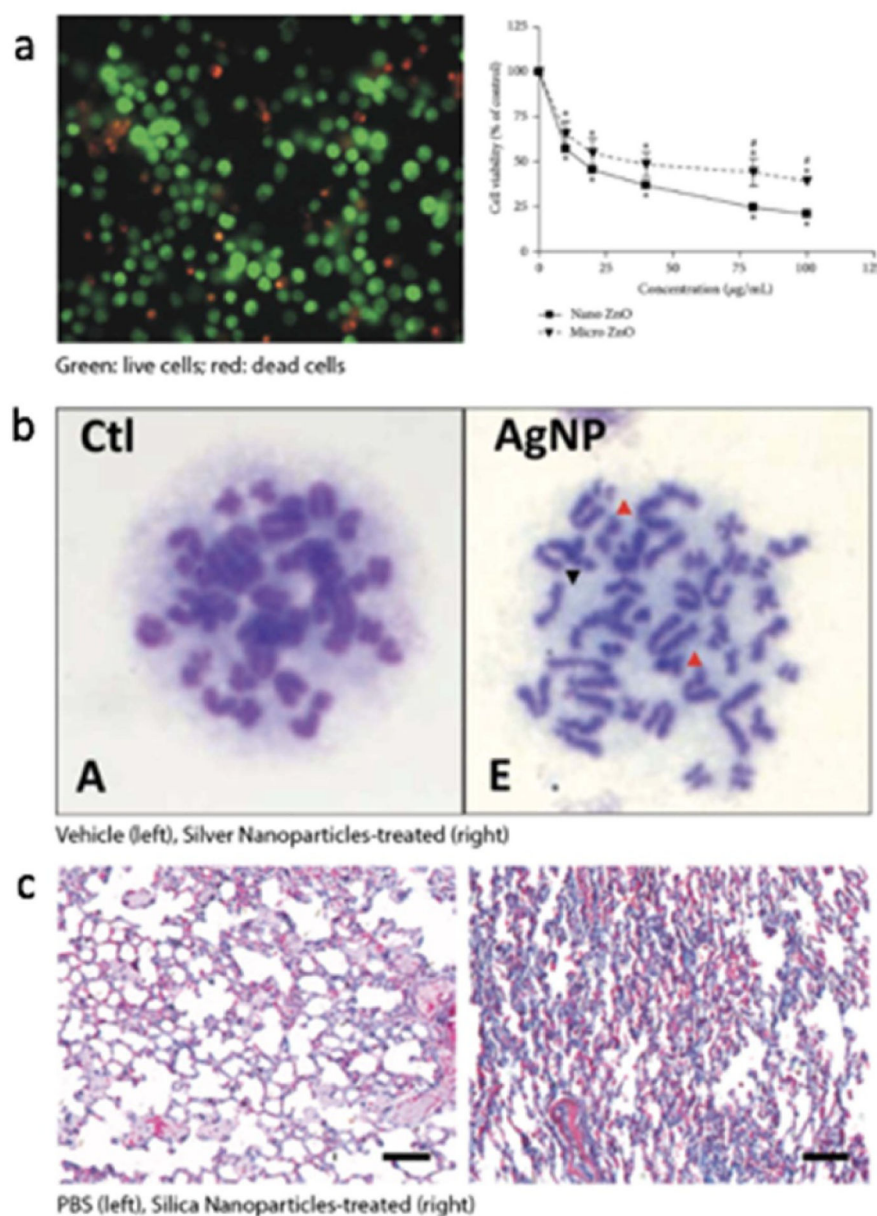


Figure 2.

Examples of NP effects such as cytotoxicity, genotoxicity, and fibrosis. (a) Nano ZnO is reported to cause more cell death of THP-1 cells (human monocytes) than micro ZnO. The picture on the left shows THP-1 treated with nano ZnO. Reprinted with permission from Sahu *et al.* *Journal of Nanoscience*, 2016, Article ID 4023852 (Hindawi Publications) [116]. (b) AgNP causes chromosome aberration in rat bone marrow cells (red arrow shows breakage, black arrow shows gap). Reprinted with permission from Wen *et al.* *PLOS ONE*, 2017, v.12(9) ([plos.org](https://doi.org/10.1371/journal.pone.0173852)) [117]. (c) Mice administered with 10 nm SiNPs show densified ECM in the lung tissue. Representative images of treatment with PBS (left, control) or 10 nm SiNPs (right) are shown. Reprinted with permission from Wang *et al.* *ACS Nano*, 2017, 11:1659–1672 (ACS Publications) [118].

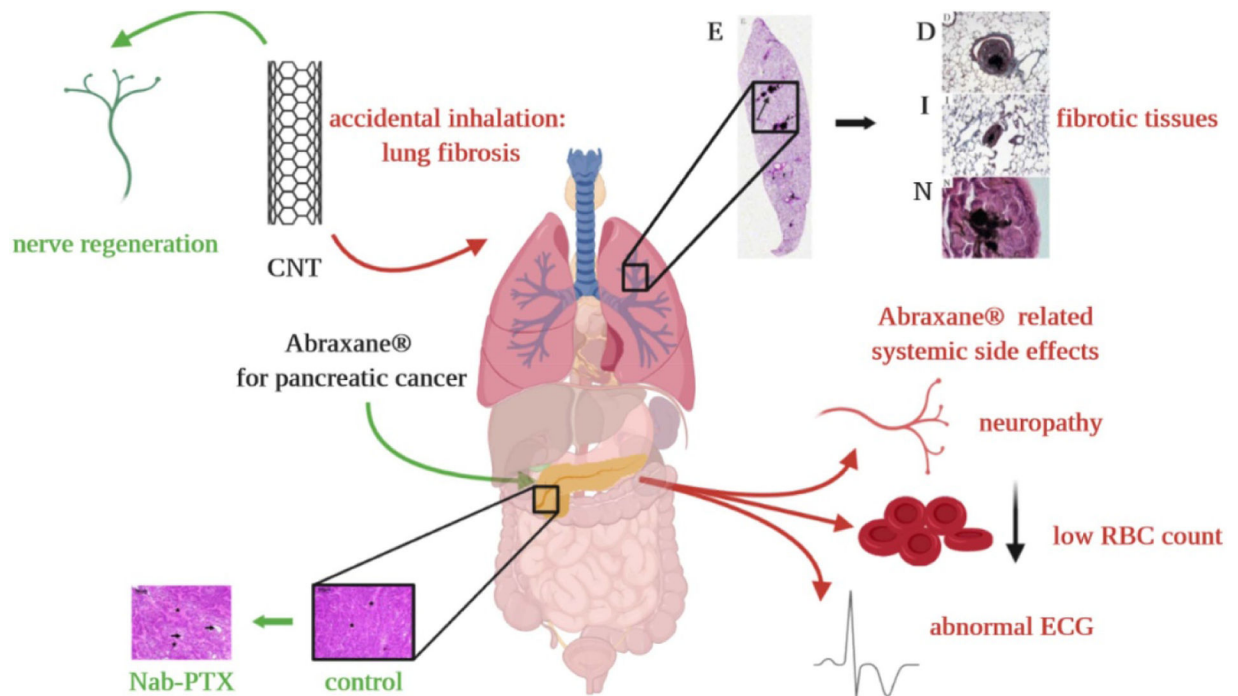


Figure 3.

NMs used to target specific organ systems or specialized tissue often carry unintended effects at levels of production, administration, and delivery. For instance, the synthesis of non-functionalized CNTs, while intended to locally promote neural attachment, foster neuronal growth, and cell membrane incorporation, pose an inhalation risk which could lead to cytotoxic pulmonary inflammation, tumorigenesis, and necrosis. After 2 mg CNT intratracheal administration (E; macroscopic view), hematoxylin/eosin-stained lung tissue displays CNT dispersion. CNT-induced pulmonary lesions are characterized by collagen rich granulomas and are seen in 10× Masson trichrome-stained bronchi (D), alveolar space (I), and in 100× hematoxylin and eosin-stained lung tissue (N). Reprinted with permission from Muller *et al.* *Toxicology Applied Pharmacology*, 2005, 3: 221–231 (Elsevier Ltd) [167]. FDA-approved Abraxane® protein-based liposome, intended as an IV administered vehicle for treating pancreatic cancer, is seen to induce peripheral neuropathy, low white and red blood count, and abnormal electrocardiogram. The bottom left set of images show hematoxylin and eosin staining of pancreatic adenocarcinoma in a murine model. The panel on the right represents untreated control tissue which shows a dense tumor stromal network. The panel on the left shows pancreatic adenocarcinoma tissue treated with NP albumin-bound paclitaxel (Nab-PTX) demonstrating a less organized architecture and a reduction in the cancer tissue components. Reprinted with permission from Rossignoli *et al.* *Theranostics*, 2019, 9(2): 436–448 [168].

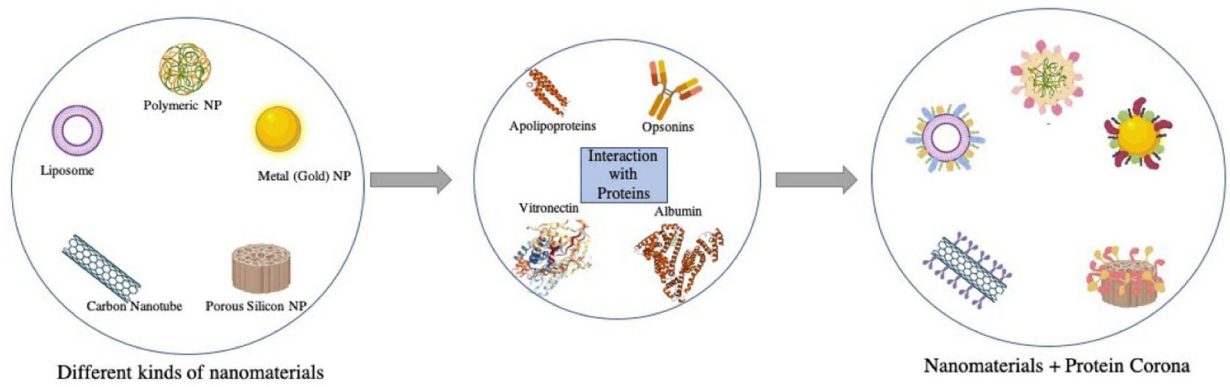


Figure 4.

Upon entering the body, NMs can interact with various proteins to create different kinds of protein coronas, which determine the biological and immunological identity of these NMs.

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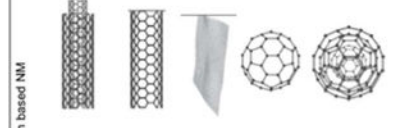



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Table 1.

NM classes, their applications, and current research or development stage.

Material category	Nanomaterials and their synthesis routes	Major Applications	Development phase (approved, clinical trial, experimental)
 <p>Polymer</p>	<ul style="list-style-type: none"> - Nanoparticles/Micelles: Synthesized via polymerization from monomer then emulsion, or interfacial polymerization to create nanoscale Or - Dispersion from preformed polymer then solvent evaporation or nanoprecipitation to create nanoscale [24,25] - Polymer-drug/protein conjugates: PEGylation [24] 	<ul style="list-style-type: none"> - Controlled drug delivery - Nucleic acid delivery - Modifications with surface receptors for specific targeting - Can be stimuli responsive to pH, temperature [24] 	<ul style="list-style-type: none"> - Targeted immunomodulatory therapies are experimental [26] - Multiple drug encapsulated polymeric micelles and nanoparticles in clinical trials [24] - PEG/polymer conjugated drugs on the market include: Cimzia, Adagen [27]
 <p>Metal/Metal Oxides</p>	<ul style="list-style-type: none"> - Noble metals: Silver and gold - Reactive metals: Copper and Aluminum - Magnetic metals: Iron, Nickel, Cobalt, Manganese: electrochemical, sonochemical, thermal decomposition, laser ablation, chemical reduction [28] 	<ul style="list-style-type: none"> - Contrast agents for imaging - Cancer detection - Antimicrobial therapy - Targeted drug delivery - Vaccine adjuvants [28] 	<ul style="list-style-type: none"> - Nanosilver for wound dressing - Gold nanomaterials in some clinical trials, not approved [29] - Aluminum hydroxide and aluminum phosphate approved as vaccine adjuvants [30] - Iron oxide for imaging in clinical settings [28] - Iron oxide is approved for iron deficiency [27] - Nanosilver (iron oxide) for glioblastoma in clinical trials [29]
 <p>Carbon based NM</p>	<ul style="list-style-type: none"> - CNTs: graphite vaporization through arc discharge or laser ablation, chemical vapor deposition from hydrocarbons, high pressure conversion of carbon monoxide (HiPco), etc. [31] - Graphene flakes: mechanical/chemical exfoliation, hydrothermal self assembly, CVD, nanotube slicing, reducing GO etc. [37] - Fullerenes: graphite vaporization through arc discharge or laser ablation, CVD from hydrocarbons, pyrolysis of dicyclics, CO₂ reduction, etc. [39] - Others - Carbon nano onions (CNOs): catalytic decomposition of methane [42] - Nanodiamonds: High pressure, high temperature method, detonation synthesis, ultrasonic cavitation, CVD, etc. [43,44] 	<ul style="list-style-type: none"> - Therapeutic delivery - Photothermal agents - Diagnostic and imaging tools - Tissue engineering scaffolds - Neuron - Prosthesis applications [32,33] - Biomimicry - Tissue engineering, suggested for cardiac repair [32,36] - Bioimaging, drug delivery, photothermal therapy [38] - Radical scavenging, anti-cancer photodynamic therapy, drug delivery, water soluble CO may help reduce inflammation in atopic dermatitis [40,41] - Functionalized CNOs seem very biocompatible, deemed as promising theranostic agents [45,46] - Drug delivery, imaging, anti-bacterial agent, bioresorbable scaffolds [47,48] 	<p>None approved yet, most are experimental, clinical trials for CNTs were only aimed at scanners for tumor imaging [34]</p>
 <p>Self assembled materials</p>	<ul style="list-style-type: none"> - PEGylated liposomes: sonication of phospholipids in water, extrusion, Mozafari method, microfluiding, etc. [49, 50] - PEGylation: post insertion or pre insertion [51] - Non-PEGylated liposomes: same as PEGylated liposomes without any PEG insertion - Protein based Nanoparticles: emulsification, desolvation, complex coacervation, electrospray, etc. [55,56] 	<ul style="list-style-type: none"> - Usually considered very 'safe' for different types of therapeutic load delivery (hydrophilic/hydrophobic), several modifications possible (anionic, cationic, zwitterionic), some formulations cause immediate hypersensitivity [49,50,52] - Non-PEGylated liposomes are much less popular, some combinations are good alternatives compared to PEGylated counterparts [54] - Drug/gene delivery, increasing antibiotic efficacy [55-57] 	<p>Some approved (10 till 2017), 33 investigational drugs [53]</p> <p>NA</p> <p>Some approved [53]</p>
 <p>Nano-Macro</p>	<ul style="list-style-type: none"> - Substrates with nanorods: nanomolding [58, 59] - Nanoporous surfaces: anodization of alumina [62,63] - Non-specific nanostructured surfaces: laser based nanosculping [65] 	<ul style="list-style-type: none"> - BMCs: rod dimension dependent macrophage polarization, stem cell regulation [58,60] - Alumina for osteogenesis; pore size dependent macrophage modulation [64] - Nanostructured Ti: topography dependent macrophage phenotype regulation [66] - Textured silicone breast implants: nanostructured (sub-micron) surface leads to fewer complications than microtextured surface [67] 	<p>Mostly breast implants have been marketed (some models asked to recall owing to increased risk of breast implant-associated anaplastic large cell lymphoma) [61]</p>
 <p>Ceramics</p>	<ul style="list-style-type: none"> - Oxides-silica, titanium, zirconium: Microemulsion: Precipitation, hydrothermal synthesis [68] - Specific nanocrystals 	<ul style="list-style-type: none"> - Drug and gene delivery - Bio-sensing - Diagnostic agent - Antidote agents [68] - Hydroxyapatite for bone growth stimulators 	<p>Cornell dots (silica) in clinical trials for fluorescence imaging [29]</p> <p>Some (4) approved as of 2017</p>

PEG: polyethylene glycol; NM: nanomaterials; CNT: carbon nanotube; CVD: chemical vapor deposition; BMG: bulk metallic glass.

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Table 2.

Polymer based: fluorescent microscopy of PLGA NPs (green) in macrophages. Reprinted with permission from Kalluru *et al.* Journal of Cell Science, 2013, Vol. 126, Pg. 3046 [239]. Carbon based: SEM image of multi-walled CNT and alveolar macrophage. Reprinted with permission from Kasai *et al.* Particle and Fibre Toxicology, 2016,13:53 (Springer Nature) [240]. Metal/metal oxide based: TEM image of magnetic NPs in alveolar macrophages. Boris A. Katsnelson *et al.* ISRN Nanotechnology,2012, Vol.2012, Article ID 143 613 (Hindawi) [241]. Nanoliposomes: U-118 MG cells treated with nanoliposome encapsulated doxorubicin. DAPI stain shown in blue and nanoliposomes shown in red. Reprinted with permission from Lin. *et al.* PLoS

ONE, 2014, Vol.9, Issue 7 (PLOS) [242]. Nanopatterned BMG: cell on micro/nano patterned Pt-BMG. Reprinted with permission from Wang *et al.*, ACS Applied Bio Materials, 2018, Vol. 1, Issue 1, Pg. 51–58 (American Chemical Society) [243].

Material Type	Material Subtype	Modification/ Specification/ Loading	Cell Type	Interacting Method	Cellular Response	References
Polymer Based	PLGA	Coated with DEC205 mAb	Dendritic Cells	Receptor mediated endocytosis	Increased IL-10 production (tolerance induction)	[234] and [235]
		Coated with CLEC 9A mAb	CD4+ T Cells	Receptor mediated endocytosis	MHC II antigen presentation and Treg expansion	[236]
Carbon Based	PBAE	Loaded with IL-12	Macrophages	Endocytosis followed by endolysosomal escape	M2 to M1 polarization	[237]
		Anti-K mAb	B Cells	BCR binding and signaling	Rare antigen CD4+ T cell expansion	[238]
		1-4mm diameter and 0.5-2um length	Macrophages	TLR activation/ NF-kB signaling	Chemokine, CCL3 and CCL5 upregulation	[88]
		1-4mm diameter and 0.5-2um length	B Cells and T Cells	Endocytosis	IL-6 and TNF-a secretion; CNT aggregate formation	[229]
Metal/Metal Oxide Based	Multi Walled Nanotubes	CMC functionalization	Monocytes	Endocytosis	Down regulated anti-inflammatory response and upregulated proinflammatory response	[239]
		NA	Macrophages	Endocytosis or interact/express integrins, carbohydrate receptors, toll-like receptors, nucleotide oligomerization domain-like receptors, Fc receptors, and scavenger receptors	Proanti-inflammatory cytokine production; toxic compound production; pre-apoptosis membrane component translocation	[240] and [241]
		NA	Macrophages	NA	Inflammation biomarker expression	[242]
		Loaded with mesoporous silica nanoparticles	Macrophages	NA	M2 polarization and secretion of anti-inflammatory factors	[243]
		NA	Macrophages	NA	ROS production and down regulation of RhoA; alterations of migration, microtubule acetylation, spreading and podosome formation	[244]
		NA	Neutrophils	NA	Induction of neutrophil extracellular traps	[201]
		TGF-b loaded and synthesized amphiphilic organic ligand protected	T Cells	Endocytosis	Enhanced T cell cytokine polyfunctionality	[245]
		Radiolucide-embedded	Dendritic Cells	NA	Induced dendritic cell maturation and antitumor immunity towards cervical cancer	[246]
		NA	Macrophages	NA	Macrophage activation; enhancement of the phagocytic and antigen processing abilities	[247]
		NA	T Cells	NA	Enhanced IFN-γ secretion and T cell mediated immune responses	[247]
Nanopiposomes	Oleoyl liposomes	NA	Dendritic Cells	Interact with CD14	CTL response; increased expression of CCL2 and IL-12	[248]
		NA	CD8+ T Cells	NA	Activation of naive CD8+ T cells	[248]
Nanopatterned BMG	DOPE	NA	Antigen Presenting Cells	Endocytosis	Antigen delivery to MHC molecules	[249] and [250]
		NA	Fibroblast	NA	Alteration of morphology and focal adhesion density; reduced Rho-A and collagen-I production	[251]
Pt-BMG	Pt-BMG	NA	Macrophages	NA	Morphological changes; reduced M1 expression of pro-inflammatory cytokines and chemokines; enhanced phagocytic ability; reduced fibrous capsule thickness; reduced FRB formation; increased blood vessel formation	[58]
		NA	Endothelial Cells (HUVEC)	NA	Morphological changes	[251]