Biocompatibility of Engineered Nanoparticles for Drug Delivery.

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Review

Biocompatibility of engineered nanoparticles for drug delivery

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ABSTRACT

The rapid advancement of nanotechnology has raised the possibility of using engineered nanoparticles that interact within biological environments for treatment of diseases. Nanoparticles interacting with cells and the extracellular environment can trigger a sequence of biological effects. These effects largely depend on the dynamic physicochemical characteristics of nanoparticles, which determine the biocompatibility and efficacy of the intended outcomes. Understanding the mechanisms behind these different outcomes will allow prediction of the relationship between nanostructures and their interactions with the biological milieu. At present, almost no standard biocompatibility evaluation criteria have been established, in particular for nanoparticles used in drug delivery systems. Therefore, an appropriate safety guideline of nanoparticles on human health with assessable endpoints is needed. In this review, we discuss the data existing in the literature regarding biocompatibility of nanoparticles for drug delivery applications. We also review the various types of nanoparticles used in drug delivery systems while addressing new challenges and research directions. Presenting the aforementioned information will aid in getting one step closer to formulating compatibility criteria for biological systems under exposure to different nanoparticles.

Contents

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1. Introduction

Nanoparticles have the potential to revolutionize a wide range of medical diagnostic and therapeutic interventions such as diagnostic imaging [1–3], photothermal therapy [4], nucleic acid delivery [5–7], implantable devices, and of particular interest in this article, drug delivery [8]. In the last several years, drug delivery research has witnessed remarkable growth due to the utilization of nanoparticles as "controlled release reservoirs" for the targeted delivery of drugs for combating many diseases [9]. To ensure an effective and safe use of nanomaterials for medical applications, the interaction between a material and the biological system of interest must be studied and characterized. Furthermore, studies of a material's biocompatibility must be conducted with particular focus on the environment in which the biomaterial will be placed in [10]. In drug delivery, it is crucial to evaluate a nanoparticle's biocompatibility to ensure safe drug release and minimize cytotoxicity. Indeed, a thorough evaluation of the factors that affect the biocompatibility of nanoparticles is central and possibly a first key step for the safe delivery of drugs. It is not surprising then that biocompatibility evaluation of engineered nanoparticles for drug delivery applications has been expanded from being primarily investigated in a laboratory setting to being applied in the multi-billion dollar pharmaceutical industry [11].

It is now well known that the inherent physical and chemical properties of nanoparticles (size, shape, surface characteristics) as well as the environment it comes into contact with, can dictate a nanoparticle's degree of biocompatibility [12–14]. For instance, the route of a material's delivery into the body such as intravenous or oral intake will induce differential immune reactions [12]. The immune reaction cascade is initiated with the adsorption of opsonins to the surface of nanoparticles. Opsonins are proteins – such as immunoglobulins or complement proteins – that bind to microbes and foreign substances and in doing so, aid their clearance via phagocytosis. Opsonin adsorption, enhanced by the hydrophobicity of a particle's surface, can present nanoparticles as foreign substances and increase their uptake by the phagocytic cells of the reticulo-endothelial system (RES) [15,16] which is obsolete terminology for mononuclear phagocytic cell (MPS). It is worth mentioning that RES was first proposed by Aschoff in 1924) [17]. In his terminology, macrophages (histiocytes) as well as reticulum cells and reticuloendothelia (phagocyticendothelia) are main member of RES system [17]. In opposition to this theory, van Furth and colleagues offered the concept of the mononuclear phagocyte system (MPS) and proposed that all macrophages – those come into sight of inflammatory foci as well as those exist in tissues upon normal stable conditions – are derived from monocytes as a result of pro monocytes differentiation [18,19]. However, Kiyoshi Takahashi reviewed the concepts of RES and MPS and their related experimental data in detail [20].

The important point is that this uptake in turn determines the route of particle internalization [21–23] and consequently dictates the fate of nanoparticles in the body [24]. This process is one of the biological barriers to nanoparticle-based controlled drug delivery [25]. All of these together, highlight the importance of surface effects for nanoparticles to be used as a carrier for drug delivery.

A number of studies have reported that the response of biological systems to nanoparticles is specific to its surface properties rather than its mass [26–30]. For example, Nel et al. provided the theoretical and methodological framework that describes the biophysico-chemical interactions at the interface of nanoparticle surface and the biological environment, including contact with cells [31]. As reported in most studies, nanoparticles with no surface modification are mostly taken up by phagocytic cells, which may cause undesirable interaction between nanoparticles and the immune system, and lead to a decrease in the drug's bioavailability and increase toxicity in the host. Consequently, the question of whether nanomedicine tools could mark an end to the necessity for "smart" drug delivery system remains upon understanding of the concept of biocompatibility and represents a major area of interest in the field of drug delivery.

In this article, an overview of the mechanisms that describe the fate of nanoparticles upon administration into the body is first reviewed. In particular, some of the most recent works on a nanoparticle's impact on biocompatibility in the scientific literature are surveyed. Second, the different types of nanoparticles commonly used as carriers in drug delivery are addressed. This allows for the advancement of nanoparticles for targeted drug delivery as well as prediction of the possible toxicological reaction to such nanomaterial (biocompatibility).

2. What is biocompatibility?

Biocompatibility first drew the attention of researchers between 1940 and 1980 in the context of medical implants and their interaction, both harmful and beneficial, with the body. Only recently, within the past two decades, has this term been formally defined under its conceptual denotation rather than practical description [10]: "The ability of a material to perform with an appropriate host response in a specific situation" [32]. The three dogma which play important roles in this definition are that a material has to perform its intended functions and not merely be present in the tissue, that the induced reaction has to be proper for the intended application, and that the nature of the reaction to a particular material and its suitability may be different from one context to another [33]. In 2010, Kohane and Langer explained biocompatibility in the context of drug delivery and defined biocompatibility as "an expression of the benignity of the relation between a material and its biological environment" [11]. However, some researchers have expanded that definition by denoting acceptable functionality of a biomaterial in a given biological context as important [11]. As such, Williams has reviewed the biocompatibility concept for long-term implantable medical devices and tissue engineering product in details [10].

In general, high degree of biocompatibility is achieved when a material interacts with the body without inducing unacceptable toxic, immunogenic, thrombogenic, and carcinogenic responses (Fig. 1). There are a number of relevant factors that should be considered for evaluation of biocompatibility. First, biocompatibility is highly anatomically reliant which leads to the fact that the reactions to particular materials are different from one location to another. For instance, biodegradable polymeric-based nano- and microspheres – such as those based on poly(lactic-*co*-glycolic acid) (PLGA) – in general make a well-characterized, subjectively mild tissue reaction, whereas the same particles introduced in the loose connective tissue surrounding nerves cause fairly strong acute inflammations [11,34–36]. Therefore, another fact that one must be cognizant of is that, if a biomaterial for a particular application can cause an adverse effect in a specific tissue type, it will not necessarily provoke the same response if used for a different application or in a different tissue type. Second, and in an interrelated perspective, the biomaterials' intrinsic characteristics exclusively will not determine whether that particular material is biocompatible or not. For instance, PLGA nanoparticles that have a rapid clearance from the body, do not usually cause peritoneal adhesion, whereas PLGA microparticles which stay longer in peritoneal cavity, do cause peritoneal adhesions [11,37]. Therefore, the

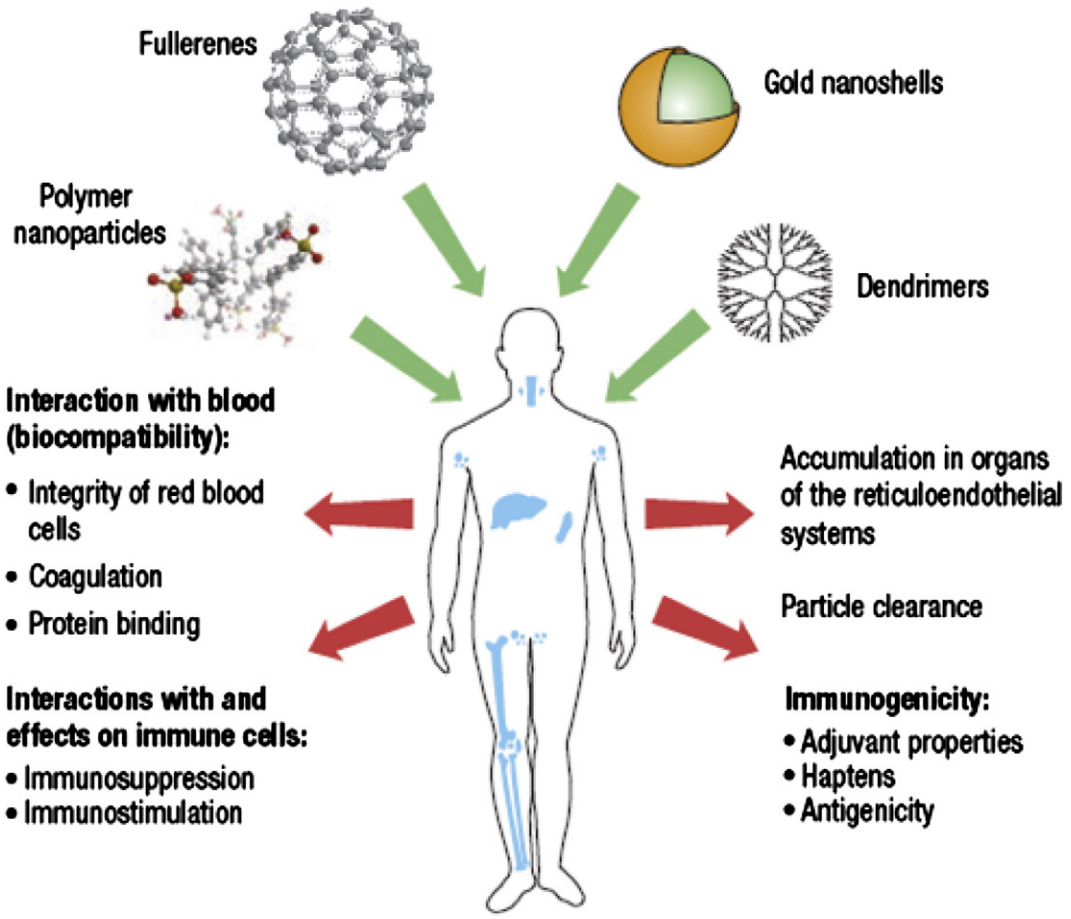

Fig. 1. When nanoparticles interact with the body, a variety of responses may occur. These include alterations to the immune system or interaction with blood, among others. These reactions vary significantly with nanoparticle composition. For example, gold nanostructures may interact differently with the body when compared to polymeric particles. For this reason, nanoparticles have to be evaluated individually or "on a case-by-case basis" [38] to better understand their effect on the body. Adapted by permission from Macmillan Publishers Ltd: Nature Nanotechnology [12], copyright (2007).

exposure half-life is another important factor that deserves consideration. Third, biocompatibility is a relative matter that depends on the risk-benefit ratio, and relies on a subjective declaration since, in general, inflammation would totally vanish over time, and the neighboring tissues do not exemplify a good proof of damage.

Last, but perhaps most important, the lack of adequate data regarding biological processes in response to foreign materials, as well as the insensitive nature of the methods available for biocompatibility [35,36,39–49] has limited the understanding of the biocompatibility of materials. All this together highlights the necessity of biocompatibility evaluation of biomaterials in a case-by-case and tissue- and application-specific manner. Bringing all these together, we can conclude that biocompatibility of materials depends on their structure, formulation and many other factors as described above and can refer to a local or total effect on the organism. Accordingly, using biocompatible materials in an absolute sense would be misleading [35,36,39–49]. Here, we will present some of the known data regarding biocompatibility and nanoparticles in the context of drug delivery.

2.1. Immunocompatibility

Immunocompatibility or the study of the immune response to a biomaterial, prosthesis, or medical device, as a subcategory of biocompatibility, represents a major area of interest. While factors such as the interaction with blood components, particle accumulation, and clearance in organs are indeed important, alterations to the immune system cannot be ignored. Nanoparticles have the potential to either stimulate or suppress the immune system, a property that may positively or adversely affect the function of a particle for particular applications.

2.1.1. Immunostimulation

As various compounds or materials are introduced into the body, the immune system recognizes them as foreign and elicits a multi-level immune response. When this occurs, the activity of one or more components of the immunoregulatory complexes is directly enhanced, and immunostimulatory effects such as flu-like symptoms and hypersensitivity to unrelated allergens are observed [50]. Chamberlain and Mire-Sluis have described the molecular structure, architecture of folding motifs, degradation products, formulation, package purity, and stability of pharmaceuticals as factors responsible for immunostimulation [51]. Furthermore, Rihova reports that factors such as dose, route and time of administration, mechanism of action, and site of activity – all of which are extrinsic to the material – are also critical in immunostimulation [52].

As part of immunostimulation, nanoparticles have displayed adjuvant properties. Adjuvants are substances that enhance the body's immune response to an antigen. In the context of cancer, pharmaceuticals are considered adjuvants when they, by stimulation of the immune system, suppress secondary tumor formation following treatment. Stieneker et al. showed that poly(methylmethacrylate) (PMMA) nanoparticles, when used as an adjuvant in human immunodeficiency virus (HIV) 2 whole-virus vaccine, were able to produce an antibody response in mice that was 100 times stronger than the traditional aluminum hydroxide or aqueous control vaccine [53]. Caputo et al. showed that novel biocompatible anionic microspheres are suitable and efficient storage and delivery systems for HIV-1 Tat protein for

vaccine applications that preserve protein conformation and activity [54]. In particular, they have shown *in vitro* that anionic nano- and microspheres attach the HIV-1 Tat protein and guard it from oxidation; therefore, rising the "shelf-life" of the Tat protein vaccine [54,55]. In addition, in this group, *in vivo* biocompatible and novel surfactant-free polymeric core–shell nanoparticles and microparticles were developed [54–56]. These particles reported to be able "to accommodate in their shell high amounts (antigen loading ability of up to 20%, w/w) of native proteins, mainly by ionic interactions, while preserving their activity" [57]. However, recent progress in the development of HIV-1 Tat-based vaccines [58] from basic science to clinical trial [59] has been reviewed elsewhere.

In a similar study by Castignolles et al. using rabies vaccine showed that lipid-coated polysaccharide nanoparticles increased antibody response, and hence vaccine efficacy, fourfold [60]. Works by Diwan et al. and Cui et al. suggest that nanoparticles exhibit adjuvant properties by enhancing antigen uptake and stimulating antigen-presenting cells [61,62]. While the mechanisms of nanoparticle-induced adjuvant properties are not fully understood, its proven effectiveness for use in vaccines has generated a great deal of interest.

One of the critical components of immunostimulation is inflammation, a non-specific immune reaction whereby signaling molecules called cytokines are secreted to recruit immune cells to the location where foreign material exists. This recognition is triggered by the core composition and surface properties of the particle. Of these properties, surface charge plays a particularly important role; generally, a positively charged particle is more apt to cause inflammation than a neutral or negatively charged particle. This fact was corroborated by Tan et al., who showed that an anionic particle did not cause the secretion of cytokines while a cationic particle did [63]. Diwan et al. provided further evidence of nanoparticle-induced inflammation. They showed that oligonucleotides bound to PLGA-based nanoparticles caused a larger amount of cytokine production and induced more T-cell proliferation than the naked oligonucleotide [64]. Foreign material is dealt with and cleared from the body in a variety of ways. Nanoparticles can be engineered to resemble pathogens so they are dealt with in an equivalent fashion. One method of doing this is by modifying nanoparticles with Toll-like receptor (TLR) ligands, which are recognized by the immunity system's dendritic cells. In one study, mannose was applied to modify the surface of particles, stimulating the particle's uptake by mannose receptors, a common mechanism for pathogen neutralization [65]. However, there is a tremendous amount of work involved in focusing micro particle based immunostimulation against cancer cells.

2.1.2. Immunosuppression

Immunosuppression is described as the down-regulation or prevention of the activation of the immune system. Since the early 1960s, immunotoxicologists have continued to catalogue the immunosuppressive ability of drugs as well as chemicals. Immunosuppression has its drawbacks such as increasing susceptibility to infections caused by bacteria, viruses, fungi, and yeast [66], as well as the development of neoplasms (most commonly skin cancers and lymphomas) [67]. While immunosuppression is undesirable in some instances, it has proven useful in the treatment of autoimmune diseases and has facilitated the acceptance of foreign tissues in organ transplant patients. As with immunostimulation, factors such as drug dose, pathway into the body, time of administration, the mechanism of action, and site of activity will affect the body's response to an immunosuppressant [52].

Nanoparticles have been shown to produce immunosuppressant properties. For instance, Shaunak et al. reported that when human macrophages and dendritic cells were exposed to the bacterial endotoxin, Generation-3.5 polyamidoamine (PAMAM) dendrimer-glucosamine conjugates – which are produced from partial modification of carboxylic acid terminated PAMAM dendrimers with glycosamine – were able to significantly inhibit cytokine- and chemokine-induced inflammation with a novel immunomodulatory and antiangiogenic properties. Interestingly, no hematological toxicity was apparent, suggesting that the dendrimer conjugates may be able to treat and prevent the formation of scar tissue [68]. In another work, PLGA nanoparticles containing collagen type II suppressed arthritis-induced inflammation in a mouse model [69]. A comparable study observed similar results using PLGA nanoparticles functionalized with betamethasone in rats [70]. In a similar mouse model, autoimmune diabetes was inhibited [71]. Cromer et al. reported that amino terminated generation-5 PAMAM dendrimers modified with 2-hydrohyhexyl groups protected against fatal sepsis and *in vivo* and *in vitro* cytokine secretion caused by bacterial lipopolysaccharide [72].

In the case of allergies, the induction of immune tolerance is considered desirable. For instance, Ryan et al. showed that polyhydroxy C60, a type of water-soluble fullerene, was able to inhibit hypersensitivity reactions both *in vitro* and *in vivo* [73]. In similar cases, it was reported that nanoparticles suppressed type I and type II allergies to common environmental and food allergens [74–78]. In this scenario, however, data conflicting with the concept of desirable effects of nanomaterials have been also presented. For instance, Zogovic et al. investigated the influence of nanocrystalline fullerene C60 on tumor progression and reported that nanoC60, "in contrast to its potent anticancer activity *in vitro*, can potentiate tumor growth *in vivo*, possibly by causing NO-dependent suppression of anticancer immune response" [79].

2.2. PEGylation

The characteristics of a material's surface are a primary factor in the determination of the biocompatibility of that material within the body [12]. This fact was recognized by Abuchowski et al., who in the 1970s, were the first to introduce the covalent bonding of poly(ethylene glycol) (PEG) to a drug or therapeutic protein in a process known today as PEGylation [80]. Later on, in 1984, de Gennes described two main regimes or conformations that PEG chains can obtain which are called mushroom and brush conformation — depending on grafting density [81]. If the grafting density is low, the PEG polymer is assumed to be in the mushroom regime. If the density is high the PEG polymers are assumed to be in the brush regime [82]. The degree of surface coverage and distance between graft sites will depend on the molecular weight and the graft density of the PEG polymer [83]; thus, requiring careful attention. Early work with PEG grafted nanoparticles pursued primarily from drug delivery [84–87]. Davis and Abuchowski, as one of the first reporters on PEGylation, described covalent attachment of methoxy-PEGs (mPEGs) of 1900 and 5000 Da to bovine serum albumin and to liver catalase [88,89]. It is now well known that PEGylation holds many attractive properties; for instance, it has been shown to increase a drug's half-life within the body, prolonging the activity of the drug, and thus reducing the dosing frequency [90]. In addition, in drug-delivery applications, PEG grafted nanocarriers decrease MNP uptake and augments circulation time versus uncoated counterparts (11). PEG's ability to prolong the circulation lifetime of the carrier (10) has been credited principally due to its physical properties [83,90,91] which in turn can cause the reduction or prevention of protein adsorption. To this point, Allen et al. addressed the question of how surface of a liposome protected with PEG molecules of different molecular weights would differ from a PEG-free liposome [83]. Their work was based on a previous approach established by Torchilin and Papisov on 1994 [92].

Needham and Kim reported that PEG of a selected molecular weight and graft thickness prevents the adsorption of certain proteins to a surface [83,90,91,93]; yet, there is not much evidence that exist for reduction of total serum protein binding due to surface PEGylation of carrier. Ahl et al. has shown that PEGylation increases a colloidal carrier's stability *in vivo* by its steric effect which acts as a barrier for aggregation [94]. Other studies have suggested that PEG endorse binding of specific proteins that mask the carrier and cause "dysopsonization" [95,96] as well as existence of attractive interactions between poly(ethylene glycol) and proteins [96,97].

Not surprisingly, PEGylation can have ability to control the physical behavior and biological performance of nanocarriers formulations and as a result substantially change their biocompatibility. Consequently, PEGylation dramatically reduces the immunogenic response to a substance's surface, including a reduction in protein adsorption (Fig. 2) [98], as well as a reduction in platelet aggregation, neutrophil activation, hemolytic activity, and coagulation [12]. However, PEGylation also carries several disadvantages that must be considered. Recent works indicate the formation of PEG-specific antibodies, which clear the particle (along with the drug) from the body, thus reducing its effectiveness [99]. In addition to this, obstacles include possible side reactions, incomplete PEGylation and the need for drug-specific tailoring [100].

2.3. Nanoparticle interaction with blood

The surface properties of nanoparticles can greatly affect their compatibility while in the blood stream. Interestingly, blood constituents can react immunologically to render nanoparticles and their drug complexes inactive. For instance, Gref et al. [84] report that in the blood stream, macrophages rapidly clear nanoparticles that lacked surface modification to prevent the adsorption of opsonins. For this reason, preclinical examination of nanoparticle biocompatibility must include studies of hemolysis, platelet aggregation, coagulation time, complement activation, leukocyte proliferation, and uptake by macrophages [12].

Therefore, evaluation of possible toxic effects of immediate exposure of nanoparticles would be the first critical step that one would be considered. We know that erythrocytes exist in a larger volume portion of the blood than mononuclear phagocytic cells. Thus, nanoparticle that injected intravenously would encounter red blood cells (RBC) before MNP cells; consequently, examination of haemolysis is an instrumental part of preclinical studies of nanoparticles [13]. Many authors reported hemolytic effects of different nanoparticles in the literature — as many of the studies have been conducted with blood to see the early toxic effects of nanoparticles. As a result, a number of mechanisms for drug-mediated haemolysis have been recommended, yet the true mechanism has not been clearly identified. It is now well known that surface properties (especially surface charge) play an important role for nanoparticles and can directly damage erythrocyte membranes. For instance, in the presence of certain concentrations of unprotected primary amines (positive charge), red blood cell damage was observed on the surface of poly-amidoamine, carbosilane, polypropylene imine, and poly-lysine [101–106]. However, deeper understanding and knowledge on how the particulate nature of blood would affect a nanoparticle will help for better design of nanoparticle-based drug delivery system. In this regards, Tan et al. provided the theoretical and methodological framework that help to understand how interactions between blood cells – with and without red blood cell – and NPs influence the particle motion and binding [107]. They reported enhance nanoparticle dispersion as well as 50% increased nanoparticle binding upon exposure to RBC. Another study also presented erythrocyte as a vital contributor to the process of transport and primary meeting of lymphocytes to the vascular wall [108]. However, there are other studies in the literature which reported mathematical or theoretical modeling of RBC on blood flow [109,110] which indirectly would influence nanoparticles efficacy in drug delivery system.

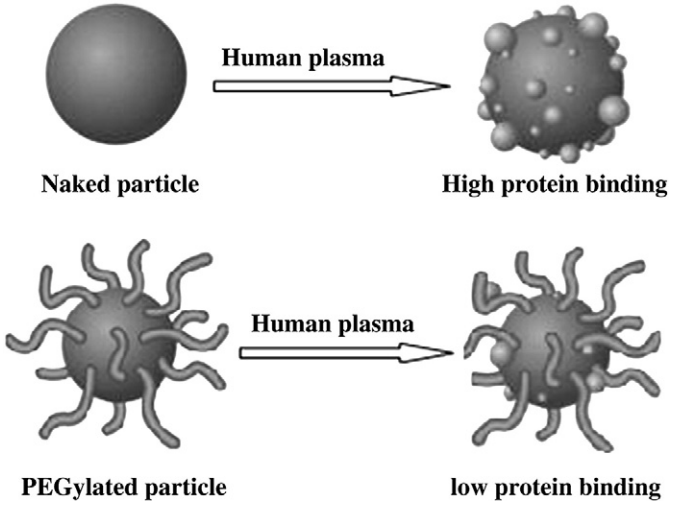

Fig. 2. PEGylated nanoparticles are able to avoid clearance from the blood stream by repelling protein adsorption, thus prolonging nanoparticle circulation time within the body.

The complement system and its activation are major characteristics of the general host response to biomaterials, including nanoparticles. Complement activation is described as the recognition, opsonization, and clearing of pathogens and foreign material by approximately 35 typically dormant proteins present within blood (either solubilized in blood or located on the surface of blood cells) [100]. The complement system can be triggered by any of three different pathways: the classical pathway, alternate pathway, and lectin pathway. These pathways are activated by different criteria: the classical pathway by specific antibodies found on the surface of the intruding material, the alternate pathway by the identification of certain microbial surface structures, and the lectin pathway by mannose residues found on microbial glycoproteins and glycolipids which are identified by mannose-binding lectin (MBL), a protein found in blood plasma [52]. Understanding a material's effect on the complement system is crucial to understanding the immunological response it may trigger. For this reason, reducing a surface's tendency for complement activation has been the subject of widespread interest [111].

2.4. Biodegradability

Biodegradable nanoparticles have been used for targeted drug delivery, vaccines and a range of other biomolecules. Generally, the clearance of nanoparticles is often a desirable goal after its introduction into the body and performance of their function. Biodegradable nanoparticles, *i.e.* those which are digested internally and subsequently cleared from the body, are often preferred over non-biodegradable particles (*e.g.* metal colloids, ceramics) [13,21,52,112], for they do not require future removal [113]. Biodegradable nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic biodegradable polymers [114]. A few of the most comprehensively employed biodegradable polymer for preparation of nanoparticles include Poly-D-L-lactide-co-glycolide (PLGA), Polylactic acid (PLA), Poly-ε-caprolactone (PCL), Chitosan and Gelatin [114]. The selection of the base polymer is dependent on different designs and end purpose criteria. Anil Mahapatro and Dinesh K Singh in this regards indicated that it depends on many factors such as 1) size of the desired nanoparticles, 2) properties of the drug (aqueous solubility, stability, etc.) to be encapsulated in the polymer, 3) surface characteristics and functionality, 4) degree of biodegradability and biocompatibility, and 5) drug release profile of the final product [114]. Besides, the biodegradation possibly will be affected by the experimental conditions: experimental models, implantation or therapeutic sites, and animal species [115,116].

Non-biodegradable nanoparticles however, are reported to accumulate in the mononuclear phagocytic cell (MPS) such as the liver and spleen, giving rise to potentially toxic side effects [21]. Further research is needed to fully comprehend how the body, specifically the immune system, deals with non-biodegradable nanoparticles [12]. In addition, it has been suggested that careful consideration should be employed for the use of non-biodegradable nanoparticles as treatment of non-terminal diseases for which there are alternative methods of treatment. This is because accumulation within the mononuclear phagocytic cell system may not be reversible, leading to the potential for lifelong side effects [117].

3. Nanoparticles as drug carriers

Nanoparticles used as drug carriers are submicron-sized particles ranging 100–1000 nm. Cristina Buzea et al. defined nanoparticles as "particles with at least one dimension smaller than 1 micron and potentially as small as atomic and molecular length scales (~0.2 nm)" [118].

Many organizations have now defined nanoparticles as the particles which should have a size below 100 nm in at least one orthogonal direction. In fact, it is not easy to track expansions in the field of nano technology since the multidisciplinary nature of this field request a similar diversity of definitions in respect to each specialty or scientific discipline. In this regards, Fred Klaessig et al. reported the dispute facing terminology and nomenclature efforts and listed the suggested upper boundary for the term *nanoscale* along with the organization, references and qualifications [119]. Another example of such is the publication by the U.K. House of Lords Science and Technology Committee titled, "Nanotechnologies and Food" [120] and recommended:

> …We recommend … that any regulatory definition of nanomaterials … not include a size limit of 100 nm but instead refer to 'the nanoscale' to ensure that all materials with a dimension under 1000 nm are considered [119,120].

"The recommendation is that the term *nanoscale* have an upper boundary of 1,000 nm for the purpose of food regulations, rather than the ISO and ASTM International determinations that scientific usage is 100 nm." [119].

However, nanosized particles or nanoparticles used for drug delivery hold great promise for their feasibility as pharmaceutical carriers and can be prepared using a wide range of materials such as polymers, lipids, viruses, and organometalic compounds; therefore, their use in medicine is predicted to spread rapidly in the coming years [121]. Studies indicate that nanoparticle-drug complexes have the ability to mitigate toxicity and side effects associated with raw pharmaceuticals such as chemotherapy drugs [12,121], by allowing for targeted drug release and improved solubility through various methods such as encapsulation, micellization, and protein cage architecture [122].

Indeed, the potential for more precise localization and reduced toxicity of therapeutic drugs is encouraging. However, evidence suggests that nanocarriers themselves may pose a toxicological risk to patients beyond that of the taxied chemicals [121]. De Jong and Borm have summarized some of the adverse toxicological responses observed over the past decade, which include lung inflammation, platelet aggregation in blood, and impaired mitochondrial function in cells [121].

As can be imagined, the toxicological effects vary with nanoparticle composition. The material composition may include metals and inorganic particles such as gold, silver, and metal oxides [38], polymer-based materials such as PLGA, and lipid-based particles such as nanoliposomes, solid lipid nanoparticles, and nanoemulsions. Each substance exhibits its own inherent physicochemical properties such as surface charge, hydrophobicity, solubility, size, shape, and aggregation tendencies which can be engineered to trigger different biological responses [8,12]. While the influence of such parameters on biocompatibility is well known in some instances (Fig. 3) [12], investigations involving newer nanoparticle designs are still underway. As expected, the manipulation of these properties for the purpose of function and biocompatibility represents a prominent area of study in nanomedicine [8].

3.1. Types of nanoparticles

Nanoparticles exist in a wide variety of sizes, shapes, and compositions (Fig. 4) [123]. Nanoparticle-bound pharmaceuticals in their many forms can be found at various stages of the pharmaceutical pipeline; some have been approved for clinical use, while others are being tested and progressed through the approval process [12]. In the following section, the authors have chosen to focus on the nanoparticles that have been widely investigated for drug delivery applications. This section also outlines a variety of approaches to nanoparticle structure and composure, both viral and non-viral.

3.1.1. Carbon-based polymers

Carbon-based polymers such as fullerenes, carbon dots, nanodiamonds, and nanofoams also represent a prominent area of nanoparticle research. Of these, fullerenes are well established and consist of C60, single-walled nanotubes, and multi-walled nanotubes. Carbon nanotubes have been proposed for use in a variety of contexts ranging from structural reinforcement of existing materials [124] to drug carriers [100]. Carbon nanotubes are simple layers of graphite rolled in a tubular shape capable of exhibiting a single- or multi-walled morphology [121,122]. Their cell-penetrating and conjugative properties make them a contender for *in vivo* drug delivery applications [100]. In addition, surface functionalization can render typically heterogeneous nanotubes water-soluble [122]. With regard to biocompatibility, carbon nanotubes have been shown to activate the complement system through the classical and alternate pathways [100]. Furthermore, it was found that carbon nanotubes might, in some cases, over-stimulate the compliment system, resulting in inflammation and granuloma formation [12,100,121]. Additional factors that may inhibit carbon nanotube use in humans include evidence of oxidative stress [38,121,122], apoptosis [122], toxicity due to metal residues from nanotube synthesis [38], lipid peroxidation, mitochondrial dysfunction, changes in cell morphology, and platelet aggregation [121]. In contrast to this, some reports indicate that an inflammatory response does not occur when carbon nanotubes have been purified [125]. Although the ambiguity of carbon nanotube toxicity and wide array of toxicological responses certainly warrants caution, the contradictory data on the toxic effects of carbon nanotubes also suggests a need for further research. Therefore, it is believed that modification and/or coating of carbon nanotube-based biomaterials would enhance their ability as suitable carriers for applications such as drug delivery. For instance, targeted and controlled doxorubicin delivery using modified single wall carbon nanotubes have been reported [126]. In addition, Hong-Xuan Ren et al. summarized the data on the toxic effects of single-walled carbon nanotube with different treatments and suggested on a standard evaluation of the effects of carbon nanotube on the cells, organs, or an entire organism [127]. Furthermore, recently, there have been published reports focusing specifically on carbon nanotubes-based biomaterials utilized in biomedical applications that clarify the importance of material modifications to fully realize their maximum potential [128–131].

Graphene is a material with a one-carbon atom thick, single layer sheet structure that occurs in nature in the form of graphite. Graphene can be used for a different range of biomedical applications due to its flexible chemical structure blend with its inherent properties. Therefore, graphene has become a potential candidate for multifunctional biomedical purposes such as biosensors [132,133] and drug delivery [134]. For instance, Hu et al. have reported about good antibacterial activity of graphene oxide (GO), recommending its potential for drug delivery in ophthalmology application [135]. A year later, in 2011, Zhang et al. showed that modified GO could be used for targeted drug delivery and controlled release in the tumor therapy [136]. Almost at the same time, Yang et al. showed that GO-based composites decreased reticuloendothelial system accumulation and remarkably enhanced tumor passive targeting effects [137].

Recently, Yan et al. investigated the *in vitro* and *in vivo* intraocular biocompatibility and cytotoxicity of graphene oxide (GO) [138] knowing the fact that eye is a particular organ with the presence of the blood–ocular barrier which makes it important in targeted medical therapies such as ocular tumor-related treatments. Therefore, they investigated novel drug delivery and controlled release systems in ophthalmology and reported that GO has favorable biocompatibility for retinal pigment epithelial cells with minimal adverse effects on cell viability and morphology in long-term cultures [138]. Furthermore, biocompatibility and

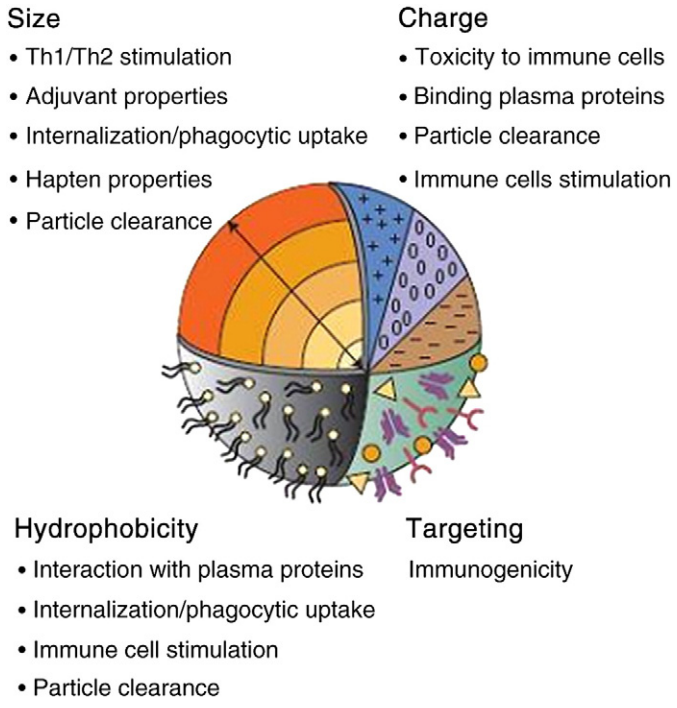

Fig. 3. The properties of nanoparticles such as size and charge determine their effect on the body. Adapted by permission from Macmillan Publishers Ltd: Nature Nanotechnology [12], copyright (2007).

toxicity of GO on A549 cells has also been evaluated, suggesting that "GO does not enter A549 cell and has no evident cytotoxicity" [139]. Nevertheless, GO could cause a dose- and size-dependent oxidative stress in cell and a trivial loss of cell viability at high concentrations. These data together suggests that overall, GO has an adequate safety profile, for drug delivery application, further supported by the positive growth of cells on GO films [139].

Although there are studies that address the biocompatibility of graphene, GO and their modified versions [140–144], a great deal of research is still required in the near future prior to their application in the clinical settings. Furthermore, there is active, ongoing research in the role of carbon nanotubes in the delivery of chemotherapeutic agents while limiting systemic toxicity [145].

3.1.2. Polymeric nanoparticles

Polymeric nanoparticles include synthetic polymers, natural polymers (*e.g.* proteins), and pseudosynthetic polymers (such as synthetic polypeptides are broadly used for drug delivery [146]. Polymer architecture, composition, backbone stability, as well as water solubility are important factors which specify the effectiveness of drug-delivery carriers [147]. In this section, a selected group of polymeric nanoparticles and dendrimers that have been the most commonly used in drug delivery applications are reviewed.

Currently we know that polymer architecture dictates the carrier's physicochemical properties, drug loading effectiveness, drug-release rate and biodistribution [147]. Polydispersity character of polymer, defined as the heterogeneous combination of chains of altering lengths [148], makes them of particular significance for biological properties which are molecular mass dependent [149]. Polymers have been found to be able to provide a sustained release of encapsulated drugs, protect drugs from the body's enzymatic and degenerative conditions, provide targeting capabilities from a tendency for passive accumulation in tumors, and display adjuvant characteristics, meaning that it may help prevent subsequent cancer attacks. In addition, they can be used to overcome the poor aqueous solubility of certain drugs such as chemotherapeutics [150]. Despite all of these benefits, polymers are frequently taken up by the immune cells and hence the immunocompatibility of these materials must be carefully considered. [151].

It has been reported that polycations may not only be cytotoxic, but can also induce hemolysis and complement activation [148]. It has also been observed that polyanions are less cytotoxic, but still can induce anticoagulant activity and cytokine release [148]. Despite these reports, there are some reports that address the compatibility of polymers for *in vivo* applications [152]. Other studies have shown that nanoparticles made from N-(2-hydroxypropyl) methacrylamide (HPMA) were able to mitigate many of the inherently toxic effects of the popular anti-cancer drug, doxorubicin [99]. Furthermore, it was found that HPMA-bound doxorubicin triggered anti-cancer immunity in mice; up to 80% of cured mice were able to survive a fatal dose of cancer cells independent of further treatment [52]. With regard to biocompatibility, evidence suggests that HPMA does not induce a significant response within the body, leading researchers to believe that HMPA copolymers are indeed "immunologically safe" [99].

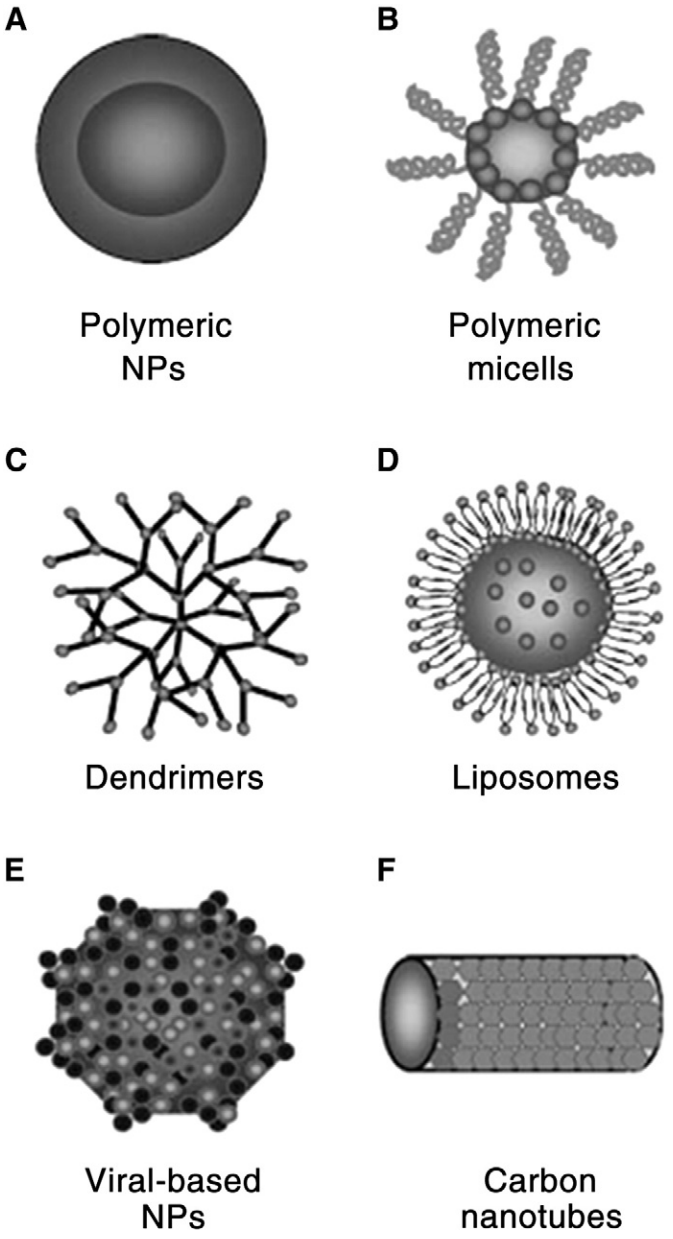

Fig. 4. Various types of nanoparticles including polymeric nanoparticles, micelles, dendrimers, liposomes, viral vectors, and carbon nanotubes. Adapted by permission from the American Association for Cancer Research [123], copyright (2008).

Another type of polymeric-based particle that can be utilized as carriers for drug delivery systems is PLGA micro- or nanoparticles [35]. These particles are known as clinically proven biodegradable and biocompatible materials [40]. One area in which they have been widely investigated is in the formulation of the chemotherapeutic drug, paclitaxel (Taxol®) [153]. In addition, they represent an innovative approach to adjuvant therapy in vaccination by presenting vaccine antigens [36,39]. Studies have reported that these polymeric particulate delivery systems [35] can present antigens and trigger specific humoral and/or cellular responses [39,41,42], highlighting the importance of their size in the resulted outcomes [43–45]. For instance, microparticles trigger a humoral-mediated immune response whereas nanosized range PLGA particles activate cell-mediate immune responses [46,47]. Not surprisingly, it is not easy to predict the phagocytic behavior subsequent to particles' uptake. Nicolete et al. produced PLGA nano- and microparticles devoid of any encapsulated bioactive. They then examined these particles' uptake by macrophages as well as their effect *in vitro*, on the production of proinflammatory cytokines, TNF-α and IL-1β [35]. They have reported that PLGA microparticles of size 6.5 μm, attached to the cell's surface at 2 and 4 h incubation times and a few could be seen inside the cells when compared to nanoparticles [35]. Danhier et al. have reviewed the beneficial usage of PLGA-based nanoparticles both *in vitro* and *in vivo* as a therapeutic strategy in different diseases [48]; they also reported on the characteristics of PLGA-based nanoparticle that makes them a promising candidate for targeted and untargeted drug delivery. Poly(lactic acid) (PLA) polymers have also been used in drug delivery [49,154]; however, due to their slow degradation rate, PLA polymers have not been broadly used, compared to PLGA polymers [48]. PLA was used for surface modification of organic microsphere poly(hydroxyethyl methacrylate) (PHEMA) [155]. PLA modified microspheres showed a better anti-tumor effect as well as increased loading capacity in compare with unmodified one [155].

In general, one of the areas in which more work needs to be done on the development of methods for visualization of polymer-based nanoparticles. Even highly sensitive methods such as scanning- and transmission electron microscopy are limited in their capability for reliable visualization of polymer-based nanoparticles within cells, compelling the need for indirect, assay-based methods to examine nanoparticle cellular uptake by phagocytes [12].

3.1.3. Dendrimers

Dendrimers are highly branched polymers whose shape, size, branching length, density, and surface functionality can be controlled and are well defined [9]. Originating from a nanosized core, polymeric branches of high specificity are grown outward, forming cavities and cages throughout the molecule [122]. These channels and closed structures allow for the physical entrapment or encapsulation of pharmaceuticals [9]. In addition, negatively charged drugs may associate themselves through electrostatic interactions with amine groups within the dendrite [9]. Furthermore, drugs can be chemically attached to surface groups on the polymeric structure [9,121,122]. Dendrimers are susceptive to surface group modification and can be tailored to facilitate targeting and improve biocompatibility [9,121,122]. For instance, dendrimers with positively charged surface groups are likely to cause cell lysis [9]. Dendrimers, like most nanoparticles used for drug delivery, aim to mitigate the inherently toxic effects of unbound drugs through targeting and subsequent accumulation in tumors; PEGylation abets or assist this process [9]. On the subject of toxicity, dendrimers cannot be classified as consistently safe or unsafe. Research suggests that dendrimers must be evaluated on a case-by-case basis to classify their particular chemistry's biocompatibility. A lack of research and clinical trial in this field deters generalization regarding safety [121]. Contrarily, there are known correlations between the properties of dendrimers and their functionality and biocompatibility. For instance, size influences both solubility and cytotoxicity, and an increase in generation number leads to an increase in both of these properties [9]. Lastly, dendrimers, possess antitumor, antiviral, and antibacterial properties [9], along with the capacity to enhance membrane permeability [9]. These intrinsic properties have sparked interest in dendrimers for bacterial cell killing and trans-membrane transport applications [121].

Micelles act by encapsulating material within its walls. Contrarily, a micelle functions on the premise of its amphiphillic monolayer. The inner core of micelles is typically hydrophobic enabling successful encapsulation of insoluble drugs, while its hydrophilic outer core renders the encapsulated material soluble [123]. Self-assembled polymeric micelles have recently attracted attention due to their special characteristics, such as high loading capacity and improved solubility of drugs, decreased systemic unfavorable effects, enhanced permeability and retention (EPR) effect which results in their accumulation at the tumor site, and lastly, their possible modification of physicochemical characteristic [37,156–158]. However, with all of the advantages, the controlled and smart release of therapeutics from traditional polymeric micelle carrier remains a challenge. Currently, nanocarriers are replaced with traditional micelle systems, since they can stably encapsulate and release therapeutics at a targeted site as a result of external stimuli such as pH, temperature, redox, and light [159–162]. However, because of their toxicity, only a small number of them have moved to the clinical studies [163] such as the pH-responsive polymeric micelles [164–169]. Therefore, to overcome the toxicity of the carriers, polymeric segments composed of polymers with better compatibility such as poly(ethylene oxide) and biodegradable polymers like polyesters, are employed to form micelles in aqueous solutions [163]. Lee et al. designed and synthesized a new class of hyperbranched double hydrophilic block copolymer of poly(ethylene oxide)-hyperbranched-polyglycerol (PEO-hb-PG) with enhanced biocompatibility, increase water solubility, and improved biodegradability after delivery of the drug [163].

3.1.4. Lipid-based nanoparticles

Lipid-based nanoparticles such as liposomes represent another category of popular drug-carrying nanostructures. Liposomes, not to be confused with micelles which are characterized by monolayers, are generally composed of one or more bilayers of an aliphatic lipid molecule arranged to form a vesicle. This vesicle formation allows for the encapsulation of drugs, vaccines, or other materials within its walls or entrapment within its layers, depending on the material to be delivered [122]. A number of liposome-based formulations have gained approval for the treatment of cancer, infections, and meningitis, with prospective applications such as therapeutic vaccines currently under development [170]. These include liposomal-based therapeutics containing the antifungal, amphotericin B (Abelcet®), chemotherapeutic drug doxorubicin (Myocet®), immunopotentiating reconstituted influenza virosome (Epaxal®) [171]. Liposomes have been categorized as those which have been designed to evoke an immune response to a contained antigen and those whose surface have been coated with PEG or a similar polymer to mitigate or suppress immune response. In general, liposomes with positively charged surfaces are more prone to eliciting an immune response than negatively charged or neutral particles [172]. A possible downside to liposomes as pharmaceutical carriers is their selectivity with respect to functionally compatible drugs. In some cases – liposome-entrapped cisplatin, for instance – the particles are unable to release the encapsulated drug at a rate sufficient to trigger antitumor activity, despite passive accumulation at tumor sites. Nevertheless, doxorubicin-entrapped liposomes of the same composition produced effective antitumor properties, corroborating the aforementioned selectivity [122].

Solid lipid nanoparticles (SLNs) mainly consist of solid lipids, which also possess properties such as biocompatibility, biodegradability and low-toxicity. SLNs are described as colloidal particles of highly purified triglycerides, complex glyceride mixtures, or waxes stabilized by a surfactant. These are lipids whose nature allows them to remain solidified at room and body temperature [173]. When regarded as a drug carrier, SLNs have undergone studies with a wide variety of pharmaceuticals ranging in structure and chemical properties [174,175]. SLNs have the

advantages of both the "soft" drug carriers such as emulsions and liposomes and polymeric nanoparticles [15].

SLNs are versatile in their methods of drug incorporation; drugs can be loaded into the particle's core or shell, between lipid layers and fatty acid chains, in the particle's imperfections, or dispersed molecularly throughout the particle's matrix [173]. Despite this versatility, SLNs feature a low drug loading capacity. Additionally, SLNs may undergo polymorphic transition during storage and administration; this causes gelation, size increase, and drug expulsion [173,174]. Undesired lipid-based particles such as micelles and liposomes as well as crystalline drug structures may also be formed within the complex, threatening the purity of the SLN colloid [174]. Conversely, SLNs allow for a variable rate of drug release and targeting within the body, provide protection to the encapsulated drug [173], and avoid the use of harmful organic solvents, and have potential for large-scale production as a result of a streamlined production process [174]. Another advantage lies in SLN composition. Since they are made from physiological compounds, metabolic pathways are already in place within the body [174]. This anticipated biocompatibility has been corroborated through *in vitro* and *in vivo* studies of SLN toxicity. For instance, tests indicate that SLN are less toxic than polymeric nanoparticles (PLA/GA) [175]. Bolus injections in mice also showed no acute toxicity as suggested by histopathology [173,175]. Furthermore, it has been noted that SLNs are suitable for use in any parenteral application where polymeric nanoparticles are accepted [175]. Recently, Qi et al. have provided an overview on the absorption, disposition and pharmacokinetics of SLNs [176].

In general, lipid-based nanoparticles are vulnerable to changes in temperature and osmotic pressure, among other extrinsic variables. This property, along with their inherent instability in biological media, may warrant the need for stability-enhancing alterations such as surface polymerization [9].

3.1.5. Quantum dots

The term "quantum dot" refers to a particular category of nanoparticle characterized by a crystalline structure usually composed of a semiconducting material [177]. Cadmium sulfide and cadmium selenide quantum dots are among the most popular [38]. Quantum dots (QDs) were discovered in the 1980s and are known to possess unique optical properties that make them ideal for imaging purpose [122,177]. In addition, quantum dots may be used for cancer detection and therapy [177], and computing applications where light is used to process signals [122].

In the drug delivery scenario, "fluorescent semiconductor nanocrystals", quantum dots, valuable features, such as small size, flexible surface chemistry, and wonderful optical properties, make QDs not only a supreme plan for the broad characterization of nanocarrier behavior (6) but also allowing their use within almost any nanocarrier – with minimal effect on overall characteristics – and drug release at both cellular and systemic levels [178]. Like all nanoparticles proposed for use in the human body, quantum dots are being tested for biocompatibility. The innate properties of the material will determine factors such as adsorption, distribution, metabolism, excretion, and toxicity, as well as the environmental conditions in which the particle is placed [121]. Studies have shown that quantum dots themselves may induce toxic effects such as damage to plasma membranes, mitochondria and nuclei [121]. For this reason, and for the purpose of targeting, these nanoparticles are often surface-coated; this may, however, induce additional toxicity. Furthermore, it was found that the quantum dot's toxicity is influenced not only by its surface chemistry, but also by its core material [121]. However, appropriately coated and passivated QDots do not show acute toxicity *in vivo* [179] and in rhesus monkey [180] regardless of the possible release of toxic chemicals such as cadmium, Cd [181,182] and production of reactive oxygen species (5). Modified quantum dots would permit brief nanocarrier screening without non-specific unfavorable effects [178]. P. Zrazhevskiy discussed about reducing long-term QDot toxicity [183]. Such properties would have made quantum dot platform to be a potential candidate for clarifying, *in vivo and in vitro*, mechanisms of nanoparticle targeting, intracellular uptake, and trafficking [178]. This in turn would ease assessment of the nanocarrier behavior in a range of drug delivery applications as well as contribute for design of novel nanotherapeutics, such as "NP-based antigen delivery vectors for immunotherapy" [178].

As toxicity is inherent to traditional quantum dots, the search for less harmful materials is ongoing and of great interest [177].

3.1.6. Metallic nanoparticles

Metallic nanoparticles hold potential for use in both diagnostic imaging and targeted drug delivery [184]. These nanoparticles are often delivered in solid colloidal form and aim to increase the therapeutic index of anticancer drugs through passive or active targeting while mitigating toxic effects by limiting drug exposure to healthy cells and tissues. Metal-based particles hold the potential to carry large drug doses as well as increase its circulatory half-life [184]. Additionally, surface modification is possible due to a large surface area-to-volume ratio [184], the effects of which have been discussed in previous sections.

The use of colloidal gold in medicine can be traced back to the 1920s for the treatment of tuberculosis [38]. Since then, colloidal gold nanoparticles have been widely researched as drug and gene delivery vehicles [121]. They can be synthesized in a variety of forms (*e.g.* rod, dot) [121] and are easily detectable within micromolar concentrations, warranting their use in imaging applications [38]. With regard to biocompatibility, cells have been shown to intake gold nanoparticles without cytotoxic effects [38,121]. Lai et al. demonstrated a median lethal dose (LD_{50}) of over 5 g/kg of body weight using a nanogold suspension with a particle diameter of 50 nm [185]. Metallic nanopoarticles, including colloidal gold, continue to be actively investigated for the purpose of drug delivery and other applications. Research in this field is expected to grow over the next few decades [184].

4. Regulatory agencies

It was previously mentioned that drug delivery systems, no matter how attractive they seem, hold no weight unless they are considered adequately biocompatible. The same is true without approval from a regulatory agency such as the FDA or the European Medicines Agency (EMEA). These two qualifiers are often a function of one another. While existing guidelines awkwardly govern the use of nanomedicine, additional regulations are required to address the properties specific to nanomaterials, be it immune system or surface chemistry modification. As novel applications of nanotechnology in medicine and requests for approval continue to flow from research institutes worldwide, the need for nanotechnology-specific regulatory guidelines is made even more obvious. Regardless, standardized guidelines have yet to be established. On the whole, continued *in vitro* and *in vivo* testing is required to build a database of knowledge on the subject of nanoparticle biocompatibility. Only then, after sufficient scientific evidence, will regulatory agencies put forth the exhaustive effort of developing new guidelines [117].

5. Conclusions and prospects

This review was intended to provide an overview of recent findings of biocompatibility for several different nanoparticles. Biocompatibility is a word that is used broadly within biomaterial science, but there is still a great deal of uncertainty about its meaning as well as about the mechanisms that collectively constitute biocompatibility. Effective and biocompatible drug delivery systems based on nanoparticles as a carriers has been the dream of scientist for many years. Although we are still far from our ultimate goal of biocompatible drug delivery, progress which points to the growing importance of this research area in related to human health has been made. As biomaterials are being used in increasingly diverse and complex situations, with applications now involving tissue engineering, invasive sensors, RNA interference (siRNA)

delivery and of particular interest to this review paper, drug delivery, uncertainty over the mechanisms of, and conditions for, biocompatibility is becoming a serious obstacle to the development of new techniques.

Evidence has shown that several different nanoparticles have been used as a carrier for drug delivery system [9,123,170]. The problem remains, however, that nanoparticles' applications are still limited by their unknown biocompatibility properties which may cause their quick removal by the immune systems. Recently, the knowledge about nanoparticle interaction with components of the immune system has increased. But, still many questions such as particle immunomodulatory effects (immunostimulatory and immunosuppression) remains to be completely addressed. Indeed a more detailed investigation and deeper understanding of mechanistic studies are required to enhance our knowledge about the physicochemical properties of nanoparticles that describe their special interaction with the immune system.

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