6

Limitations for Nanomedicines

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Outline

Introduction

Nanomedicine – utilization of nanotechnology/nanomaterials for the diagnosis, Prognosis, treatment, and prevention of various fatal diseases [1]. Nanomedicines, comprising sub-micro-meter-sized carriers (nanocarriers/nanoparticles), are meticulously fabricated to enhance the biodistribution of trapped/ loaded compounds [2]. This enhancement is achieved through more effective and selective delivery to the diseased or infected site (site-specific drug delivery) or by skillfully administering their movements and taking them away from normal and healthy organs and tissues [3]. The overarching goal of this technology is to refine the equilibrium between the efficiency and toxicity of therapeutic compounds [3, 4]. Nanoparticles manifest a synergy of physical (e.g., size, morphology, shape, homogeneity, and lamellarity), chemical (e.g., composition, Surface modification, Phase transition temperature, and surface), and biological (e.g., Loaded compounds i.e., drug molecules and surface loaded ligands) characteristics, collectively determining their *in vivo* behaviour [5].

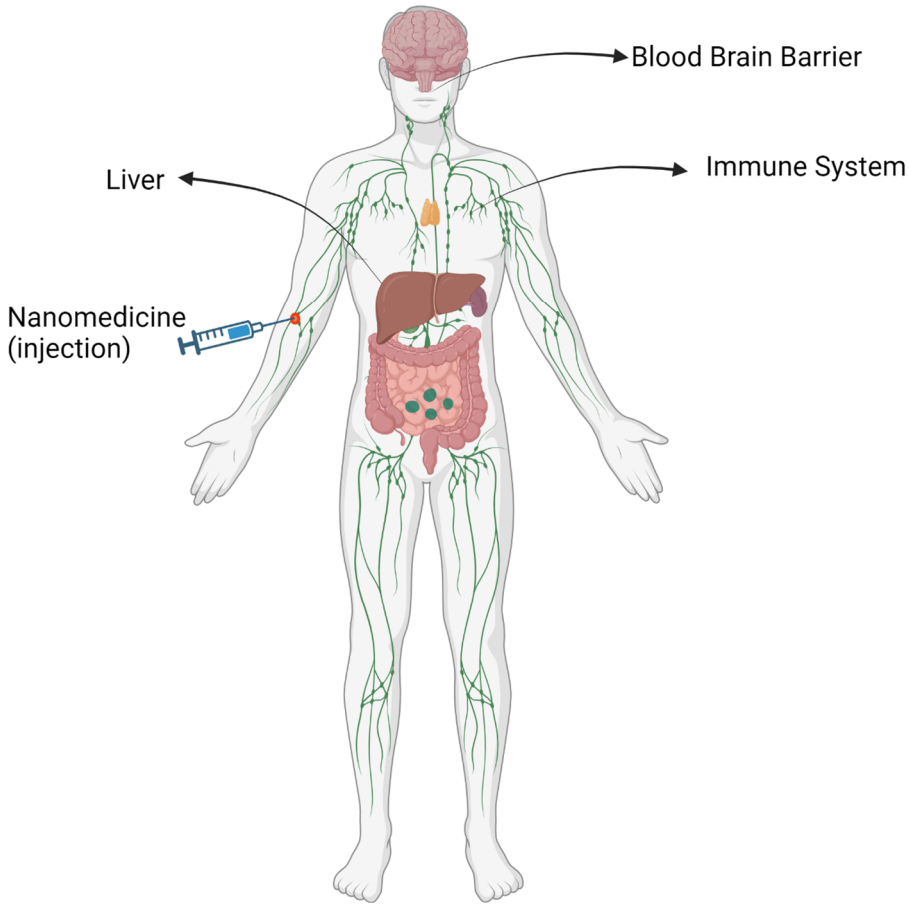

FIGURE 6.1
Possible Hindrances that may Limit the employability of Nanomedicines.

Regardless of substantial strides in drug delivery platforms and technologies over recent decades, the clinical translation of nanomedicines has advanced gradually [6, 7]. It is proposed that effective development of nano-based medicines i.e., nanomedicine, necessitates a disease-driven methodology, departing from the conventional formulation-driven viewpoint where the emphasis lies on drug delivery system engineering [8]. This shift demands a profound comprehension of the intricate bonds between the technology and biosciences. This includes discernment of the impact of disease pathophysiology on nanomedicine accumulation, efficacy, retention, distribution, and retention, as well as deciphering the correlation between *in vivo* animal behaviour in comparison to humans and properties of drug delivery systems [7]. Figure 6.1 shows possible hindrances that nanomedicine has to face inside the human body.

Furthermore, there are arising doubts in the minds of the scientific community about the nanoparticles/nanocarrier behaviour and other questions like aspects that can limit the use of nanocarriers as medicines. These can be a range of hindrances that a nanocarrier would have to face before it can do its action, although these hindrances and obstacles have a chance to be avoided when the surfaces of these carriers are functionalized with some sort of ligand, anti-body, or other biomolecules, as discussed in chapter 4, still the real behaviour needs keen observation and plenty of research.

Scientists and researchers have listed a few types of major hindrances that nanomedicine could face, during its journey with the living body, i.e., the Blood Brain Barrier – commonly short-formed as BBB by the researchers, their accumulation inside an organ, Immune System, and scale-up of the nanocarriers (i.e., their large-scale production) [9].

In this chapter, we will be discussing these major hindrances that could limit the use of nanocarriers and their commercialization.

Limitations faced by Drug loaded Nanocarriers

While drug-loaded nanocarriers have significantly transformed the landscape of nanobiotechnology, offering efficient drug delivery solutions, particularly in cancer research, they encounter several challenges upon introduction into a diseased body [1]. The foremost obstacles faced by nanocarriers are the immune system (IS), accumulation inside an organ, and the blood-brain barrier (BBB) [2]. Although these factors dictate the movement of nanocarriers within a living body, nanocarriers are also associated with potential toxicities against the IS and BBB. Although a lot of research is being carried out in this region, the questions, and issues still remain at large and need more concentration of the researchers. We also know that nanocarriers and their action may also be largely affected by their size, morphology, and shape, as discussed earlier in Chapter 1, it is very difficult to predict their exact behaviour. With that it is also very difficult to synthesize nanoparticles/nanocarriers in the same size range with an exact size distribution, therefore, a focus is required.

Immune System (IS) and Nanocarriers

The body's immune system serves as a protective mechanism [10], tasked with safeguarding the body's integrity against invading foreign substances, including chemicals, pathogens, bacteria,

viruses, and other potential threats to the human body [11]. The immune system is composed of both soluble components and cellular collections, forming a comprehensive and intricate defence system. Upon detecting a pathogen, the immune system initiates a detailed and complex response. For example, if a bacterium enters the body, the immune system analyses its molecular structure, assesses the tissue damage it causes, and mobilizes various innate immune system (IIS) cells to eliminate the threat. Furthermore, dendritic cells (DCs) have the capability to detect and respond to the damage caused by pathogens, gathering samples from the invading pathogenic cell, and transporting it to the lymph nodes. Subsequently, within the lymph nodes, DCs present antigens to T cells, which play a crucial role in eliminating the pathogenic cell [12].
The immune system can be classified into two primary categories: i) the innate immune system (IIS), and ii) the adaptive immune system [13]. Herein, we will discuss a little about these two types of immune system, in brief.

Innate Immune System

The innate immune system (IIS) is acknowledged as the primary safeguard against invading pathogens like bacteria and viruses within the body. It comprises diverse cell types predominantly originating from the bone marrow, encompassing lymphoid and myeloid cells. Lymphoid cells encompass innate lymphoid cells and natural killer cells (NK), while myeloid cells consist of dendritic cells (DCs), neutrophils, macrophages, and monocytes. In addition to its pivotal role as the first line of defense, the IIS is crucial for maintaining tissue equilibrium [14].

Adaptive Immune System

Where the IIS is fast and broad in operation, it is a range of broader countermeasures, while the AIS is slow and takes fewer targets. This may last from days to weeks so that AIS can mount a focused response against each invading pathogen [15]. On the other hand, AIS relies on special white blood cells, especially the T- and B-lymphocytes. AIS consists of different types of cells, such as CD4 T cells, CD8 T cells, B cells, natural killer (NK) cells, and γδ T cells, including a subtype of cells that are called T cells, such as Th1, Th2, T regulatory (Treg) cells, and Th22 cells [16].
Macrophages are essential cells of the innate immune system that perform many roles in the body of the organism, such as support in development, maintenance of homeostasis, regeneration of tissues, and, together with this, providing immunity [17]. This traditional perspective, however, was refuted by more recent research: it was found that while circulating monocytes in the blood were to be responsible for only a small fraction of them, the majority originate from other sources in such organs as the brain, liver, kidney, lung, and heart [18]. This could mean that the macrophages can be activated in different ways to result in two phenjsons: M1 (proinflammatory) and M2 (anti-inflammatory). This pattern, therefore, modulates to balance the inflammatory and anti-inflammatory responses in great hosts of conditions in health and disease [19].
Hence, they have been designed in such a way that they will interact with monocytes/macrophages, for they play a crucial role in the mechanism of diseases. Correspondingly, the surface of the particles is appropriately functionalized to provide the targeting of macrophages with specific surface ligands, such as monoclonal antibodies, peptides, or small molecules, able to bind to receptors overexpressed on macrophages. The effects nanoparticles have on monocytes/macrophages may vary from stimulation of bone marrow and increasing mobilization of monocytes to modulation of the

permeability of microvessels and polarization of cells. Nanoparticles can reach the site of localization more precisely and avoid the immune system by using the natural properties of monocytes/macrophages [20].

Neutrophils, essential components of the innate immune system, play a crucial role in combating extracellular pathogens during acute inflammation [21]. Accounting for 50–70% of circulating leukocytes, neutrophils have a segmented nucleus and cytoplasm enriched with granules and secretory vesicles [21]. Their maturation involves passing through stem cell, mitotic, and postmitotic pools in the bone marrow under the regulation of specific transcription factors, proteins, and receptors. Neutrophils are recruited during inflammation through adhesion molecules, contributing to the elimination of pathogens via phagocytosis, degranulation, and the release of neutrophil extracellular traps. These front-line troopers not only scavenge invaders but also influence innate inflammation and contribute to the adaptive immune reaction. Researchers have designed various nanoparticles to target neutrophils, modulating the inflammatory microenvironment and alleviating inflammation in disease models. Mechanisms include the modulation of neutrophil migration, depletion of inflammation-related neutrophils, neutrophil-based drug delivery, and neutrophil biomimetic techniques.

Immune cells originate from hematopoietic stem cells (HSCs) located in the bone marrow. HSCs undergo differentiation, leading to the formation of common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) cells. CMP cells produce diverse subtypes of cells that include granulocytes, macrophages, megakaryocytes, and erythrocytes. On the other hand, CLPs form in various kinds of lymphocytes in which T and B lymphocytes form the most important constituent of adaptive immunity [22].

T cell maturation is centralized to the thymus once the pluripotent progenitors from the bone marrow migrate to seed the thymus. The maturation process includes complex steps, such as the gene rearrangement of T cell receptor genes and the surface expression of molecules like CD3, CD4, and CD8. The mature T lymphocytes include effector T (TE) cells, regulatory T (Treg) cells, and memory T (TM) cells, each further divided into CD4+ and CD8+ based on the surface markers [23]. Among the T lymphocytes, CD4+ T cells are helper T (Th) cells, regulators, and controllers of diverse types of immune responses by producing cytokines. On the other hand, CD8+ T cells, also known as cytotoxic T lymphocytes (CTL), mediate cellular immunity. Treg cells serve as suppressors and reduce the effecting actions. TM cells are responsible for immune memory and form the most important part of the immune response [24]. B cell maturation initiates in the bone marrow, and upon activation by antigens in peripheral lymphoid tissue, B lymphocytes can differentiate into plasma cells, producing antibodies and initiating humoral immunity [10]. Memory B cells, another product of B cell differentiation, enable a rapid response upon encountering the same antigen again [25]. Lymphocytes play a pivotal role in adaptive immune responses, and various nanoparticle platforms have been developed to modulate the immune system by targeting these cellular components for the management of inflammatory diseases. Mechanisms include inducing T cell-related immune tolerance, depleting auto-reactive T lymphocytes, and modulating B cell-related immune responses. Since the primary function of IS is to eliminate the recognized foreign bodies, therefore, a weak IS can affect the quality of life gravely. Furthermore, due to large surface to volume ratio, nanocarriers may cause immunotoxicity also. Major contributors to these toxicity issues of nanocarriers are associated to their shape, size, larger surface area and specially their Reactive Oxygen Species (ROS) production property. ROS are known to cause oxidative stress on cells causing them to start apoptosis [26]. Furthermore, these nanocarriers also cause inflammation, since they are regarded as a foreign

body by the immune system. It is also known that nanoparticles with larger size the 100 nm are easily eliminated by the immune cells [27].

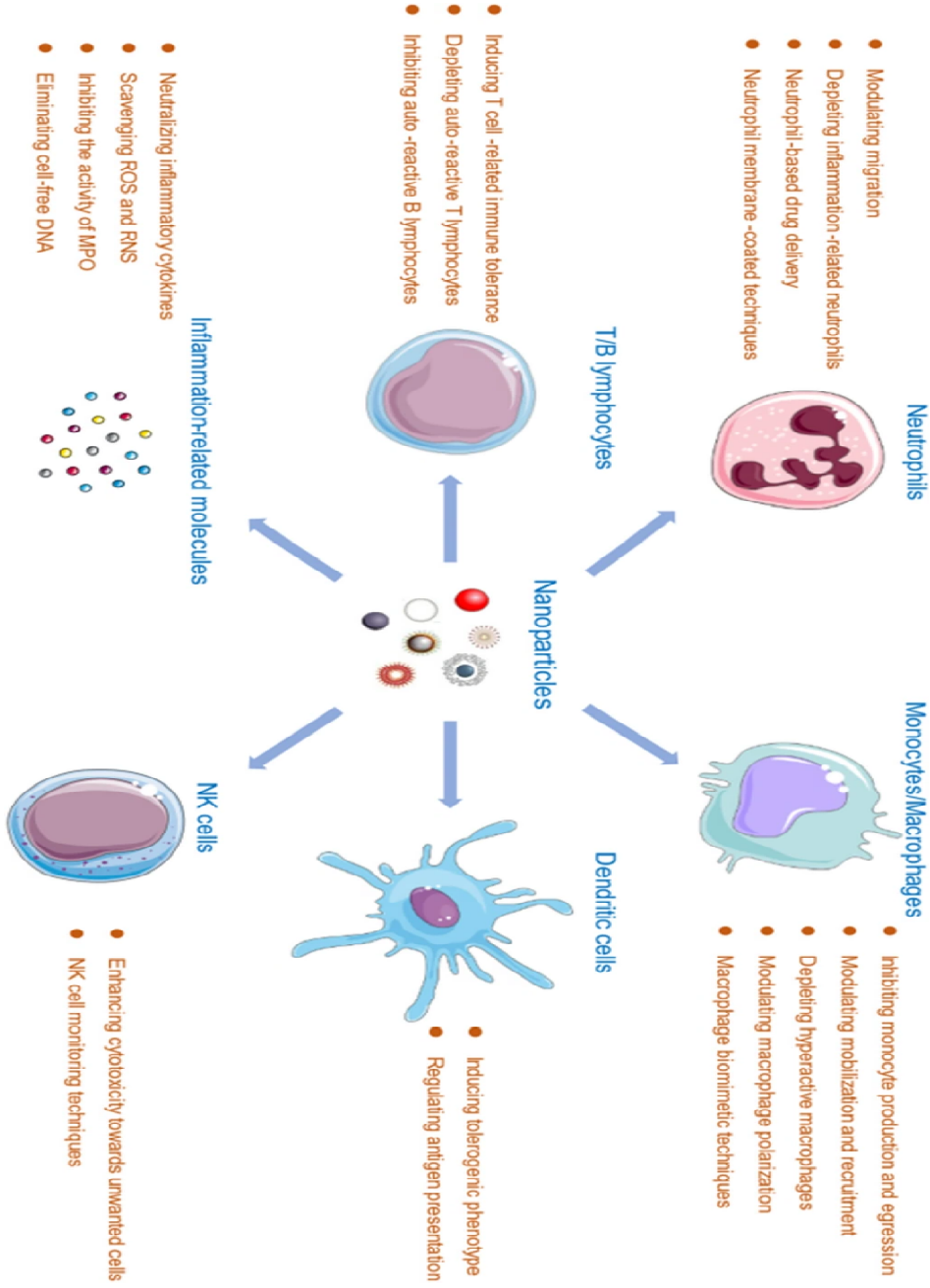

FIGURE 6.2
Potential interface between nanocarriers and parts/components of immune system.

Figure 6.2 shows schematics of nanocarriers/ nanoparticle's interaction with immune system (Figure adopted from [20], published under open access, Creative Commons (*CC*) License). Various types of nanocarriers are used to deliver drugs to targeted cites including carbon, polymer and metal and metal oxide based nanocarriers to be more common. Furthermore, there is vast gap needed to be filled by research to know how nanoparticles do behave when they come in contact with human blood. Immuno-toxic impacts of nanocarriers are broad spectrum based on the route of administration and size; the shape of nanocarriers, ranging from lungs, systematic damage, to the liver, and acute inflammation. If the nanocarriers are introduced intravenously, the nanocarriers can come in undesired contact with various blood components, including, Red Blood Cells (RBCs), White Blood Cells (WBCs), macrophages, and blood proteins which can alter the blood components, resulting in posing a threat to biocompatibility, safety, and biodegradability of the drug-carrying nanoparticles [28].

Scale-Up

Nanotechnology involves the integration of technology with various other disciplines, such as nano-chemistry, nanoelectronics, nanobiotechnology, nanomedicine, and more [29, 30]. Presently, a significant number of researchers are drawn to nanotechnology due to the profound alterations in material properties when they reach the nanoscale [31]. Many researchers are particularly focused on biomedical applications, aiming to develop nanomaterials capable of delivering drugs to specific body parts, thereby treating diseases with reduced systemic toxicity [32]. However, before utilizing these materials for their enhanced properties, they must be brought down to the nanoscale.

Two primary approaches are employed to achieve this: Bottom-Up and Top-Down [33]. In the Bottom-Up approach, atoms or molecules are assembled to reach the nano size, while the Top-Down approach involves breaking down bulk-sized materials or chemically reducing them to the nano scale range. Various methods, such as chemical, biological, physical, or biochemical degradation, are used in the Top-Down approach to obtain materials of nanoscale range [34]. Although controlling materials at the atomic or molecular scale is challenging and resource-intensive, breaking down bulk materials is comparatively easier.

The physical synthesis of nanomaterials follows the employment of various expensive and energy consuming instruments i.e., arc discharge, chemical vapour deposition, LASER ablation and many others. These instruments are quite sensitive and dangerous to operate since, LASERs can cause optical damage to the user, and they are so expensive to use that these methods can not be recommended or suitable for using them at industrial level. Furthermore, these methods lack control over the nanoparticle size and shape.

Compared to physical methods for nanoparticles/nanocarriers development, chemical synthesis methods and quite inexpensive, time saving, energy saving and come in handy, since they do not cover as much space as the instruments used in physical synthesis. In the chemical synthesis. There are various approaches, namely co-precipitation method, sol-gel and many more. The co-precipitation method, a widely used chemical method, is employed to obtain nanomaterials from bulk materials [35]. In brief, a precursor salt is used which is reduced with the help of a reducing agent, and at their birth, after reduction of precursor salt, the nanoparticles are very reactive on their surface, due to which they tend to attract each other and increase in size. To overcome this issue of agglomerations, a capping agent (most commonly a surfactant) is used, which has a hydrophobic end

and a hydrophilic end, which arrange themselves over the nanoparticles surface, to keep them in a particular size range. The chemical synthesis of nanoparticles comes with a few advantages i.e., quick synthesis, better control over size and shape, morphology. But on the contrary, this method has drawbacks, including the generation of bio- and environmentally toxic by-products [1].
Since discussed previously, the green chemical approach has various benefits over both chemical and physical synthesis methods, but it also needs use of one chemical at least, i.e., precursor salt. Being less toxic, economic and environmental and eco-friendly, this approach has a major issue also. That is consumption of a lot of plant material. Furthermore more, in this approach, the plant extract may be replaced with bacteria or fungi, but then there is issue of bacteria generating resistance against the nanomaterials and become stronger.
While generating materials at the laboratory scale for small-scale research is straightforward, scaling up the production to an industrial scale presents challenges [36]. Issues faced during scaling up synthesis include economic constraints, manpower, safety concerns, toxicity to workers, and significant environmental impacts. Additionally, controlling the size and shape of nanocarriers/nanoparticles is challenging due to the sensitivity of minor pressure and temperature changes, making it unlikely to reproduce identical materials under the same conditions [37]. The %yield of production also poses a hindrance to scaling up nanocarrier production, as the raw material costs vary. **Figure 6.3** shows various scale-up issues, Figure adopted from [38], published under open access, Creative Commons (*CC*) License).

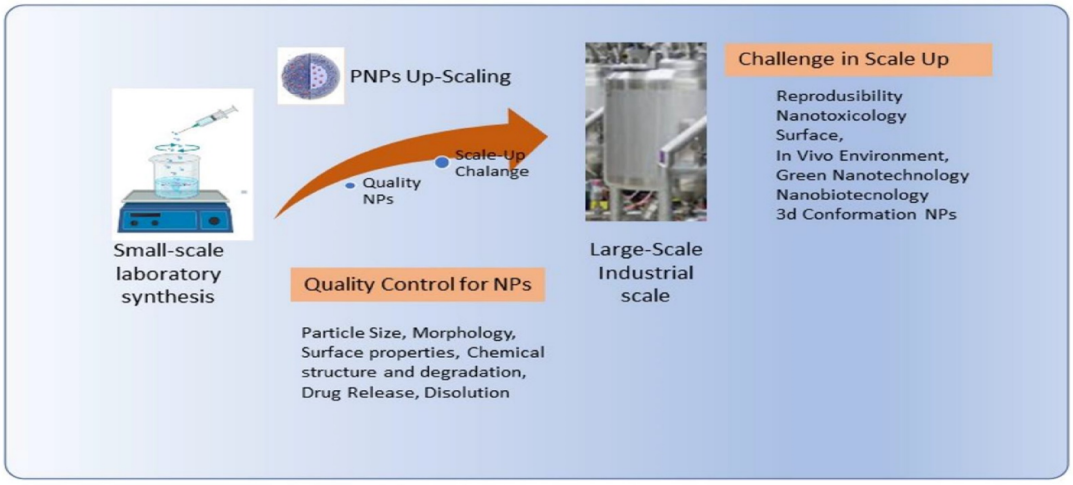

FIGURE 6.3
Possible Scale-Up issues for nanotechnology.

Despite the possibility of initiating nanocarrier production, concerns about the toxicological impacts on workers persist. Safety, bio- and environmental degradability of nanocarriers/nanoparticles remain unclear, discouraging approvals for large-scale production [39]. The degradation of nanomaterials in the environment is yet to be fully understood, including their behaviour, penetration, and shelf life before complete degradation. Furthermore, their interaction with microorganisms, animals, and plants in the environment requires careful attention. These combined

factors impede the development and scaling up of nanomaterials/nanocarriers for use in the medical industry.

Blood Brain Barrier (BBB)

Nanocarriers prove advantageous not only for delivering medicine to tumors in lower parts of the body but also for addressing upper body regions, such as the brain, owing to their small size and modified physiological, chemical, optical, and mechanical properties. Research into drug delivery for various neurodegenerative diseases is underway, focusing on nanocarriers' ability to traverse the blood-brain barrier (BBB) [40]. The BBB serves as a protective barrier, preventing larger or foreign substances and blood components from contacting the brain and central nervous system [41]. Due to the BBB's highly selective and semi-permeable nature, certain drugs intended for brain treatment face challenges. The primary functions of the BBB include maintaining transport regulation, tissue function, and protection of the brain and CNS [41]. Its structure comprises the capillary basement membrane, CNS endothelial cells, pericytes, and astrocytes [41].

Although progress has been made in understanding nanocarrier passage across the BBB in animal studies, their potential toxic impacts on the brain, CNS, and BBB remain incompletely understood. In one investigation, the bioavailability of PLA PEGylated nanocarriers across the BBB and penetration into neuronal cells has been noticed in zebrafish. It has reported crossing the BBB effectively but further studies were to be carried out for the overall evaluation of the toxicity of nanocarriers and nanoparticles. They may interfere with close contacts of endothelial cells, crossing the BBB. Recurrent or prolonged exposure to the penetrating BBB substances, causing possible damage to tight junctions, can result in dysfunction, uncontrolled material flux, and toxicity within the brain and CNS. An associated study on PEGylated PLA nanocarriers hatched zebrafish eggs successfully but incited drug-loaded nanoparticle-related safety problems for them to cross BBB [42]. This further goes to show that in an experiment using Silver (Ag) and Copper (Cu) nanoparticles on rat models, the blood-brain barrier (BBB) had been breached, and this consequently led to changes in myelin and glial cells, which indicate possible injury to nerve cells [43]. Taken together, these hurdles do not allow the use of nanoparticles in drug delivery to reach wide acceptance until the general research resolves toxicity and safety concerns, especially to the blood-brain barrier (BBB) and the central nervous system. In another experiment, one of the brain endothelial cell models, bEnd.3, was used in order to evaluate the size of nanoparticles that would cross the blood-brain barrier and would thus be able to exert an effect on brain tissues. The BBB is composed of endothelial cells joined by tight junctions and end-feet astrocytes covering the surface of the capillaries. The model of bEnd.3 represents only the first barrier between blood and brain. It is very important in influencing the probability of penetration of the BBB. The group found that the intracellular uptake of Au NPs exhibits a clear dependence on Au NPs size. The total gold content per cell was measured for various Au NPs sizes (20, 50, 70, and 110 nm). The results reveal that 70 nm Au NPs yield the highest gold uptake per cell (0.21 ± 0.03 ng, approximately 90% of the Au NPs), while 20 nm Au NPs exhibit a lower uptake of 0.12 ± 0.03 ng (around 50% of the Au NPs) per brain endothelial cell. Statistical analysis using a T-test indicates a significant difference (P value < 0.05) among the different sizes [44].

Upon quantitatively measuring the total gold amount bound to a single cancer cell using flame atomic absorption spectroscopy (FAAS), calculations were performed to determine the exact number of

nanoparticles and GNP surface area per cell. Evaluation of the total free surface area revealed that 20 nm GNPs had the maximum free surface area per cell, with a decrease observed as GNP size increased. A T-test on these results also showed a significant difference (P value < 0.05) among the different sizes [44].

The BBB allows normal transition through various mechanisms, including diffusion transport, carrier systems, and receptor-mediated endocytosis. In the context of this study, the entrance of gold nanoparticles (GNPs) into these cells signifies potential penetration through the BBB. It's important to note that while this in vitro model provides insights into normal BBB behavior, it may not accurately represent abnormal conditions such as those observed in brain tumors. **Figure 6.4** shows various types of nanoparticles and nanocarriers developed and the surface functionalizations that have been employed for nanoparticles go across BBB (Figure adopted from [45], published under open access, Creative Commons (*CC*) License).

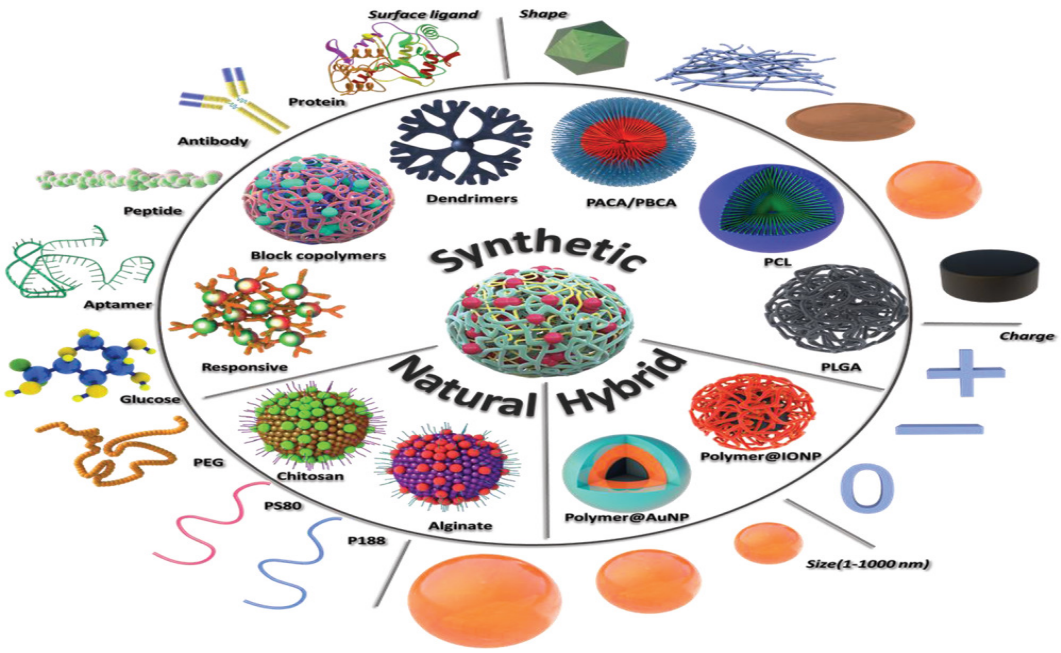

FIGURE 6.4
Nanoparticles and their Functionalization to cross BBB, (Figure adopted from [45], published under open access, Creative Commons (*CC*) License).

Stability of Nanomedicines

In addition to the challenges posed by the blood-brain barrier and the immune system, another significant issue that continually perplexes scientists and the scientific community regarding the utilization of nanomaterials for treatment, diagnosis, and prognosis purposes is the stability of nanoparticles as nanomedicines. It is well-established that nanoparticles necessitate a stabilizing

agent on their surface to prevent agglomeration and maintain stability. Ideally, a stabilizing agent possesses a hydrophobic head and a hydrophilic tail, aligning itself onto the nanoparticle surface to ensure stability. The stability of nanoparticles is commonly assessed through techniques such as UV-Vis Spectrophotometry, Surface Plasmon Resonance (SRP), and other spectroscopic methods. It has been indicated from the laboratory experiments that surface modifications of the nanoparticles tend to improve the stability of nanoparticles. For example, Sharma et al. have done work in which they synthesized Iron Oxide (hematite phase) nanoparticles coated with a range of agents like PVP, Citrate, and Starch. The capped nanoparticles were subjected to a thermal stability analysis compared with that of the uncapped Iron Oxide nanoparticles. The result was a more thermal effect on the capped particles than on the uncapped ones. In this connection, it is clear that the aspect of surface modification on increasing nanoparticle stability could be greatly important in providing important insights into prospective medical application [46].

They are found in various stability, which is affected by temperature, pH, and thermal conditions. Das et al. observed that the stabilization of the Au nanoparticles under study was effected by the stabilizers bovine serum albumin, aspartic acid, and citrate. The research noted the stability of the Au nanoparticles to these conditions, and the condition included changes in pH values and salt concentrations. The present study was also included to assess the toxicity of these Au nanoparticles on MRC-5 human fibroblast cell line. A series of tests by the research team concluded that all synthesized Au nanoparticle samples were minimal in their toxicity. However, stability presented quite different behaviors. In general, AuNPs stabilized with aspartic acid could show tendencies of aggregation, which became evident due to the loss of surface functionalization, especially in higher pH and salt concentrations. On the other hand, citrate-stabilized Au NPs showed poor stability with a tendency to grow at very high salt concentrations. It was noted that the Au nanoparticles coated on bojsonlution into the protein were very stable, dependent only on the cohesion of the chains. These reports give the insight into the nuanced attributes of stability of differently stabilized Au NPs, which are required in a deeper understanding of their functioning under diverse environmental and application conditions [47].

However, under some substrates or chemical species, the nanoparticles might exhibit stable behavior within the limits of a cuvette or a petri dish in a lab. Such dynamic environment experienced in human or animal bodies is a great contrast to such controlled conditions. The body pH levels and many other things present great challenges to these nanoparticles as they navigate through the complex biological milieu. Surface modifications of NPs with ligands, antibodies, or proteins are therefore implemented so that the NPs bear such specificity. Whereas it is an interaction of stabilizing surfactants with these biomolecules, the more pressing question is: what is the interaction of the surface-modified nanoparticles, biomolecule or the surfactant, with the human blood and their stability in this complex bioenvironment? Key considerations include whether nanoparticles will maintain stability upon introduction into the body or if their surface stabilization may be compromised, leading to a change in their target specificity. Addressing these questions requires extensive research to comprehensively understand how nanoparticles behave within the physiological context and whether their surface modifications withstand the challenges posed by the intricate and dynamic environment of the human or animal body.

Clearence of Nanomedicines

Another critical factor that can impede the utilization of nanoparticles as nanomedicines is their clearance rate from the human or tested animal body. There are 2 major ways of nanoparticles clearance, hepatic or renal [48]. As Shown in **Figure 6.5** (figure adopted from [48], published under open access, Creative Commons (*CC*) License). It is well-established that nanoparticles, like other compounds such as drugs or biomolecules, inevitably encounter the liver in some capacity. The liver, being a vital organ responsible for detoxification, plays a pivotal role in clearing toxins from the body. In the scenario where a naked, uncapped nanoparticle manages to enter the body and evade the immune system, persisting within the bloodstream, the likelihood of it bypassing the liver is quite low. The liver is adept at recognizing and intercepting foreign entities, directing them to be excreted from the body. Thus, the liver's efficient clearance mechanisms pose a significant obstacle for nanoparticles, underscoring the challenges in achieving sustained circulation and targeted delivery within the complex physiological environment.

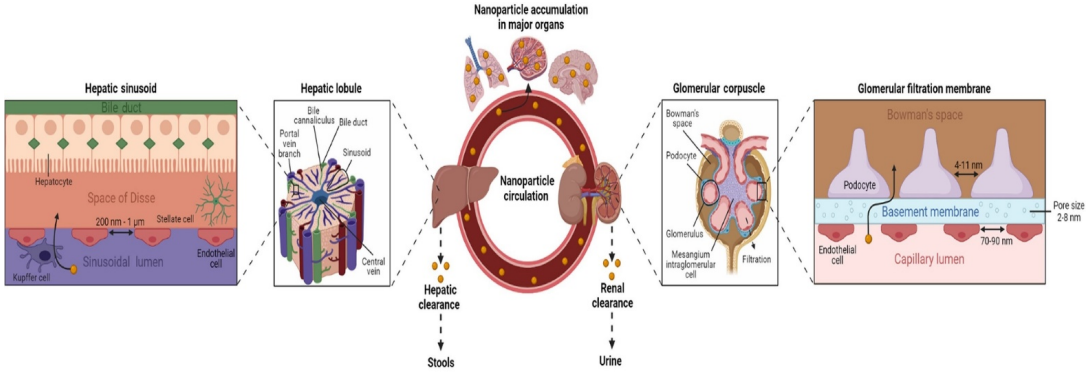

FIGURE 6.5
Two Possible Pathways for Nanoparticles to clear the body i.e., Renal and Hepatic (figure adopted from [48], published under open access, Creative Commons (*CC*) License).

Certainly, advancements in technology have enabled scientists to explore innovative ways of introducing various biomolecules and compounds that facilitate the unhindered movement of nanoparticles within the body. This is fundamental to the field of nanomedicine, aiming to enhance the retention time and bioavailability of drugs and nanoparticles for improved therapeutic outcomes. The challenge, however, lies in the effective clearance of nanoparticles from the body once they have served their purpose. If nanoparticles are unstable, there is a risk of them becoming uncapped in the bloodstream, potentially being directed to the liver for excretion. On the contrary, stable nanoparticles that successfully reach the target site and deliver their cargo raise questions about their behavior post-cargo release. Understanding whether stable nanoparticles accumulate at the site of delivery or are efficiently cleared from the body after releasing their payload is a critical consideration for scientists and researchers. These factors play a pivotal role in determining the safety and efficacy of nanoparticles as a delivery system in medicine. As research progresses, addressing these complexities will contribute to the responsible and effective utilization of nanoparticles in medical applications.

Loynachan, C. N., and group synthesized Au nanoclusters and studied them for their potential to treat diseases like cardiovascular disease and cancer. The size of synthesized Au nanoclusters was 11.3nm on average and they found that almost 73% of the nanoparticles were leaving the mouse body via urination within 1 hour of introduction. The study on urine was conducted with the help of colorimeter [49]. Although this study was promising that nanoparticles, largely, clear the body, but then it raises question on the bioavailability of the nanomedicines.

Regulatory and Ethics

While the FDA and the European Medicines Agency (EMA) have approved several nanomedicine products for cancer therapy, there is currently a lack of specifically implemented guidelines for drug products containing soft materials by these regulatory bodies. The absence of such guidance means that regulatory decisions regarding nanomedicine therapeutics are made through individual assessments of benefits and risks, resulting in a time-consuming process that demands expertise in innovative technologies. This situation may lead to significant regulatory delays. The regulatory challenges in nanomedicine also play a crucial role in the advancement of state-of-the-art technologies for characterizing and monitoring the quality of nanomedicine products, in addition to the clinical trials and approval processes. There is a pressing need for comprehensive guidelines addressing the characterization and quality control of nanomedicine products. Encouragingly, progress is being made in this area, with the gradual establishment and enhancement of definitions, guidelines, and cooperation efforts. For instance, the FDA released the guidance for industry titled "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology" in June 2014, defining nanomaterials as engineered materials with at least one dimension between 1 and 100 nm [50]. Furthermore, United States Environmental Protection agency (U.S. EPA) defines nanomaterials as, "…chemical substances that are solids at 25 °C and atmospheric pressure and that are manufactured or processed in a form where the primary particles, aggregates, or agglomerates are in the size range of 1–100 nm (nm) and exhibit unique and novel characteristics or properties because of their size. The proposed rule would apply to chemical substances containing primary particles, aggregates, or agglomerates in the size range of 1–100 nm in at least one dimension." [51]. Whereas European Union (EU) defines Nanomaterials as, "Nanomaterial is a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm." [52]. According to these definitions, a material can be defined or classified as nanomaterial if it has size distribution in 1 – 100nm, even in the agglomerated form, it can be the general definitions, while according to EU, if, at least 50% of the agglomerated materials have size distribution on 1 – 100 nm size range, they are defined as nanomaterials. Collaboration initiatives, such as those between the FDA and the European Technology Platform on Nanomedicine (ETPN), along with partnerships with the Nanotechnology Characterization Laboratory (NCL) and European Nano-Characterization Laboratory (EU-NCL), aim to facilitate regulatory reviews and in-depth characterizations of nanomedicine products. The critical demand for regulatory agencies in the field of nanomedicine therapeutics is to refine and standardize requirements for approving safe nanomedicine products. Additionally, the development of more advanced and multifunctional tools is necessary, even if it complicates the approval process.

For That is about all about the materials, but plastics, have been found in various sizes also, as the number of publications on the hazardous impacts of micro plastics, have increased and the microplastics have become a point of discussion in the scientific community. Now, the question is, if the plastics can reach micro size range, is it possible that they can also reach the nano scale range? and if so, what will be the societal implications? How will they impact human health and our ecosystem? And furthermore, how will they impact the living things, i.e., plants, animals, aquatic life? There have been various investigation sand concerns about the nano-plastics and there have been various regulatory authorities that have given definitions on the nano-plastics. The United States National Oceanic and Atmospheric Administration (US-NOAA) defines microplastics as, "Plastic fragment with size less than 5mm in length" [53], while the United Nations (UN) and International Standards Organization (ISO) defines the microplastics as "non-soluble plastic particles with size in-between 1 – 5 mm, while nano-plastics are plastic particles with size less than 1µm" [54], while European Food Safety Authority (EFSA) defines nanoparticles as the plastic materials with external dimensions between 1 – 100nm [55].

Although these definitions are in contradiction to one another, still, they provide us with a rough definition, but there should be one definition, as the definition of nanomaterials is standard throughout the globe. Furthermore, there is a grave need of authorities that define and keep a check on the practices being followed around the globe for the production, use and their introduction to the environment and analyse the toxic impacts of these nanomaterials and nano-plastics around the world.

Conclusion

Although nanoparticles have been highly appreciated for their biomedical applications and their excellent drug delivery capabilities. Initially naked nanoparticles were being employed for their biomedical potential but due to their potential toxicity and possible accumulation inside the organs, encouraged scientists to develop nano-carriers, which take the medicine to a specific target. Although it decreases the toxicity by many folds, yet there is need for in depth analysis and evaluation of various factors that could cause potential toxicity.

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