Nanoparticles types and properties – understanding these promising devices in the biomedical area

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ABSTRACT

Biomaterials field has evolved with the development of novel nanostructures. Specifically, nanoparticles (NPs) can be designed to obtain nanodevices for drug delivery, imaging and/or diagnosis in medicine. The study of the different types of NPs, as well as their properties represents a crucial knowledge to develop suitable NPs for a particular purpose.

Keywords: nanoparticle size, nanoparticle polydispersity index, nanoparticle shape, nanoparticle surface electrical potential, nanoparticle types, nanoparticle applications

1. Introduction

Currently available methods for drug delivery are characterized by daily or more frequent administrations. Despite several routes may be used to deliver the drug, not all of them are suitable for all types of molecules. In fact, oral route cannot be used to deliver drugs that may be degraded in the digestive tract. Frequently, the available methods for drug delivery have low solubility in aqueous media, reducing the bioavailability of the drug. The recurrent side-effects are also a problem of these methods. Then, various alternative candidates to effectively and safely deliver drugs have been investigated.

Biomaterial devices have been tailored at a micro- and nanoscale to be used in medicine. The devices can be produced with different shapes (i.e. particles, fibers, rods, discs) for drug delivery of a variety of molecules.

Microparticles (MPs), defined as particles whose size ranges from 1 to 1000 μm have been studied. They can be administered by different routes, such as intranasally, subcutaneously or orally. Additionally, they have high specific surface area, when comparing with macroparticles, which ensures high quantity of functional groups for chemical reactions. Stable suspensions containing MPs characterized by narrow size dispersion and presenting constant size along time, can be obtained. Gellan-gum, chitosan or dextran are just examples of a broad range of biomaterials used to produce these MPs. MPs can be tailored to avoid clearance mechanisms from the body, by increasing for example their hydrophilicity. Moreover, they can be targeted to specific anatomical sites of the human body. When used as drug delivery devices, MPs are able to entrap efficiently active agents, taking into account the hydrophobic or hydrophilic drug character, protecting the drug from undesired premature degradation and interactions with the biological environment.

Nanoparticles (NPs) are regarded as nanoentities whose size ranges from 10 to 1000 nm. Besides sharing all the above-mentioned MPs advantages, NPs also have the capability to overcome several limitations of MPs. In fact, due to their smaller size, they present much higher specific surface area, because the total surface area of a particle is inversely proportional to its diameter. Moreover, this reduction in size enables an easier administration. As a matter of fact, NPs are more suitable for parenteral administration, while MPs are commonly used as implants, since the capillaries have diameters in the range of 5 - 6 μm. Additionally, the uptake efficiency of nanostructures into mice gastrointestinal tissue was reported to be 15 – 250 times higher than that of particles with sizes in the range of 1 - 10 μm. Furthermore, NPs have sizes in the same range of the entities controlling basic cellular functions. In addition, some properties are size-dependent and only found in the nanoscale, such as optical response or magnetism, allowing their use also as diagnosis/imaging devices.

2. NPs properties

The properties of the NPs influence their behavior in vivo. Particularly, morphological properties like shape and size, can influence NPs circulation and targeting within the body. These properties are also responsible for variations in the degradation rate of NPs and drug release kinetic. The shape and size of NPs are also responsible for specific cell signaling. NPs surface properties and the presence/absence of targeting ligands can also influence NPs behavior within a biological system. Being all these NPs properties somehow related, it is difficult to define which of them will ensure a particular biological effect. Additionally, small variations in only one of these properties can, in fact, potentiate enormous changes in the other NPs performance.

2.1 Size and polydispersity index

Ideally, NPs should be in circulation until they reach the target anatomical site. However, the immune system can contribute to their elimination, due to NP recognition by the reticulum endothelial system (RES). Alternatively, mechanical filtration by the lungs, liver, kidneys or spleen can lead to NPs clearance. Size, among other properties, is very important regarding particle elimination. A schematic representation of the distribution of particles with different sizes after intravenous administration is presented on Figure 1. Studies have reported that MPs larger than 7 μm are filtered mechanically, being entrapped in the capillary network of the lungs. Particles with diameters in the range of 0.1 - 7 μm are detected by the RES in the liver or spleen, being phagocytized by Kupffer cells or by spleen macrophages, respectively. If the particle
polydisperse suspension can lead to unexpected important parameter because the presence of a given suspension. Basically, the suspension contains particles with increasingly different sizes as the value of the PDI increases. NPs PDI, besides NPs size, is a very important parameter because the presence of a polydisperse suspension can lead to unexpected variations in the NPs behavior.

Figure 1: Expected body distribution of intravenously administration of particles with different sizes.

Size also affects the rolling velocity, diffusion and adhesion of particles. In fact, it was shown that larger microspheres roll faster than smaller microspheres. Regarding NPs adhesion, as their size increases, their attachment rate decreases. This fact can influence cellular internalization of NPs because this phenomenon can be influenced by a previous step of particle adhesion to cells. This can be explained by the need of specific and essential interactions in order to endocytosis occur. Works dedicated to study the influence of the size of gold NPs in the cell internalization have inconsistent results. While some researchers argue that smaller gold NPs are better internalized than larger NPs, others claim that, no linear correlation exists and that there is an optimal NP size for cellular internalization. This incongruence might be explained by the use of different cell types with different biological characteristics.

NPs diameter and surface area also have a fundamental role in drug delivery applications, if the drug release relies on NPs matrix degradation. For instance, poly(lactic-co-glycolic acid) (PLGA) NPs and MPs were used to assess the kinetics of degradation and release of loaded proteins. At initial time points, smaller particles presented faster degradation and protein release rates than larger particles.

Additionally to the size, it is important the polydispersity index (PDI) of NPs suspensions. This index gives information about the sizes of NPs present in a given suspension. Basically, the suspension contains particles with increasingly different sizes as the value of the PDI increases. NPs PDI, besides NPs size, is a very important parameter because the presence of a polydisperse suspension can lead to unexpected variations in the NPs behavior.

2.2 Shape

Recent reports have suggested a significant role of particle shape (sphere, ring, disc) in the circulation of NPs, distribution within the body, cellular uptake and general in vivo behavior. The influence of the shape in the NPs transport within the human body was already evaluated in several works. While spherical particles move freely, particles with irregular geometry present much higher probability to align or tumble in vessels bifurcations or filtering organs. For a spherical particle to pass through the spleen, it must have less than 200 nm in diameter. However, if it is a disk-shape with a diameter around 7 \( \mu \text{m} \) and 150 \( \mu \text{m} \) in height, it can pass through this organ. Additionally, it was already shown that nanospheres and nanocylinders are internalized more promptly than longer filaments, in vitro. The circulation time after intravenous injection also depends on the shape. Spherical and cylindrical NPs were compared after intravenous injection in mice, and the results showed that cylinders are able to persist longer in circulation. The interactions of albumin, present in the blood, with gold NPs is also dependent on NPs shape. Cubic gold NPs were able to induce strong unfolding effects in albumin than the spherical counterparts. Recent studies have also shown that particles having identical chemical composition but different shapes have different cytotoxicities: nanowires proved to be more toxic than spherical NPs. A recent review covers the influence of non-spherical NPs in the delivery an anti-tumor drug.

2.3 Surface properties

Distinct surface properties, such as hydrophobicity and surface charge, have been used to characterize NPs. Hydrophobicity has huge relevance since it influences NPs clearance from the body due to RES action. Indeed, a decrease in the hydrophobicity leads to reduction of the nonspecific interactions with proteins. As a consequence, phagocytosis by macrophages is diminished.

NPs surface electrical potential, also known as zeta potential, is the potential of a particle or molecule due to its charge in a certain medium and gives the tendency for the particles to undergo aggregation. In fact, as the absolute value of this surface electric potential increases, the repulsion between NPs is intensified. Positively charged NPs are more nonspecifically internalized than their neutral or negatively charged counterparts. Specifically for both dendritic cells and macrophages uptake, similar conclusions were reported. In addition to charge-dependent, the tendency for internalization is also cell-dependent.

2.4 Targeting

After administration, it is desired that NPs, contrarily to other alternative therapies, have the ability to target a specific anatomical site (Figure 2), in order to reduce side effects over healthy tissues. To achieve this goal, targeting approaches, either passive or active, can be
used. In the passive targeting, it is exploited the physicochemical/structural characteristics of the target site used to adjust the NPs properties. As an example, NPs size can be reduced enough to allow taking advantage of the enhanced permeation and retention mechanism present in tumors, characterized by their characteristic leaky vasculatures. On the other hand, in the active targeting, molecules are linked to the surface of the NPs to produce actively targeted NPs. These molecules can be ligands, monoclonal antibodies, engineered antibody fragments, proteins, peptides, carbohydrates, nutrients, or aptamers. The active targeting mechanism takes advantages of the highly specific interactions between the molecule present in specific tissues, cells or organelles within the body and a molecule linked at the surface of the NPs.

Micelles are stable due to their hydrophilic shell and have prolonged circulation time in the blood.

A dendrimer is morphologically characterized by a branched structure grown from one or more cores. The size of these NPs is easily controlled by the number of generations that are allowed to grow over these cores. Dendrimers present difficulties regarding drug incorporation and release, being their synthesis quite time-consuming.

Liposomes are vesicles made entirely of lipidic compounds. The most common are the unilamellar liposomes whose size usually ranges from 100 to 800 nm. These spherical structures are made of amphiphilic compounds and present high production cost and content leakage. As main advantages, they are completely biodegradable, compatible, non-toxic and non-immunogenic.

Compact polymeric NPs are nanostructures made entirely of natural or synthetic polymers. They are usually more stable than liposomes allowing sustained localized drug delivery for weeks, with reduced drug leakage. In these polymeric nanostructures, the therapeutic agent can be eventually linked covalently. Alternatively, it can be adsorbed at the NPs surface or dissolved or entrapped within the NPs structure (nanospheres) or encapsulated inside a polymeric shell (nanocapsules).

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An intermediate type of NPs is the core-shell polymer-lipid hybrid NPs. In its structure a biodegradable hydrophobic polymeric core and a lipidic outer monolayer are present. Alternatively, an inner polymeric core surrounded by an external lipid bilayer can be used. Core-shell polymer-lipid hybrid NPs bring together complementary characteristics of both structures, namely higher stability, enhanced drug encapsulation yield and superior in vivo cellular delivery efficacy. As drug delivery systems, the drug is usually encapsulated in the polymeric core, whereas the lipid outer layer reduces water diffusion rate, slowing down the kinetics of drug release. Alternatively, hybrid NPs can be composed of an inorganic core surrounded by an

Figure 3: Main types of NPs used in various applications; image not at scale.
organic shell, namely a metallic core surrounded by a polymeric shell.

Quantum dots, nanometric multifunctional inorganic fluorophores used in imaging, detection and targeting, are luminescent semiconductor crystals. They are made of elements from groups II–VI or III–V, being their structure generally based on cadmium sulfide (CdS) and cadmium selenide (CdSe), that can be highly toxic. As advantages, compared to traditional fluorophores (organic dyes and fluorescent proteins), quantum dots present a broad absorption range and narrow emission spectra. In fact, they have tunable size emission with different wavelengths over a broad range of the light spectrum. Additionally, quantum dots present high photostability, being remarkably resistant to photobleaching. The use of quantum dots is based on their unique chemical and physical properties, achieved due to their size and highly compact structure.

NPs can also be simply made of carbon molecules with various highly symmetric and stable forms, called fullerenes (allotrope of carbon). Buckminsterfullerene (C_{60}), the most well-known fullerene, is a rigid icosahedron with 60 carbon atoms. In its structure, single bounds form pentagons and double bounds form hexagons. Fullerenes disadvantages, such as the low solubility in organic solvents, are overcome by their unique optic, electric and magnetic properties (such as superconductivity), rendering them important devices in medical diagnosis and imaging.

Inorganic materials, such as gold, silver, platinum and silica, can also be used to produce NPs. Inorganic NPs can be prepared by different methods, forming a highly ordered and rigid three-dimensional arrangement with metal or covalently linked atoms. Their properties, such as size and shape, are almost not influenced by the in vivo conditions, unlike organic NPs. However, inorganic NPs drawbacks have to be taken into account. In the case of metallic NPs, the impossibility to load drugs into their structure and their possible negative effects in blood have to be considered. However, their high potential as magnetic responsive nanoentities is of great importance and has been extensively reviewed in the literature. On the other hand, silica NPs have associated a cytotoxic effect related with an increase of reactive oxygen species levels and decrease of glutathione levels.

4. Biomedical applications

NPs have been produced to deliver drugs, proteins/peptides and genes, to be used in various biomedical areas including cancer therapy and vaccination. In fact, NPs can be used in various administration routes, such as oral, nasal, parenteral or intracellular, representing and efficient and effective improvement over current methods.

As drug delivery devices, NPs have been widely explored as biodegradable carriers due to their great advantages over conventional systems such as: enhanced delivery (increase the stability of pharmaceutical agents), use of lower amounts of expensive drugs, extended drug bioactivity by protecting it from environmental effects in biological media and more effective treatment with minimal side effects. Ideally, these drug delivery nanosystems are characterized by a single dose treatment, allowing a controlled drug release profile along time, characterized by a drug concentration within the therapeutic window. This will allow surpassing the multiple dosages causing a variable concentration, which characterize commonly available therapies (Figure 4).

The mechanism behind the release of the therapeutic compound from the NPs may be mediated by surface or bulk erosion, chemical or biological degradation, diffusion through the pores or release from the surface. Additionally, also an external stimulus can trigger the drug release. Sometimes, more than one of these mechanisms occurs simultaneously. Detailed information on the mechanisms and factors/parameters affecting drug release from specific types of NPs are reported elsewhere.

Figure 4: Concentration profile of common therapies (red line) and controlled release along time (green line).

Additionally to the characteristics already mentioned for drug delivery systems (i.e. biocompatible, non-toxic, controlled release of the loaded compound), in the case of gene delivery, NPs needs also to ensure high transfection efficiencies. Genetic material, such as DNA or siRNA, was incorporated into NPs, allowing to protect the genetic material from nuclease degradation. Similarly to drugs, the genetic material should be released in a controlled way (characterized by a suitable dose during a certain time period), in order to ensure controlled gene expression. The genetic material needs to be entrapped within the matrix, being released due to the combined effects of diffusion and matrix degradation.

Certainly for gene therapy and sometimes for drug/protein delivery, NPs need to be internalized by the target cells to obtain an intracellular action. Two main groups of endocytic pathways for the internalization of nanocarriers have been identified: phagocytosis and pinocytosis. Among this last pathway, receptor-mediated endocytosis (clathrin-mediated endocytosis or caveolin-mediated endocytosis), clathrin/caveolin-independent endocytosis and macropinocytosis have been described in the literature (Figure 5). The cellular uptake of NPs depends on the surface charge, size, shape or cell type. Indeed, while phagocytosis is typically related with the uptake of large particles, pinocytosis is...
associated with the uptake of fluids and solutes. On the other hand, while phagocytosis is related to cells with phagocytic capacity (macrophages, neutrophils, monocytes and dendritic cells), pinocytosis is a more general phenomenon, occurring in all types of cells.

**Figure 5:** Cell-NPs interaction due to antibody-antigen.

As important as the overall stability and bioactivity of the loaded cargo, its delivery in the biological location (specific cells or tissues) is also required. Taking advantage of the receptor-mediated endocytosis, NPs can be tailored to deliver intracellularly their cargo to a specific population of cells (Figure 6). Exploitation of the unique cell surface markers present in a specific cell population allows promoting drug delivery. In fact, loaded NPs can be surface modified with antibodies, peptides and other disease-targeting moieties for disease treatment, as referred previously.

Beyond their use in therapy, NPs can also be utilized in combined functionalities, like theranostics (therapy + diagnosis). Several examples are already available in the literature. For instance, polymers like PLGA and PCL have been used for theranostics purpose. For example, hybrid NPs containing a polymeric part (PLGA) loaded with a drug (doxorubicin for cancer treatment) and a magnetic part (magnetic nanocrystals used as ultrasensitive magnetic resonance imaging (MRI) probes) linked to specific antibodies (responsible for the targeting action) allow the treatment, targeting and imaging of breast cancer. In other work, PCL NPs were prepared by a double-emulsion process in which a drug (Stilbene) and iron oxide NPs (used as imaging devices) were included to obtain the final formulation.

**Figure 6:** Schematic representation of the main types of endocytosis.

5. **Conclusions**

Despite the tremendous evolution in the NPs field in the last years, a lot of improvements are still needed to routinely use those medical devices. For instance, knowing NPs size after their production following certain process conditions is a real obstacle to develop highly reproducible therapies. Additionally, the complete study of how a variation in the production parameters affects NPs properties is another limitation. The creation of NPs enabling the release of a specific compound in both spatial and temporal controlled way is still an open problem. The complexity involved in all these studies has hampered the development of efficient and suitable NPs for specific biomedical applications. The development and implementation of a totally automatic equipment able to surpass the inherent variability of the operator and applicable to all materials and conditions can be a possible solution to increase NPs reproducibility. Another issue regarding NPs production is the scale-up. Indeed, some of the NPs production methods cannot be easily performed in an industrial scale needed to create a product available in the market. As important as all just-mentioned issues is the creation of a laboratorial platform able to mimicking the in vivo environment specific of a living organism. It will be a powerful and unique tool in the NPs development, since in vitro studies lack several biological cues and events that can influence the performance of NPs. This could avoid the use of animals in a first stage and render NPs testing with less ethical constraints.

6. **References**


