REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

A Brief Review on Nanomedicine and the Therapeutic Applications of Gold Nanoparticles

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Abstract

Nanoparticles have gained a great interest due to their pharmaceutical and medical uses. The applications of nanoparticles rely on the small particle size, surface charge, intensity and bioavailability of nano-sized particles. The nano-biotechnologies in which the nanoparticles are used as carriers for active ingredients can provide more specific drug targeting and delivery, reduction in toxicity while maintaining therapeutic effects, greater safety and biocompatibility, and faster development of new safe medicines. Different types of nanoparticles are widely used today; including zinc oxide nanoparticles (ZnONPs), silver nanoparticles (AgNPs), titanium dioxide nanoparticles (TiO2), silica nanoparticles (SiNPs) and gold nanoparticles (AuNPs). The AuNPs have different types, gold nanoshells; gold nanocages, gold nanosphere (AuNSs) and gold nanorods (AuNRs). Much research has been carried out on the medical applications of AuNPs. The AuNPs now have many pharmaceutical and medical applications because of their investigated and proved antibacterial, anticancer, anti-inflammatory and antioxidant activities.

Keywords: Drug delivery; nanomedicine, gold nanoparticles.

1. Introduction

Nanomedicine is the science of diagnosing, treating and preventing disease with the use of molecular biology combined with nanotechnology, which is an emerging sub-discipline **(Datta et al., 2006)**. Nanotechnology is a collective term for technologies where the characteristic size of the end product ranges between one and one hundred nanometers **(Taniguchi, 1974)**. The prefix "nano" derives from the Greek word for "dwarf". One nm is equal to one billionth of a meter, or about the width of 6 carbon atoms or 10 water molecules **(Whitesides, 2003)**.

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-100 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained **(Kommareddy et al., 2005)**. Nano-capsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while

nano-spheres are matrix systems in which the drug is physically and uniformly dispersed **(Langer, 2000)**.

Recently, biodegradable polymeric nanoparticles (PNPs), particularly those coated with hydrophilic polymer such as poly ethylene glycol (PEG) known as long circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time and target a particular organ **(Lee and Kim, 2005)**. They can act as carriers of DNA in gene therapy, and might be able to deliver proteins, peptides and genes **(Bhadra et al., 2002)**.

2. Characterization of Nanoparticles

Nanoparticles characterization is based on the size, morphology and surface charge, using such advanced microscopic techniques as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). There are several factors like size, distribution and average particle diameter that affect the physical stability and the *in vivo* distribution of the nanoparticles (Sonavane et al., 2008). Certain properties like surface morphology, size and overall shape are measured by electron microscopy techniques **(Schrand et al., 2010)**.

2.1. Particle Size

The smaller the size of nanoparticles the larger the surface area, resulting in fast drug release. Significant drug release could be caused by exposing to the particle surface area. On the other side, slow diffusion of larger particles occurs inside the nanoparticle drugs. Therefore, smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion, that's why there is a mutual compromise between maximum stability and small size of nanoparticles **(Singh and Lillard, 2009)**.

2.2. Surface Charge

The interaction of nanoparticles with biological environment, e.g. mitochondria, is determined by surface charge and intensity. Zeta potential is a major determinant for colloidal material stability, which is an indirect measure of the surface charge that can be obtained by evaluating the probable difference between the outer Helmholtz plane and the surface of shear. Thus, zeta potential of colloidal based dispersion assists in evaluating its storage stability. Zeta potential values are aiming to ensure stability, avoid aggregation of the particles and evaluate surface hydrophobicity and the nature of encapsulated material within the nanocapsules or coated onto the surface **(Pangi et al., 2003)**.

2.3. Surface Hydrophobicity

Recent studies on analytical tools for surface property analysis of nanoparticles are still under investigation. One of the modern techniques is X-ray photon correlation spectroscopy; not only does it determines surface hydrophobicity, but also it can identify specific chemical groups on the nanoparticles surface **(Sharma, 2017) (Figure 1)**.

2.4. Drug Release

Release of nanoparticles could be measured by drug loading capacity. It is defined as the amount of drug bound per mass of polymer, or in another term it is the moles of drug per mg polymer, or it could be also calculated as percentage relative to the polymer. Different techniques like UV spectroscopy or high-performance liquid chromatography (HPLC) are used to determine this parameter. Methods that are used for drug release analysis are also corresponding to drug loading assay which is more often assessed for a period of time to evaluate the drug release mechanism **(Khanbabaie and Jahanshahi, 2012)**.

3. Bioavailability of Nanoparticles

Physicochemical and molecular complexity of drugs and *in vivo* inaccessibility of most drug targets presents the most challenging concern to deliver specific drugs to their site of action at therapeutically relevant levels. Drug targeting has evolved as the most desirable but elusive goal in drug delivery science **(Jia, 2005)**.

Poor drug solubility makes it very difficult to perform high-throughput screening of compounds for potential drug effects. Therefore, there is an urgent need for intelligent drug formulations to achieve sufficient bioavailability. Many different approaches have been developed to overcome the solubility problem of poorly soluble drugs including solubilisation, inclusion compounds, and complexation **(Jia, 2005)**. An alternative to these methods is drug nanoparticle formulation. The basic advantage of nanonization is increases in surface area and concentration gradient of these poorly soluble compounds followed by an increased dissolution rate of the compounds

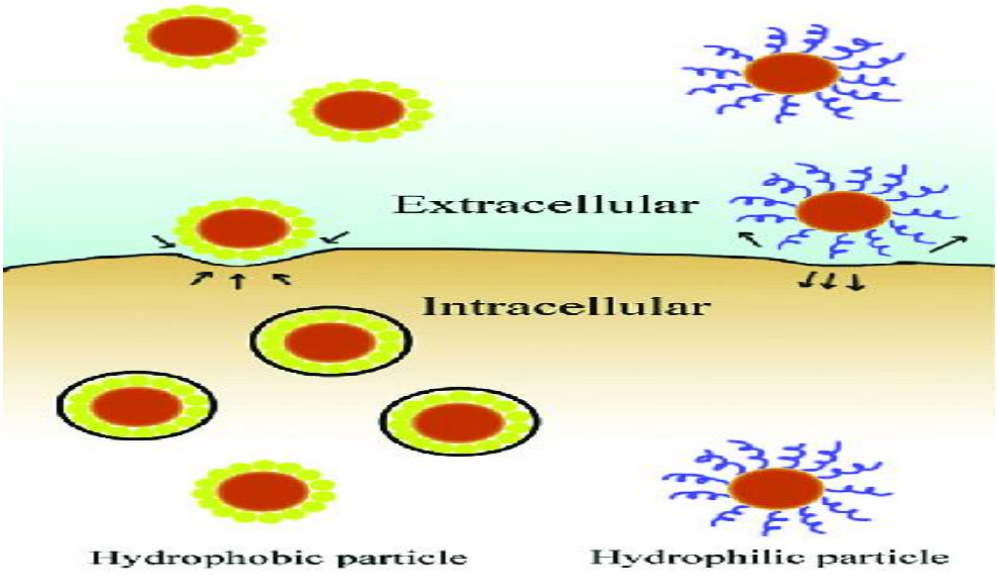

Figure 1: Nanoparticles with different hydrophobicity present different affinity with the cell membrane **(Kou et al., 2013)**

according to the Noyes-Whitney equation **(Noyes and Whitney, 1897)**. In addition, the saturation solubility is also increased after nanonization. All these may benefit oral bioavailability of poorly soluble drugs by enhancing drug transport through a gut wall into the systemic circulation.

4. Nanoparticles and Drug Delivery

Drug delivery and related pharmaceutical development in the context of nanomedicine should be viewed as science and technology of nm scale complex systems (10–100 nm), consisting of at least two components, one of which is a pharmaceutically active ingredient **(Duncan, 2003; Ferrari, 2005)**, although nanoparticle formulations of the drug itself are also possible **(Baran et al., 2002; Cascone et al., 2002; Duncan, 2003; Kipp, 2004)**.

The whole system leads to a special function related to treating, preventing or diagnosing diseases, sometimes called smart-drugs or theragnostics **(LaVan et al., 2003)**. The primary goals for research of nano-biotechnologies in drug delivery include:

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility, and
- Faster development of new safe medicines.

The main issues in the search for appropriate carriers as drug delivery systems pertain to the following topics that are basic prerequisites for design of new materials. They comprise knowledge on (i) drug incorporation and release, (ii) formulation stability and shelf life, (iii) biocompatibility, (iv) bio-distribution and targeting and (v) functionality. In addition, when used solely as carrier, the possible adverse effects of residual material after the drug delivery should be considered as well. In this respect biodegradable nanoparticles with a limited life span as long as therapeutically needed would be optimal **(Gaurav et al., 2012)**.

4.1. Cellular and Intracellular Targets

For drug delivery, not only organ or cellular targeting is of importance but also the fate of the nanoparticles within the cells. Particles generally end intracellular in endosomes or lysosomes followed by degradation. For activity of the encapsulated drugs release into the cytosol is needed. However, for nanoparticles of about 20 nm; also cellular uptake without contribution by endocytic mechanisms was demonstrated **(Edetsberger et al., 2005)**.

Chemical characteristics such as surface charge may also determine the fate of nanoparticles in cells. Surface functionalization of AuNPs with PEG resulted in efficient internalization in endosomes and cytosol, and localized in the nuclear region **(Shenoy et al., 2006)**.

Poly D, L-lactic-co-glycolic acid (PLGA) nanoparticles were found to be ingested by cells by endocytosis. The escape from these endosomes into the cellular cytoplasm was suggested to be caused by a change in surface charge form negative to positive of the PLGA nanoparticles resulting in cytoplasmic delivery of the incorporated drugs **(Panyam et al., 2002; Konan et al., 2003)**.

Surface modifications of nanoparticles offer possibilities for medical applications like drug targeting in terms of cellular binding, uptake and intracellular transport. Carbohydrate binding ligands on the surface of biodegradable and biocompatible PLGA nanospheres were found to increase cellular binding **(Weissenböck et al., 2004)**.

Such increased adherence may lead to an enhanced activity of the drug presented as or incorporated in nanoparticles. Coupling specific proteins such as antibodies to the nanoparticle surface may enable a more specific immunologically directed targeting of the particles **(Nobs et al., 2004; Prinzen et al., 2007)**.

4.2. The Brain as a Target for Drug Delivery

From several perspectives the brain is a challenging organ for drug delivery. First, the incidence of degenerative diseases in the brain will increase with the aging population. Secondly, the blood brain barrier (BBB) is well-known as the best gatekeeper in the body toward exogenous substances **(Pardridge, 2007)**.

Generally, pharmaceuticals including most small molecules do not cross the BBB. The endothelial barrier is specifically tight at the interface with the brain astrocytes and can in normal conditions only be passed using endogeneous BBB transporters resulting in carrier mediated transport, active efflux transport and/or receptor mediated transport. However, the barrier properties may be compromised intentionally or unintentionally by drug treatment allowing passage of nanoparticles **(Olivier et al., 1999; Kreuter et al., 2003; Lockman et al., 2003; Koziara et al., 2006)**.

Passage of the BBB was suggested to be possible by the toxic effect of nanoparticles (about 200 nm) on cerebral endothelial cells **(Olivier et al., 1999)**. Physical association of the drug to the nanoparticles was necessary for drug delivery to occur into the brain **(Kreuter et al., 2003)**.

When nanoparticles with different surface characteristics were evaluated, neutral nanoparticles and low concentrations of anionic nanoparticles were found to have no effect on BBB integrity, whereas high concentrations of anionic nanoparticles and cationic nanoparticles were toxic for the BBB. The extent of brain uptake of anionic nanoparticles at lower concentrations was superior to neutral or cationic formulations at the same concentrations. So, nanoparticle surface charge must be considered for toxicity and brain distribution profiles **(Lockman et al., 2004)**.

5. Therapeutic Uses of Some Nanoparticles

5.1. Titanium Dioxide Nanoparticles

Engineered nanoparticles form a major fraction of man-made nanomaterials currently escalating in both development and commercial implementation **(Shi et al., 2013)**. Among the engineered nanomaterial, titanium dioxide (TiO2) nanoparticles are one of the most highly manufactured in the world and are widely used in paints, printing ink, paper, cosmetics, pharmaceuticals, sunscreen, bio-medical ceramic and implanted biomaterials, industrial photocatalytic processes and decomposing organic matters in wastewater **(Anselmann, 2001; Lowe, 2002).**

Concerns regarding the potential health risks of these nanoparticles have been raised due to their inherent physicochemical attributes such as small size, increased surface area, conductivity and aggregation potential. Studies on the bio-distribution of TiO2 nanoparticles have indicated the liver as one of the principal sites in the body for accumulation through intentional ingestion or indirectly through nanoparticle dissolution from food containers or secondary ingestion of inhaled particles **(Meena and Paulraj, 2012; Ma et al., 2009)**. A study demonstrated that one of the TiO2 particle surface coatings produced increased pulmonary inflammation compared with other formulations containing different surface coatings **(Okuda et al., 2002)**.

5.2. Silica Nanoparticles

For silica nanoparticles, both *in vitro* toxic and non-toxic responses were observed. Both 15 nm

and 46 nm silica nanoparticles showed similar dose dependent cytotoxicity *in vitro*. There was an increase in toxicity both at increasing doses and at increasing exposure time. SiO2 exposure resulted in an increased reactive oxygen species (ROS) levels and reduced glutathione levels indicating an increase in oxidative stress **(Lin et al., 2006)**.

Chang et al. (2007) found silica nanoparticles to be toxic at high dosages as shown by reduction in cell viability/cell proliferation and by lactate dehydrogenase (LDH) release from the cells indicating membrane damage. Cells with a long doubling time were more susceptible for the cytotoxic effects of the silica nanoparticles than cells with short doubling times.

In another study, only at concentrations above 0.1 mg/ml a significant reduction in cell viability was observed In addition, an alveolar macrophage cell line (MHS) was found to be more susceptible for nanaoparticle induced cytotoxicity than a lung epithelial cell line (A549) which was suggested to be due to the phagocytic properties of the macrophage cell line **(Jin et al., 2007)**. In contrast, for cationic silica nanoparticles using amino-hexyl-amino-propyltrimethoxysilane as a surface modification, low or no cell toxicity was observed **(Kumar et al., 2004)**.

5.3. Silver Nanoparticles

Silver nanoparticles have proved to be most effective because of its good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms **(Gong et al., 2007; Rai et al., 2009)**. Silver nanoparticles are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions etc. (**Sharma et al., 2009; Rai et al., 2009)**. Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity **(Junggwon et al., 2008)**. Studies have already reported the successful biosynthesis of silver nanoparticles by plants such as *Azadirachta indica* **(Shankar et al., 2004)**, and *Capsicum annuum* **(Bar et al., 2009)**.

5.4. Alloy Nanoparticles

Alloy nanoparticles exhibit structural properties that are different from their bulk samples **(Ceylan et al., 2006)**. Bimetallic alloy nanoparticles properties are influenced by both metals and show more advantages over ordinary metallic nanoparticles **(Mohl et al., 2011).**

5.5. Magnetic Nanoparticles

Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (MRI) **(Fan et al., 2009)**.

5.6. Zinc Oxide Nanoparticles

Zinc oxide nanoparticles have widespread applications. They are used to prevent sunburn, as biosensors, food additives, pigments, resin production and electronic materials **(Catherine et al., 2003)**. Zinc oxide nanoparticles have negative effects on bacterial growth like staphylococcus and streptococcus, so they can prevent spreading of epidemic diseases as an etiological agent **(Sharma et al., 2009; Zheng et al., 2009)**. Other studies on zinc oxide nanoparticles showed that they can also inhibit *E. coli* growth *in vivo* **(Deng et al., 2009)**.

Kamel and El-Dawy (2017) demonstrated that a significant increase in gene expression of IGF-1 and GH genes were shown in groups treated with 60 and 90 mg/kg ZnONPs and these genes improve growth performance resulting in improving growth traits in broiler chicken compared to control group. While there was a significant decrease in gene expression of myostatin gene which interferes with muscle growth in the same groups compared to control group.

5. Gold Nanoparticles (AuNPs)

Metallic colloidal AuNPs are widely used, can be synthesized in different forms (rods, dots), are commercially available in various size ranges and can be detected at low concentrations. Cells can take up AuNPs without cytotoxic effects **(Connor et al., 2005; Shenoy et al., 2006)**. For biomedical applications, they are used as potential carriers for drug delivery, imaging molecules and even genes **(Kawano et al., 2006)**, and for the development of novel cancer therapy products **(Hirsch et al., 2003; Hainfeld et al., 2004; Loo et al., 2004; O'Neal et al., 2004; Radt et al., 2004).**

Gold solutions are also used to prepare nanoshells

Figure 2: Different shapes of AuNPs **(Bolaños et al., 2019)**.

composed of gold and copper, or gold and silver to function as contrast agents in MRI **(Su et al., 2006)**, and gold-silica for photothermal ablation of tumor cells **(Benardi et al., 2007; Stern et al., 2007)**. *In vitro*, the non-targeted nanoshells did not show cytotoxicity for the tumor cells, whereas after binding to the tumor cells, cell death could be obtained after laser activation **(Lowery et al., 2006; Benardi et al., 2007; Stern et al., 2007)**. Also *in vivo* positive results were obtained with photothermal ablation therapy in a mouse model for colon carcinoma after intraveneous administration of PEG coated gold nanoshells of approximately 130 nm **(O'Neal et al., 2004)**.

5.1. Types of AuNPs **(Cai et al., 2016)**: Gold nanoshells, Gold nanocages, AuNSs and AuNRs **(Figure 2)**.

Gold Nanoshells: The core of gold nanoshells is made up of silica and outer surface is made up of gold. Gold controls the thickness of the shell.

Gold Nanocage: Through galvanic replacement, gold nanocage is synthesized by the reaction between truncated silver nanocubes and equeous Gold (III) chloride solution (HAuCl).

AuNSs: AuNSs are synthesized by reduction of an aqueous HAuCl by using citrate as reducing agent. Through citrates / gold ratio the size of nanospheres can be controlled. The size of nanopheres can be affected by thiol/ gold molar ratios.

AuNRs: AuNRs are synthesized by template method. They are prepared by electrocrochemical deposition of gold within the pores of nanoporous Polycarbonate template membranes. AuNRs diameter is based on the diameter of the pore of the template membrane. The AuNRs have promising applications in drug delivery and plasmonic photothermal therapy (PPTT). They have unique optical and photothermal properties and are easy to be synthesized.

5.2. Applications of AuNPs

5.2.1. Antibacterial Activity of AuNPs

The AuNPs are used as bacteria targeting particles in antibacterial therapy. The therapy targets bacteria with light absorbing AuNPs (10 nm, 20 nm, 40 nm) conjugated with specific antibodies, thus selectively kill bacteria using laser. Studies has shown the effectiveness of this method in killing Staphylococcus aureus, which is a significant human pathogen responsible for a wide range of diseases such as skin and wound infections, toxic shock syndrome, septic arthritis, endocarditis, and osteomyelitis. In this system, the bacteria damage is caused by inducing strong laser, which leads to overheating effects accompanied by the bubble- formation phenomena around clustered AuNPs **(Zharov et al., 2006)**.

5.2.2. Management of Osteoporosis

AuNPs have been shown to be the most effective material for treating bone diseases because of their potential use not only for inhibition of osteoclast differentiation but also for stimulation of osteoblast formation **(Ko et al., 2015)**. Also, AuNPs exhibited no *in vivo* toxicity. Previous reports have shown that 30 nm AuNPs did not cause any significant damage to internal organs, despite their accumulation in the liver, spleen, and kidney after intraperitoneal injection **(Heo et al., 2014)**.

5.2.3. Anticancer Activity of AuNPs

AuNPs have the advantage of delivering drugs specifically onto the target sites with unique features, including tunable surface characteristics and microenvironment stability, along with negligible side effects **(Tom et al., 2004)**. AuNPs accumulate in specific sites, which could be utilized to trace the path of cancer cell **(Qian et al., 2008)**. They provide microscopic probes for the study of the cancer cell. Their photo physical properties can be exploited for drug release at

remote place **(El-Sayed et al., 2006)**. They acquire several advantages that enhance their potential as important agents in nanotechnology. In addition to their absorption and scattering properties, AuNPs can absorb light and switch it into heat. This property can be used to induce killing of cancer cells though protein denaturation and induction of apoptosis. Photothermal therapy also allow for monitoring of the process that eventually will lead to the death of the cancer cells **(Huang et al., 2011)**.

Accordingly, **Zharov et al. (2006)** made use of this property for photothermal therapy of Hodgkin Lymphoma. Two AuNPs-antibody conjugates; one of them was combined with an anti-CD30 receptor which binds to CD30 on the surface of L-428 Hodgkin cells and the other with an anti-CD25-receptor as a control. High killing power was achieved using appropriate does of laser irradiation and gold concentration for gold-targeted L-428 cells with little to no effect on neighboring non-targeted cancer cells. These data further support previous findings for the potential use of AuNPs as a safe modality for treatment of cancer.

5.2.4. Vaccine

AuNPs can play an important role in the vaccination field as adjuvants, reducing toxicity, enhancing immunogenic activity, providing stability of vaccine in storage, and having great potential as carriers for the development of a great diversity of fully synthetic vaccines **(Alberto et al., 2015; Tao et al., 2015)**. Their shape and size can affect immunological responses *in vivo* and *in vitro* **(Niikura et al., 2013)**. Moreover, they are able to penetrate blood vessels and tissue barriers and to be targeted to a specific cell by means of specifically functionalized molecules **(Popescu and Grumezescu, 2015)**. Moreover, AuNPs can be packaged inside virus-like particles generated by heterologous expression of viral structural genes that are powerful tools in vaccine development **(Freivalds et al., 2014)**.

5.2.5. Anti-inflammatory Activity of AuNPs

AuNPs-dependent down-regulation of IL-1 B – induced inflammatory reactions. This has been addressed both in vitro (cell culture) and in vivo. Furthermore, **Sumbayev et al. (2013)** suggests possible clinical implications of AuNPs against different types of widely distributed IL-1 B-dependent autoimmune disorders (rheumatoid arthritis, scleroderma, psoriasis). According to **Tsai et al. (2007)** AuNPs decreased the levels of proinflammatory cytokines and macrophage infiltration in a model of arthritis. **Dohnert et al. (2012) and Victor et al. (2012)** have demonstrated the anti-inflammatory action of AuNPs in the treatment of tendonitis and muscle damage in animal models.

5.2.6. Antioxidant Activity of AuNPs

BarathManikanth et al. (2010) demonstrated that AuNPs were antioxidative agents that inhibited the formation of ROS and scavenged free radicals to improve antioxidant defense enzymes. AuNPs elicited important action against oxidative damage in biomolecules, including the addition of free SH groups associated with the decreased profile of antioxidant **(Paula et al., 2015)**.

Mehanna et al. (2022) also demonstrated that the effect of AuNPs is shape and dose dependent. The repeated 5 days IV 50 nm AuNRs doses over 15 days showed a significant antioxidant effect, with no considerable toxicity.

5.3. Biodistribution of AuNPs

Biodistribution studies in animal models have shown that following intravenous injection, AuNPs are rapidly cleared form the bloodstream by the reticuloendothelial system, mainly accumulating in the liver and spleen. Consequently, short plasma half – life and prolonged tissue retention constitute major obstacles for clinical use of AuNPs **(Dykman and Khlebtsov, 2011)**.

Zhang et al. (2010) reported that the administration of 2.2 mg/kg bw/day AuNPs caused them to appear in the blood and in bone marrow cells. Gold was found in the liver, kidneys, blood, lungs, heart, brain and spleen. In addition, approximately 0.05% of the administered gold was foundin 24 h urine, suggesting this as a route of elimination.

De Jong et al. (2010) indicated that TEM detection of AuNPs may only be achievable within certain levels of accumulation, since AuNPs may not be evenly distributed in the tissues, which emphasizes the importance of employing different endpoints to assess NPs effects.

5.4. Toxicity of AuNPs

AuNPs have increasingly been investigated as inert carriers for medical purposes. Humans are exposed to gold from various sources. Non-oral sources include jewelry and during the manufacturing of gold containing products **(Hamilton and de Gannes, 2011)**. Oral sources include food, dental fillings, tobacco and pharmaceuticals **(Wittsiepe et al., 2003)**.

Regarding genotoxicity, *in vitro* studies indicated that AuNPs induce DNA damage in mammalian cells, including DNA strand breaks and chromosomal damage. *In vivo*, AuNPs induced genotoxic effects in Drosophila melanogaster, however, adequate genotoxicity studies in mammals are lacking **(Hadrup et al., 2015)**.

AuNPs are significantly more toxic when entering cells via endocytosis as opposed to those mainly entering through energy independent mechanisms directly into the cytosol, and that for a large set of metal containing nanoparticles, their toxicity is mainly ascribed to them in situ degradation and intracellular release of toxic ions **(Stefania et al., 2014)**.

6. Conclusion

Nanoparticles now can be used in different medical applications including diagnosis, preventing and treating diseases. The therapeutic effect varies according to the particle size, shape, dose and rout of administration. Through the potential use of nanotechnology in nanomedicine, full attention is needed to safety and toxicological issues. Further clinical studies are required to assess the safety of certain nanoparticles before large scale production and therapeutic usage due to the little available data about toxic health hazards of nanoparticles *in vivo* and *in vitro*.

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