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Review Article

Nanotechnology and Drug Delivery: Recent Applications and Future Challenges

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HIGHLIGHTS

- Advances in nanotechnology have revolutionized fabrication and development of novel materials with diverse theranostic applications.
- Gold nanoparticles (GNPs) have been nominated as promising candidates in delivery of protein and peptide drugs.
- A deep understanding of biological responses to bare nanoparticles and their complex form is a prerequisite to produce low risk hybrid nanocarrier systems.

ABSTRACT

Today, nanotechnology has paved the way for developing a new generation of delivery systems for potential drugs, proteins, peptides, and genes of interest. This effort reviews a couple of recent reports on application of gold nanoparticles in protein/peptide delivery, with a glance at toxicological aspects of nanoparticles and potential challenges in the upcoming future.

Keywords:
Gold nanoparticles
Nanocarrier systems
Peptide delivery
Toxicity

Introduction

Recent reports have witnessed unprecedented growth of research and applications in nanoscience and nanotechnology (Huang et al., 2011). There is increasing optimism that nanotechnology will bring remarkable advances in diagnosis and treatment of different diseases (Peer et al., 2007; Godin et al., 2010; Brambilla et al., 2011; Huang et al., 2011). So far, a variety of biomedical applications has been proposed for nanomaterials including drug delivery, *in vitro* and *in vivo* molecular diagnostics, nutraceuticals, biomedical imaging, biocompatible materials of improved properties (Figuerola et al. 2010; Huang and El-Sayed 2010; Jayakumar et al., 2010; Thanh et al. 2010; Lev Dykman et al., 2012; Tohidi Moghadam and Ranjbar, 2015; Mohseni et al. 2016), and the list could go on and on.

Drug delivery and related pharmaceutical development in the context of nanomedicine is considered as science and technology of hybrid systems at nanometer scale, consisting of a pharmaceutically active ingredient (Duncan, 2005). However, at times, it is possible to formulate the candidate drug at nanoscale, without using nanoparticles or nanomaterials as the carrier system (Duncan, 2005). Either in the complex form or nanoformulation, the overall system plays a critical role in developing functions related to treating, preventing or simultaneous diagnosis and treatment of diseases, i.e. theranostics (Jong and Borm, 2008). The primary goals of nanobiotechnologists in drug delivery research are drug targeting and delivery with high specificity, low toxicity (meanwhile maintaining therapeutic effects), more safety and biocompatibility, and also development of new safe medicines.

To design new materials of therapeutic characteristics and appropriate carrier systems for successful delivery

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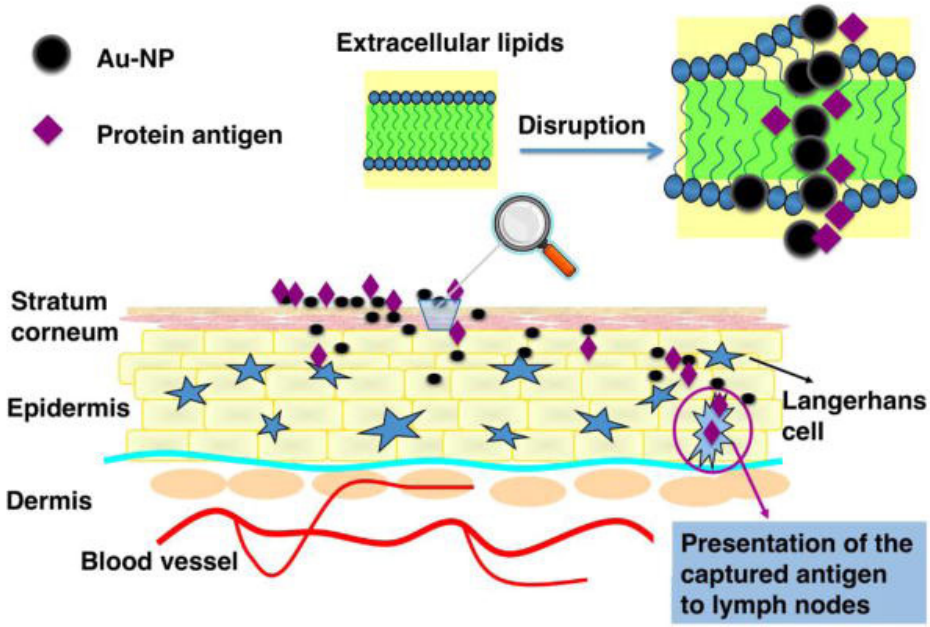

Figure 1. Transcutaneous antigen delivery mediated by GNPs (Huang et al., 2010).

to the targeted area, a number of factors still make the achievements challenging. Such issues comprise knowledge of (i) drug incorporation and release, (ii) formulation stability and shelf life (iii) biocompatibility, (iv) biodistribution, targeting, and (v) functionality (Borm and Muller-Schulte, 2006; Jong and Borm, 2008). Moreover, when nanoparticles are used as carrier systems, possible adverse effects might occur, which should be carefully considered. Sometimes the residual material itself might induce toxicity after delivering the drug to the sight of interest. In this respect, biodegradable nanoparticle, as well as biocompatible one with a limited life span (as long as therapeutically needed) would be optimal (Jong and Borm, 2008). Another important issue in the context of drug/protein/peptide/gene delivery by nanoparticles has risen due to the fact that nanoparticles might induce a number of alterations in the structure of biomolecules. Once the structure of biomolecule has undergone irreversible structural/conformational changes, it might severely lose its function prior to reaching the targeted area for desired therapeutic action. So far, results of many reports have encouraged possibility of using nanoparticle based drug delivery systems. On the other hand, fundamental investigations on the structure and function of biomolecules with various nanoparticles as carrier candidates are scarce (Tohidi Moghadam et al., 2011). It is also worth noting that the final fate of nano drug delivery systems with fruitful function and efficacy critically depends upon the type of nanoparticle, its size, surface charge, morphology, etc. (Chakraborty et al., 2011; Tohidi Moghadam et al., 2011; Tohidi Moghadam et al., 2012; Dai et al., 2016).

Based on evidences of fruitful applications of nanotechnology in medicine, this mini review has focused on a number of recent reports on utilization of gold nanoparticles, as promising candidates in delivery of protein and peptide drugs, as well as an overview of other nanomaterials in the context of drug delivery systems and their future challenges.

Percutaneous delivery of peptide drugs

Huang et al. have reported an interesting nanoscale interfacial phenomenon mediated by gold nanoparticles (GNPs). Co-administration of protein drug with 5 nm GNPs enabled skin permeation and percutaneous delivery of the drug (Huang et al., 2010). The researchers suggested that the phenomenon might occur due to the nano-bio interaction with skin lipids and the consequent induction of transient and reversible openings in the stratum corneum. They also noticed that upon simultaneous utilization of GNPs, the protein drugs could penetrate the skin barrier and migrate into the deep layers. Therefore, co-administration of a potential drug with the skin-permeable GNPs can mediate proteins across the skin barrier. The results of this effort highlighted a simple, yet effective method of overcoming the skin barrier for percutaneous protein drug delivery (Fig. 1).

More importantly, the method is considered to be a non invasive strategy for developing novel vaccine delivery techniques. With topical co-administration of antigens and gold nanoparticles, robust immune responses were shown in the tested animals. As a consequence, achieving a needleless and self-administrable transcutaneous vaccination can be expected in the upcoming future.

Peptide cancer vaccines delivery

It has been noticed that gold nanoparticles are promising vehicles for cancer immunotherapy, with demonstrated efficacy in immune delivery and innate cell stimulation. Nevertheless, their potency for delivery of peptide cancer vaccines and *in vivo* applications needs to be deeply assessed. Almedia et al. assumed that the immune distribution and adjuvant qualities of GNPs could facilitate delivery of the ovalbumin (OVA) peptide antigen and the CpG adjuvant, enhancing their therapeutic effect in a B16-OVA tumor model (Almedia et al., 2015). As anticipated, GNPs-mediated delivery of OVA (GNPs-OVA) and CpG (GNPs-CpG) increased the efficacy of both agents, with strong antigen-specific responses. Furthermore, the researchers found that using GNPs-OVA delivery without CpG was sufficient to promote significant antigen-specific responses, leading to subsequent anti-tumor activity and prolonged survival in both prophylactic and therapeutic *in vivo* tumor models. Such enhanced therapeutic efficacy might be attributed to the adjuvant effect of peptide coated GNPs, as the complex system induced inflammatory cytokine release when cultured with bone marrow dendritic cells (Almedia et al., 2015). The significant therapeutic effect of GNPs-mediated peptide delivery system (without the use of adjuvant) evidenced that AuNPs are effective peptide vaccine carriers with possibility of decreasing adjuvant doses for safer vaccination (Almedia et al., 2015).

Nanoparticle mediated laser transfection of proteins

Molecular medicine has faced many challenges for efficient and targeted delivery of proteins into mammalian cells. To overcome the problems, nanotechnology is proposed to have a key solution. Nowadays, nanoparticle-laser interactions are widely used in cell manipulation processes. In the light of large cross section of plasmonic nanoparticles and ability to strongly absorb electromagnetic radiation at a characteristic wavelength, Heinemann et al. presented a new protein transfection technique via laser scanning of cells incubated with gold nanoparticles (Heinemann et al., 2014). The plasmonic effects of the gold nanoparticles upon absorption of laser radiation induced a transient permeabilization of the cellular membrane, allowing proteins to enter the cell. The researchers tested this strategy to monitor possibility of delivering green fluorescent protein (GFP) into mammalian cells. Heinemann et al. noticed that GFP was delivered with an efficiency of 43%, maintaining a high level of cell viability.

Furthermore, functional delivery of an apoptosis mediating protein i.e. Caspase 3 was tested in several cellular assays (Heinemann et al., 2014). The researchers claimed that compared to conventional protein transfection techniques such as microinjection, gold nanoparticle mediated laser transfection enables high-throughput transfection of about 10000 cells per second. Moreover, a well-defined point in time of delivery is guaranteed, providing detailed temporal analysis of cellular pathways and protein trafficking (Heinemann et al., 2014).

Functional nano-capping agents for targeting drug delivery

Recently, Ganchao Chen et al. designed a novel stimuli responsive drug delivery system, using mesoporous silica nanoparticles (MSNs), benefiting from its high porosity, good tunability and ease of functionalization (Chen et al., 2016). End-capped MSNs has the potential to display "zero premature release" prior to reaching target tissues or cells, whereas it shows controlled drug release upon exposure to specific internal/external stimuli. So far, numerous MSN-based controlled release systems have been developed using variety of pore-blocking agents, such as macromolecules, nucleic acid, polymers, inorganic nanoparticles (INPs), proteins, or peptides. Once the drug carrier reaches the target, control of the payload release is usually achieved via specific stimuli, either internal stimuli such as the presence of certain biomolecules (e.g. ATP or enzymes), redox potential, pH, or external stimuli such as light, temperature, ultrasound, etc. (Chen et al., 2016). The researchers designed a hierarchically constructed pH-responsive nano-carrier utilizing peptide functionalized gold nanoparticles as the gate-keeper for targeting drug delivery (Fig. 2). They used gold nanoparticles as MSNs' pore capping agent due to good biocompatibility, facile modification with thiol containing ligands or peptides, and desirable physicochemical features for constructing multi-functional nano-carriers (Chen et al., 2016). The researchers functionalized MSNs via a pH sensitive linker with negative charge, which degrades in the acidic environment in endo/lysosome. On the other hand, GNPs were coated with oligo-Lysine containing peptides to hold positive charge, and RGD peptide to enhance the targeting efficiency towards cancer cells (Chen et al., 2016). Under neutral condition, the pores of MSNs were decorated and capped by a layer of gold nanoparticles and peptide to inhibit premature leakage. Upon endocytosis, the acidic environment of the endo/lysosome (pH 4.0-6.0) induced rapid hydrolysis of the linker, resulting in a charge

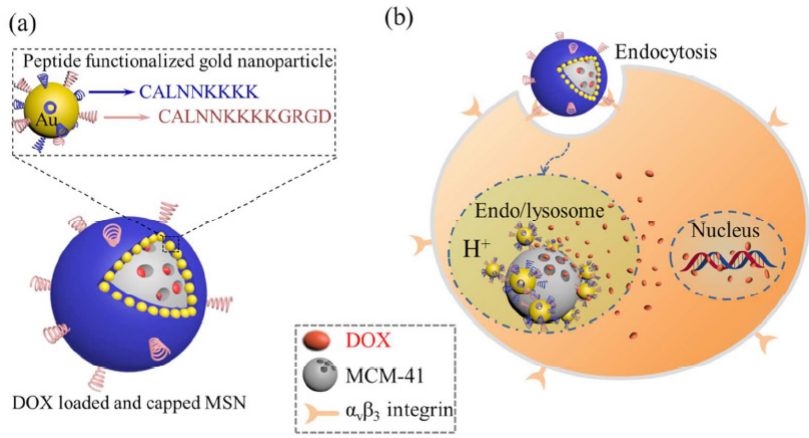

Figure 2. A new pH-responsive drug delivery carrier; (a) Schematic illustration of the GNPs coated with mixed peptides and the drug loaded and end–capped MSN with RGD peptide residue for cancer cell targeting. (b) The cellular uptake and intra-organelle removal of the gate-keeping GNPs due to charge reversal under acidic condition (Chen et al., 2016).

reversal of the MSNs and removal of the nano-capping agent and release of the entrapped drug (Doxorubicin). The incorporation of RGD peptide facilitated targeting delivery to $\alpha_v\beta_3$ integrin overexpressing cancer cells (Chen et al., 2016). The system designed in this report can serve as a platform for developing diversified multifunctional nanocomposites with variety of functional inorganic nanoparticles and bioactive peptides (Chen et al., 2016).

Future challenges

So far, a considerable amount of data has been released on the toxicity of engineered NPs, such as fullerenes (Yamakoshi et al., 2003; Oberdörster 2004; Sayes et al., 2005; Lovern and Klaper, 2006), carbon nanotubes (Shvedova et al., 2003; Lam et al., 2004; Warheit et al., 2004; Sayes et al., 2006; Monteiro-Riviere et al., 2005), dendrimers (Svenson and Tomalia, 2005; Duncan and Izzo, 2005), quantum dots (Hardman, 2006; Cho et al., 2007), and (Derfus et al. 2004; Hoshino et al., 2004; Kirchner et al., 2005; Hoshino et al., 2007).

Nevertheless, this data is mainly based on a limited category of nanoparticles (Oberdörster, 2004). In most studies, the nanoparticles have been used as a model of ambient air particle toxicity. One of the more general conclusions is that nanoparticles tend to be more toxic than larger particles with the same chemical composition. For nanoformulations used in drug delivery systems, most reports have mainly focused on taking proper strategies to reduce toxicity of the incorporated drug; whereas the possible toxicity of the nanocarrier itself has not been considered. In particular, it is possible that residues of a typical treatment may harbor potential local and/or systemic toxic responses.

The use of Nanotechnology in developing a new generation of drug delivery system is set to spread rapidly. The pharmaceutical industry has been seriously seeking novel nanoformulations strategies, using nanoparticles to reduce toxicity and side effects of the conventional drugs. Fortunately, it has been realized that nanocarrier systems themselves might impose unknown risks to the patient and severely interrupt the overall diagnostic and therapeutic procedures. The types of hazards that could be introduced by nanoparticles are still beyond our knowledge border. Very little is known about the fundamentals of the interaction of nanoparticles with living cells, organs, and organisms. A deep understanding of biological responses to bare nanoparticles and their complex form is a prerequisite to develop low risk drug nanocarrier systems in the future.

Competing Interests

The author declared that there are no competing interests.

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