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Nanotechnology and the diagnosis/treatment of leishmaniasis.

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Review
Revisión

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RESUMEN

Objetivos: Este trabajo pretende actualizar la situación actual en el diseño de nanoplataformas contra la leishmaniasis. En este sentido, especial atención merecen los nanotransportadores de fármacos diseñados para ser administrados al paciente a través de las vías de administración parenteral, tópica y oral. Asimismo, se discuten las posibilidades que ofrecen las técnicas o estrategias de formulación más avanzadas en el diseño de estas nanoplataformas biomédicas. Finalmente, también se dedica especial atención a la utilización de estos nanosistemas en la administración de vacunas y en el diagnóstico de la leishmaniasis.

Material y Métodos: Con este fin, se utilizaron las páginas Web *PubMed*, *HCAplus*, *Thomson* y *Registry* como principales fuentes para la búsqueda de los trabajos de investigación más interesantes publicados sobre la materia. La información así obtenida fue cuidadosamente analizada, resaltando aquellos resultados preclínicos más relevantes en cuanto al desarrollo de nanomedicamentos contra la leishmaniasis, y considerando también los nanosistemas transportadores de vacunas y las nanoplataformas de utilidad en el diagnóstico de esta enfermedad.

Resultados y conclusiones: La nanotecnología es utilizada para mejorar el diagnóstico y tratamiento de la leishmaniasis. El objetivo es, en todos los casos, la mejora de la selectividad por el parásito de los fármacos, vacunas y moléculas utilizadas como agentes de contraste en técnicas de imagen, especialmente cuando este microorganismo se encuentra localizado en el interior de macrófagos y neutrófilos. Con esta interesante nanoherramienta, se puede también obtener una significativa reducción en la aparición y severidad de la toxicidad asociada a las técnicas de diagnóstico y tratamiento de la leishmaniasis. Es evidente que sólo con un inteligente diseño de estos nanosistemas se logran los mejores resultados de diagnóstico y terapia de la enfermedad.

PALABRAS CLAVE: Diagnóstico, Farmacoterapia, Leishmaniasis, Nanopartícula, Transporte de Fármacos.

ABSTRACT

Aim: The review article updates the current state of the art in the engineering of nanoplatforms against leishmaniasis. Special attention is devoted to the development of drug nanocarriers to be given to patients through the parenteral, topical, and oral routes of administration. Challenges and opportunities coming from advanced formulation methods/strategies introduced in the design of these nanosystems are emphasized. Finally, particular attention is also given to the use of nanoparticulate systems for vaccine delivery and for the diagnosis of the disease.

Materials and Methods: To that aim, the Web sites of *PubMed*, *HCAplus*, *Thomson*, and *Registry* were used as the main sources to perform the search for the most significant research articles published on the subject. The information was then carefully analyzed, highlighting the most important preclinical results in the development of nanomedicines against leishmaniasis, as well considering vaccine delivery systems and nanoparticulate-based diagnosis.

Results and Conclusion: The introduction of nanotechnology into the leishmaniasis arena is intended to optimize both the diagnosis and treatment (drug/vaccine therapy) of the disease. The objective is always to improve the selectivity of the imaging molecules or drugs/vaccines toward the parasite, especially when it is located inside phagocytic cells and neutrophils, while keeping to a very minimum the toxic side effects. Of course, only the wise engineering of the nanoparticulate delivery system will assure the best diagnostic/therapeutic outcomes.

KEY WORDS: Diagnosis, Drug Delivery, Drug Therapy, Leishmaniasis, Nanoparticle.

INTRODUCTION

Leishmania spp. are protozoan parasites belonging to the Trypanosomatidae family. These parasites are digenetic, and complete their life cycle in two different hosts: dogs (zoonosis, principally caused by *L. infantum*) and humans [anthroponosis, primarily caused by *L. donovani* (visceral leishmaniasis), and *L. tropica* (cutaneous leishmaniasis)][1]. Once inside blood of the hosts (when they are bit by the sand flies of the two genera of the subfamily *Phlebotominae*: *Lutzomyia* spp. and *Phlebotomus* spp.), the parasites infect phagocytic cells and neutrophils. Dissemination of the disease inside the host is then the consequence of the replication of the parasite as amastigote inside the phagolysosomes of macrophages. In this respect, pharmacotherapy failure is commonly due to the existence of amastigotes inside macrophages[2].

Leishmaniasis currently affects ≈ 12 million people in the World. This severe disease is characterized by a mortality rate of ≈ 60 thousand people per year, being diagnostized 2 million of new cases per year. In addition, leishmaniasis is considered as endemic in ≈ 98 countries[3].

Current drug therapy against leishmaniasis is based on the use of ≈ 25 active agents, i.e., meglumine antimoniate, sodium stibogluconate, amphotericin B, pentamidine, miltefosine, and paramomycin, to cite just the most representatives. Their safety and effectiveness is considered to be widely heterogeneous[4]. Generally speaking, all these drug molecules are characterized by a limited efficacy (given their inability to attack the parasites inside macrophages), by a severe toxicity, and by the development of drug resistances by the parasite[5]. Probably, the most important mechanism of drug resistance is related to an increased expression of P-glycoprotein, an efflux pump that minimizes the drug concentration inside the parasite structure[6]. Additionally, the leishmania ATP-binding cassette (ABC) transporter PGPA can confer antimony resistance to the parasite by sequestering intracellularly thiol-metal conjugates. In this line, PRP1 is an ABC protein determining pentamidine resistances and antimony cross-resistances, when overexpressed in *L. major*[7]. Unfavorable pharmacokinetics and pharmacodynamics, and the lack of sensitivity of the parasite to drug molecules are also hypothesized to be responsible for pharmacotherapy failure[8].

In the disease arena, the search for alternative molecules to improve the management of the disease has continued with bisphosphonates, e.g., risedronate (against visceral leishmaniasis)[9] and pamidronate (against cutaneous leishmaniasis)[10], with limited success[11]. Another example is the immunomodulator imiquimod, which activates the release of nitric oxide (the major mediator of the leishmanicidal activity in macrophages), when in contact with monocytes/macrophages[12]. Nicotinamide[13], the family of silent information regulator (SIR2) proteins[14], the family of arylimidamides[15], and plant non-specific lipid transfer proteins (nsLTPs)[16], have also been proposed against leishmaniasis. Preclinical and clinical investigations have analyzed the potential introduction of these drugs into the clinic.

Recently, and in order to beat the very important challenges of drug therapy against leishmaniasis, it has been proposed the introduction of nanotechnology in the management (diagnosis and treatment) of the disease[1]. Numerous research reports have highlighted the benefits coming from the association of drug molecules with nanoparticulate delivery systems (formulation of the so-called nanomedicines) against leishmaniasis: improved efficacy and minimized toxicity[17,18]. Ideally, such alliance is expected to result in a specific accumulation of the drug inside macrophages containing the parasite, and in a prolonged exposure of the parasite to these active agents. Additional benefits may come from the improvement of the pharmacokinetics of drug molecules, and from the significant reduction in the formation of toxic drug degradation compounds (thanks to the protection of the drug inside the nanocarrier). The association between the drug and the delivery system may also facilitate the overcoming of drug resistances[19,20].

This review article is devoted to the analysis of the most significant advances in the engineering of nanomedicines against leishmaniasis. Challenges and opportunities coming from the use of such targeting strategies to deliver drugs to the parasite will be emphasized. Finally, the use of nanoparticulate systems for vaccine delivery and for the diagnosis of the disease is also considered.

METHODS

To that aim, the Web sites of Thomson, PubMed, HCAplus, and Registry were used as the main sources to perform the search for the most interesting research articles published on the subject. The information coming from this search was carefully analyzed to compile and discuss the most significant *in vitro* and *in vivo* results in the development (and preclinical/clinical use) of nanomedicines against leishmaniasis, also considering vaccine delivery systems and nanoparticulate-based diagnosis.

RESULTS AND DISCUSSION

The use of nanotechnology-based strategies for the diagnosis

and pharmacotherapy of leishmaniasis is revolutionizing the current management of this severe disease[1,21].

Drug delivery systems against leishmaniasis

Nanomedicine is considered to be the medical application of nanotechnology to human health[1]. Nanoparticulate systems have offered very promising results in drug delivery to severe diseases[22]. Basically, they are biodegradable nano-sized particles composed of organic and/or inorganic materials[8]. Habitually, drug nanocarriers are based on lipids (e.g., liposomes, niosomes, solid lipid nanoparticles), and/or polymers like poly(D,L-lactide) (PLA), poly(D,L-lactide-*co*-glycolide) (PLGA), chitosan, poly(ε-caprolactone) (PCL), poly(alkylcyanoacrylates), and their copolymers. Numerous research reports have shown that they are capable of enhancing the drug efficacy, while reducing drug toxicity[22]. This is generally the consequence of the optimization of the pharmacokinetics and pharmacodynamics of drug molecules. Table 1 compiles some illustrative examples of (advanced) drug delivery systems that have been engineered for the treatment of leishmaniasis.

Generally speaking, all these drug delivery systems (or nanomedicines) can be given to patients through the parenteral, topical, and oral routes of administration. Ideally, advanced formulation methods/strategies are introduced in the design of these systems to assure the best therapeutic effect.

The parenteral route of administration

Different (advanced) drug delivery systems have been proposed for the treatment of leishmaniasis through the parenteral route of administration. Among them, liposomes are the most interesting platforms for the efficient delivery of anti-leishmania drugs. Generally speaking, they are micro- or nano-vesicles displaying one or more bilayers of lipid molecules (capable of entrapping hydrophobic drugs) that enclose an aqueous compartment (where hydrophilic drugs are loaded). An appropriate design and engineering can optimize the *in vivo* fate and, thus, the efficacy and safety of the liposome-based drug therapy. However, *in vitro* and *in vivo* stability aspects related to these drug delivery systems can limit their clinical use[18].

Recently, different liposome-based formulations have entered the leishmaniasis arena, being marketed (Fungizone®, Abelcet®, Amphocil®, and AmBisome®, to cite just a few). They have been proposed against visceral leishmaniasis (also known as kala-azar) and cutaneous leishmaniasis[23,24]. Despite their efficacy against leishmaniasis, such liposomal systems are not frequently used against the disease in developing countries, given their high economic cost.

AmBisome® is a liposomal formulation of amphotericin B that has been approved for clinical use by the United States Food and Drug Administration (FDA)[18]. It is also under clinical use in the countries bordering the Mediterranean Sea, e.g., it was approved by the Spanish Drug Agency (AEMPS). The liposomal medicine is used in the treatment of visceral leishmaniasis and, more recently, of cutaneous leishmaniasis[24]. It is very safe and highly effective against primary visceral leishmaniasis in endemic areas of *L. infantum* and *L. donovani* (countries bordering the Mediterranean Sea, Central and South America, and South of Asia, i.e., India, Bangladesh, and Nepal). In fact, AmBisome® has been recommended as first line treatment of leishmaniasis by the World Health Organization (WHO) Expert Committee on the Control of Leishmaniasis[18].

More recently, liposomes loaded with meglumine, paromomycin, and miltefosine have been developed against cutaneous leishmaniasis[17]. The drug-loaded liposomes were synthesized by following a double emulsion method combined with a lyophilization process, and they were evaluated in BALB/c mice infected by *L.*

Table 1. Representative examples of drug delivery systems that have been developed for the efficient treatment of leishmaniasis.

Drug delivery system	Drug molecule	References
Liposomes	Amphotericin B, meglumine, paromomycin, miltefosine	17,23-25
Emulsomes	Amphotericin B	26,27
Solid lipid nanoparticles	Paramomycin	28
Apolipoprotein-stabilized phospholipid nanodisks	Amphotericin B	29
Polymeric nanoparticles	Amphotericin B, pentamidine	30-32
Cyclodextrins	Meglumine	20,33
Nanosuspensions	Amphotericin B	34
Gold nanoparticles	Quercetin	35

major. Remarkably, only the liposome-based carrier loaded with miltefosine was capable of reducing significantly the number of amastigotes and the skin lesion size, compared to control groups.

Niosomes have also been proposed as drug delivery systems against leishmaniasis[36]. They are non-ionic surfactant-based liposomes formulated by using mixtures of cholesterol and non-ionic surfactants (e.g., sorbitan esters and polyoxyethylene alkyl ethers), which are biodegradable, non-toxic, and more stable and cheaper than liposomes. Furthermore, niosomes don´t need special preparation conditions (e.g., low temperature, vacuum, or nitrogen atmosphere) to be produced and storage. In fact, they are stable against oxidative processes.

Alternatively, apolipoprotein-stabilized phospholipid nanodisks have been formulated as efficient nanocarriers for amphotericin B[29], which is characterized by a poor solubility in water and an important toxicity. This drug delivery system exhibits a significant solubility in water, absence of an aqueous core, and a mean diameter between 8 and 20 nm[29]. The efficacy of the amphotericin B-loaded nanodisks was evaluated in BALB/c mice infected by *L. major*. It was concluded that the intraperitoneal administration of the nanoformulation (dose: 5 mg/Kg) significantly reduced the size of the lesion and the parasite load.

Finally, polymeric nanoparticles have also been synthesized as efficient drug nanocarriers against leishmaniasis[2]. For instance, chitosan nanocapsules were loaded with amphotericin B by following a deposition technique based on the formulation of a polymer mold nanoemulsion[30]. *In vitro* and *in vivo* experiments demonstrated an effective internalization of the nanoparticles by infected macrophages, thus optimizing the drug activity. More interestingly, complementary immunoadjuvant chemotherapy (based on the up-regulation of tumor necrosis factor *a*, interleukin-12, and nitric oxide synthase; and the negative regulation of transforming growth factor β, interleukin-4, and interleukin-10) significantly augmented the therapeutic effect displayed by the drug-loaded nanoparticles.

The topical route of administration

In comparison to systemic therapy, the use of topical formulations facilitates the administration of anti-leishmania agents, can reduce the adverse drug effects, and it is considered as a cost-effective approach to the treatment of leishmaniasis[37]. However, little has been done up to now to improve drug absorption through this route.

In fact, it has been merely proposed the use of some permeation enhancers, e.g., ethanol. For example, topically applied dispersions of amphotericin B-loaded liposomes (Fungizone®) in 5 – 25% ethanol have been found more effective in the reduction of the lesion size (cutaneous leishmaniasis) compared to controls[25]. In another investigation on the treatment of cutaneous leishmaniasis, it was demonstrated an important increase of the skin permeation of paromomycin-loaded unilamellar liposomes when they were associated with the permeation enhancer methyl benzethonium chloride[19].

The oral route of administration

Numerous drug molecules are characterized by inadequate water solubility, this determining their reduced oral absorption (and bioavailability). In order to overcome the problem of the reduced bioavailability of these active agents when administered orally, it has been proposed the use of drug delivery systems. As an example, the formulation of chitosan-based mucoadhesive nanosuspensions (hydrogels) can prolong the exposure time of gastrointestinal mucosa to released drugs (thus augmenting their oral absorption)[38]. In this line, amphotericin B has been formulated as a nanosuspension for the treatment of visceral leishmaniasis (*L. donovani*). Concretely, the nanosuspension was prepared in an aqueous solution of Tween® 80, Pluronic® F-68, and sodium cholate, by using a large pressure homogenization. When this nanosuspension was orally administered, it reduced by 29% the parasite load[34].

Cyclodextrins are another promising system for the efficient oral delivery of anti-leishmania drugs, thus enhancing their oral bioavailability[2]. For example, meglumine antimoniate-loaded β-cyclodextrins have been prepared by merging both compounds in water at a 1:1 molar ratio, and subsequently warming at 55 ºC for 48 hr. In this formulation method, the final freeze-drying step determines the formation of the supramolecular nano-assembly[20,33]. *In vivo* experiments demonstrated that cutaneous lesions caused by *L. amazonensis* were significantly less developed by animals daily receiving an administration of meglumine antimoniate-loaded β-cyclodextrins (32 mg/Kg), compared to those treated with the free drug (120 mg/Kg) and control animals treated with saline.

Finally, micelles of miltefosine (hexadecylphosphocholine) and amphotericin B have recently been developed against leishmaniasis[39]. It was found that these molecular assemblies are characterized by an improved paracellular permeability, thus resulting in an optimized oral bioavailability.

Finally, miltefosine (hexadecylphosphocholine) is still the only oral drug clinically used to treat visceral leishmaniasis and cutaneous leishmaniasis[39]. It has been proposed its inclusion in combination drug therapy regimens to obtain a

synergic therapeutic effect. Unfortunately, drug molecules to be included in such regimens are characterized by a limited oral bioavailability. To beat the challenge, micelles of miltefosine and amphotericin B have recently been developed against leishmaniasis[40]. It was found that these molecular assemblies can improve the paracellular permeability of the drugs, thus resulting in an optimized oral bioavailability.

Ligand-mediated drug delivery

Despite the previously commented drug delivery systems can offer promising preclinical (and clinical) results in the treatment of leishmaniasis, a much better selectivity of the nanomedicine for the infected cells is still a challenge. To face the problem, it has been considered the introduction of active targeting strategies in the development of these drug nanocarriers[1]. Such strategies are mainly based on the engineering of nanoparticulate systems surface decorated with (bio)molecules capable of binding specifically to ligands (over)expressed by those cells infected by the parasite. Concretely, macrophages display onto their surface many receptors recognizing carbohydrate molecules, e.g., galactose, mannose, glucose, and/or fucose. Thus, the surface functionalization (generally by chemical conjugation) of the nanomedicines with these biomolecules is expected to maximize their targetability toward the parasite[41].

For instance, amphotericin B-loaded lipid nanospheres have been optimized by surface functionalization with mannose moieties[42]. *In vivo* experiments were carried out to define the benefits of this strategy in BALB/c mice infected by *L. donovani*. Remarkably, it was found that this nanoformulation exhibited a more quickly distribution to the liver and spleen, thus resulting in a higher leishmanicidal activity.

In this way, O-palmitoyl mannan moieties have been conjugated to the surface of amphotericin B-loaded trilaurin-based emulsomes (nano-sized lipid particles) stabilized by soya phosphatidylcholine. The nanomedicine reported *in vivo* a greater inhibition of *L. donovani* of macrophages in spleen[26]. Similar encouraging results were obtained with tripalmitin-based emulsomes surface functionalized with the same biomolecule[27].

Vaccine delivery systems against leishmaniasis

Leishmaniasis is considered one of the few parasitic diseases likely to be controllable by vaccination. Despite several generations of vaccines have been developed, i.e., live-attenuated to recombinant, synthetic, and even naked deoxyribonucleic acid (DNA) vaccines, limited protection against the parasite has been obtained. Probably, inoculation of plasmid DNA coding for parasite antigens is the more promising strategy, given its capability of inducing humoral and cellular immune responses[43].

As a promising alternative to current vaccination techniques, vaccine delivery systems have been engineered. Interestingly, they can improve the interaction between antigens and dendritic cells or macrophages. For instance, these nanoparticulate systems can be based on cationic solid lipid nanoparticles (being loaded with cysteine proteinase genes)[44,45], PLGA nanoparticles (being loaded with plasmid DNA encoding the kinetoplasmid membrane protein-11[46], or autoclaved *L. major*[47]), or cationic liposomes (being loaded with soluble Leishmania antigens)[48,49], to cite just some interesting examples.

In a recent investigation, solid lipid nanoparticles were loaded with leishmanial cysteine proteinase type I, and then investigated in C57B/6 mice suffering from leishmaniasis[44]. Remarkably, after intraperitoneal vaccination with the nanoformulation, a strong antigen-specific T-helper type 1 immune response was induced in comparison with control groups. In fact, it was described that the lymph node cells showed a reduction on parasite burden, a higher generation of interferon-gamma and inmmunoglobulin G2a, and a lower production of interleukin-4.

PLGA nanoparticles have been loaded with plasmid DNA encoding the kinetoplasmid membrane protein-11 to obtain protection against cutaneous leishmaniasis caused by *L. braziliensis* in terms of lesion development and parasite burden[46]. It was found that this nanostrategy induced an immune cell response, the presence of both pro-inflammatory and anti-inflammatory cytokines, thus resulting in an intense reduction in the parasite load.

Nanotechnology-based approaches for the diagnosis of leishmaniasis

The need for a (very) fast, effective, and non-invasive diagnosis of leishmaniasis remains as a challenge up to now. Regarding imaging techniques, it is accepted that contrast agents may increase their sensitivity when loaded to nanoparticulate delivery systems, thus facilitating the diagnosis of previously undetectable diseases[50].

Nanotechnology is expected to help in the design of more efficient techniques permitting a specific, non-invasive, and cost-sensitive detection of the disease. Concretely, nanotechnology may contribute to the development of improved biosensors and biomarkers for the detection of chemical or biological materials, such as, nucleic acids, proteins, and ions[50,51]. As a result in leishmaniasis, nanotechnology will optimize the possibilities of serology-based diagnosis techniques [i.e., enzyme-linked

immunosorbent assays (ELISA), hemaglutination (HA) tests, and immunoblotting and rapid diagnostic tests (TDR)], serological and molecular biology approaches [e.g., loop-mediated isothermal amplification (LAMP) test], and proteomics [matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) technique, surface-enhanced laser desorption mass spectrometry time ionization flight (SELDI-TOF MS) technique, liquid chromatography combined with mass spectrometry (LC-MS-MS), isotope-coded affinity (ICAT) technique, and isotope labeling for relative and absolute quantification (iTRAQ) technique], to cite just some representative examples.

For instance, it has been investigated the clinical use of magnetic beads to improve the serum peptide profiling by MALDI-TOF MS[52]. It was demonstrated the applicability of the test to detect the infection by *L. donovani* in mice, with ≈ 94% accuracy, sensitivity of ≈ 92%, and specificity of ≈ 97%.

It was also developed a chronocoulometric DNA sensor for the accurate diagnosis of kala-azar which is based on a sol-gel film synthesized nanostructured nickel oxide (NiO) deposited on an indium tin oxide (ITO) coated glass electrode[53]. This is a DNA electrochemical biosensor based on nucleic acid hybridization, which is characterized by a great simplicity, speed, miniaturization, sensitivity, selectivity, and cost-effectiveness for detecting specific DNA sequences or mutant genes. In the structure of the biosensor, the single-stranded DNA (ss-DNA) probes were immobilized on the surface of the transducer (the NiO-ITO electrode). It was concluded that the sol-gel derived NiO nanostructured film was an excellent matrix for the immobilization of DNA and the development of genosensors.

In line with the later investigation, it has been engineered a nanostructured NiO-based DNA biosensor for the molecular detection of kala-azar[54]. Ultraviolet-visible spectrophotometry, Fourier transform infrared spectrometry, X-ray diffraction, and scanning electron microscopy studies demonstrated the correct and complete formation of the biosensor. Response studies were performed to the biosensor by using differential pulse voltammetry in the presence of methylene blue (a redox mediator), given its easy association with guanine bases of free ss-DNA. It was found a linear response in the range of concentrations between 2 mg/mL and 2 pg/mL, complementary to the target genomic DNA (DNA of the parasite). Interestingly, the bioelectrode demonstrated a high specificity in the differentiation between DNA from *L. donovani* and DNA of a healthy person, with the aid of an electrochemical technique, i.e., differential pulse voltammetry (DPV, or differential pulse polarography, DPP).

Nanoporous niobium oxide films have also been prepared for the (label-free) electrochemical detection of DNA hybridization events[55]. Multiwall carbon nanotubes/nano-zirconium dioxide (ZrO_2)/chitosan-modified glassy carbon electrodes were also developed for the electrical detection of DNA hybridization[56].

Fluorescence techniques (involving the use of organic fluorophores and fluorescent proteins combined with nanoparticulate systems) may be used in the diagnosis of leishmaniasis, but they have further been adapted to the *in vitro* and *in vivo* characterization of the cellular uptake and retention of nanoparticles, and to the control of the nanoparticle biodistribution in animal models[21].

Regarding the former use, fluorescent nanomaterials are beginning to overcome many of the problems associated to image-based fluorescence with conventional fluorophores [i.e., poor skin/tissue penetration, weak and short-time *in vivo* imaging, and high susceptibility to photodegradation (due to stimulating light energies)]. Hence, they have opened up the application of fluorescence imaging techniques to disease diagnosis, and to gain further insights into the disease progression at the systemic, cellular, and molecular levels[21].

Semiconductor quantum dots [QDs, nanometer-scale particles usually made from combinations of heavy metals, such as, cadmium selenide (CdSe), cadmium telluride (CdTe), indium phosphide (InP), or indium arsenide (InAs), with distinctive optical characteristics (e.g., high photostability, and more efficient and selective *in vivo* imaging)] can replace conventional organic fluorophores in fluorescence imaging[21]. Concretely, they have been used to label cellular components, track movement and cell differentiation, image vasculature and lymph nodes, and visualize the tissue distribution of nanomaterials[57]. Unfortunately, QDs are considered toxic, since they are made of heavy metals (such as, cadmium). Finally, QDs can also concentrate into mononuclear phagocyte system (MPS) organs for prolonged periods of time, thus causing adverse side effects[58].

Alternatively, fluorescent nanomaterials may also be prepared by encapsulating organic fluorophores into nanoparticulate systems, e.g., silica nanoparticles. As a result, the properties of the fluorophore are generally improved (increased signal intensity and, hence, the sensitivity of the assay will be enhanced, while the resistance to photobleaching will also be optimized)[59,60]. Silica-based nanosystems are particularly interesting because of their

low toxicity and high potential for surface modification (Ligand-mediated fluorophore delivery)[59].

CONCLUSIONS

Nanotechnology-based approaches to the efficient management of leishmaniasis are principally devoted to the optimization of both the diagnosis (for a more selective, non-invasive, and cost-sensitive technique) and the drug (and vaccine) therapies (for an improved therapeutic effect), while reducing the associated toxicity.

Despite the interesting preclinical (and clinical) results that have been obtained up to now, the limited selectivity of the nanoparticulate-based systems toward the parasite is still a challenge. To face the problem, it is clearly needed the introduction of both passive (formulation of long-circulating nanoparticulate systems) and active (design of nanoparticles surface functionalized with biomacromolecules for ligand-mediated delivery, and/or synthesis of nanoplatforms by using stimuli-sensitive materials) targeting strategies when engineering these nanoplatforms for leishmaniasis diagnosis and treatment. Moreover, the combined use of these strategies in nanoparticle development is expected to facilitate a more selective (and intense) biodistribution of the imaging molecules or drug/vaccine toward the parasite.

Finally, the complete introduction into the clinic and long-term use of all these nanoparticulate systems additionally relies on a better knowledge of the disorders causing the disease, and on the elucidation of nanotoxicity aspects. Probably, a definitive step toward the perfect management of leishmaniasis may come from the development of theranostic nanoplatforms (for a combined disease diagnosis and drug/vaccine therapy).

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