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Review article

Nanomaterials for diagnosis, treatment, and prevention of human cutaneous leishmaniasis: A review

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ABSTRACT

Leishmania parasites are the organisms responsible for one of the most important tropical diseases, leishmaniasis. This neglected disease mainly affects populations in developing or underdeveloped countries, causing nearly one million new cases per year. This article focuses on the cutaneous form of the disease. Common antileishmanial medications have several disadvantages, such as low efficiency, high toxicity, several adverse effects, resistant strains, long treatments, and high costs. As a result, first- and second-line treatments are insufficient. Therefore, there is a need for new antileishmanial agents and strategies, most of which utilize nanotechnology. While novel nano-drug delivery devices can transport antileishmanial drugs to target cells, reducing secondary toxic effects, several advances in nanotechnology and photonics pursue activation of leishmanicidal mechanisms once they reach their target. Here is a summary of recent nanotechnology approaches to the treatment, diagnosis, and prevention of human cutaneous leishmaniasis, including promising techniques still in development.

1. Introduction

Leishmaniasis is a neglected tropical disease caused by a protozoa parasite. There are three main forms of the disease: cutaneous, mucocutaneous and visceral. This work will focus on the cutaneous form. Cutaneous leishmaniasis is widespread in tropical and subtropical regions and affects mainly the poorest populations. It is estimated that there are between 600,000 and one million new cases each year [1]. For this reason, it is important to learn about this disease and its characteristics.

Leishmania is transmitted by female sandflies of the *Phlebotominae* subfamily (more than 30 species known as leishmaniasis vectors). They bite their host and use the blood to produce their eggs. The epidemiology of leishmaniasis depends on the type of parasite and sand fly species, the ecological environment of transmission, past and current human exposure to the parasite, and human behavior. There are about 70 animal species including humans that are natural reservoirs of *Leishmania* [1]. It is important to

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emphasize that this fact depends on the country where transmission occurs. For example, there are many countries where humans are not considered reservoirs for Leishmania.

All forms of this protozoan infection share three common pathogenic features: they target the tissue-resident macrophages that support intracellular replication of the parasites; human immune-inflammatory response controls how the disease manifests and progresses; and persistent tissue infection is typical [2].

However, there is a wide range of clinical characteristics for cutaneous leishmaniasis. The condition can either create a small number of lesions (localized cutaneous leishmaniasis) or a significant number of ulcers (diffuse cutaneous leishmaniasis). In addition, mucous membranes in the nose, throat, and mouth may be destroyed as a result of mucocutaneous leishmaniasis. Some species, including *Leishmania donovani, L. infantum, L. chagasi,* and *L. major*, can also enter the viscera and result in the potentially fatal condition known as kala-azar. The *Leishmania* species largely determines the clinical features of cutaneous leishmaniasis in immunocompetent patients, as it is shown in Fig. 1 [3,4].

Responding to this group of diseases with the most appropriate course of therapy, requires accurate identification because the disease's outcome varies depending on the species [5]. Therefore, when diagnosing cutaneous leishmaniasis, two main difficulties arise: identifying the specific form of *Leishmania* and its corresponding causative microorganism, and differentiating it from other skin diseases with similar clinical characteristics such as typhoid, malaria, and tuberculosis [6].

At present, diagnosis of cutaneous leishmaniasis relies on clinical characteristics (supported by epidemiological data) and laboratory tests including microbiological, cytological, and histological examinations [7]. The diagnostic methods that are commonly used involve identification and quantification of parasites, but their sensitivity, specificity, ease of use, and feasibility in field settings limit their utility in clinical practice [6]. For this reason, medical experts commonly treat patients without properly identifying the species instead of making educated guesses based on the patient's geographical context and local epidemiology [3].

In turn, current treatments for this group of diseases fall into two large groups: First- and second-line. First suggested by Vianna in 1912 [8], pentavalent antimonial solutions (Glucantime®, Pentostam) are nowadays the first-line treatment. Although it is a treatment with a slow response, it sometimes leads to incomplete healing, the disease may reappear in the first six months; 30-days daily application of intramuscular injections of pentavalent antimonials is painful and traumatic; and it leads to severe side effects. Frequently, all these reasons make the treatment impractical for the affected population, mostly rural, and the addition of the general remoteness of health centers, ends up in interrupted therapies.

Furthermore, second-line treatments consist mainly of Amphotericin B (AmB), Pentamidine, Miltefosine and Paromomycin (PM), among others. These alternative treatments are expensive and have significant levels of toxicity in addition to the high hydrophilicity of the active substance that cannot be compensated by conventional excipients.

Despite the facts described above, the development of new treatments is historically slow, mainly because it presents poor market prospects for the private sector. The need to develop treatments to replace the conventional one and provide a better therapeutic effect, with a decrease in side effects, and at an affordable cost fall mainly on government agencies, academic institutions, and public-private joint ventures [9].

Another way to prevent this infection is through vaccination. Although some progress has been made in the leishmanial diagnosis and treatment, so far there is no human vaccine available for this group of disease. Even though several research groups have been working on potential vaccines against leishmaniasis, the variety of Leishmania species and the complexities of the parasite-host interaction make it difficult to develop effective long-term immunity [10].

Consequently, current and future strategies for the control of CL need to focus on new tools and their availability in rural and more deprived areas [11]. Nanoparticles are used in biology, the environment, medicine, and other fields and could help eradicate infectious diseases transmitted by vectors, such as leishmaniasis [12]. From all the aforementioned, it is very important to develop alternative drugs, therapies and prevention by adopting novel strategies, such as nanomedicine, that can effectively control this disease. In this review, we highlight the works of the last 5 years on the prevention, diagnosis, and treatment of cutaneous leishmaniasis using nanotechnology and its tools, including promising techniques still in development.

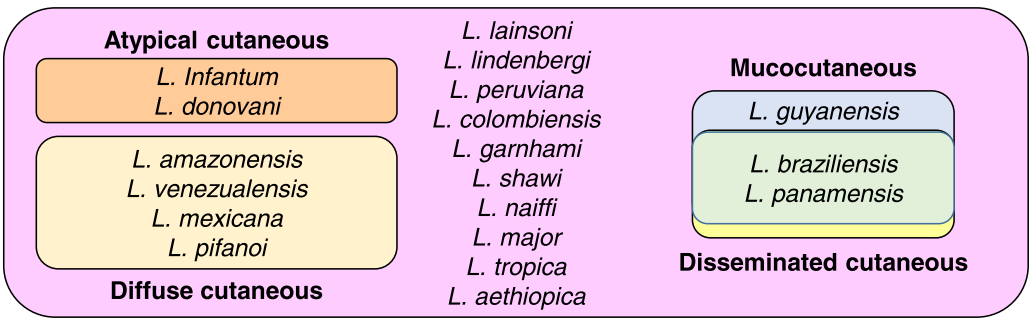

Fig. 1. *Leishmania* species according to the clinical forms of cutaneous leishmaniasis.

2. Diagnosis of cutaneous leishmaniasis

The clinical manifestations of leishmaniasis are usually different enough to make a differential diagnosis in endemic areas; there may be papules, plaques, ulcers, or nodules, depending on the type of leishmaniasis and the stage of the disease. A dermatoscopic evaluation may be useful to distinguish cutaneous leishmaniasis from other clinically similar lesions [13]. In any case, early laboratory diagnosis is the key to an adequate choice of specific treatment and thus controlling disease evolution [13]. However, when choosing a diagnostic test, infrastructure and resources are frequently more important than test accuracy [13].

Due to its high specificity and cost-effectiveness, parasitological diagnostics are still considered the gold standard in the diagnosis of leishmaniasis. This is typically accomplished by histopathological examination of an *in vitro* culture of fixed or parasitic tissue from suspicious lesion material. Although in the past ten years the SYBR Safe fluorescent dye has been suggested, the microscopic diagnosis of CL is established by direct detection of amastigotes in biopsy smears, scrapes, or smears stained with Giemsa [14].

On the other hand, parasite culture requires great technical experience, it is prone to contamination, it is time-consuming, and it presents low sensitivity [15,16]. An improved method, in which samples are recovered without needles, is called microculture [13,16]. It is less expensive and easier to use, and with high sensitivity and specificity [17], although it cannot discriminate between species.

One of the indirect methods mostly used is the Montenegro skin test because of its ease of use and high sensitivity, which is based on a marker of delayed cellular immune response. Although, the major disadvantages include the need for culture facilities to produce the Montenegro antigen, the difficulty in maintaining *Leishmania* culture, and the inability of the test to distinguish between past and current infections [1,6,13].

We could also find serological and molecular diagnosis in this group of methods. The first one is limited for CL due to their low levels in the samples [18,19] and the second one fulfills the previous drawback and enables genus-specific and species identification but, the efficacy of the assay depends on the amplifying target, the sample being tested, and the handling skills [6].

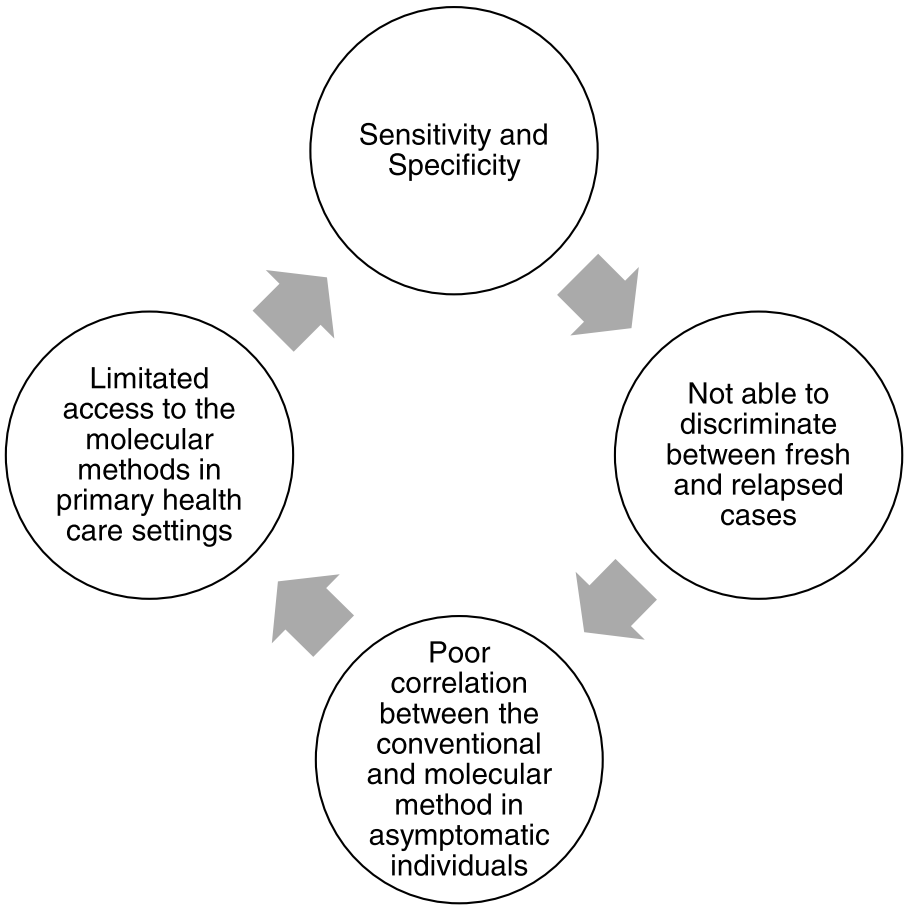

Fig. 2. Challenges of *Leishmania* diagnostics: Limitations for the detection of *Leishmania* parasites using current conventional diagnostics. Adapted from Gedda et al. [6].

2.1. Limitations of current leishmaniasis diagnosis

A big obstacle in the clinical management of leishmaniasis is the vulnerability of primary health care (PHC) services in many affected countries with challenges like: shortage of capital resources, uncompromised system, limited infrastructure, few human capital, and uncontrolled population growth.

Although there are several techniques available to validate CL cases, they are all far from being ideal, and these shortcomings have been highlighted in Fig. 2. Microscopic examination and serological tests are fast and cheap approaches. Because of their great sensitivity and specificity, they are considered to be one of the best techniques for parasite detection, but in endemic areas, PHCs cannot employ them effectively. The reliability of these testing tools is highly variable and relies on the sampling technique and the technical abilities of the test performed by the physicians and laboratory personnel [6].

Even molecular diagnostic techniques have some limitations, such as counting dead parasites DNA (in DNA-based PCR), need for expensive and sophisticated instruments, temperature-controlled settings and highly skilled laboratory personnel [6].

2.2. New approaches from nanotechnology

Numerous research groups have concentrated on developing new tactics for efficient and affordable diagnostic techniques. Despite these efforts, diagnostic methods are not yet effective, with limitations in terms of cost, sensitivity, specificity and difficulty of use in the field.

According to the systematic review of existing publications about diagnostic methods by Pena et al., PCR showed the highest accuracy for leishmanial diagnosis and its use should be encouraged in clinical practice. ELISA is recommended for the screening of suspicious cases, but cross-reactions should be considered [20].

For these reasons, many of the recent proposals regarding diagnostic innovation correspond to modifications to the PCR technique: nested PCR [21], qPCR [22,23], LAMP PCR [24–27] and RFLP-PCR [28]. These new techniques, together with existing ones, are likely to be available for diagnosis to both specialized and remote primary health centers soon [29,30].

Modifications were also made to immunoassays such as the immunohistochemistry test for CD1a [31–33].

Regarding our topic, nanotechnology offers a new paradigm for various medical diagnoses with unique properties to solve the challenges we have pointed out in this review. However, there are significant drawbacks in translating it into clinical applications [6]. The combination of nanoscience and nanotechnology with existing molecular biology tools enables the fabrication and application of various structures, among which we highlight metal and metallic nanoparticles, quantum dots, and superparamagnetic nanoparticles due to their physicochemical properties.

Modifications in techniques employing diverse nano-objects were tested (Table 1): the combination of magnetic beads and cadmium selenide quantum dot probes for the detection of specific surface antigens of *Leishmania* (proteins) and DNA [34], the use of a detection system based on the dual effect of immobilization and electrocatalysis of quantum dots in copper and zinc ferrites [35] and systems to obtain an efficient distinction between cutaneous leishmaniasis and other diseases such as Chagas disease using biosensors based on PAMAM dendrimers and proteolysosomes [15].

Other studies carried out were based on the detection of nucleic acids with gold nanoparticles [36], a very sensitive and cost-effective alternative that can detect even the asymptomatic infection. Another recent gold-nanoparticle-based method performs a serological detection due to aggregation when antibodies recognize a promastigote-surface peptide antigen conjugated to the nanoparticle [37]. Gold hierarchical nano-leaflets have been synthesized by the electrodeposition method marked with a DNA probe by immobilization with DNA detection capacity for *L. major* [38], while gold nanoparticles have been synthesized for detection of the genomic DNA that may be useful for the differential diagnosis of *L. major* from other non-*Leishmania* species [39].

Another nanodiagnostic development was based on the analysis of volatile organic compounds in exhaled breath with an array of specifically designed chemical gas sensors [40]. This test consists of six sensors with different metal nanoparticles (Au or CuNPs), NPs core size, and a molecular organic ligand. This sensor test was 98.2% accuracy, 96.4% sensitivity, and 100% specificity. Interestingly, one of the sensors developed from CuNPs with a 2-mercaptobenzoxazole ligand, gave results for the differentiation of cutaneous leishmaniasis in humans that were highly sensitive, accurate, and specific. In addition, nine potential breath indicators were discovered by analyzing breath samples using gas chromatography coupled with mass spectrometry. Although the results showed a promising diagnostic method, it is necessary to increase the n.

Although the platform of nanodiagnosis for leishmaniasis is improving constantly and there are many of the techniques used in VL

Table 1
Diagnostic techniques according to detection method, *Leishmania* strain, nanoparticle of choice and reference.

Nanoparticle	Detection	*Leishmania* strain	Refs.
QDs and magnetic beads	DNA and its specific surface antigens	*Leishmania* spp.	[34]
QDs	Electrochemical genosensor	L. *major*	[35]
AuNP	Nucleic acids detection	L. *donovani,* L. *martiniquensis and* L. *orientalis*	[36]
AuNP	Nucleic acids detection	*Leishmania* spp.	[37]
Au Nanoleaflets	Electrodeposition	L. *major*	[38]
AuNP	Nucleic acids detection	L. *major*	[39]
Au and CuNPs	Volatile organic compounds in exhaled breath	Human cutaneous leishmaniasis	[40]

diagnostic likely to be adapted to CL [6] which helps even more CL investigations, more funding for the development of nano-diagnostics is needed to encourage researchers to investigate towards the CL elimination.

3. Treatment of cutaneous leishmaniasis

Conventionally, CL has been treated with highly toxic pentavalent antimonial solutions (Glucantime®, Pentostam) since 1912 when Vianna suggested this therapy [8] after Dr. Leishman discovery about the causative agent of the disease in 1903 [41]. Nowadays, there are first and second-line treatments with known disadvantages that make it difficult to continue treatment from high costs to adverse effects even more painful than the disease itself.

Another reason why many of the treatments mentioned and other antiparasitic treatments fail, lies in the inability of the active substance to reach a particular type of tissue and, even more, in the lack of selectivity towards a specific cell type. In CL, the parasitized cells are located in the deep part of the epidermis. At a late stage, the parasites live inside the fibroblasts of the dermis, even after a clinical cure. The particular location of infected cells makes it difficult for active substances to reach them, both from the upper area (topical route) and the lower one (intravenous and intramuscular routes).

Despite the facts described above, the development of new treatments is historically slow, mainly because it presents poor market prospects for the private sector. The need to develop treatments to replace the conventional ones and provide a better therapeutic effect, with a decrease in side effects, and at an affordable cost fall mainly on government agencies, academic institutions, and public-private joint ventures [9].

Consequently, current and future strategies for the control of CL need to focus on new treatments and their availability in rural and more deprived areas [11]. Nanoparticles are used in biology, the environment, medicine, and other fields and could help eradicate infectious diseases transmitted by vectors, such as leishmaniasis [12]. From all the aforementioned, it is very important to develop alternative drugs and therapies by adopting novel strategies, such as nanomedicine, that can effectively control this disease.

3.1. Approaches for nanotechnology-based therapies

Drug delivery methods developed for the treatment of infectious diseases have changed dramatically in recent years. Thanks to the advances in biotechnology and genetic engineering techniques, numerous therapeutic methods have been developed for specific diseases. Many efforts were made to effectively administer these therapeutic modalities [42]. Advances in nanotechnology are part of the new approaches that the treatment of leishmaniasis needs.

Nanotechnology intervenes at two levels in the treatment of this infection. First in the design of nano-pharmaceutical delivery systems for common and conventional drugs. This method has received remarkable attention in the field of drug development due to the improved pharmacokinetic properties and efficacy, low toxicity, high objective administration effect, high concentration release of controlled system medications, and prolonged systemic circulation life [43]. The second approach is conventional drug nanonization or production of new drugs on the nanoscale that may be phagocytosed by macrophages as foreign bodies, resulting in specific delivery for *Leishmania* parasites into these cells. Niosomes, polymeric and metallic nanoparticles, ethosomes and liposomes (Table 2) are tentative strategies for the direct administration of drugs to the parasitophorous vacuole where *Leishmania* resides [44].

3.1.1. Niosomes

Niosomes constitute a drug delivery system produced through hydration of non-ionic surfactants [45]. They are biodegradable, comparatively less toxic than pentavalent antimonials, more stable, of relatively low cost, and an alternative to liposomes [42]. Due to their properties, these nanoparticles were applied for two extremely important uses against cutaneous leishmaniasis – prevention and therapy – mainly in endemic areas. We would focus on the last one.

In recent years, nano niosomes loaded with miltefosine and ketoconazole were prepared by thin-film hydration, and it was found that the release of the formulations showed improved and controlled drug dissolution [46]. In a recent study of thioxolone niosomes, an immunomodulatory role was identified in enhancing the Th1 cytokine and inhibiting the Th2 cytokine profile, meaning that it could be used for the treatment of anthroponotic cutaneous leishmaniasis [47].

In a recent research work, Anjum et al. co-encapsulated amphotericin B and pentamidine in niosomes and transported them via chitosan gel. The system showed a more prolonged release of both drugs with respect to the same individually. An increase in skin penetration, a higher percentage of inhibition and a lower IC50 against *L. tropica* promastigotes were recorded, as well as the safety of the nanosystem [48].

It must be noted that most studies and advances in the use of niosomes in antileishmanial therapy focus on visceral leishmaniasis, maybe due to the fact that niosomes are mostly used in subcutaneous and intradermal formulations.

The evolution of niosomal medical administration technology is still at an early stage, but this type of delivery system, as noted above, has shown promising results in antileishmanial therapy [42].

3.1.2. Metal and metallic oxide nanoparticles

The development of silver (AgNP) and gold (AuNP) nanoparticles for the treatment of parasitic illnesses is well documented [49–51]. These particles are significant in this golden age of nanotechnology due to their eye-catching nanoscale structures and high aspect ratio [52–54]. The size, shape, thermal and optical properties of these nanoscale metals are different from those of the raw material, which makes the nanoparticles (NP) ideal for use in theranostic applications.

Metallic NPs have a deep impact in their dynamic role in the emerging area of nanotechnology. This is due to the wide variety of

Table 2
Mentioned nanotherapies according to the nanoparticle selected, the drug delivered, the *Leishmania* strain, the method of administration, the model selected, and the reference.

Nanoparticle	Drug delivered	*Leishmania* strain	Administration	Model	Refs.
Niosome	Miltefosine and ketoconazole	L. *major*	*In vitro*	Murine macrophage cells (J774-A1 ECACC, n°91,051,511) and Promastigote form of L. *major*	[46]
	Tioxolone	L. *tropica*	*In vitro*	Murine macrophage cells (J774 A.1 ATCC®TIB- 67™) and Promastigote form of L. *tropica*	[47]
	1. Niosomal AmB and pentamidine incorporated into a chitosan gel	L. *tropica*	*In vitro*	Promastigote form of L. *tropica*	[48]
Metal and metallic nanoparticles	AuNP obtained from *Eucalyptus camaldulensis*	L. *major*	*In vivo*	L. *major*-infected BALB/c mice	[67]
	Cannabis sativa mediated AuNP	L. *tropica*	*In vitro*	*Amastigote form of* L. *tropica*	[68]
	AgNP obtained from quercetin / *Artemisia aucheri*	L. *major*	*In vitro*	Promastigote form of L. *major*	[73]
		L. *major*	*In vivo*	L. *major*-infected BALB/c mice	
	AuNP/AgNP obtained from *Sargentodoxa cuneata*	L. *tropica*	*In vitro*	Murine macrophage cells (J774) and Promastigote form of L. *tropica*	[69]
	AuNP obtained from *Maytenus royleanus*	L. *tropica*	*In vitro*	Promastigote form of L. *tropica*	[70]
	4′,7-dihydroxyflavone-functionalized Au and AgNPs	L. *donovani*	*In vitro*	Promastigote and axenic amastigote form of L. *tropica*	[71]
	TiO_2AgNPs with *Nigella sativa* oil	L. *tropica*	*In vitro*	Murine macrophage cells (J774) and Promastigote form of L. *tropica*	[72]
	AgNPs obtained from *Fusarium oxysporium*	L. *amazonensis*	*In vitro*	Murine peritoneal macrophage cells and Promastigote form of L. *amazonensis*	[74]
	Cur@AgNPs	L. *major*	*In vitro*	Promastigote form of L. *major* and amastigote form of L. *major*	[75]
			In vivo	L. *major*-infected BALB/c mice	
	Chrysin conjugated AuNPs	L. *donovani (MHOM/IN/80/DD8)*	*In vitro*	Murine macrophage cells (J774) and Promastigote form of L. *donovani (MHOM/IN/80/DD8).*	[76]
	AgNP with amphotericin B obtained from *Isatis tinctoria*	L. *tropica*	*In vitro*	Promastigote form of L. *tropica*	[77]
	Meglumine antımoniate-TiO_2AgNPs	L. *tropica and* L. *infantum*	*In vitro*	Murine macrophage cells (J774.A1) and Promastigote form of L. *tropica* and L. *infantum*	[78]
	Anacardic acid and Cardol AgNPs obtained from *Anacardium occidentale*	L. *braziliensis*			
(MHOM/BR/94/H-3237)	*In vitro*	Murine macrophage cells (J774. A1), Amastigote form and Promastigote form of L. *braziliensis*			[79]
	V. officinalis and *V. tenuisecta* mediated ZnONPs	L. *tropica*	*In vitro*	Promastigote form of L. *tropica*	[80]
	Chitosan-TiO2-glucantime NPs	L. *major*	*In vitro*	Murine macrophage cells (J774) and Promastigote form of L. *major*	[82]

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Table 2 (*continued*)

Nanoparticle	Drug delivered	*Leishmania* strain	Administration	Model	Refs.
	Superparamagnetic iron oxide nanoparticles (SPIONs)	–	*In vitro*	Murine-immortalized blood macrophages RAW 264.7	[83]
Liposomes	Liposomal Amphotericin B (AmBisome)	L. *major*	*In vivo*	L. *major*-infected BALB/c mice	[91]
	Meglumine antimoniate liposomes (Glucantime)	L. *major*	*In vitro*	Amastigote form and Promastigote form of L. *major*	[92]
			In vivo	L. *major*-infected BALB/c mice	
	Meglumine antimoniate stearylamine-bearing liposomes (Glucantime)	L. *major*	*In vitro*	Murine macrophage cells (J774.A1) and Promastigote form of L. *major*	[93]
			In vivo	L. *major*-infected BALB/c mice	
	Miltefosine liposomes	L. *major*	*In vitro*	Murine macrophage cells (J774.A1) and Promastigote form of L. *major*	[94]
			In vivo	L. *major*-infected BALB/c mice	
	Rifampicin-laded nanotransfersomes (RIF-loaded NTs)	L. *tropica* KWH23	*In vitro*	Murine peritoneal macrophage cells and Promastigote form of L. *tropica*	[95]
			In vivo	L. *tropica*-infected female BALB/c mice	
	Topical Nanoliposomal Amphotericin B (SinaAmpholeish)	L. *major* and L. *tropica*	*In vitro*	Promastigote form of L. *major* (MRHO/IR/75/ER), amastigote form of L. *major* and macrophage B10 cells infected with GFP-expressing L. *tropica* parasite	[96,97]
			In vivo	L. *major*-infected BALB/c mice	
			In vivo	Rabbit model - Draize test and eye irritation test	[98]
			Phase I Clinical Trial	Healthy Volunteers	[99]
	Liposomal Amphotericin B incorporated into a chitosan gel	*Leishmania* spp.	In vitro	*Leishmania* spp. promastigotes	[101]
Nanoemulsions	Nanoemulsions containing α-copaene of *Copaifera paupera* Oleoresins	L. *amazonensis* and L. *infantum*	*In vitro*	Promastigote forms of L. *amazonensis* and L. *infantum*	[105]
	Lavandula angustifolia and *Rosmarinus Officinalis* Essential Oils based nanoemulsions	L. *major* (MRHO/IR/75/ER)	*In vitro*	Murine macrophage cells (J774) and Promastigote form of L. *major*	[106]
	Copaiba- and Andiroba-based nanoemulsions	L. *amazonensis* and L. *infantum*	*In vitro*	Promastigote forms of L. *amazonensis* and L. *infantum*	[107]
	Amphotericin B loaded Triglyceride-rich nanoparticles mimicking chylomicrons (TGNP-AB)	L. *amazonensis*	*In vitro*	Murine macrophage cells (J774) and Promastigote form of L. *amazonensis*	[108]
			In vivo	L. *amazonensis*-infected BALB/c mice	
	Nanoemulsions charged with Amphotericin B	L. *amazonensis*	*In vitro*	Murine macrophage cells (RAW 264.7) and Promastigote form of L. *amazonensis*	[109]
Solid lipid nanoparticles (SLN)	Paromomycin loaded solid lipid nanoparticles	L. *major* and L. *tropica*	*In vitro*	Human monocyte cell line THP-1 and Promastigote forms of L. *major* and L. *tropica*	[115]
	Paromomycin loaded solid lipid nanoparticles	L. *major*	*In vivo*	L. *major*-infected BALB/c mice	[116]
	Paromomycin loaded solid lipid nanoparticles	L. *tropica*	*In vivo*	L. *tropica*-infected BALB/c mice	[117]
	Amphotericin B loaded solid lipid nanoparticles	–	*In vivo*	White New Zealand rabbits	[118]

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Table 2 (*continued*)

Nanoparticle	Drug delivered	*Leishmania* strain	Administration	Model	Refs.
Nanostructured lipid carriers (NLC)	Curcumin loaded NLCs	L. *tropica* Skin permeation studies	*In vitro*	Promastigotes and axenic amastigote like cells (AALCs) forms of L. *tropica*	[121]
Ethosomes	Ethosomes charged with PRM and/or TFL-A	–	*In vitro*	Human Skin cells	[123]
	Nitazoxanide-loaded transethosomes in chitosan gel	L. *tropica*	*In vitro*	Peritoneal macrophages and Promastigote form of L. *tropica*	[124]
		–	*Ex vivo*	Wistar rats	
	2. Mannosylated imiquimod-terbinafine co-loaded transethosomes	L. *major*	*In vitro*	Intracelular amastigotes of L. *major*	[125]
			In vivo	L. *major*-infected BALB/c	
Lipid biomimetic delivery systems	*Artemisia absinthium* EO nanocochleates and *Bixa orellana* and *Dysphania ambrosioides* EO nanocochleates	L. *amazonensis*	*In vitro*	Intracellular amastigotes of L. *amazonensis* and non-infected peritoneal macrophage	[127, 128]
			In vivo	L. *amazonensis*-infected BALB/c	
Polimeric nanoparticles	Hydroxymethylnitrofurazone charged dextran-coated poly (n-butyl cyanoacrylate) nanoparticles	L. *amazonensis*	*In vitro*	Murine macrophage cells (J774.A1) and Promastigote form of L. *amazonensis*	[137]
	Chalcone CH8-loaded poly (lactic-co-glycolic acid) microspheres	L. *amazonensis*	*In vitro*	Murine peritoneal macrophage cells	[142]
			In vivo	L. *amazonensis*-infected BALB/c mice	
	Amphotericin B loaded-PLGA nanoparticles	L. *major*	*In vitro*	Human monocyte cell line THP-1 and Luciferase-expressing Lm:pSSU-int/LUC Promastigote form of L. *major*	[143]
			In vivo	L. *major*-infected BALB/c mice	
	Amphotericin B loaded-PCL nanoparticles	L. *tropica* KWH23 and L. *donovani*	*In vitro*	L. *tropica* KWH23 and L. *donovani* infected Murine macrophage cells (J774)	[144]
	PM-loaded into mannosylated CS (MCS) nanoparticles using dextran (PM-MCS-dex-NPs)	L. *major*	*In vitro*	Human monocyte cell line THP-1 and Promastigote forms of L. *major*	[145]
	Nitric oxide-loaded chitosan nanoparticles (NONPs)	L. *amazonensis* (MHON/BR/73/2269, transgenic line expressing luciferase)	*In vitro*	Bone marrow-derived macrophages (BMDM) from BALB/c mice and Promastigote form of L. *amazonensis*	[146]
	Chitosan-Coated Poly(isobutylcyanoacrylate) Nanoparticles	L. *major*	*In vitro*	Promastigote, axenic amastigote and intramacrophage forms of L. *major*	[147]
			In vivo	L. *major*-infected female BALB/c mice	
	Co-loaded rifampicin and pentamidine polymeric nanoparticles	L. *tropica*	*In vitro*	Promastigote form of L. *tropica*	[148]
Other nanoparticles	Bovine serum albumin nanoparticles containing amphotericin B	L. *amazonensis*	*In vitro*	Murine macrophage cells (J774.A1) and Promastigote form of L. *amazonensis*	[149]
			In vivo	L. *amazonensis*-infected BALB/c mice	
	Amphotericin B andd Betulinic acid charged Anionic Linear Globular Dendrimers	L. *major*	*In vitro*	Murine peritoneal macrophage cells and Promastigote form of L. *major*	[150, 151]
			In vivo	L. *major*-infected BALB/c mice	
	Cisplatin-bonded carbon nanotubes	L. *major*	*In vitro*	Murine peritoneal macrophage cells and Promastigote form of L. *major* / L. *major*-infected BALB/c mice	[152]
	AmB-Zn/Al LDHs	L. *major*	*In vitro and in silico*	Murine macrophage cells (J774.A1), intramacrophage amastigote and Promastigote form of L. *major*	[153]
	ZnS QDs	L. *major*	*In vitro*	Macrophage cells, intramacrophage amastigote and Promastigote form of L. *major*	[154]

applications in different fields, especially nanomedicine. These NPs have caught the attention of physicians thanks to their potential application in the treatment of different complex diseases [55], highlighting their antimicrobial and anticancer potential [56,57]. Among the explicit characteristics of metallic NPs are their size, shape, and high surface/volume ratio that makes them perfect for many biological applications, including antileishmanial drugs [58,59]. Previously, NPs were synthesized primarily by physical and chemical methods. Due to the many disadvantages observed in traditional synthesis methods, the current research approach has shifted to biological methods that have played an essential role in the synthesis of metal NPs. Therefore, researchers have focused on natural materials for green synthesis [60], mainly in the use of active ingredients that originate from metabolic products in fungi, bacteria and plants [61–63]. Nevertheless, Shakeel et al. recently developed a chemically synthesized combination of polymeric NPs (see Section 2.3.5 below) with AgNPs as the active agent in terms of antiparasitic effect [64]. They formed a mannosylated thiolated chitosan-coated AgNP (MTC-Ag) loaded emulgel to treat CL. MTC-Ag had a positive charge, a size of approximately 115 nm, and slow release of silver ions. They were effective in inhibiting intracellular amastigotes, while being much less cytotoxic for macrophages than AgNP alone..

For the application of metallic NPs, colloidal suspension stability plays a crucial role. Stability may be compromised due to the aggregation of unstable NPs or their spontaneous growth that limits their widespread applications by destroying their favorable characteristics [65,66].

Some works related to the search for antileishmanial therapies using biogenic metal nanoparticles are highlighted below.

In the work of Torabi et al., AuNPs were produced from the methanolic extract of *Eucalyptus camaldulensis* through the reduction of aqueous $AuCl_4$ in a size range of 1.25–17.5 nm with an average size of 5.5 nm. These were evaluated in the BALB/c mouse model for cutaneous leishmaniasis (CL) caused by *L. major* Iranian strain [67]. Other green-synthesized gold NPs were obtained by Hameed et al. using *Cannabis sativa* extract for the reduction of gold to its bioactive form. These were characterized, finding that their antimicrobial and antifungal capacity is noteworthy compared to AmB, while their antileishmania activity was verified, evaluated in L. *tropica* [68].

Ahmad et al. demonstrated the antileishmanial activity of the biosynthesized silver (3–8 nm) and gold (15–30 nm) NPs of *Sargentodoxa cuneata*, showing IC_{50} values of 4.37 and 5.29 μg/ml respectively [69]. Their antileishmanial activity was tested for 96 h in L. *tropica* promastigotes. The study concluded that both biogenic AgNP and AuNP were significantly leishmanicidal; however, biogenic AgNP demonstrated a maximum inhibition of 95.45% after 48 h of incubation. In another study by this same research group, biosynthesized AuNP (30 nm) obtained from *Maytenus royleanus,* which showed to inhibit 75% of the growth of L. *tropica* promastigotes after 72 h of incubation. It is also interesting to note that the activity decreased somewhat after incubation, which the authors attribute to the loss of AuNP gold ions [70]. Recently, 4′,7-dihydroxyflavone-functionalized gold and silver NPs showed ROS-mediated toxicity on promastigotes and amastigotes from *L. donovani* strains cultivated *in vitro* [71] In other studies, the antileishmanial effect of nontoxic concentrations of TiO_2-AgNPs with oil combinations of *Nigella sativa* was tested in promastigote and amastigote macrophage culture systems and found to have a significant antileishmanial effect on each life form of *L. tropica* parasites [72]. The latter results from the fact that TiO_2Ag nanoparticles have a much stronger inhibitory effect on microorganisms compared to the separate use of silver and TiO_2 nanoparticles, as shown in other works. The high antimicrobial activity of TiO_2-AgNPs can be explained by the combination of silver and TiO_2 nanoparticles, which have different effects on microorganisms [62]. Furthermore, other ecofriendly AgNPs were obtained by Alemzadeh et al. from *Artemisia aucheri*, which lowered the inflammatory response and enhanced wound healing [73].

Among the works using fungal metabolic products, there is a study conducted by Fanti et al. investigating the action of biogenic silver nanoparticles (AgNp-bio) against promastigote and amastigote forms of *L. amazonensis.* These nanoparticles were obtained by reducing silver nitrate using *Fusarium oxysporium* enzyme nitrate reductase. The results showed that AgNp-bio has a direct effect on the forms of *L. amazonensis.* AgNp-bio acts on the immunomodulatory capacity of infected macrophages, reducing infection without inducing the synthesis of inflammatory mediators, since continuous stimulation can generate and aggravate leishmanial lesions [74].

Concerning metal nanoparticles as drug delivery systems, Badirzadeh et al. combined the known antiparasitic effects of curcumin by conjugating it to the surface of silver nanoparticles (Cur@AgNPs). These NPs showed antiparasitic activity on both promastigotes and amastigotes without affecting mouse macrophages [75]. Raj et al. improved the performance of chrysin, an antioxidant compound previously tested as leishmanicidal, by attaching it to gold nanoparticles [76]. Ahmad et al. demonstrated the enhanced antileishmanial activity against *L. tropica* by linking AgNP to amphotericin B using an aqueous extract of *Isatis tinctoria*, better known as woad leaves. Biogenic AgNP conjugated with amphotericin B showed IC_{50} values of 2.43 μg/μL compared to pristine AgNP (10–20 nm), which reached an IC_{50} value of 4.2 μg/μL. The aqueous extract of *L. tinctoria* showed moderate activity of 43% inhibition after 48 h incubation with promastigotes, while biogenic AgNP and amphotericin B adsorbed on AgNP showed 73% and 85% inhibition, respectively [77]. After completing this research, Abamor et al. investigated the antileishmanial efficacy of TiAgNps-meglumine antimoniate combinations at nontoxic doses to develop a new treatment method that minimizes the toxicity of pentavalent antimonials while maximizing their efficacy. These were tested in promastigote and amastigote macrophage cultures of *L. tropica* [78]. Green-synthesized silver nanoparticles from cashew nutshell extracts also showed promising results on amastigotes *in vitro* [79].

In the case of ZnO nanoparticles, Sumaira et al. described their biosynthesis using leaf extracts of *Verbena officinalis* and *Verbena tenuisecta* and examined them through their leishmanicidal potential against *L. tropica* [80]. *V. officinalis* synthetized from ZnO NPs showed more potent leishmanicidal activity compared to *V. tenuisecta* ZnO NPs due to their smaller size and increased phenolics doped onto its surface. These results can be a step forward towards the development of novel compounds for this disease. Further investigations regarding the toxicity of this kind of nanoparticles are needed to be used as guidelines for safer use of ZnO-based nanomaterials in topical treatments [81].

Finally, Varshosaz et al. worked with nano-assemblies of TiO_2 and chitosan nanoparticles loaded with glucantime. This design permits to take advantage of their synergistic effects and improve the toxic effects of glucantime on *Leishmania* parasites. They found

that, at a concentration of 50 μg/ml and after 72 h of exposure, nano-assemblies helped decrease the proliferation of promastigotes and amastigotes of *L. major* by 13 and 4 times, respectively, compared to glucantime alone [82].

In a more recent study, low-cost superparamagnetic iron oxide nanoparticles (SPION) were synthesized together with coconut water in order to study the ability of macrophages to internalize them. These presented a high value of intrinsic energy loss and were not cytotoxic for mammalian cells [83]. While they were not explicitly evaluated in *Leishmania*, the group established a possible scope for parasitic diseases that involve macrophages in their infection processes.

In summary, the mechanisms of antileishmanial activity through biogenic metal NPs that stand out are the ROS generation and the inhibition of leishmania proliferation [62]. In addition, drug toxicity reduction due to the lower concentrations required when using nanoparticles in the treatment of leishmaniasis should not be forgotten. These NPs may be used to treat cutaneous leishmaniasis due to the positive results they have shown in previous therapies.

3.1.3. Liposomes

Liposomes are colloidal nanoparticles that were discovered in 1965 by Bangham et al. [84]. They found that certain lipids (phospholipids and cholesterol) can form bilayers in presence of excess water enclosing aqueous compartments which can be used for delivery of drugs to the skin, especially in the treatment of several dermatological disorders [85]. The versatility of liposomes lies primarily in their ability to incorporate hydrophilic, hydrophobic, and amphiphilic molecules into their structure. Also, their physical properties can be easily modified. Finally, they can be functionalized with antibodies or other ligands with the aim of targeting a specific site [86] or with probes to guide the nanosystem to the region to be treated [87].

In the nineties, an interesting liposomal formulation emerged looking for greater direct skin penetration: the ultradeformable liposomes or transferosomes [88]. These are a type of liposomes with the ability to reach the inner layers of the skin, below the *stratum corneum* (SC) thanks to changes in their deformable membrane.

Liposomes is one of the drug delivery techniques that should be considered in the search for treatments with fewer side effects against cutaneous leishmaniasis.

Although several topical and lipid-based formulations are developed and used in various types of studies [89,90], there is only one commercial topical formulation available for CL treatment, which consist of liposomal amphotericin B (L-AMB) commercialized by Gilead Sciences S.L. (AmBisome®). Encouraged by the high cost of AmBisome in the market, several groups continued with the evaluation of liposomal amphotericin B for topical application, such is the case of Wijnant et al., who compared the cutaneous pharmacokinetics of L-AMB with AmB in murine models of *L. major* [91].

To add, there is an increase in the interest of different groups in researching and improving the development of new treatments for this disease through liposomal delivery.

In a work from the last decade, formulations for topical use composed of liposomes containing meglumine antimoniate (MA), with or without the presence of oleic acid, were developed by Moosavian Kalat *et al.* They evaluated the therapeutic effects of these liposomes on *L. major*-induced lesions in BALB/c mice. In the treated mice, the size of the lesions was significantly smaller than those in the control groups (exposed to unloaded liposomes or buffer only). Nevertheless, the size of the lesions showed increments after the end of the treatment, remaining smaller in comparison to those on the control animals [92]. This same research group also proposed the addition of stearylamine (SA) in the structure of the liposomes, as it has antileishmanial activity by itself, showing that SA liposomes encapsulated with MA could be useful for the topical treatment of CL, but further investigation is needed [93].

Two of the most recently published works are about antileishmanial drug-loaded liposomes and nanotransferosomes with promising results. In one of them, carried out by the group led by Dr. Kavian, miltefosine-loaded liposomes were topically applied to BALB/c mice previously infected with *L. major*. 4% liposomal miltefosine was the best concentration for skin penetration *ex vivo* and for leishmanicidal activity *in vivo*, in comparison to a cream formulation of miltefosine and other liposomal concentrations [94]. The other work carried out by Rabia et al. used rifampicin (RIF) as the active pharmaceutical ingredient in nano transfersomes formulated in a chitosan gel [95]. Both *in vivo* study and analysis carried out by flow cytometry showed that the nanoformulation was more effective in promoting apoptosis and also achieving toxicity on leishmania than the free RIF. In all, this transporter that combines transfersomes into the chitosan matrix appears promising as a vehicle for application on skin to deliver proven antileishmanial molecules for CL.

A very promising treatment seems to be the recently approved formulation for CL treatment named SinaAmpholeish, from Iran. It consists of Amphotericin B at a 0.4% concentration, loaded in nano-liposomes for topical application [96]. The research group first formulated liposomes containing 0.1, 0.2, and 0.4% AmB (SinaAmpholeish) and characterized their size, entrapment efficiency, long-term stability, and skin penetration properties. The *in vitro* effectiveness of SinaAmpholeish was tested on *L. tropica* and *L.* major, while it also proved to be effective in curing lesions in an *in vivo* model (BALB/c infected by L. major) [97]. On the other hand, irritancy assessment was performed in a rabbit model, by the Draize test [98]. A clinical trial (phase I) was carried out in healthy volunteers to assess safety [99], concluding that a scheme of two daily applications is appropriate for further phase II testing because it did not cause adverse effects.

Although there are no research articles about phase II clinical trials and safety studies published, they are described in the SinaAmpholeish 0.4% brochure that could be found in Exir Nano Sina web page, a company that was founded by Dr. Jaafari and that produces the drug [100]. According to phase II, both SinaAmpholeish alone and in combination therapy trials showed effectiveness over 92%. Meanwhile, safety studies showed no adverse topical effects with an exception of two patients with extensive lesions that had a tolerable burning sensation. This formulation represents the initial promise about the potential of liposomes in the treatment of the disease and shows the important role of research groups that have worked in this field for years. Finally, a recent work from Gürbüz Çolak et al. included liposomal amphotericin B in a chitosan gel matrix for sustained release [101].

Therefore, the comprehensive studies carried out so far demonstrate that the more we study and use these nanoparticles, the better

therapies will be developed.

3.1.4. Other lipid nanosystems

Other lipid-based carriers for drug delivery such as ethosomes, nanoemulsions, solid lipid nanoparticles (SLN), or second-generation SLN —also known as nanostructures lipid carriers (NLC)— caught attention for their potential to encapsulate drugs that are hard to dissolve in water, while being biocompatible and biodegradable [102]. These particles share similarities with other nanosystems such as liposomes, in terms of lipid nature, and with polymeric nanoparticles, in terms of solid matrix structure. However, they also have unique characteristics that help overcome some of the disadvantages associated with both systems mentioned above, such as liposome stability problems or the potential toxicity associated with some polymers and solvents used in the preparation of polymeric nanoparticles [86].

Nanoemulsions. Nanoemulsions (NE) are emulsions in which the size of the droplets is smaller than 100 nm. Therefore, they can be used as drug carriers for the treatment of diverse diseases that develop in the skin, delivering lipophilic molecules throughout the stratum corneum [103]. Moreover, NE are made up of biocompatible compounds, and their preparation and their scale-up are relatively simple processes that render high amounts of loaded drug [104].

In the work of Rodrigues et al., based on the ethnopharmacological evidence on the activity against leishmaniasis of oleoresins from *Copaifera spp*, the effects of crude extracts and oleoresin fractions of two specimens of *Copaifera paupera* on strains of *L. amazonensis* and *L. infantum* were evaluated. The best activity against both of them was exhibited by oleoresins high in α-copaene (38.8%). This molecule —a sesquiterpene— was isolated and then assessed, causing *in vitro* toxicity on the same Leishmania species. Then, NEs carrying the active oleoresin fractions were prepared to be tested on promastigotes from the same strains, with the α-copaene-loaded NE (named "nanocopaen") exhibiting the highest antileishmanial activity in both cases [105].

In the same line of using active principles from plants, we could find the use of different oils with antileishmanial activity for preparation of nanoemulsions or as an active principle.

In the first case, we find the work of Shokri et al. where they evaluated antileishmanial effects of *Lavandula angustifolia* and *Rosmarinus officinalis* medicinal plant's essential oils and nano-emulsions on *L. major* compared to the effect of meglumine antimoniate [106]. The nano-emulsions of both plants showed better performance *in vitro* than essential oil on the mean infection rate of the macrophages and the number of amastigotes per infected macrophage. While the free lavender oil was more effective than the MA, both NE of essential oils performed better than the free essential oils. *In vivo* testing in a murine model is yet to be assessed.

De Moraes *et al*. developed nanoemulsions as delivery systems for copaiba and andiroba oils (namely "nanocopa" and "nanoandi") to test their effects on *L. infantum* and *L. amazonensis* [107]. Nanocopa and nanoandi presented toxic activity against promastigotes of both species, and they also reduced infection by amastigotes in macrophage cultures. When they were tested *in vivo* in a murine model (infected with parasites from *L. amazonensis*), parameters such as the parasite burden, lesion sizes, and histopathology showed significant promising values.

In a recent work from Brazil, researchers tested safety and efficacy of a formulation of amphotericin B loaded in nanoparticles rich in triglycerides (TGNP-AB) —intended to mimic chylomicrons— in a mouse model infected with *L. amazonensis*. The TGNP-AB performance showed many advantages compared to free AmB: On one hand, cytotoxicity on macrophages was reduced. On the other hand, it was more effective in preventing the infection of macrophages, in reducing the number of amastigotes per infected cell, and it was also more effective against promastigotes. Hepatotoxicity in the host decreased. The lesion sizes and parasitic burden were also lowered by the treatment. In sum, its leishmanicidal activity was notable, while toxicity on the host was lower even at high doses [108].

In one of the last studies, Dos Santos Matos et al. have developed oil-water NE containing AmB by varying the presence of polysorbate 20 as co-surfactant, and the amount of copolymer surfactant (poloxamer 407) used in the aqueous phase. *In vitro* antileishmanial activity using *L. amazonensis* promastigotes was evaluated. The best AMB-NEs were submitted to stability tests and these formulations presented excellent results after a year under refrigeration, keeping the conservation of the drug content higher than 95%. AMB-NEs displayed slow and controlled AmB kinetic release and low skin permeation [109] becoming a promising nanosystem that is expected to reach clinical trials.

Solid lipid nanoparticles (SLNs). Solid lipid nanoparticles were introduced in 1990 [110]. They are composed of a solid lipidic core surrounded by surfactants, with sizes below 1000 nm. In order to achieve the regulated release of the active ingredients, the lipid matrix of the core is crucial [111]. SLNs have appeared as an advantageous alternative to other delivery systems due to specific features such as the possibility of loading both lipophilic and hydrophilic active principles, their improved physical stability, and their low cost and relatively easy scale-up in comparison to liposome production [112]. The small size of SLNs improves contact between them and the SC, thus increasing the amount of active principle that can actually penetrate the skin [113].

Many research groups proposed this nano-drug delivery system for the treatment of CL. In particular, the Department of Immunotherapy and Leishmania Vaccine Research in Iran worked with SLNs loaded with paromomycin from their formulation and characterization [114] to their *in vitro* [115] and *in vivo* [116,117] evaluation. Researchers obtained a stearic acid-based formulation through microemulsion. Paromomycin-loaded SLNs were significantly more effective than free paromomycin ($P < 0.05$) to stop *in vitro* propagation of *L. major* and *L. tropica*. After *in vivo* results, the authors concluded that SLNs were safe and effective against leishmaniasis by improving the performance on paromomycin on parasites and changing the immune response to Th1.

Butani *et al*. designed and developed SLNs loaded with amphotericin B for topical use with antifungal purposes, but also mentioning

the potential use in antileishmanial therapies. Results found that the system was useful to overcome the low solubility of the active principle in aqueous media and to improve its therapeutic efficacy [118].

It is significant to highlight that the most of leishmaniasis research efforts using this delivery mechanism concentrate on creating vaccines for topical, subcutaneous, or parenteral injection to treat visceral leishmaniasis. Therefore, we hope to see the evolution of the previously mentioned works as well as the occurrence of new ones.

Nanostructured lipid carriers (NLCs). These nanoparticles are considered as the second generation of SLNs, with improved features due to a less organized lipid nucleus that allows a more stable and long-lasting loading of active principles [119]. The main focus of the production of this transporter, concerning leishmaniasis, used to be the oral delivery of drugs to target the lymphatic system [120], with no topical application strategies intended for cutaneous manifestation [102].

Last year, Riaz et al. chose curcumin as an antileishmanial drug [121] and loaded NLCs (Cur-NLCs) were prepared and characterized for *in vitro* drug release, *ex vivo* skin permeation and deposition studies.

Ethosomes. Ethosomes are lipid nanoparticles mainly composed of phospholipids and ethanol. In theory, they are a useful delivery system for skin administration because of the action of ethanol on the lipid organization of the SC. Ethosomes (and transethomes, which also include an edge activator in their membrane) are more flexible than conventional liposomes and more stable than other flexible entities such as ultradeformable liposomes, and they can deliver active principles to the deepest skin layers and even to the systemic circulation [122]. Nevertheless, few works have proposed this nanosystem for the treatment of CL. For example, Silva [123] developed ethosomes loaded with paromomycin (PRM), trifluralin analogs (TFL-A), or both together as a new strategy to treat CL. Most of the tested formulations showed acceptable values in size and Z-potential, and high encapsulation efficiency, with nonspecific-toxicity reduction. Khalid et al. [124] have recently developed nitazoxanide-loaded transethosomes for topical administration against CL in order to minimize the side effects associated with oral intake of the drug. The formulation was suspended in a chitosan gel and demonstrated safety, non-irritating properties, and the ability to penetrate the skin of albino Wistar rats. *In vitro* studies also showed anti-promastigote activity. Last, in another work from the previous group, Jamshaid et al. conducted a just-published study [125] in which they co-encapsulated imiquimod and terbinafine in mannosylated transethosomes that were then loaded into a chitosan gel. Their design maximized drug load and macrophage targeting. The results were very promising, as they demonstrated *in vitro* synergistic action against *L. major* amastigotes and reduced lesion size in infected BALB/c mice.

Lipid biomimetic delivery systems. Biomimetic systems comprise structures designed to mimic other analogous structures present in living organisms. So far, this type of system has mostly been tested for the treatment of visceral leishmaniasis. Although Alonso et al. presented in 2012 [126] some biomimetic lipid vesicles loaded with miltefosine and pointed out its potential use in cutaneous leishmaniasis, the research line was apparently abandoned. The recent literature on biomimetics for cutaneous leishmaniasis includes only studies from one group that uses nanocochleates, which are compounds consisting of a lipid bilayer that wraps around a central core in a spiral shape. Tamargo and Machín's research group presented nanocochleates formulated with different essential oils that demonstrate antileishmanial activity [127,128]. In 2017, they evaluated the antileishmanial activity of essential oil (EO) from *Artemisia absinthium* and, in 2019, essential oils from *Bixa orellana* and *Dysphania ambrosioides*, in all cases using nanocochleates as carriers. While *in vitro* assays were inconclusive, *in vivo* assays using intralesional application in BALB/c infected models showed some reduction in lesion size and inhibition of infection progression.

3.1.5. Polymeric nanoparticles

Polymeric nanoparticles have been proposed as a passive drug administration system due to the long time they remain in circulation and their rapid elimination by phagocytes, thus improving drug action and reducing required doses [129]. Polymeric nanoparticles are solid, colloidal particles, with sizes below 1 μm, made of biocompatible polymeric matrix [130]. The drug can be dissolved, retained, or adsorbed in the matrix, or even solubilized in the oil nucleus of the so-called nanocapsules [131]. Biodegradable matrices include synthetic polymers like poly (alkyl cyanoacrylate) (PACA), polylactide (PLA), Poly (butyl cyanoacrylate) (PBCA), Poly Lactic-co-glycolic acid (PLGA), Polycaprolactone (PCL), poly (glycolic acid) (PGA), poly (amino acid) and natural polymers such as gelatin, albumin, chitosan and alginate [132]. Polymeric nanoparticles can be useful in therapies for diseases like leishmaniasis as they can trespass biological barriers and be captured by cells in infected tissues [133,134]. In particular, chitosan has been reported to have antileishmanial activity [135].

The main benefit of polymeric nanoparticles in comparison to liposomes is their ability to tolerate physiological tension, *i.e.*, higher biological stability [136]. Several methods have been employed to load antileishmanial drugs in polymeric nanoparticles, such as polymerization in emulsion or microemulsion [137], interfacial polymerization [138] and polymerization by precipitation [139]. Their main disadvantages are related to problems in biodegradability and the presence of toxic traces from organic solvents [140].

Polymeric NPs are also attractive as a potential single-dose treatment for CL via intralesional injection; NPs up to 6 μM can be phagocytosed by infected macrophages, while bigger particles or aggregates can act as a depot for sustained release in the infected area [141]. By applying a single subcutaneous chalcone injection loaded into PLGA NPs, the effect over *L. amazonensis* parasites on BALB/c mice was enough to control growth [142], and this performance was better than the achieved by three applications of the free drug or Glucantime®.

Ammar *et al.* generated PLGA nanoparticles loaded with AmB but they were examined using *in vivo* and *in vitro* tests against *L. major*. The obtained results suggested that AmB NPs improve AmB delivery and may be used for local treatment of CL [143].

In one of the most recent works, Saqib et al. developed amphotericin B loaded-biopolymer of polycaprolactone (PCL) nanoparticles varying parameters, such as concentration of PCL and surfactant Poloxamer 407, to optimize the formation. With a mean hydrodynamic particle size of 183 nm with a spherical morphology and an encapsulation efficiency of 85%, these nanoparticles showed promising results according to *in vitro* assays, although, *in vivo* assays as well as the establishment of safety profiles are needed [144]. In the work of Esfandiari *et al.*, paromomycin-loaded mannosylated chitosan nanoparticles using dextran (PM-MCS-dex-NPs) were synthesized and characterized [145]. The findings demonstrated that PM-MCS-dex-NPs significantly impacted both life phases of *L. major* parasites, especially in the amastigote form. Another work studied chitosan nanoparticles loaded with nitric oxide (NONPs) to assess their antileishmanial performance on intracellular amastigotes and promastigotes of L. *amazonensis* (MHON/BR/73/2269, transgenic line expressing luciferase) [146]. NONPs were able to achieve a sustained death of parasites for both infective forms with no toxicity on macrophages.

A promising nano-vehicle was tested by Malli et al. They investigated whether poly (isobutyl cyanoacrylate) nanoparticles coated with chitosan could provide intrinsic antileishmanial activity after topical application [147]. Tests against *L. major* parasites (both *in vivo* and *in vitro*) showed that the nanoparticles combined or not with amphotericin B-deoxycholate have antileishmanial activity. Finally, in the work of Khan et al. [148], two combined antibiotics were loaded into a carbopol-based gel formulation of polymeric nanoparticles of chitosan, sodium tripolyphosphate, and Tween® 80 for topical application, with promising results in term of skin penetration, sustained drug release, uptake by macrophages, and better antiparasitic effect.

These mentioned works show the increasing relevance of polymeric nanoparticles development in nanomedicinal approaches for antileishmanial therapies and all the work that has to be done until they reach the market.

3.1.6. Other nanoparticles

There are some well-known, cutting-edge nanosystems that are just starting to be considered for CL treatment. Thus, a few works are emerging where systems such as proteic NPs loaded with bovine serum albumin [149], dendrimers [150,151], carbon nanotubes [152] are used as carriers for antileishmanial drugs, among others.

In the first case, amphotericin B was nanoencapsulated in bovine serum albumin (BSA) nanoparticles. Its activity was tested against *L. amazonensis in vitro* and *in vivo*. AmB-BSA nanoparticles were more effective against amastigote than promastigote forms and showed significant decrease in the lesion thickness at the infected footpad of male BALB/c mice. It also did not show tissue toxicity compared to commercial AmB-deoxycholate. The research group suggests that this nanosystem has more therapeutic advantages than conventional AmB-D therapy for treating cutaneous leishmaniasis.

Regarding the use of dendrimers, Mehrizi et al. studied Amphotericin B and Betulinic acid loaded into Anionic Linear Globular dendrimers. Both nanoformulations were completely nontoxic against BALB/c mouse-derived peritoneal macrophages after 48 h incubation and against female BALB/c mice. The use of 20 μg/mL concentration of AD and BD had lower *L. major* promastigote killing effects compared to the use of chitosan NPs (61 and 73%, respectively) and amastigote inhibition of 52 and 61%. Even though the research group recommends chitosan formulations, dendrimer systems promise another possible drug delivery platform for cutaneous leishmaniasis to be tested with other antileishmanial agents.

In turn, cisplatin-bonded single-walled carbon nanotubes (CP-SWCNT) and cisplatin-bonded multi-walled carbon nanotubes (CP-MWCNT) were tested against *L. major* amastigote and promastigote and their cytotoxicity against macrophages alone. In comparison with free cisplatin and Glucantime(R), CP-MWCNT showed considerable antileishmanial activity on amastigotes of *L. major* with a selective killing effect on infected macrophages as well as greater efficacy, while CP-SWCNT were found to be not completely safe, and affect both macrophage and amastigotes.

Another promising nanocarriers are layered double hydroxides (LDHs). In the case of Bahraminejad et al. research, they synthesized and characterized Zn–Al layered double hydroxides as a drug delivery system for amphotericin B. This system showed higher efficacy against intra-macrophage amastigotes of *L. major* parasites compared to conventional amphotericin B, although it was less effective against the promastigote form. Furthermore, AmB-Zn/Al LDHs were taken up more quickly by macrophages via endocytosis, had appropriate antioxidant activity, had higher apoptosis level and less necrotic level than AmB and stimulated anti-leishmaniasis immune responses associated with Th1 and Th2 cells. It is of great importance that this system be evaluated *in viv*o and in other Leishmania species [153].

Meanwhile, another nanoparticle that is often used in photodynamic therapy was tested as an antileishmanial agent. Zinc sulfide (ZnS) quantum dots (QDs) were eco-friendly synthesized using green tea extract. ZnS QDs were tested against Leishmania major promastigotes, macrophages and L. major infected macrophages. These nanoparticles exhibited higher efficacy, better biocompatibility, and eco-friendly advantages compared to meglumine antimoniate and showed a significant effect on the gene expression profiles of Th1 and Th2. Although it appears to be a promising nanoparticle against this disease, it needs further study regarding application and biodistribution due to the size and characteristics of this type of nanoobject [154].

Most of these nanosystems and nanoparticles are interesting approaches to continue testing in other Leishmania species to later continue its study in preclinical models and clinical trials.

The use of nanoparticles as photodynamic therapy effectors or as delivery systems for those effectors will be considered in the following section.

4. Photodynamic therapy for leishmaniasis

Photodynamic therapy (PDT) is based on the photodynamic effect, which consists in generating reactive species upon energy transfer from a photosensitive molecule that has been light-irradiated to biological acceptors (generally oxygen) that can lead to cell

Table 3
Mentioned PDT nanotherapies according to the nanoparticle of choice, PDT effector, *Leishmania* strain, method of administration, model of choice, and year of publication.

Nanoparticle	PDT effector	Irradiation source	*Leishmania* strain	Administration	Model	Refs.
Metal and metallic oxide nanoparticles	ZnO based nano-formulations	Visible light	L. *tropica*	In vitro	Fresh human macrophages - Promastigote form of L. *tropica* - Brine shrimp assay	[81]
			–	*In vivo*	BALB/C male mice	
	Fe-, Zn-, or Pt- doped TiO_2NPs	Visible light	L. *amazonensis*	*In vitro*	Murine macrophage cells infected with L. *amazonensis*	[163]
	Anatase and Rutile-NPs	UVA and UVB light	L. *major*	*In vitro*	Promastigote form of L. *major*	[164]
	TiO_2ZnNPs with hypericin	Visible light and dark conditions	L. *amazonensis* MPRO/BR/1972/ M1841-LV-79	*In vitro*	Murine peritoneal macrophages infected with and Promastigote form of L. *amazonensis*	[165]
				In vivo	L. *amazonensis*-infected BALB/C mice	
	Trigonella foenum-graecum-mediated ferromagnetic iron oxide nanorods (FIONs)	LED light and dark conditions	L. *tropica*	*In vitro*	Murine peritoneal macrophages infected with and Promastigote form of L. *tropica*	[166]
Liposomes	UDL-Cl-AlPc	Visible light	L. *(V.) braziliensis*	*In vitro / In vivo*	Human leukemia THP-1 cells, murine fibroblast NIH/3T3 cells and Murine macrophage cells (J774) infected with and Promastigote form of L. *(V.) braziliensis* / L. *(V.) braziliensis*-infected BALB/c mice	[169]
	UDL-Cl-AlPc	Visible light	–	*In vivo*	Wistar rats	[170]
	L-Cl-AlPc	Visible light	L. *amazonensis*	*In vivo*	L. *amazonensis*-infected BALB/c mice	[171]
Nanoemulsions	ZnPc	Visible light and dark conditions	L. *amazonensis* and L. *infantum*	*In vitro*	RAW 264.7 cells infected with and Promastigote form of L. *amazonensis* and L. *infantum*	[172]
	Cl-AlPc	Visible light and dark conditions	L. *(V.) braziliensis*	*In vitro*	THP-1 cells, promastigote and intracellular amastigote form of L. *(V.) braziliensis*	[174]
				In vivo	Healthy and L. *(V.) braziliensis*-infected BALB/c	
Quantum dots	CDs and Ga@CDs	–	L. *donovani* and L. *major*	*In vitro*	Human leukemia THP-1 cells infected with L. *donovani* promastigote (GFP) parasites. L. *donovani* promastigotes, L. *donovani* promastigotes chromosomes (Ld1S-GFP), and L. *major* promastigotes	[177]
			–	*In vivo*	BALB/c mice	

death [155]. Initially intended for cancer treatment, its horizon has been broadened to other diseases due to its versatility and advantages, such as low invasiveness, absence of systemic toxicity, high selectivity, better functional and cosmetic results, and the ability to be repeated on the same site. Furthermore, when the photosensitizer is excited, it could also generate fluorescence. Thus, many photosensitizers are said to have theranostic potential, since they can act as a therapeutic and diagnostic tool. This has led to coining the term "photodynamic diagnosis" in addition to PDT [156].

PDT requires a photosensitizer, oxygen and light that can be well absorbed by the photosensitizer to render —through different photo-oxidation pathways— the highly cytotoxicity of reactive and singlet oxygen species [157–159]. Ideal photosensitizers for topical PDT should accumulate at the site of action and produce short periods of photosensitivity, long activation wavelengths (which are more penetrating and innocuous) and high quantum yields of singlet oxygen [158,160]. The low cost of some photosensitizers and light sources, and the absence of reports of parasite resistance to PDT, raises its potential benefit in the treatment of tropical diseases [155]. Nevertheless, the specificity of PDT is a disadvantage when the photosensitizer cannot penetrate the skin adequately, as the half-life of singlet oxygen and the distance it can diffuse are very short [161] and thus the photosensitizer must be present at the site of action before its activation. To overcome this difficulty, nanosystems can be used as photosensitizer carriers [162] as well as nano-objects that act directly as PDT effectors (*e.g.*, some metallic nanoparticles and quantum dots) can be formulated (Table 3).

4.1. Nanoparticles for PDT

4.1.1. Metal and metallic oxide nanoparticles

The intrinsic antileishmanial properties of these nanoparticles were covered in Section 3.2.2. Nevertheless, some works have studied their toxicity after irradiation.

Lopera *et al.* developed 100-nm aggregates of 20-nm sized TiO_2 NPs doped with Fe, Zn or Pt, in which the doping allows a shift in the absorption spectra to the visible range, thus allowing ROS production with visible light irradiation which was lethal to L.

amazonensis amastigotes nests in murine macrophages culture [163].

Other two works on TiO_2NPs showed interesting findings. In Dolat et al., the synergistic effect of UVA and UVB radiation on the promastigotes of L. *major* was investigated in the presence of two crystalline forms of TiO_2— Rutile and Anatase— nanoparticles (Anatase and Rutile-NPs) [164]. They discovered that the combination of UV light and TiO_2-NPs can effectively kill L. *major* promastigotes, although more *in vivo* studies are required. While in Lopera Sepulveda et al., Zn was added to TiO_2 nanoparticles (TiO_2ZnNPs) and synthesized with hypericin (HY) in order to increase its photodynamic activity in the visible light spectrum [165]. Both *in vitro* and *in vivo* assays indicate that this nanosystem could be a new alternative to current therapies.

Another work using ZnO NP is the case of Nazir et al., which assessed antileishmanial activity *in* L. *tropica* promastigotes from clinical isolates upon irradiation of a set of doped ZnO nanoparticles (using carbon, nitrogen, or carbon plus nitrogen as dopers). After *in vitro* toxicity in Artemia salina and human-derived macrophages —that was low in general—, the NP with the best performance (in this case, the nitrogen-doped ZnO NP) were also tested *in vivo* upon administration by the intraperitoneal or the topical route in mice to assess toxicity. The absence of toxic effects when applied topically appears promising towards their use in CL [81].

Under another recent study, the promastigote and amastigote forms of L. *tropica* were exposed to ferromagnetic iron oxide nanorods obtained from plants (named FIONs), in photodynamic treatment [166]. Based on their *in vivo* applications in the treatment of other diseases, *in vitro* antileishmanial activity was evaluated under light-emitting diode (LED) light and dark conditions. FIONs showed very interesting antileishmanial properties, and they should be further investigated in an *in vivo* model. Other recent work presented a conjugation of gold nanoparticles with a specific anti macrophage antibody that showed a good therapeutic effect on BALB/C mice after irradiation with red light [167].

4.1.2. Liposomes for PDT

The incorporation of PDT effectors in liposomes for topical treatments is extensively covered in literature. In this review we will continue focusing on the recent works.

Pereira Ribeiro *et al*. encapsulated a chloroaluminium phthalocyanine (Cl-AlPc) in conventional liposomes made of l-α-dipalmitoylphosphatidylcholine (DPPC) and cholesterol and tested it in mice infected with L. *amazonensis* together with oral administration of miltefosine [168]. After irradiation with a 670 nm laser, a dose of 100 J/cm^2 achieved a significant reduction in the number of parasites, while the hind paws of mice in which the infection was produced after intradermal injection, returned to their original diameter after 20 days of treatment.

In the case of Escobar et al., the group has been working on many formulations varying the PDT effectors loaded into ultradeformable liposomes since 2007 and tested on promastigotes and intracellular amastigotes of many leishmanial strains. The results obtained previously allowed them to test some of it in experimental models. Ultradeformable liposomes loaded with Cl-Al formulation was not useful to treat BALB/C mice infected with L. *braziliensis* [169], although, in parallel the group concluded that the main pitfall could be the retention of the phthalocyanine in the *stratum corneum*, after assessing biodistribution in healthy Wistar rats [170].

In a more recent study, Caldeira Lopes et al. discovered that systemic pentavalent antimony was not as efficient at reducing the parasite load in the lesion and spleen of animals infected with L. amazonensis. Instead, chlorine-aluminum phthalocyanine liposomes made of egg phosphatidylcholine were [171].

4.1.3. Other lipid nanosystems for PDT

Few works involving lipidic nano dispersed systems of non-liposomal nature for PDT in CL have been published to date. For example, De Siqueira *et al*. developed a 30-nm sized nanoemulsion of clove essential oil and a polymeric detergent in water as a possible way to deliver a Zn phthalocyanine (ZnPc) by the topical route [172]. In this case, the essential oil used was rich in eugenol to take advantage of its leishmanicidal properties. The formulation was effective *in vitro,* both in darkness and after irradiation, but better results were obtained when irradiated, against L. *infantum* and L. *amazonensis* promastigotes, and on amastigotes of L. *infantum* in infected RAW 264.7 cells. The same research group developed a similar delivery system but using niosomes containing ZnPc. While not tested in PDT, it could be an interesting proposal [173]. In turn, another nanoemulsion-based PDT nanosystem was developed loading paromomycin sulfate and using Cl-AlPc as a photosensitizer. This was found to have a better mouse skin penetration profile compared to paromomycin aqueous solution, while it did not significantly improve the free photosensitizer profile. The results of the *in vitro* tests showed that the doubly charged nanosystem has an effect on both promastigotes and intracellular amastigotes, being greater in the latter [174].

Finally, this nanosystem plus or without PDT was unable to reduce CL lesions on L. *(V.) braziliensis* infected mice, but it is reasonable to suggest that modifications on the therapeutic protocol of even combination of PDT with other antileishmanial agents can be studied in an attempt to have better outcomes.

Another example is the use of curcumin-loaded NLC of Riaz's research group [121]. Although they did not test its PDT activity, according to Pinto et al., curcumin photodynamic activity could be an interesting application for these nanoparticles [175].

4.1.4. Quantum dots and other nano-objects

First described in 1981, quantum dots (QDs) are semiconductor nano-crystals whose size go from 2 to 10 nm, that have revolutionary optic properties due to their quantum confinement, like high fluorescence quantum yields, modulable light emission and high photochemical stability. Their field of application has been continuously growing in many different technological niches, including therapies and diagnostics for diverse diseases. Originally, QDs were made of metallic cores, more recent works explored their synthesis from natural sources through ecological friendly methods, giving birth to the so-called Carbon Quantum Dots [176]. To date, QDs were related to CL with diagnostic perspectives, like in the previously mentioned works by Andreadou [34] and Heli [35]. There is one work

that studied the potential of carbon dots (CDs) and gallium-doped carbon dots (Ga@CDs) dispersed in a commercial ointment against *L. major* and *L. donovani* [177]. The formulated ointments with CDs and Ga@CDs exhibited higher activity against both *Leishmania* species, with a minimal concentration of 30 μg/mL for CDs/Ga@CDs, compared with a commercial counterpart. Experimental evidence from *in vitro* and *in vivo* studies with mice and healthy cells have shown that CDs are completely harmless. Nevertheless, the utility of these CDs for photodynamic therapy was not evaluated, although they show potential for bioimaging and antileishmanial use.

The theranostic potential of QDs as fluorescent markers and PDT effectors for the treatment of skin diseases continue to attract the attention, in particular for cancer treatment [176], and it was logical to expect that theranostic applications for CL strategies would soon emerge. This supposition can be extended to other NPs involved in ROS generation after irradiation that are also being explored for their theranostic potential, *e.g.*, silicon nanoparticles that also have shown effective skin penetration [178].

5. Current approaches on nanotechnology-based vaccines

Beyond the efforts made to find an effective vaccine against cutaneous leishmaniasis, the results obtained so far do not guarantee improved immunogenicity and safety for human immunization. These are the reasons why few vaccines have reached clinical trials. The main obstacles that have not yet been overcome by conventional developments are mainly the adverse effects and the great differences between *Leishmania* species and the type of consequences they cause in patients. Additionally, the lack of a good adjuvant and an effective delivery system that can safely enhance the immune response and allow future standardization, are the main problems that highlight the need for innovative vaccine development. Liposomes and polymeric nanoparticles appear as nanotechnological tools involved in the development of vaccines over the last years.

The assessment of a liposomal formulation of an adjuvant based on the well known toll-like receptor agonist, imiquimod, was carried out by Mehravaran et al. [179]. In their study, they use BALB/c mice infected with *L. major* as the model to try soluble *Leishmania* antigens in presence of imiquimod as adjuvant in distearoyl phosphatidylcholine liposomes (DSPC). Mice were injected and the results were taken by: following the immune reaction through the levels of cytokines and IgG, analyzing lesion development through footpad swelling and measuring parasite load in individuals. All in all, these assays conclude that this adjuvant encapsulated in DSPC liposomes induces Th1 immune response and triggers protection against *L. major*. On the other hand, liposomes were also studied as carriers for a vaccine based on gene delivery. Hezarjaribi et al. [180] proved that a liposome made of cationic lipid dioleoyl trimethylammonium propane (DOTAP), dioleoyl phosphoethanolamine (DOPE), and cholesterol is able to admit the load of a specific plasmid (pcLACK) with the aim to produce an effective nanoliposomal vaccine against *L. major*. This plasmid has the Leishmania homolog of receptors for activated C-kinase gene that, from previous studies, is known for its susceptibility to the immune system and is effective on entering in cells. While the stability of the plasmid in the liposome is good, the effectiveness of the system to prevent CL still needs to be proved. Jafari et al., [181] also used DOTAP liposomes, in this case to deliver a whole leishmania lysate. After subcutaneous administration, BALB/c mice were exposed to *L. major* promastigotes, and immune responses were developed, but the authors remark the need for an adjuvant to enhance Th1 response. Another type of liposomes are the so-called archaeosomes, which are based on lipids from archaebacteria and exhibit adjuvant properties as they are immunostimulatory. In the study by Higa et al. [182], ultradeformable archaeosomes and liposomes containing total antigens from *L. braziliensis* promastigotes solubilized with sodium cholate were formulated to develop a topical vaccine, rather than an injectable one. Although both liposomes and archaeosomes penetrated the skin of BALB/c mice, only archaeosomes were taken up by the antigen-presenting cells, making them interesting candidates for topical nanovaccination.

One of the latest studies carried out by Ayari-Riabi et al. [183] aimed to sort out the concerns of an efficient adjuvant. In their work they developed a poly (D,L-lactide) nanoparticle with the ability to adsorb a recombinant H2B histone from L. *major* (H2B/PLA) to improve the response in the immune system. They compared this formulation with one containing a traditional adjuvant and noticed that the humoral response (IGg1/IgG2) remained similar. However, when challenged both with an isolated strain from a human lesion, mice injected with the nanoparticles showed an improvement in symptoms avoiding ulceration while the spread of parasites stopped. Therefore, they conclude that H2B/PLA is an easy manufacturing injectable with an effective adjuvant activity. In other work, Tabatabaie et al. [184] tested dendrimers and poly (methyl methacrylate) nanoparticles as adjuvants for a thiol-specific antioxidant (a predominant antigen in L.*major*) recombinant plasmid DNA. Both nano-adjuvants induced targeted immune responses and reduced parasite burden in Leishmania-infected BALB/c mice, compared to the control without the dendrimer or the NPs. Katebi et al. [185] assayed different parameters of the immune response against combinations of poly lactic-co-glycolic acid NPs (PLGA NPs) with soluble antigens of *Leishmania* encapsulated with agonists of both TLR1/2 and TLR7/8, adsorbed and encapsulated in the PLGA NPs, respectively. The findings indicate that all of the combinations, including their soluble form, were effective in overcoming the lack of stimulation that L. *major* typically generates on macrophages to establish a chronic infection. However, encapsulation increased the duration and strength of the response, suggesting that these delivery formulations may be suitable for a therapeutic vaccine. Additionally, the effects of PLGA NPs as enhancers of immunogenicity against recombinant cysteine peptidase A and B from L. *major* were investigated by Noormehr et al. [186]. The recombinant proteins were conjugated to the nanoparticles and injected into BALB/c mice in different groups to compare free antigens with the PLGA-conjugated. The results showed that parasite spread was effectively controlled, macrophages induced nitric oxide production, and an increase in IFN-γ cytokine was measured in splenocytes when the nanoformulation vaccine was injected, in contrast to the injection of the free antigens. Finally, the uttermost novelty in terms of nano-vaccine candidates is described in the work of Margaroni et al. [187]. The researchers started with a vast *in silico* proteomic analysis of the *L. infantum, L. major,* and *L. braziliensis* species to produce a multi-epitope chimeric protein (LeishChim) that binds MHCI and MHCII molecules. LeishChim was encapsulated into nanoparticles of poly(D,L-lactide-co-glycolide) with monophosphoryl lipid A as adjuvant and was found to induce a specific cellular immune response in BALB/c mice. While this development shows promise as an

effective vaccine against various types of Leishmania, further translational studies are necessary.

6. Conclusions

Despite numerous methods of diagnosis and treatment for leishmaniasis, no single method has emerged that is fast, free of side effects, and accessible. While SinaAmpholeish is a promising candidate to replace current first-line treatments, its high cost -as with most nanomedicines on the market- is a major drawback, especially for low-income populations. However, the incorporation of nanotechnology has brought significant advances in leishmaniasis research and offers promising options for controlling the cutaneous form of the disease. The use of lower doses of purified drugs or biomaterials at the nanoscale could lead to lower costs and toxicity. Advances in nano-theranostics and the development of nano-vaccines for prevention could play a fundamental role in meeting this clear need, particularly in populations affected by or exposed to disease. By enabling diagnosis, treatment, and prevention, these techniques could reduce costs and improve other factors such as waiting time and stress caused by treatment. Although challenges remain, such as staff training and logistics, continued research in this area is critical to improving the lives of millions of people affected by this disease. In summary, the use of nanotechnology in the diagnosis, treatment, and prevention of leishmaniasis could be beneficial to all sectors involved in the healthcare system due to moderate synthesis and development costs, accessibility to vulnerable areas, and minimal side effects.

Declaration of Competing Interest

On behalf of all the co-authors of this manuscript, I declare that there are no conflict of interest regarding this review.

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