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REVIEW

Nanomedicine advances in toxoplasmosis: diagnostic, treatment, and vaccine applications

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Abstract Toxoplasmosis is an infectious disease caused by the intracellular parasite *Toxoplasma gondii* that affects about one third of the world's population. The diagnosis of this disease is carried out by parasite isolation and host antibodies detection. However, the diagnosis presents problems in regard to test sensitivity and specificity. Currently, the most effective *T. gondii* treatment is a combination of pyrimethamine and sulfadiazine, although both drugs are toxic to the host. In addition to the problems that compromise the effective diagnosis and treatment of toxoplasmosis, there are no reports or indications of any vaccine capable of fully protecting against this infection. Nanomaterials, smaller than 1000 nm, are currently being investigated as an alternative tool in the management of *T. gondii* infection. This article reviews how recent nanotechnology advances indicate the utility of nanomaterials in toxoplasmosis diagnosis, treatment, and vaccine development.

Keywords Nanotechnology · Nanoparticles · Nanomedicine · *Toxoplasma gondii* · Infection

Introduction

Toxoplasmosis is an infectious disease, caused by the obligate intracellular protozoan *Toxoplasma gondii*. Cats are the definitive hosts, with humans, other mammals, and birds being intermediate hosts (Robert-Gangneux and Dardé 2012). Toxoplasmosis therefore has a zoonotic characteristic, affecting around one third of the world's population, with higher rates in South and Central America and Continental Europe (50–80%) (Al Nasr et al. 2016; Saadatnia and Golkar 2012).

The severity of toxoplasmosis partly depends on the *T. gondii* strain. However, severity is primarily determined by the host immune response, with the infection usually being asymptomatic in immunocompetent individuals. In immunocompromised individuals, infection may result in neurological, ocular and systemic complications (Machala et al. 2015). In pregnant women, transplacental transmission allows *T. gondii* infection to have serious deleterious effects in the embryo, including neonatal malformations and stillbirths, as well as miscarriage, which depends on the gestational period (Oz 2014).

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The diagnosis of toxoplasmosis can be carried out by a number of methods, including isolation of blood or body fluid tachyzoites; parasite histological determination in tissue; and protozoan DNA determination by polymerase chain reaction and by antibody detection using serological tests. The latter is the most currently used method, providing serological evidence of immunoglobulin (Ig)G, IgM, IgA, and IgE antibodies specific to *T. gondii* antigens. However, these tests may display sensitivity and specificity problems, leading to false-positive or false-negative results (Zhang et al. 2016).

Currently, the most effective treatment of toxoplasmosis is the combination of pyrimethamine and sulfadiazine, which have a synergic action, disrupting the biosynthesis of folic acid. However, this therapy has significant side effects, including hypersensitivity, bone marrow suppression, and teratogenic effects (Petersen 2007) In cases of ocular toxoplasmosis, the administration of an anti-inflammatory is required in addition to conventional treatment, thereby decreasing the spread of retinal damage (Morais et al. 2016).

In congenital toxoplasmosis, spiramycin is used alone or, depending on the gestational stage, combined with sulfadiazine and pyrimethamine. The latter combination of treatments produces toxic effects, including bone marrow suppression, which can be attenuated by the adjunctive use of folinic acid (Antczak et al. 2016).

Pharmaceutical treatment of toxoplasmosis that produces desirable effects, such as a drug with the ability to cross biological barriers, reach a specific target, and minimize toxic side effects (Briones et al. 2008) is not yet available. Consequently, it has been necessary to focus toxoplasmosis management on prevention and control, while optimizing available treatments and driving the development of an effective vaccine.

Although a vaccine developed with live and attenuated tachyzoites of the strain S48 (Toxovax) is available for use in sheep, there are no reports or indications of any effective vaccine to prevent and control toxoplasmosis in humans or other animals. Research studies have investigated the immunological effects of an array of types of vaccines types in different animals, including live, killed, and attenuated antigens, as well as DNA vaccines. Such work has utilized different *T. gondii* antigens and an array of adjuvants (Liu et al. 2012). This work highlights the need for further investigations of toxoplasmosis, including in the improvement of diagnosis and treatment, especially the need for effective and affordable vaccines.

In this context, advances in nanomedicine are promising, allowing the use of nanotechnology in the management of toxoplasmosis. In this article, nanomaterials are highlighted. Characteristics of nanomaterials include a size less than 1000 nm with a core that is either metallic, polymeric, or lipidic in nature (Gutiérrez 2016). Nanoparticles are used in the diagnosis of various infections. In the diagnosis of toxoplasmosis, nanoparticles may improve test sensitivity and specificity (Hegazy et al. 2015; Li et al. 2015), while in treatment, nanoparticles may act as drugs carriers. In such a role, they can modulate pharmacokinetics, increase bioavailability, and target release, while reducing toxicity (Khalil et al. 2013). Furthermore, the use of nanomaterials is also applicable to vaccine development, increasing their efficiency, improving the availability of antigens to antigen presenting cells, and optimizing T helper (Th)1 response (Torres-Sangiao et al. 2016).

Given the clear need for improvements in the management of toxoplasmosis and the many benefits of nanomedicine, the aim of this review is to present the biological advances in the use of nanomedicine that are relevant to diagnosis, treatment, and vaccine improvements in toxoplasmosis.

Methodology

This study reviews the use of nanomedicine in toxoplasmosis diagnosis, treatment and vaccine development. For the identification of relevant studies, a search was conducted using PubMed (US National Library of Medicine), Latin American and Caribbean Health Sciences (LILACS), the electronic version of Index Medicus (MEDLINE), Science Direct, and Web of Science. The search terms used for research in all databases were as follows: "(nanoparticul* OR nanocapsul* OR nanomedicine* OR nanocarrier* OR nanotechnology OR liposome* OR nanoemulsion* OR nanomaterial* OR nanosuspension*) AND (toxoplasma*)". Database researches were limited to English language articles published until February 2017.

General aspects of nanomaterials

Nanotechnology is the science and engineering area dedicated to materials with diameters in the range of tens or hundreds of nanometers. The size of these particles allows different communications with biomolecules on cellular surfaces and intracellularly, where they can influence several physicochemical and biochemical cellular properties (Salata 2004).

Different types of nanomaterials are used in nanomedicine applications, including liposomes, which are small spherical vesicles formed from phospholipids or cholesterol. Nanoparticles can be divided into two main classes, nanocapsules and nanospheres. These are composed of different materials, including metals, lipids, and polymers (Gutiérrez et al. 2016).

Briefly, nanocapsules are systems constituted of vesicles in which the compound or molecule is confined in an aqueous or oily cavity coated by a membrane of the material used. Nanospheres are characterized by housing the compound or molecule in a dispersed manner into particles in the matrix (Prabhu et al. 2015).

Table 1 Nanomaterials used in toxoplasmosis diagnosis studies

References	Nanomaterial	Assay	Biological sample	Detection
Jiang et al. (2015)	Gold nanoparticles	DFICT	Serum (cat and dog)	Antibodies (IgG)
Wang et al. (2004a)	Gold nanoparticles	LPEIA	Serum (rabbit)	Antibodies
Jiang et al. (2013)	Au-Fe_3O_4	Electrochemical immunosensor	Serum (human)	Antibodies (IgM)
Li et al. (2015)	Goldmag nanoparticles modified with poly methacrylic acid	LFIA	Serum (human)	Antibodies (IgM)
Hegazy et al. (2015)	Magnetic nanoparticles	Capture enzyme-linked immunosorbent assay	Serum (human)	Antigens (SAG1)
Wang et al. (2004b)	Silica nanoparticles	Piezoelectric direct immunoassay	Serum (rabbit)	Antibodies (IgG)
Yang et al. (2009)	Quantum dot	Microarrays	Serum (human)	Antibodies
Miao et al. (2011); Xu et al. (2013)	CdTe/Ni mQDs	DNA Sensing System	–	DNA
He et al. (2015); Xu et al. (2011)	CdTe/Fe_3O_4 QDs	DNA Sensing System	–	DNA

DFICT dynamic flow immunochromatographic test, *LPEIA* latex piezoelectric immunoagglutination assay, *LFIA* lateral flow immunochromatographic assay, Au-Fe_3O_4 magnetic gold nanoparticles (goldmag), *CdTe/Ni mQDs* quantum dots synthesized with nickel nanoparticles, *CdTe/Fe_3O_4 QDs* quantum dots synthesized with magnetic nanoparticles of Fe_3O_4, *SAG1* surface antigens of *T. gondii* 1, *IgG* Immunoglobulin G, *IgM* Immunoglobulin M, *DNA* deoxyribonucleic acid

Metallic nanoparticles are based on well-defined small noble metal clusters in the state of zerovalent. Most common are the silver nanoparticles, which have antimicrobial and anti-inflammatory activity as well as utility in imaging with imagenology and in forming conjugates with antibodies, while iron nanoparticles have magnetic properties and potential in the management of tumorigenesis (Edmundson et al. 2014).

Nanoemulsions are different to metallic nanoparticles. Nanoemulsions are heterogeneous systems consisting of two immiscible phases, oil in water (O/W), in which the particle core is composed of water, and water in oil (W/O), in which the particle core is composed of oil. In addition, it is important to mention the solid lipid nanoparticles that have a lipid core in the solid state. These two types of lipid nanomaterials allow the controlled release of drugs or molecules (Mukherjee et al. 2009).

Polymeric nanoparticles are solid systems that present biocompatibility and biodegradable characteristics, where the therapeutic agent can be dissolved, absorbed, or encapsulated in the polymer matrix. These nanomaterials may contain synthetic polymers, such as poly(D,L-lactic-*co*-glycolic acid) (PLGA), polyethylene glycol (PGE), or polyester biobeads, as well as being natural, such as alginate, insulin, or chitosan (Chan et al. 2010).

In general, nanomaterials can act in a direct way on a microorganism or as carriers. In the latter role, drugs or molecules can be directed to the target (especially intracellular pathogens), thereby enhancing drug/molecule bioavailability and stability as well as controlling its release, enhancing its activity, and/or avoiding its degradation and decreasing its toxicity (Gutiérrez et al. 2016; Khalil et al. 2013). Due to the different types and forms, the versatility of nanomaterials provides advantages with potential for application in diagnosis, treatment, and immunizations against various infectious diseases, including toxoplasmosis (Torres-Sangiao et al. 2016).

Nanomedicine in the diagnosis of toxoplasmosis

Nanomaterials can enhance and/or replace the diagnostic methods available and commonly used in toxoplasmosis (Hegazy et al. 2015). This section reviews data on the utility of gold, nickel, magnetic, and quantum dots nanoparticles in the diagnosis of this parasitic disease (Table 1).

Several studies have reported the use of gold nanoparticles in the development of new diagnostic tests for *T. gondii* infections (Li et al. 2015; Jiang et al. 2013, 2015; Wang et al. 2004a). For example, a dynamic flow immunochromatographic test (DFICT) using gold nanoparticles of ~15 nm, conjugated to staphylococcal protein A (SPA), has been developed for the serological diagnosis of infection in dogs and cats. Anti-*Toxoplasma* IgG antibodies from the sera of dogs and cats positively react with the conjugate forming a complex gold-SPA-antibody. The DFICT has a sensitivity and specificity of approximately 92 and 93.1%, respectively, similar to the ELISA test. However, the time for performing the DFICT is shorter than the ELISA and requires 10 to 20 times less samples versus conventional immunochromatographic test (CIT) (Jiang et al. 2015). Similarly, Jiang et al. (2013) also reported that the use of an electrochemical immunosensor using magnetic gold nanoparticles (Au-Fe_3O_4) (goldmag) and graphene sheets was effective in the detection of IgM anti-*T. gondii*. The goldmag, labeled with anti-IgM horseradish peroxidase (HRP), improved the reduction to H_2O_2, increasing the sensitivity of the immunosensor. This way, the proposed immunosensor showed good selectivity, stability, and reproducibility, with a recovery of about 98.8 to 101.2%, thereby detecting IgM anti-*T. gondii* in human serum samples with precision and accuracy.

In 2015, Li et al. (2015) employed a lateral flow immunochromatographic assay (LFIA) for detection of

specific IgM to causative agents of infections during pregnancy, namely TORCH (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex virus infections). This LFIA using goldmag nanoparticles modified with polymethacrylic acid (PMAA), conjugated with IgM anti-human, obtained 100% sensitivity and specificity, plus the ability to simultaneously detect multiple pathogens.

Other nanomaterials can be used in serological diagnosis, including a piezoelectric direct immunoassay for the detection of specific IgG antibodies to *T. gondii* in infected rabbit's sera. This method comprises silica nanoparticles with antigen of the parasite on a polymerized plasma film (PPF) forming PPF-SiO_2. This test improved the amount of *T. gondii* antigens (TgAg) immobilized and their immunoactivity compared to other commonly used methods. The immunoassay using PPF-SiO_2 showed sensitivity to detect IgG similar to ELISA similar and higher than the dot-immunogold test, with a detection limit in the dilution of 1:1535 (Wang et al. 2004b).

With the aim of improving the latex piezoelectric immunoagglutination assay (LPEIA), Wang et al. (2004a) reformulated this existing technique for the detection of anti-*Toxoplasma* antibodies in the infected rabbit's serum, passing the use in latex place, gold nanoparticles ~10 nm coated with *T. gondii* antigens. This assay, using gold nanoparticles, had sensitivity for detection of antibody titers in dilutions up to 1:5500 of samples, displaying results comparable with the ELISA, without the need for washing and separation steps.

Although nanoparticles are used to detect antibodies, Hegazy et al. (2015) developed a capture enzyme-linked immunosorbent assay using polyclonal antibodies coupled to magnetic nanoparticles (IMB-ELISA) with the intention of detecting surface antigens of *T. gondii* 1 (SAG1) circulating in human serum samples. The authors demonstrated that IMB-ELISA showed higher sensitivity (98%) and specificity (96.4%) than the ELISA-sandwich (sensitivity 92% and specificity 92.7%).

In 2009, Yang et al. (2009) using photoluminescent nanomaterials, the quantum dot, developed microarrays for detection of antibodies against antigens TORCH, obtaining a sensitivity and specificity greater than 85%, with rates similar to the gold standard test, ELISA. However, this microarray is proposed as a test to be used in screening, as it is fast (about 20 min) and requires ten times less antigen when compared to ELISA.

Besides serological tests, other *T. gondii* diagnostic tests include the detection of genetic material by means of DNA probes with nanoparticles. Miao et al. (2011) developed a probe of quantum dots synthesized with nickel nanoparticles (CdTe/Ni mQDs) of ~15 nm, with magnetic and fluorescent properties from fluorescence resonance energy transfer. In 2013, Xu et al. (2013) also synthesized molecular beacon probes with nickel nanoparticles of ~20 nm. These two studies showed that the probes had high sensitivity, specificity, and rapid detection of the target DNA of *T. gondii*.

Magnetic nanoparticles of Fe_3O_4 are also being used to develop probes for DNA detection. Xu et al. (2011) developed quantum dots synthesized with magnetic nanoparticles of Fe_3O_4 (CdTe/Fe_3O_4 QDs). The authors showed that these probes were capable of increasing the fluorescence intensity, thereby enhancing the sensitivity and specificity of target DNA detection. He et al. (2015) employed CdTe/Fe_3O_4 conjugated with synthetic oligonucleotides of *T. gondii*, which showed good sensitivity and specificity, exhibiting a detection limit of 8.339 nM.

Thus, the use of nanomaterials is capable of assisting and improving the detection of antibodies, antigens, and DNA of *T. gondii*, with quick and easy handling techniques, presenting good sensitivity and specificity, indicating a potential to act in the screening and diagnosis in different stages of toxoplasmosis.

Nanomedicine in the treatment of toxoplasmosis

Nanotechnology involves useful drug delivery systems for improving the pharmacokinetic profile of drugs. This means that there is an increase of drug bioavailability by increasing the solubility area, the stability, the dissolution rate, and the surface, as well as modulating therapy and permeability of the drug action through the absorption into membranes, thereby lowering the drug doses required (Verma et al. 2003).

In order to improve therapy against infection caused by *T. gondii*, research employing nanotechnology has also shown promising results. Treatment with metallic nanoparticles based on silver and gold, as well as polymeric, lipid nanoparticles, liposomes, and nanosuspensions associated with molecules or drugs is reviewed below (Table 2).

Gaafar et al. (2014) analyzed the properties of polymeric nanoparticles of chitosan (CSNP), a natural amino polysaccharide derived from chitin, and silver nanoparticles (AgNP), as well as the association (CSNP + AgNP) in a murine model of toxoplasmosis. Treatment of Swiss mice, infected with tachyzoites of *T. gondii* virulent strain RH with AgNP and CSNP + AgNP, reduced the number of parasites in the liver and spleen. Furthermore, this treatment provided the paralysis of motion and caused deformity in tachyzoites of the peritoneal exudate, with formation of multiple grooves, irregular papules, and large projections, with disorganized conoid, observed by scanning electron microscopy. These results were not observed in mice treated only with CSNP. All infected groups that received the treatment with nanoparticles presented an increase in serum IFN-γ levels, especially in the AgNP and CSNP + AgNP groups.

Similarly, in 2015, Anand et al. (2015) reported the effect of oral administration of lactoferrin (BLF), a glycoprotein with

Table 2 Nanomaterials associated with molecules or drugs for toxoplasmosis treatment studies

Reference	Nanomaterials	Drugs/molecules
Gaafar et al. (2014)	Silver nanoparticles	–
Pissuwan et al. (2007)	Gold nanorods	Conjugated with anti-*T. gondii*
Pissuwan et al. (2009)	Gold nanospheres	Conjugated with anti-*T. gondii*
Gaafar et al. (2014)	Polymeric nanoparticles of chitosan	–
Anand et al. (2015)	Polymeric nanocapsules of alginate-chitosan	Lactoferrin
Dalencon et al. (1997); Sordet et al. (1998)	Polylactide (PLA)polymeric nanocapsules	Atovaquone
Mellors et al. (1989)	Liposomes	Recombinant IFN-γ
Tachibana et al. (1990)		Stearylamine and phosphatidylcholine
El-Zawawy et al. (2015a, b)		Triclosan
Si et al. (2016)		Usnic acid
Leyke et al. (2012)	Core-shell latex nanoparticles	–
Dunay et al. (2004); Schöler et al. (2001)	Nanosuspensions	Atovaquone
Shubar et al. (2011)		Atovaquone + sodium dodecyl sulfate
Shubar et al. (2009)		Atovaquone + apolipoprotein E
Pissinate et al. (2014)	Core lipid nanocapsules	Pyrimethamine
Prieto et al. (2006)	Drug-dendrimer complex	Sulfadiazine
Bottari et al. (2015a)	2-Hydroxypropyl-β-cyclodextrin	Resveratrol
Bottari et al. (2015b, 2016)		Sulfamethoxazole-trimethoprim

iron sequestering properties and activity against various microorganisms, and polymeric nanocapsules of alginate-chitosan (polymers with high biocompatibility and biodegradability) loaded with BLF (BLF-NC) in BALB/c mice infected with tachyzoites of *T. gondii*. The treatment with BLF-NC increased the bioavailability of BLF in tissue. The treatments reduced inflammation and parasite load, as well as increasing the intracellular production of reactive oxygen species (ROS) and nitric oxide (NO) in the liver and spleen, this being more prominent in the BLF-NC group. The authors also showed high levels of Th1 cytokines in the sera of mice treated with the nanocapsule. Thus, the production of Th1 cytokines, ROS, and NO collaborated to killing parasites in the tissues and helped in the survival of the animals until 25 days after infection.

Given their biocompatibility, gold nanoparticles are of great interest in experimental photothermic therapies. This is due to their ability to be conjugated with antibodies and their capacity to strongly absorb light from lasers, allowing them to have utility against infections caused by *T. gondii* (Jain et al. 2006). The efficacy of gold nanoparticles conjugated with anti-*T. gondii* antibodies and irradiated with laser light has been shown in a number of studies (Pissuwan et al. 2007, 2009). Pissuawan and colleagues carried out experiments investigating the utility of gold nanorods ~39.5 nm (Pissuwan et al. 2007) and gold nanospheres ~20 nm (Pissuwan et al. 2009) nanoparticles conjugated with antibodies specific to the *T. gondii* surface antigen of the RH strain. These conjugates were able to bind the tachyzoites surface and when irradiated with laser light enhanced parasite killing in vitro, in an effect that dose-dependent upon levels of laser irradiation. These authors also demonstrated that pretreatment of tachyzoites with gold nanospheres conjugated with anti-*T. gondii* reduced the infection capability in Chinese hamster ovarian epithelium cells (CHO-K1) (Pissuwan et al. 2009).

Besides the chitosan and metallic nanoparticles, core-shell latex nanoparticles with size ~213.4 nm have also been used, being able to inhibit the growth of free tachyzoites of the RH strain of *T. gondii* and decrease the number of infected macrophages in the J774-a1 macrophage line (Leyke et al. 2012).

Liposomes, which are being widely used as carriers of drugs and/or molecules, both hydrophilic and lipophilic, are another technological advance (Johnston et al. 2007). Liposomes may act directly or indirectly on the microorganism, activating the immune system. Mellors et al. (1985) showed that recombinant IFN-γ (rIFN-γ) incorporated into liposomes were ten times more potent in activating macrophages to produce H_2O_2 and improve anti-*Toxoplasma* activity of the free rIFN-γ, as well as prolonging its effects. In 1990, Tachibana et al. (1990) reported the protective effect and therapeutic efficacy of liposomes consisting of stearylamine (SA) and phosphatidylcholine (PC) liposome, the SA/PC-liposome, against infection caused by *T. gondii*. Treatment of tachyzoites of the RH strain with the SA/PC-liposome was able to induce cell swelling and structural

changes, as well as killing about 95% of the parasites in vitro. The authors also showed that SA/PC-liposome treatment before or after infection of ICR mice increased the survival of mice by 80 and 70%, respectively, over 30 days versus controls (Tachibana et al. 1990).

Other compounds, such as triclosan (TS), an inhibitor of the enzyme enoyl-acyl reductase carrier protein, possess the ability to inhibit the growth and survival of apicomplexan protozoan in vitro (Mcleod et al. 2001), although their therapeutic use is limited by low solubility features as demonstrated by Vandhana et al. (2010). However, liposomes can increase biological potential of TS (Tang et al. 2010). This was confirmed in experiments by El-Zawawy et al. (2015a) using Swiss mice infected with *T. gondii* cysts of the ME49 strain treated with TS and TS-liposomal, with a 20 and 70% decrease in infectivity, respectively. The analysis was done by scanning electron microscopy and showed irregularities, including compressions and ledges in *T. gondii* cysts in treated groups. Transmission electron microscopy, in the treated groups, showed bradyzoites presented with a partially disintegrated wall, extensive vacuolization and membrane discontinuity. Furthermore, TS-liposomal treatment reduced the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) compared to the TS group.

In another study, El-Zawawy et al. (2015b) reported that Swiss mice, infected with tachyzoites of *T. gondii* and treated with TS (150 mg/kg/day) and TS-liposomal (100 mg/kg/day), showed no behavioral changes. Animals of infected and untreated group showed a decrease in food intake and lethargic behavior. Tachyzoite number was decreased in the peritoneal fluid and liver smears of all treated groups; however, a significant reduction of the parasitic load was observed on TS-liposomal group compared with the TS group.

Usnic acid (Durazo et al. 2004; Erba et al. 1998), a natural compound found in some species of lichen of the genus Usnea, has a variety of biological effects (Pramyothin et al. 2004), including against protozoa (Ingolfsdottir 2002). Due to its toxicity and low solubility in water, the utilization of liposomes can improve transport to target cells. The analysis of the therapeutic effects of usnic acid and usnic acid-liposome (~130 nm) were performed by Si et al. (2016) in Swiss mice infected intraperitoneally with tachyzoites of the *T. gondii* RH strain. The authors verified higher survival rates in mice treated with liposomal usnic acid compared to usnic acid.

Atovaquone is a drug used in the management of toxoplasmosis, with some evidence of potential efficacy against *T. gondii* infection. However, atovaquone also presents with low solubility, which reduces its bioavailability. Therefore, some studies have assessed the bioavailability and anti-*T. gondii* activity of atovaquone when incorporated within nanotechnology conjugates (Dalencon et al. 1997; Dunay et al. 2004; Schöler et al. 2001; Shubar et al. 2009, 2011; Sordet et al. 1998).

Atovaquone nanosuspensions (ASNs) have been utilized for efficacy in models investigating the reactivation of latent *T. gondii* infection (murine interferon regulatory factor 8 knockout), which results in the development of toxoplasmic encephalitis (Dunay et al. 2004; Shubar et al. 2009, 2011). Dunay et al. (2004), showed that intravenous treatment of mice, with ASN 10 mg/kg, increased the drug concentration in the blood and afforded 100% protection of mice from reactivated acute infection, with maintenance on atovaquone orally for 7 days preventing the development of toxoplasmic encephalitis, with no evidence of parasites and inflammatory foci in the brains and livers of animals. However, the association of ASN with apolipoprotein E, an amino acid residue that binds to low density lipoprotein receptor (LDLr) did not improve the passage over the blood-brain barrier nor the treatment activity in vivo, but rather, increased ASN accumulation in brain endothelial cells (Shubar et al. 2009).

Sodium dodecyl sulfate (SDS) enhances the transport of molecules across epithelial barriers and stabilizes nanoparticles loaded with drugs. Shubar et al. (2011) showed that oral treatment with ASN coated with SDS (ASN/SDS) was able to increase the bioavailability and reduce the inflammation, number of parasites, and DNA concentration of *T. gondii* in the animals' brains, in a model of infection reactivation. Interestingly, IFN-γ knockout mice can be used to develop another type of latent infection reactivation by *T. gondii*. Using this model with macrophages infected with tachyzoites of the strain BK, Schöler et al. (2001) showed ASN to reduce the number of infected cells and parasites per cell. These authors also showed that intravenous ASN 10 μg/mL treatment with ASN 10 μg/mL increased survival, while decreasing the parasitic load and inflammatory infiltrate. The therapeutic and protective effects were observed at a ten times lower dose of ASN versus free atovaquone.

Other researchers have encapsulated atovaquone with polymeric nanocapsules of poly lactic acid (PLA) in the treatment of experimental murine toxoplasmosis (Dalencon et al. 1997; Sordet et al. 1998). Swiss mice infected with *T. gondii* COUL strain were treated orally with PLA polymeric nanocapsules (210 nm) containing atovaquone for 10 days at 15 mg/kg/day. The mice receiving this treatment had higher survival rates, reduced parasitic load in the liver, and absence of parasites in the blood and brain (Dalencon et al. 1997). Sordet et al. (1998) also showed the therapeutic effect of PLA nanocapsules loaded with atovaquone (206 nm), in acute and chronic experimental toxoplasmosis. The treatment of mice, with nanocapsules containing atovaquone, improved drug activity, increasing survival and reduced the parasite burden versus free atovaquone, in models of acute and chronic infection.

Pissinate et al. (2014) showed core lipid nanocapsules loaded with pyrimethamine (PYR-LNC) to increase the survival of CF1 mice infected with *T. gondii* tachyzoites of the RH strain via an intraperitoneal route. The biodistribution of conjugate

complexes has also been investigated. Prieto et al. (2006) analyzed the resistance and stability of the drug-dendrimer complex (ramified nanostructures) by determining its release in vitro, in association with its anti-*Toxoplasma* activity. This study showed that the free sulfadiazine was released in 90 min, whereas the SDZ-DG4.5 complex (anionic dendrimer) retained 98% of SDZ over 24 h, and the SDZ-DG4 complex (cationic dendrimer) retained about 85%, demonstrating a significant control of drug release. Furthermore, SDZ-DG4 0.03 μM decreased kidney lineage cell (Vero) infection by 60%, with SDZ-DG4.5 reducing this by 25%. Such data shows that these complexes lead to slower drug release, thereby extending their temporal biodistribution (Prieto et al. 2006).

Resveratrol is a much investigated factor across a number of medical conditions, which has also been investigated in association with nanomaterials, including in the management of toxoplasmosis (Miller et al. 2009). Resveratrol can have significant anti-inflammatory and antioxidant effects. However, resveratrol is easily degraded by acids and enzymes present in the digestive tract (de la Lastra and Villegas 2007). The use of nanomaterials contributes to improving the bioavailability and activity of drugs and compounds, such as resveratrol, including by preventing their degradation.

The therapeutic efficacy of free resveratrol and resveratrol complexed with 2-hydroxypropyl-β-cyclodextrin (HPβCD), associated or not with sulfamethoxazole-trimethoprim (ST), has been investigated in murine models of experimental toxoplasmosis. Treatment with either combination of free or resveratrol-HPβCD, with ST in Swiss mice infected with tachyzoites of the VEG strain (type III), decreased the number of brain cysts and hepatic inflammatory infiltrates, which the authors propose to be driven by a synergistic interaction. It was also observed that animals treated with the combination had greater acetylcholinesterase (AChE) activity, thereby increasing acetylcholine breakdown, when compared with healthy animals, but lower AChE activity than infected and not treated animals. Furthermore, this treatment did not alter the activity of creatine kinase, an enzyme associated with brain energy metabolism, indicating resveratrol's protection against central inflammatory processes more generally (Bottari et al. 2015a). Bottari et al. (2015b) showed that treatment with free resveratrol and resveratrol-HPβCD associated with TS in BALB/c mice infected with *T. gondii* bradyzoites reduced the number of brain cysts versus controls and also reduced the inflammatory infiltrate in the liver. The free resveratrol and resveratrol-HPβCD complex did not alter the production of most proinflammatory cytokines. However, when combined with TS, levels of the Th2 cytokine, IL-10, were elevated versus infected and untreated mice.

Recently, Bottari et al. (2016) also showed synergistic effects of free resveratrol and resveratrol-HPβCD, when combined with ST in the treatment of chronic infection of Swiss mice with *T. gondii* bradyzoites. These combination treatments decreased the numbers of cerebral cysts and hepatic inflammatory infiltrates, as well as increasing the levels of advanced oxidation protein products and the total oxidation state in the brain but not in liver. A combination of resveratrol and ST increased the total antioxidant capacity (TAC) and ferric reducing ability of plasma in the brain. However, only a combination of resveratrol-HPβCD and ST increased hepatic antioxidants levels, with this combination also having the most protective effect on memory. The authors propose that this mnemonic effect arises from the gradual release of resveratrol in the brain, unlike the free form, which is rapidly metabolized. Overall, it is clear that different types of nanomaterials increase bioavailability and controlled release, as well as preventing degradation, thereby increasing drug efficacy and decreasing toxicity.

Nanomedicine in vaccine development for toxoplasmosis

The development of toxoplasmosis vaccines is another important area for nanomaterial applications, including studies investigating nanoparticles and liposomes. Nanoparticle utilization in murine models has significant effects, as the nanoparticulate systems assist in the generation of effective immune response against *T. gondii* infections (Dimier-Poisson et al. 2015; El Bissati et al. 2014).

Using transgenic mice model of HLA-B*0702-expressing human major histocompatibility complex (MHC)-I, El Bissati et al. (2014) observed that immunization with self-assembling polypeptide nanoparticles (SAPNS) ~38 nm, containing an epitope for CD8+ T cells from the dense granules 7 ($GRA7_{20–28}$) and universal epitope of CD4+ T cells (PADRE), were able to enhance IFN-γ production and reduce the parasitic load after challenge with *T. gondii* type I and II.

Similarly, Dimier-Poisson et al. (2015) confirmed the protective effect of nasal immunization of CBA/J mice with total extract (TE) of *T. gondii* antigen strain RH, encapsulated in a porous maltodextrin-based with lipid core nanoparticles (DGNP/TE). DGNP/TE (average size 88.4 nm) enhanced internalization and antigen delivery in macrophages and dendritic cells. DGNP/TE also led to a 2.5-fold increase in nuclear factor-kappa B (NFκB) versus TE alone, which was mediated via toll-like receptor (TLR)2 and TLR4 activation. DGNP/TE also increased the production of IL-1, IL-12p40, IL-6, and TNF-α by these cells. Additionally, the use of this nanoparticle stimulates humoral immunity specificity, as indicated by increased IgG production, as well as Th1/Th17 cellular responses, thereby protecting the mice in a model of acute and chronic infection by *T. gondii* strain 76 K (type II).

The first studies in 1983 and 1985 using liposomes, composed of cholesterol or nontoxic phospholipids, for the development of vaccines against *T. gondii* infections were

unsuccessful. The immunization of Swiss mice with *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP), incorporated in multilamellar liposome with the addition of 80 μg/mL parasite lysate antigen, induced the production of specific antibodies. However, there was no improvement in the microbicidal capacity of macrophages and no protection against infection by strain ME49 of *T. gondii* (Mcleod et al. 1985) Despite the increase in antibody titers, Waldeland and Frenkel (1983), there was no improvement in the protection and mortality of mice immunized with liposome and lysate antigen of *T. gondii* and challenged with tachyzoites of the strain M7741 (type III).

In contrast, Elsaid et al. (1999) showed a protective effect of liposomes carrying surface antigens of *T. gondii*. Swiss mice were immunized with *T. gondii* tachyzoites antigen of virulent strain N incorporated into liposomes and then orally infected with cysts of the low virulence strain P, which showed improved survival rates and lower levels of brain cysts.

Similarly, Tanaka et al. (2014) showed that immunization of C57BL/6 mice with profilin from *T. gondii*, a potent agonist recognized by TLR11, encapsulated in liposomes coated with oligomannose (TgPF-OML), and increased the protection against toxoplasmosis caused by the cytogenetic strain PLK (strain type II) of *T. gondii*. These authors observed improved survival rates and decreased numbers of brain cysts in animals immunized with TgPF-OML. Furthermore, it was also demonstrated that immunization with TgPF-OML increased the production of IgG, IFN-γ and decreased IL-10 compared to the animals immunized only with profilin (tgPF) or not immunized.

In 2009, Chen et al. (2009) utilized dense granule protein 4 recombinant encapsulated in liposomes (liposome-pGRA4) and only pGRA4 for immunization of C57BL/6 and BALB/c mice, prior to infection with strain ME49 and RH of

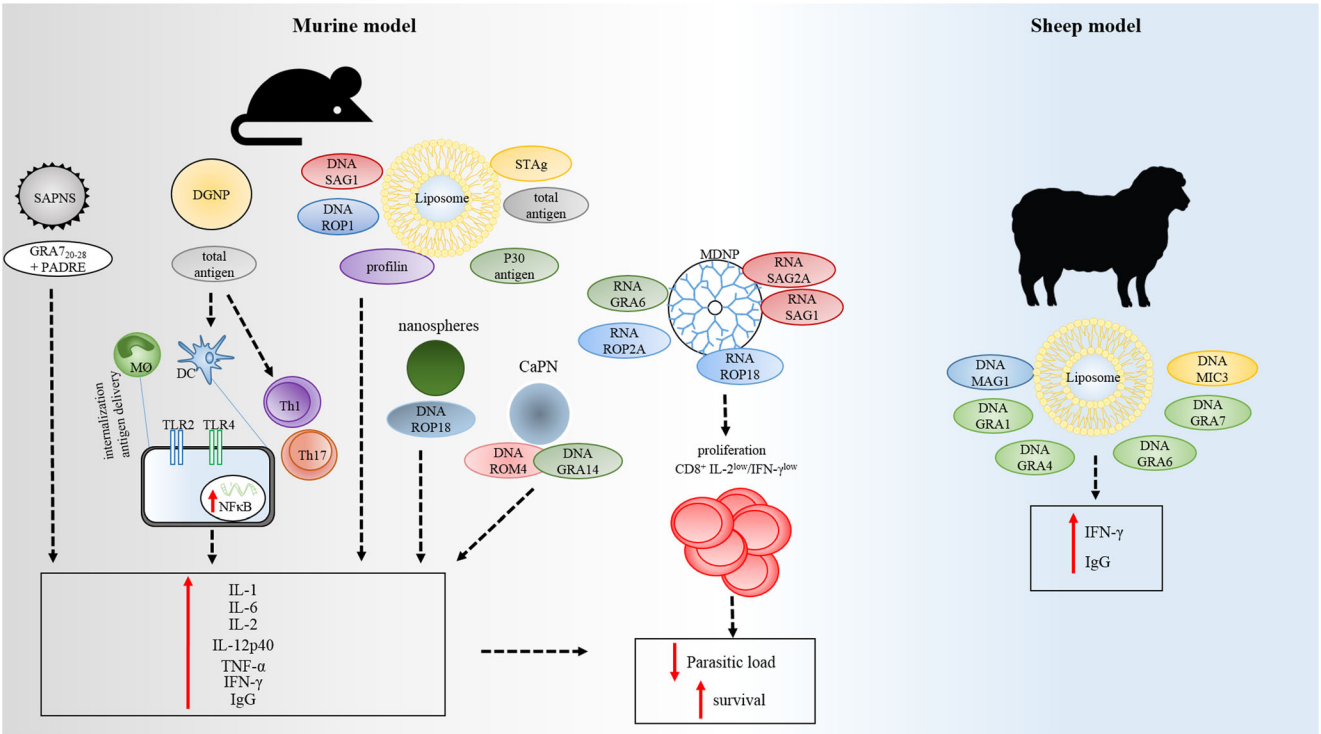

Fig. 1 Main findings about immunization using nanomaterials loaded with different *T. gondii* antigens in murine and sheep experimental models. In murine models, different types of nanomaterials can carry different *T. gondii* antigens. DGNP loaded with total antigen enhance the internalization and antigen delivery in macrophages and dendritic cells, being able to increase NFκB activation via TLR2 and 4, and activate Th1 and Th17 cells. DGNP loaded with total antigen; SAPNS loaded with $GRA7_{20–28}$ + PADRE; nanospheres containing ROP18 DNA; CaPN acting as adjuvant in combination with ROM4 DNA and GRA14 DNA; and liposomes incorporating SAG1 DNA, ROP1 DNA, profilin, P30 antígen, total antigen, and STAg can induce the inflammatory cytokines production (IL-1, IL-6, IL-2, IL-12p40, TNF-α, and IFN-γ) and specific IgG, reducing parasitic burden and increasing survival. In addition, GRA6, ROP2A, ROP18, SAG1, and SAG2A-RNA encapsulated in MDNP stimulate $CD8^{+}IL\text{-}2^{low}IFN\text{-}\gamma^{low}$ T cells, resulting in the reduction of parasites and consequently increasing the survival rate of the animals. In another model, immunization of sheep with liposomes containing MAG1, GRA1, GRA4, GRA6, GRA7, and MIC3 DNA induces the production of IFN-γ and specific IgG. *CaPN* calcium phosphate nanoparticles, *DC* dendritic cells, *DGNP* porous maltodextrin-based with lipid core nanoparticles, *DNA* deoxyribonucleic acid, *GRA* dense granule antigen, $GRA7_{20–28}$ epitope for CD8+ T cells from the dense granules 7, *IFN-γ* interferon γ, *IgG* immunoglobulin G, *IL* interleukin, *MAG* matrix antigen, *MDNP* modified dendrimer nanoparticles, *MIC* microneme antigen, *MØ* macrophages, *NFκB* nuclear factor-kappa B, *PADRE* = universal epitope of CD4+ T cells, *RNA* ribonucleic acid, *ROP* rhoptry antigen, *ROM* rhomboid antigen, *SAG* surface antigen, *SAPNS* self-assembling polypeptide nanoparticles, *STAg* soluble tachyzoites antigen, *Th* T helper cells, *TLR* toll-like receptor, *TNF-α* tumor necrosis factor α

T. gondii, respectively. The immunization with pGR4-liposome was capable of increasing the IgG2a levels serum, as well as inducing IL-2 and IFN-γ by splenocytes stimulated with pGRA4 antigen, but not IL-4. The protection granted by the use of pGRA4-liposome was improved, increasing survival rates and reduced the number of cysts compared to the immunization with just pGRA4, acting similarly in lineages C57BL/6 and BALB/c. The protective effect of P30 purified antigen of *T. gondii* strain RH, associated with liposome (P30-liposome) in toxoplasmosis caused by infection with the less virulent strain C, was demonstrated by Bülow and Boothroyd. (1991). These authors reported that Swiss mice immunized with P30 and P30-liposome presented with high antibody levels and lower mortality rate, with a mortality of 33% in the group immunized with isolated P30 and 7% in the P30-liposome group. This shows that the use of liposomes improved the response against *T. gondii*, acting as an adjuvant.

Immunization with liposomes has also been investigated in congenital toxoplasmosis by Elsaid et al. (2001). BALB/c mice were immunized with soluble tachyzoites antigen (STAg) or purified antigens by immunochromatography using monoclonal antibodies (mAb) anti-P32 (pTAg) and incorporated into liposomes. Mice between 10 and 14 days of gestation were challenged with low virulent *T. gondii* P strain, orally administered. The authors demonstrated that immunizations increased antibody levels and lymphoproliferation. The authors also found that immunization with antigens encapsulated in liposomes significantly decreased congenital transmission, as well as the mortality rate of pups.

Besides use in immunization with *T. gondii* antigens, nanomaterials may act as carriers of genetic material, such as DNA vaccines. The encapsulation of DNA-GRA1 plasmid in chitosan nanoparticles of about 400 nm showed efficiency in cellular uptake and afforded protection from DNA degradation. Although immunization of C3H/HeN mice with this nanoparticle increased specific IgG production, the predominant response was Th2, which is related to disease susceptibility (Bivas-Benita et al. 2003). In the same year, Chen et al.

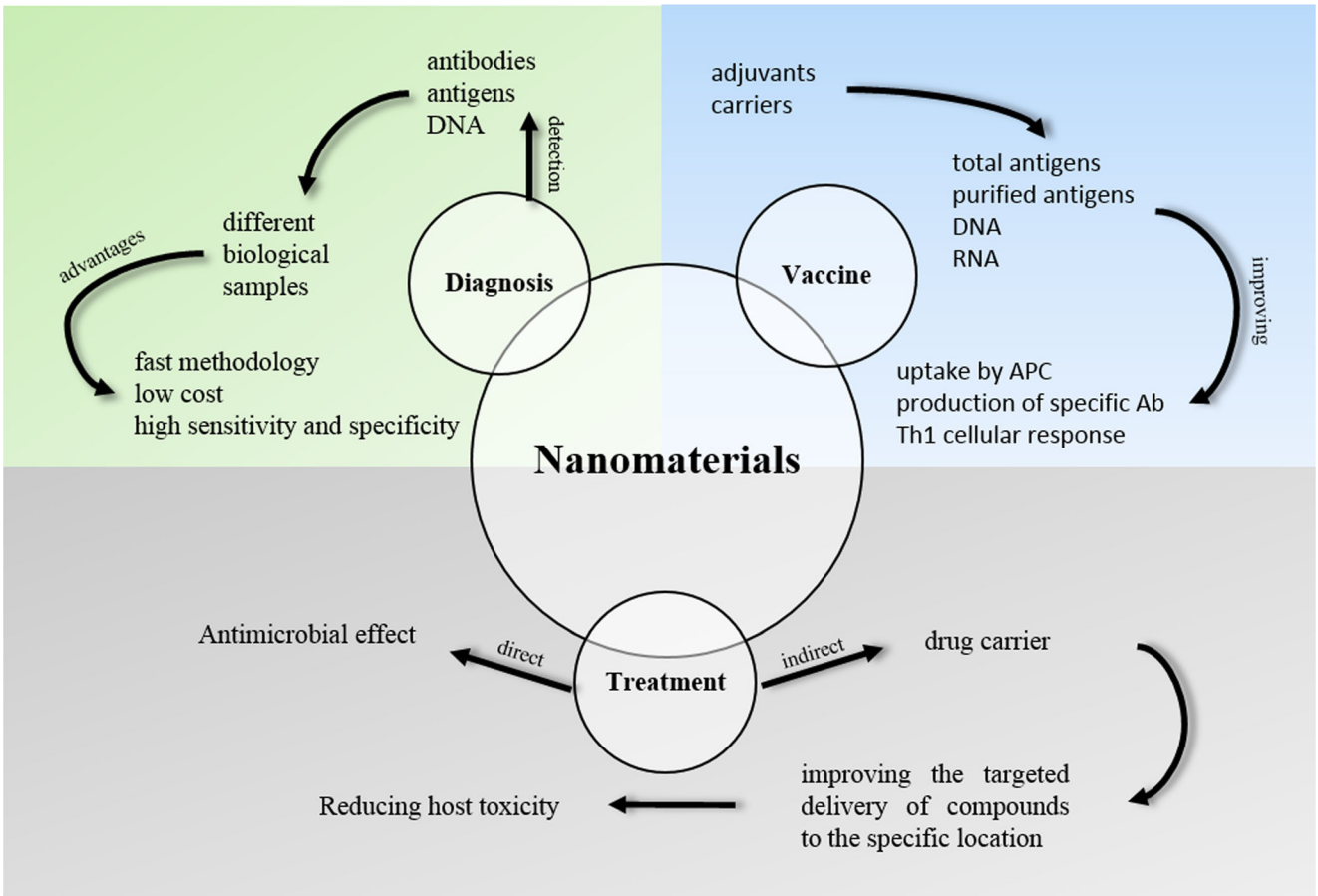

Fig. 2 Scheme summarizing the versatility and integrative use of nanomaterials in toxoplasmosis. Nanomaterials can be used in the diagnosis, treatment, and vaccines. In the diagnosis, nanomaterials can be used for the detection of antibodies, antigens, and DNA in different biological samples, presenting fast methodology, low cost, and high sensitivity and specificity. In the treatment, the nanomaterials can act of direct way, with antimicrobial effect, or indirectly acting as drug carrier, improving the targeted delivery of compounds to the specific location and reducing host toxicity. In relation to the use of nanomaterials in vaccines, these can act as adjuvants or carrying total antigen, purified antigen, DNA, or RNA, improving uptake by APC, production of specific Ab, and optimal Th1 immune response. *Ab* antibodies, *APC* antigen-presenting cells, *DNA* deoxyribonucleic acid, *RNA* ribonucleic acid, *Th1* T helper 1 cells

(2003) also showed that the murine immunization with surface antigen (SAG1) DNA and rhoptry antigen (ROP1) DNA encapsulated in liposomes, induced a cellular immune response with production of IFN-γ and IL-2 and a humoral immune response with antibodies against tachyzoites of *T. gondii*. In addition, rROP18 DNA in nanospheres administered intranasally in Swiss-Webster mice, resulted in increased humoral immune response, with production of specific IgA and IgG2a (Nabi et al. 2017). Rahimi et al. (2017) showed immunization of BALB/c mice using a combination of rhomboid 4 (ROM4) DNA, and GRA14 DNA with nanoadjuvant calcium phosphate nanoparticles (CaPNs) were able to increase the production of IFN-γ and IgG2a/IgG1 ratio, reducing the number of parasites after challenge with RH strain of *T. gondii*, increasing survival.

Other animal models may also be used for research on vaccine development based on liposomes containing plasmid with DNA of different *T. gondii* antigens: matrix antigen 1 (MAG1) (Hiszczyńska-Sawicka et al. 2010a), dense granule antigen (GRA1, GRA4, GRA6, GRA7) (Hiszczyńska-Sawicka et al. 2010b, 2011), and microneme antigen 3 (MIC3) (Hiszczyńska-Sawicka et al. 2012). The immunization of sheep with DNA plasmid of MAG1, GRA1, 4, 6, 7, and MIC3 incorporated in liposomes was able to induce IFN-γ and IgG. However, only animals, immunized with DNA of MAG1 and GRA7 with liposomes, showed an increased IgG2/IgG1 ratio, due to a Th1 response (Hiszczyńska-Sawicka et al. 2010a, b, 2011, 2012).

Besides the use of nanoparticulate systems in total antigen vaccines, protein, and DNA, research has been conducted with RNA replicons, which have a self-replicating capacity. Chahal et al. (2016) reported that the use of RNA replicon that encoded GRA6, ROP2A, ROP18, SAG1, and SAG2A as well as apical membrane antigen-1, encapsulated in modified dendrimer nanoparticles (MDNP), is capable of delivering self-replicating RNA into the cytoplasm of cells and protecting against the action of nucleases. Furthermore, the authors showed that murine immunization with MDNP-RNA, in a single dose, was able to protect against lethal infection by *T. gondii*, as well as activate and stimulate the proliferation of CD8+ T lymphocytes coupled to mild expression of IFN-γ and IL-2. Thus, the use of MDNP-RNA is a good alternative to vaccines based on genetic material, thereby avoiding possible mutagenic integration with the host DNA. These findings are summarized in Fig. 1. Moreover, nanotechnology enables improvement in vaccine development, because they act as adjuvants or carriers of different types of antigens, increasing the immunogenicity.

Conclusion

In reviewing investigations regarding the applications of nanotechnology in toxoplasmosis, the data collated above indicate that nanoparticles can be used in association with a number of other molecules, as well as with laser radiation. Such diagnostic applications allow the detection of *T. gondii* antigens, antibodies, and DNA molecules in different biological samples, providing fast methodology and low cost, coupled to high diagnostic sensitivity and specificity. Nanomaterials may be used alone or as drug carriers in the treatment of *T. gondii*. Nanoparticle utilization in treatment may be done by acting directly or by improving the targeted delivery of compounds to the specific location, while also reducing toxicity. Nanoparticle-based vaccines present promising results, with an array of benefits, including acting as adjuvants or as carriers of total antigens, purified antigens, DNA, or RNA of *T. gondii*; improving uptake by antigen-presenting cells; enhancing the production of specific antibodies; and inducing a more optimal Th1 cellular response. Figure 2 presents the versatility and integrative use of nanomaterials in the toxoplasmosis.

This review highlights the advances of nanomedicine and how nanotechnology can contribute to improvements in diagnosis, treatment, and vaccine development in the management of toxoplasmosis, as well as encouraging further research in the fields of medicine and toxoplasmosis, especially in their overlaps.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human participants or animals performed by any of the authors.

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