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Authors:

First author full name

Yunuen Avalos-Padilla[1,2,3,*]

[1]Barcelona Institute for Global Health (ISGlobal, Hospital Clínic-Universitat de Barcelona), Rosselló 149-153, 08036 Barcelona, Spain.

[2]Nanomalaria Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10-12, 08028 Barcelona, Spain.

[3]Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain.

ORCID iD: 0000-0003-4074-5624

yavalos@ibecbarcelona.eu

Second author full name

Xavier Fernàndez-Busquets[1,2,3]

[1]Barcelona Institute for Global Health (ISGlobal, Hospital Clínic-Universitat de Barcelona), Rosselló 149-153, 08036 Barcelona, Spain.

[2]Nanomalaria Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10-12, 08028 Barcelona, Spain.

[3]Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain.

ORCID iD: 0000-0002-4622-9631

xavier.fernandez@isglobal.org

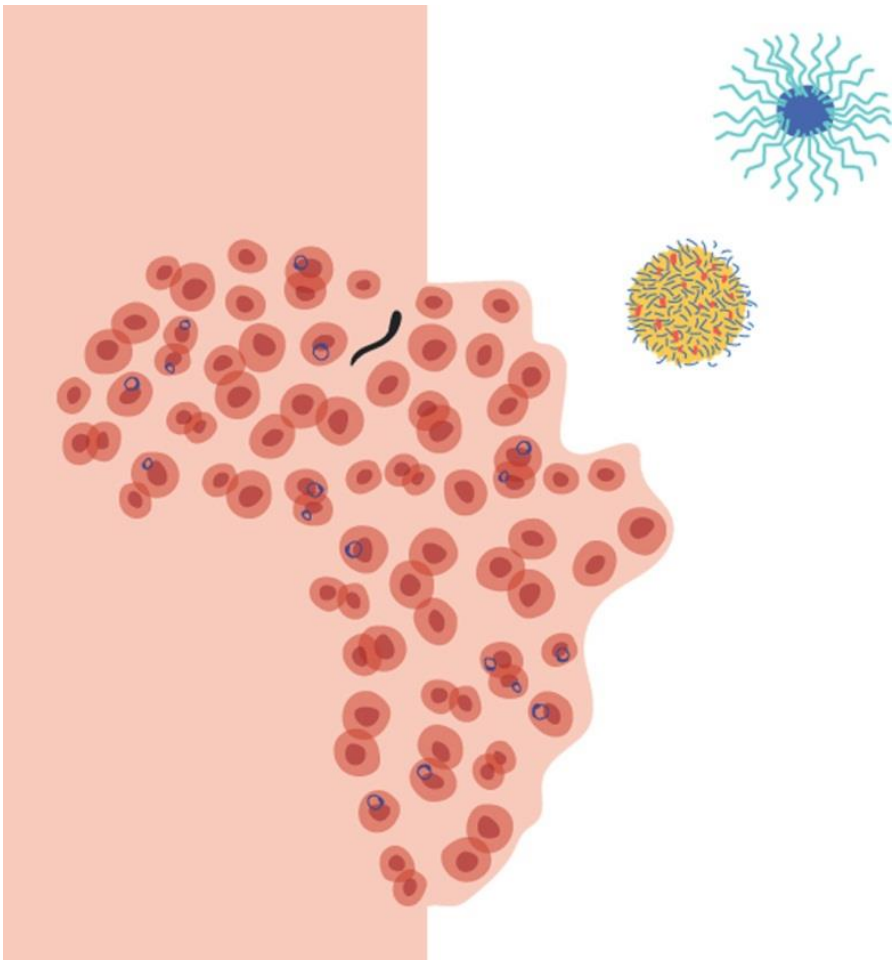
Conflict of Interest

The authors declare no relation that could be perceived as conflict of interest.

Abstract

Malaria, caused by different species of protists of the genus *Plasmodium*, remains among the most common causes of death due to parasitic diseases worldwide, mainly for children aged under five. One of the main obstacles to malaria eradication is the speed with which the pathogen evolves resistance to the drug schemes developed against it. For this reason, it remains urgent to find innovative therapeutic strategies offering sufficient specificity against the parasite in order to minimize resistance evolution and drug side effects. In this context, nanotechnology-based approaches are now being explored for their use as antimalarial drug delivery platforms due to the wide range of advantages and tuneable properties that they offer. However, major challenges remain to be addressed to provide a cost-efficient and targeted therapeutic strategy contributing to malaria eradication. The present work contains a systematic review of nanotechnology-based antimalarial drug delivery systems generated during the last 10 years.

Graphical/Visual Abstract and Caption



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1. INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium* parasites that are transmitted to the human through the bites of infected female *Anopheles* mosquitoes (Cox, 2010) (Figure 1). This disease affects low-income and tropical regions, where it can lead to severe illness. With 619 000 deaths in 2021, malaria ranks as the leading cause of death in several developing countries, where children under the age of five and pregnant women are the most vulnerable groups (World Health Organization, 2022). Worldwide, a total of 29 countries were responsible for 96% of all malaria cases. Among these, four countries held a significant share of the burden, with Nigeria leading the list at 27% of global cases. Following closely were the Democratic Republic of the Congo with 12%, Uganda with 5%, and Mozambique with 4% (World Health Organization, 2022). Collectively, these four nations accounted for nearly half of all malaria cases reported globally. Malaria is a major public health concern, and efforts to prevent, control, and treat the disease are ongoing to reduce its impact. Malaria symptoms typically include fever, chills, headache, sweating, fatigue, muscle/joint pain, nausea and vomiting, and in severe cases, anaemia, jaundice and organ dysfunction (Basu & Sahi, 2017). The prognosis for malaria depends on several factors, among which prompt treatment and availability of healthcare services are crucial for a positive outcome (Hanboonkunupakarn & White, 2022). In areas with effective healthcare systems and access to antimalarial drugs, most cases of uncomplicated malaria can be successfully treated, leading to a full recovery. However, without proper treatment, severe malaria can be fatal. In geographical areas where malaria is endemic (e.g. sub-Saharan Africa), individuals may develop partial immunity over time, which can reduce the severity of the disease in subsequent infections (World Health Organization, 2022).

Malaria also imposes a significant economic and societal burden, which includes i) direct healthcare costs associated with diagnosis and treatment, ii) productivity fall translated to economic losses for individuals and communities, iii) preventive measures carried out by governments, organizations and individuals, iv) research and development for new malaria treatments and improved diagnostic tools, and v) tourism decrease in endemic areas. However, estimating the global malaria budget is challenging due to the lack of a standardized breakdown of expenses and a comprehensive, universally comparable quantification of the disease´s economic impact on society and governments (Andrade et al., 2022). Nevertheless, it is worth mentioning that the total fund allocated to combat malaria through different initiatives has remained relatively stagnant since 2010, reaching only US$

3.5 billion in 2021 (RBM Partnership to End Malaria, 2023; World Health Organization, 2022). This amount falls considerably short of the estimated US$ 7.3 billion needed to remain aligned with the milestones set forth in the Global Technical Strategy for malaria 2016-2030 (World Health Organization, 2021). According to this document, to reach over 80% coverage of currently available interventions, investment in malaria (both international and domestic contributions) must increase substantially above the current annual spending of about US$ 3.0 billion. Total annual resources required were estimated at US$ 9.3 billion per year by 2025 and US$ 10.3 billion per year by 2030. Additionally, funding of US$ 8.5 billion is projected to be needed for research and development during the period 2021–2030, representing an average annual investment of US$ 851 million. The economic impact of malaria in Africa is estimated to be US$12 billion every year. This includes costs of health care, absenteeism, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of economic investment. Therefore, the control of this disease and its eventual eradication will have an immediate effect on improving the health and well-being of the citizens in endemic countries.

There are five *Plasmodium* species that can infect humans, among which *Plasmodium falciparum* and *Plasmodium vivax* represent the greatest threat to global health (Sato, 2021). Currently, a limited arsenal of drugs is available, with artemisinin-based combination therapies (ACTs) being the first-line treatment for uncomplicated malaria since 2005 (Bosman & Mendis, 2007). After the introduction of ACTs, malaria case incidence fell steadily until 2019. However, this progress has slowed and even reversed in higher burden countries due to the evolution of resistance in *P. falciparum*. The first evidence of resistance to ACTs was reported back in 2009 in the Thai/Cambodia border region (Samarasekera, 2009), and it has been detected in Africa (Uwimana et al., 2020), and South America (Mathieu et al., 2020). To overcome this problem, there is a critical need to develop innovative, cost-effective, nontoxic, and efficient drug delivery approaches with low propensity to stimulate the emergence of drug resistance (Gujjari et al., 2022).

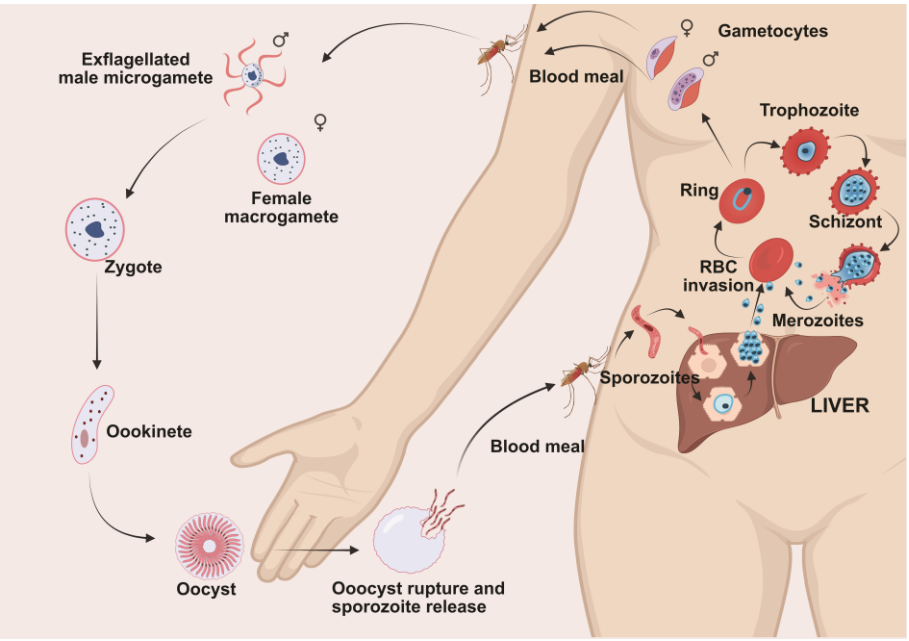

Figure 1. Schematic representation of the life cycle of the malaria parasite. Infection begins when an infected female *Anopheles* mosquito takes a blood meal and injects sporozoites into the human host. Sporozoites travel through the blood stream to the liver, where they infect hepatocytes, and mature into schizonts, which eventually rupture and release merozoites back to the blood circulation. This stage is asymptomatic and can last from a few days to a few weeks, depending on the *Plasmodium* species. The newly released merozoites infect red blood cells (RBCs), where the parasite undergoes the asexual reproduction: ring stages progress into trophozoites and schizonts, which finally rupture, releasing merozoites. This cycle of invasion, replication, and rupture causes the characteristic symptoms of malaria, including fever, chills, and anaemia. Some parasites can differentiate into sexual erythrocytic stages (gametocytes), which are ingested by a female mosquito during its blood meal. Inside the mosquito stomach, microgametes fuse with macrogametes generating zygotes, which mature to become motile and elongated ookinetes. Then, ookinetes invade the mosquito midgut wall and develop into oocysts that grow and rupture, releasing sporozoites. Finally, sporozoites reach the salivary glands of the mosquito, which is now able to inoculate parasites into a new human host, perpetuating the malaria life cycle. Modified from (Maier et al., 2019). Created with BioRender.com.

With the introduction of nanotechnology to the biomedical field, it has been observed an improvement of treatment efficacy as well as a reduction of the detrimental drug side effects in many diseases, including cancer and bacterial infections (reviewed in: (Gao et al., 2012; Mubeen et al., 2021; Munir et al., 2020; Sun et al., 2023; Wang & Huang, 2020)). The main advantages that nanoformulations offer are: i) small size and high surface-to-volume ratio, which enables them to cross biological barriers, ii) increased on-target bioavailability, reducing drug doses and time required to cure the patient, iii) minimization of unwanted side effects, and iv) possibility to functionalize their surface with the aim of improving physicochemical properties or target specificity (Borgheti-Cardoso et al., 2020; Kirtane et al., 2021; Sanità et al., 2020; Santos-Magalhães & Mosqueira, 2010; Urbán & Fernàndez-Busquets, 2014). During the last years, nanotechnology-based drug delivery systems have been extensively used to improve the physicochemical characteristics and performance of drugs in treating several infectious diseases (Kirtane et al., 2021). In the case of malaria, nanoformulations offer promising advantages for the treatment of the disease, including improved drug delivery, targeted therapy, reduced toxicity, and the potential to overcome drug resistance, making them a valuable approach in the fight against this deadly disease. The present review provides a comprehensive summary of the progress made over the past decade in the application of nanotechnology for malaria interventions. It specifically focuses on nanotechnology-based strategies that have undergone testing in *in vitro* *Plasmodium* cultures and/or *in vivo* murine models. The aim is to illustrate the diverse range of potential applications for nanomaterials in the fight against malaria.

2. CURRENT LANDSCAPE IN MALARIA TREATMENT

Artemisinin and its derivatives dihydroartemisinin (DHA), artesunate (ART), artemether (ARM), and arteether constitute the frontline in current treatments against malaria. These drugs have low toxicity and are highly effective against different malaria blood stages (Pukrittayakamee et al., 2004). Artemisinins share a common mode of action that begins with the activation of the drug by the reduced Fe^{2+} form of iron, primarily derived from heme molecules resulting from the digestion of hemoglobin by *Plasmodium* (Combrinck et al., 2013; Klonis et al., 2013; Lu et al., 2019; Meunier & Robert, 2010). Once activated, artemisinins generate highly reactive intermediates capable of causing damage and disrupt several cellular components within the malaria parasite. This disruption affects

proteins, lipids, and nucleic acids, ultimately resulting in a significant reduction in the parasite load within parasitized red blood cells (pRBCs) (Lu et al., 2019).

Since 2010, the World Health Organization (WHO) recommends the use of ACTs as a strategy to reduce resistance to antimalarial monotherapies. ACTs are based on the combination of the rapid-acting but short plasma half-life (<2 h in humans) antimalarial action of an artemisinin component with a slower-acting partner drug. According to the WHO guidelines for malaria (updated in October of 2023), patients with uncomplicated forms of the disease should be treated orally with one of the following ACTs: ARM-lumefantrine, ART-amodiaquine, ART-mefloquine, DHA-piperaquine, ART-sulfadoxine/pyrimethamine or ART-pyronaridine (Reyburn, 2010; World Health Organization, 2015, 2023), following a weight-based dose as detailed in their guidelines. Furthermore, it has been demonstrated that the administration of the artemisinin component in a 3-day course is sufficient to cover two *Plasmodium* asexual cycles. This ensures that only a small fraction of parasites remains, which can then be effectively cleared by the partner drug (World Health Organization, 2023). On the other hand, for severe malaria, the WHO recommends the administration of intravenous or intramuscular ART for a minimum of 24 h, until patients can tolerate oral medication. If ART is unavailable, ARM is recommended over quinine for the treatment of both children and adults (World Health Organization, 2023). The average cost per case varies across a range from US $1.36 (for uncomplicated malaria treated at the community level) to US $92.80 (for severe malaria cases with cerebral complications treated at district hospitals) (Masimbi et al., 2022).

However, in recent years, there has been a notable decline in the effectiveness of three-day therapeutic courses against malarial parasites (Ashley et al., 2014; Haldar et al., 2018; Krishna & Kremsner, 2013). Moreover, artemisinin and its derivatives face certain challenges, namely, low bioavailability when taken orally (Fu et al., 2021; Navaratnam et al., 2000), chemical instability in the presence of heat, moisture, and light (Jansen & Soomro, 2007), and a short half-life (de Vries & Dien, 1996), which, together with the growing drug resistance scenarios, contribute to reduce therapeutic efficacy. In general, ACT resistance manifests as delayed clearance of parasites after therapy implementation, and is mediated by mutations in the Kelch (K13) *Plasmodium* protein (Ariey et al., 2014). Moreover, resistance to some ACT partner drugs including sulfadoxine/pyrimethamine, amodiaquine (Beshir et al., 2022), mefloquine (Phyo et al., 2016), and piperaquine (Amaratunga et al., 2016), threat the advance towards malaria eradication. Unfortunately, no alternative antimalarial

drugs with safety and efficacy profiles comparable to ACTs are currently available. Therefore, there is a compelling need to advance drug delivery systems aimed at enhancing the effectiveness of current antimalarial agents and mitigating the potential development of resistance to newly discovered drugs.

3. NANOMATERIALS IN THE FIGHT AGAINST MALARIA

Nanomaterials have unique characteristics derived from their nanoscale size, which typically falls between 1 and 100 nm. They can be synthetically produced or naturally occurring, and are classified according to their morphology and structure, which largely influence the physicochemical properties of the resulting nanoformulations.. Some of their key advantages which make them attractive for approaches to combat diseases like malaria include extended drug release, improved drug solubility, enhanced bioavailability, and amenability to functionalization for targeted therapies, which contribute to reduce side effects and mitigate resistance (Patra et al., 2018). Although the initial development bill can be high, the long-term benefits of improved treatment outcomes and fewer drug doses can result in cost-efficiency advantages. In the malaria field, several classes of nanoparticles have been explored, which are detailed in the following sections.

3.1. Liposomes and other lipid-based nanoparticles

Liposomes are artificial spherical-shaped vesicles composed of one or more phospholipid bilayers (Figure 2). Due to their dual nature, liposomes have the ability to simultaneously incorporate lipophilic drugs in their lipid bilayer, and hydrophilic compounds in their aqueous core (Zangabad et al., 2018). Moreover, liposomes have several advantages for drug delivery, including their low-toxic nature, and a wide range of available surface modifications (e.g. attachment of antibodies or chemical moieties) (Torchilin, 2005) (Figure 2). For malaria treatment, liposomes have been widely explored to achieve passive and active drug targeting. Passive targeting relies on the use of conventional and long-circulating liposomes, usually functionalized with polyethylene glycol (PEG), whereas active targeting is achieved by the modification of the liposomal surface with targeting ligands (e.g. antibodies, proteins, glycolipids, peptides, carbohydrates or nucleic acid aptamers) (Memvanga & Nkanga, 2021), which usually allow binding to receptors found in pRBCs. Since the first use of liposomes in the chemotherapy of murine malaria in 1979 (Pirson et al., 1979), a large number of experimental approaches have appeared in the literature reporting significant advances in the field.

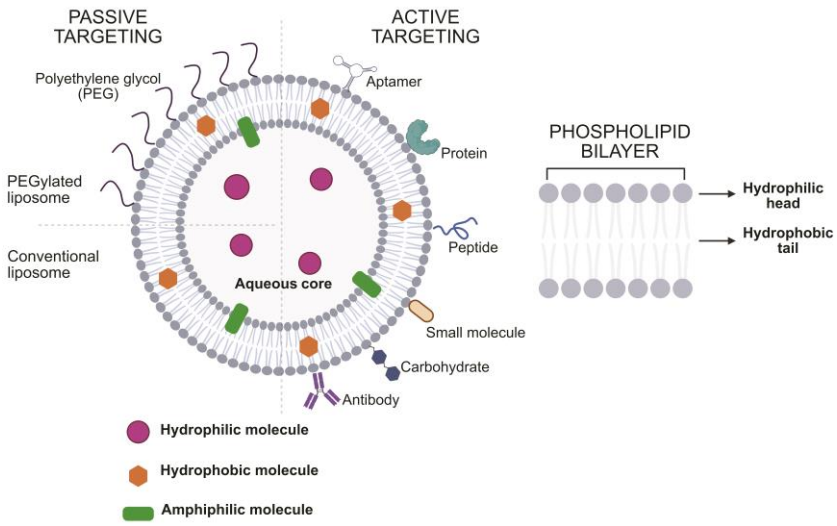

Figure 2. Structure of liposomes as drug delivery systems. Modified from (Olusanya et al., 2018). Created with BioRender.com.

Well-known antibiotics like sulfadoxine have been explored for their use in malaria treatment, but often they cause a delayed death phenotype or a mild parasite growth inhibition, which makes them inappropriate for malaria therapy when used alone (Pradel & Schlitzer, 2010). For this reason, antibiotics have been incorporated into nanoformulations to enhance their antimalarial properties, such as the anticoccidial drug monensin, a polyether ionophore with antimalarial activity against some *Plasmodium* species, including *P. falciparum* (Adovelande & Schrevel, 1996; D'Alessandro et al., 2015; Gumila et al., 1997). Ghosh and collaborators evaluated the antimalarial activity of monensin in long-circulating sterically stabilized liposomes (SSLs) with a previously tested distearoyl-phosphatidylethanolamine (DSPE)-methoxy-PEG 2000 (DSPE-mPEG2000) functionalization. Liposomes were made of soy phosphatidylcholine (SPC)-cholesterol (Chol), stearylamine (SA)-SPC-Chol, and phosphatic acid (PA)-SPC-Chol (Rajendran et al., 2016). The mean diameter of the resulting liposomes ranged from 90 to 120 nm, and their surface zeta potential (ζ) values were dependent on the incorporation of the charged lipids (SA and PA), and the PEG polymer (see Table 1 for physicochemical properties). The antiplasmodial activity in *P. falciparum* cultures of some of the formulations was significantly higher than for free monensin (half-maximal inhibitory concentration,

IC_{50} = 3.17 nM). For instance, the IC_{50} of monensin-loaded SPC-Chol liposomes was 1.11 nM, which significantly improved by the addition of 5 mol% DSPE-mPEG2000, reaching a value of 0.39 nM. On the other hand, SA-SPC-Chol drug-loaded liposomes showed an IC_{50} of 0.74 nM, which was not altered by addition of the PEG polymer. Moreover, the formulations surpassed the antimalarial activity of conventional drugs such as chloroquine (CQ, IC_{50} ~ 40 nM (Sidhu et al., 2002)) and artemisinin (IC_{50} ~ 10 nM (Tangnitipong et al., 2012)), probably due to the observed preferential incorporation of SSLs by pRBCs. The authors also tested the efficacy of their formulations in a *Plasmodium berghei* NK65 murine model of malaria. Following a modified Peter´s 4-day suppressive test (Peters, 1975), it was found that monensin-SA-SPC-Chol liposomes containing 5 mol% DSPE-mPEG2000, subcutaneously (s.c.) administered at different doses (4 mg/kg and 6 mg/kg of body weight), demonstrated superior effectiveness in reducing the parasite load compared to other formulations (median survival time of 27.5 days). Moreover, when these liposomes (5 mg/kg SA + 6 mg/kg monensin) were co-administered with free artemisinin (40 mg/kg) via s.c. injection to *P. berghei* ANKA- and NK65-infected mice, the parasite burden was completely eliminated leading to a 100% survival rate, and opening the road to alternative ACTs (Rajendran et al., 2016). In a follow-up study, Ghosh and colleagues incorporated the antibacterial drug doxycycline into long-circulating PEGylated SA-PCP-Chol liposomes and PCP-Chol liposomes (Rajendran et al., 2018) (see Table 1). Furthermore, the *in vitro* antiplasmodial activity in *P. falciparum* 3D7 showed respective IC_{50} values of 0.36 ± 0.11 µM and 0.85 ± 0.16 µM, significantly better than those of the free drug (IC_{50} = 14 ± 1.7 µM). When administered through a s.c. injection at a dose of 2.5 mg/kg per day to mice infected with either CQ-resistant or CQ-sensitive *P. berghei* strains, survival rates were significantly enhanced, with a median survival time over 40 days for both strains, whereas it was only 27 days in the group receiving the free drug (Rajendran et al., 2018). Following the same line, maduramicin, an ionophore antibiotic with good gametocytocidal activity (Sun et al., 2014) but of high toxicity and lipophilic character (Chen et al., 2018), was incorporated in SPC-Chol and PEGylated liposomes, (Raza et al., 2018). The PEGylated liposomal formulations (Table 1) not only prevented the unwanted toxic side-effects of this drug, but showed IC_{50} values of 1.25 ng/mL and 1.20 ng/mL in *P. falciparum* CQ-sensitive (3D7) and -resistant (INDO) strains, respectively, which represents almost half the values obtained with free maduramicin. Maduramicin-PEGylated liposomes were tested in a *P. berghei* ANKA murine model of cerebral malaria following the Peter´s 4-day suppressive test. It was observed

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that when treating the mice for 4 days with a 1 mg/kg dosage, PEGylated liposomal maduramicin proved to be highly effective in curing the animals (median survival time of 24 days), compared to the free drug (median survival time of 11 days). Moreover, when administered at an increased dose of 1.5 mg/kg, there was a complete clearance of blood parasitemia in mice treated with the PEGylated formulation of maduramicin, providing a promising strategy against aggressive forms of the disease (Raza et al., 2018).

Combining two drug molecules to form a dimer is an approach used to develop novel structures with better pharmacokinetic and pharmacodynamic properties than the respective parent compounds (Fujii et al., 2009; Paquin et al., 2021). In this sense, several artemisinin-based dimers have been developed and some have been found to be more potent against *Plasmodium* and less toxic than their monomeric analogs (Çapci et al., 2021; Lai et al., 2013). In 2018, Ismail *et al.* combined both dimerization and self-assembly approaches to obtain a novel amphiphilic dimeric artesunate glycerophosphorylcholine (Di-ART-GPC) molecule used to generate liposomes (Table 1). Di-ART-GPC was able to assemble in a multilamellar vesicle-type structure with an ART loading capacity of around 77.6% (Ismail et al., 2018). The Di-ART-GPC liposomes had better pharmacokinetic properties than free ART in a BALB/c model after intravenous (i.v.) administration. These included prolonged blood clearance, increased distribution volume, and a higher peak plasma concentration, and showed an enhanced antiplasmodial activity in *P. falciparum* parasites *in vitro* (IC_{50} = 0.39 nM) compared to free ART (IC_{50} = 5.17 nM), and to conventional ART-loaded liposomes (IC_{50} = 3.13 nM) (Ismail et al., 2018).

~~-a major problem for many antimalarials like artemisinin, which, to circumvent this limitation, was encapsulated in lipid-based large unilamellar vesicles. The resulting liposomal formulation had an antiplasmodial activity comparable to that of the ethanol-solubilized drug, both in *P. falciparum* cultures (IC_{50} of 13.15 nM and 14.97 nM, repectively), and in *P. berghei*-infected mice. Despite no improvement in activity was observed with these formulations, increasing drug solubility represents a first step towards improving antimalarial therapies.~~

During its life cycle, *Plasmodium* requires a high amount of phosphatidylcholine (PC) to support the production of the parasitophorous vacuole membrane (Contet et al., 2015; Itoe et al., 2014). Thus, the inhibition of PC biosynthesis represents a good target for antimalarial therapies, and, indeed, some choline derivatives that compete with choline in PC biosynthetic pathways have antiplasmodial activity

(Peyrottes et al., 2012). Based on this observation, Duan and colleagues synthesized an artelinic acid-choline derivative (AD), consisting in the endoperoxide functional group of artemisinin conjugated to an anologue of choline (Duan et al., 2020). AD was then formulated to liposomes (ADLs), and tested in *Plasmodium yoelii* BY265-infected mice in a 4-day suppression test. In all dosages assayed, the suppression of parasitemia was higher for ADLs (>90%), than for liposomes encapsulating only one of the two drugs (50 - 75%). Moreover, in a rat model, ADLs showed better pharmacokinetic features (higher total plasma levels and slower elimination rate) compared to AD in solution (Duan et al., 2020).

Oil-in-water (o/w) nanoemulsions are systems composed of oil, surfactants and co-surfactants dispersed in an aqueous phase. Jaromin *et al.* developed an o/w nanoemulsion using a concentrated form of ethyl esters of n-3 and n-6 polyunsaturated fatty acids as oil phase, for the delivery of 9*H*-3-azacarbazole (Jaromin et al., 2021), whose carbazole core is present in many biologically active compounds and possesses antiplasmodial activity (Wang et al., 2016). The generated nanoemulsion was evaluated in *in vitro* and *in vivo* studies, and found to inhibit *P. falciparum* growth. Whereas the non-encapsulated drug had IC_{50} values of almost 2000 ng/mL in *P. falciparum* D10 (CQ sensitive) and W2 (CQ resistant) strains, the encapsulated drug improved its activity to an IC_{50} of 697.0 ± 26.5 ng/mL (D10) and 528.7 ± 149.0 ng/mL (W2). Finally, *in vivo* assays demonstrated a rapid absorption of the nanoemulsions with a peak of concentration only 5 min after intragastric administration (Jaromin et al., 2021). This work constituted the first report of this type of nanoemulsions used as nanocarriers of antimalarials and represented a significant advance in the field.

~~More recently, Rajablou *et al.* encapsulated amphotericin B in DSPC/DSPE-PEG2000 micelles, which resulted in a higher parasiticidal activity compared to the free drug (IC_{50} of 2.4 µg/mL and 4.8 µg/mL, respectively).~~

Solid-lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are lipid-based nanoparticles used for the delivery of drugs and other compounds used in diagnosis and therapy. The main advantages of these nanocarriers include an increase of drug bioavailability, extended plasma half-life, and low toxicity (Viegas et al., 2023). These nanostructures are essentially composed of lipids and surfactants, but whereas SLNs are composed of a single lipid matrix that is in a solid state at room temperature (crystalline lipid core), NLCs have a more complex structure combining both solid and liquid lipids to form the nanostructure (Fonseca-Santos et al., 2020). Onwoyo *et. al.* synthesized

primaquine (PQ)-loaded SLNs (PQ-SLNs) (Table 1) and observed a parasite-suppression efficacy of 93.5% in *P. berghei* ANKA-infected mice when treated intraperitoneally (i.p.) at a 2 mg/kg/day dosage. In comparison, only 71.9% parasite clearance was observed in the group treated with the same dose of free PQ (Omwoyo et al., 2014). In a follow-up study, the group formulated DHA into SLNs and observed improved antimalarial activity both *in vitro* (with an IC_{50} of 0.25 ng/mL) and *in vivo* (with a 97.2% reduction in parasitemia at a daily dose of 2 mg/kg in a *P. berghei* murine model). The enhancement in effectiveness was 24% compared to free DHA, demonstrating the potential of these formulations in antimalarial drug delivery (Omwoyo et al., 2016).

Attama and collaborators encapsulated ARM and lumefantrine into SLNs (Table 1) and studied the *in vivo* activity of the formulations in *P. berghei*-infected mice using a standard suppressive protocol (Peters, 1975). A high parasitemia clearance (>70%) was found after oral administration of the drug-containing SLNs, which was sustained up to the 14^{th} day (Attama et al., 2016). In a different study, ARM-lumefantrine NLCs (ARM-LFN-NLCs) were formulated by Prabhu and collaborators (Prabhu et al., 2016), and tested in *P. berghei* ANKA-infected C57BL/6 mice following a modified Peter's 4-day suppressive test protocol. Results showed that ARM-LFN-NLCs i.v. injected at 0.04 mg/kg/day of ARM and 2.4 mg/kg/day of LFN dosage, completely abolished parasite growth and a 100% survival rate was observed. When these drug-loaded NCLs were tested in a murine model of cerebral malaria, treated mice showed complete resolution of the disease following the 4-day treatment. On the contrary, the group treated with artemisinin died within 14 days.

3.2. Polymer-based nanocarriers

Polymer-based nanoparticles (PNPs) have been widely used in the treatment of numerous infectious diseases (Cano et al., 2020; Landriscina et al., 2015; Mercan et al., 2022). PNPs present several advantages as drug delivery systems, such as simple manufacturing, good biocompatibility, high stability, and potential for oral delivery (Galindo-Rodriguez et al., 2005; Marasini et al., 2017), which improves patient compliance. Moreover, the physicochemical properties of PNPs (i.e., size, surface, charge, and hydrophobicity) are highly flexible due to the wide range of materials used for their synthesis. Finally, similar to liposomes, PNPs can be conjugated to targeting moieties such as antibodies, peptides or small molecules (Feng et al., 2013).

3.2.1. Polymer-drug conjugates

Polymer-drug conjugates are usually formulated with three units (solubilizing unit, targeting moiety, and a therapeutic agent) incorporated into the polymer backbone (Elvira et al., 2005; Pang et al., 2014) (Figure 3). The main advantages of these conjugates are increased drug solubility, reduced toxicity, and improved pharmacological properties (Marasini et al., 2017). Therefore, polymer-drug conjugates could help overcome some of the main obstacles in treating malaria.

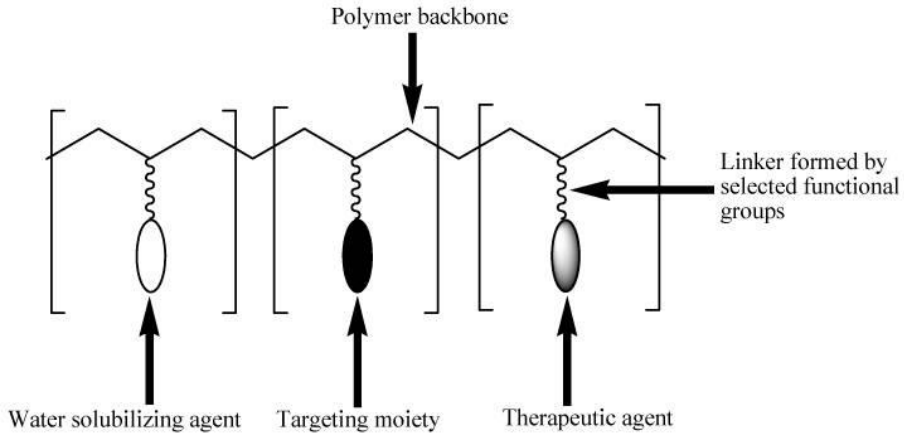

Figure 3. Schematic diagram of polymer-drug conjugates. Reproduced from (Alven et al., 2020).

Urbán and colleagues explored this approach by incorporating CQ and PQ into a poly(amidoamine) (PAA) backbone (Urbán et al., 2014). The selected PAAs, AGMA1 and ISA23 (Figure 4), showed high encapsulation efficiencies and preferential targeting (>80%) towards late forms of *P. falciparum* and *P. yoelii*-infected RBCs. Interestingly, drug-free AGMA1, and to a less extent ISA23, exhibited intrinsic antiplasmodial activities *in vitro*, which increased with polymer size. The authors hypothesized that this inhibitory activity could be mediated through the binding of AGMA1 to free merozoites thereby impeding the reinvasion process. Finally, *in vivo* studies in a *P. yoelii* 17XL model of murine malaria, revealed that 4 doses of 0.8 mg/kg of AGMA1 and ISA23, loaded with CQ and i.p. administered, induced almost 100% parasite clearance, whereas the same dose of free drug produced a reduction of only 16% in parasitemia.

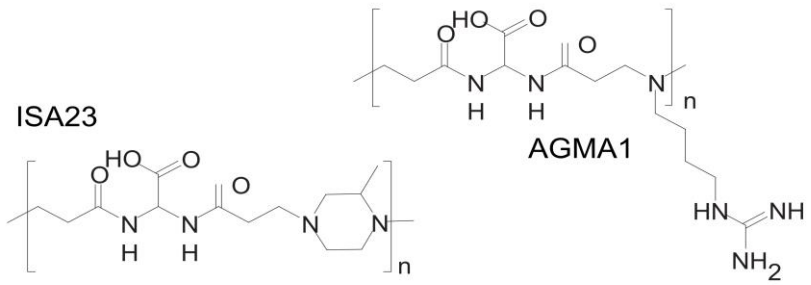

Figure 4. Chemical structures of AGMA1 and ISA23. Reproduced from (Urbán et al., 2014).

Oral administration is considered favourable because of better convenience and compliance by patients. However, the physiological barriers of the digestive system (i.e. extreme pH and enzyme degradation) limit drug absorption (Pridgen et al., 2015). When Martí Coma-Cros *et al.* incorporated a fluorescent tag (fluorescein 5-isothiocyanate, FITC) into AGMA1 and ISA23 and fed the labeled nanoparticles to *P. yoelii* 17XL-infected mice (Martí Coma-Cros et al., 2018), PAA-FITC nanoparticles were found in the mouse blood circulation 24 h after administration, and ISA23 was also present in the plasma of orally administered animals. The fluorescent signal was also found in mosquito midguts after sugar feeding to *Anopheles atroparvus* and *Anopheles gambiae*, indicating that this platform could be further exploited for oral and direct administration to mosquitoes of antimalarial drugs.
~~In a different study, Mukaya *et al.* explored the use of polyaspartamides to carry antimalarial drugs, and designed conjugates of this polymer containing platinum(II) complex and bisphosphonate. *In vitro* results indicated that some of the generated conjugates possessed a higher activity against *Plasmodium* than the control drug CQ, indicating their potential use against malaria.~~ More recently, Aderibigbe *et al.* used a polyaspartamide platform to synthesize conjugates containing pyrimethamine in combination with either 4-aminosalicylic acid or PQ (Aderibigbe et al., 2019). The release of the drugs from these formulations was low and sustained at pH 7.4, with ca. 25 % of pyrimethamine released from the conjugates over a period of 5 days. The most active conjugate (Z0, containing pyrimethamine and 4-aminosalicylic acid) (Table 1) exhibited an IC_{50} of ca. 332 ± 6 nM in *P. falciparum* cultures. Nanoformulations that exhibit a prolonged drug release represent a good opportunity to overcome resistance problems, potentially leading to more effective treatments.

However, it is important to mention that lower and fewer doses are not guaranteed solely due to this characteristic, as dosing considerations depend on several factors, including drug pharmacokinetic properties.

~~Using a different approach, Valissery *et al*. improved the water solubility of artemisinin by encapsulating it in a nanocarrier based on polycaprolactone. The generated NPs retained the antiplasmodial activity of artemisinin but the resulting IC_{50} was higher than the one for the free drug (62.74 nM vs 14.97 nM). However, when the formulation was tested on the *P. berghei* murine malaria model, the activity of the encapsulated drug was similar to the ethanol-dissolved drug, showing a parasitemia reduction of 85.5% after 9 days of treatment. Although there was no improvement in activity, the increased solubility allowed for a higher dosing regimen to be implemented.~~

Finally, Ghosh and Banerjee prepared a curcumin-(3-bromo-N-(4-fluorobenzyl)-benzo[b]thiophene-2-carboxamide) conjugate (cur-compound 6) (Ghosh & Banerjee, 2020). *In vitro* treatment of *P. falciparum* with this compound resulted in parasite clearance within 24 h with an IC_{50} of 0.5 µM, indicating an improved activity compared to free curcumin (IC_{50} = 5 µM). The nanoformulation was stable after oral administration in *P. berghei*-infected mice, and increased life span to 90 days, whereas mice that received equal oral doses of curcumin alone survived for only 10 to 16 days.

3.2.2. Micelles

Polymeric micelles are formed by amphiphilic polymers that self-assemble in solution (Figure 5). Below a specific concentration (i.e. critical micellar concentration, CMC), these polymers behave as monomers in solution, while above the CMC they assemble into micelles presenting a lipophilic core and an external hydrophilic shell (Ghezzi et al., 2021). The ease of their preparation as well as their small size and physicochemical properties place polymeric micelles as good drug delivery systems for antimalarial drugs.

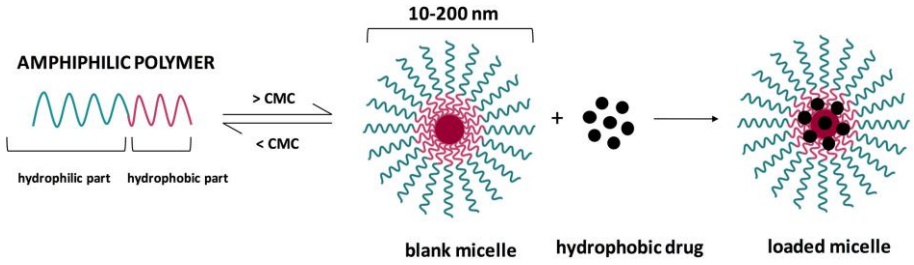

Figure 5. Schematic representation of polymeric micelles. Reproduced from (Ghezzi et al., 2021).

Plumbagin (PNL), a hydrophobic plant-derived drug with antiplasmodial effects (Simonsen et al., 2001), was incorporated in micelles made of a poly(e-caprolactone)-PEG-poly(e-caprolactone) tri-block copolymer (PCL-PEG-PCL). The resulting micelles had PEG as a hydrophilic shell and PCL as a hydrophobic core (Table 1) (Rashidzadeh et al., 2022). When PNL-loaded micelles were screened *in vivo* using the 4-day suppressive test against *P. berghei* ANKA infection in mice, those loaded with drug showed a 92.3% parasite depletion compared to empty micelles (9.2% reduction) and the free drug (30.7% reduction). Moreover, PNL-loaded micelles displayed sustained drug release behaviour and improved blood circulation time, indicating that the use of this drug delivery system could be applied to carry antimalarial hydrophobic drugs like PLN.

Similar efforts to encapsulate antiplasmodial hydrophobic drugs have been carried out by other groups. For instance, Biosca *et al.* explored the use of zwitterionic poly(butyl methacrylate-co-morpholinoethyl sulfobetaine methacrylate) (PBMA-MESBMA)-based nanoparticles, which intrinsically exhibited specific targeting to pRBCs vs naïve RBCs (Figure 6) (Biosca et al., 2021). When loaded with curcumin, the *in vitro* IC_{50} in *P. falciparum* was equivalent to that of the free drug (~5 µM), probably due to the low release of curcumin from the nanoparticles. However, *in vivo* assays in *P. yoelii* 17XL-infected mice revealed their safety for oral administration. At 1 h after ingestion PBMA-MESBMA-curcumin reached concentrations in the blood higher than for the free drug fed at the same concentration (18.0 vs. 2.1 ng/mL, respectively). Nevertheless, similar antimalarial activities were observed for the free and the encapsulated drug at longer times after oral administration.

In another study, Pestehchian and colleagues loaded pyrimethamine in Poloxamer 407 nanomicelles and tested their activity in *P. berghei* NICD-infected mice (Pestehchian et al., 2020). Free pyrimethamine, empty nanomicelles or pyrimethamine-nanomicelles were administered i.p. for 4 days at a 2 mg/kg dosage. The results showed that the encapsulated drug had a strong antimalarial activity, with a mean survival rate of 15.5 days, compared to the 5.3 days observed in the control group treated with the free drug.

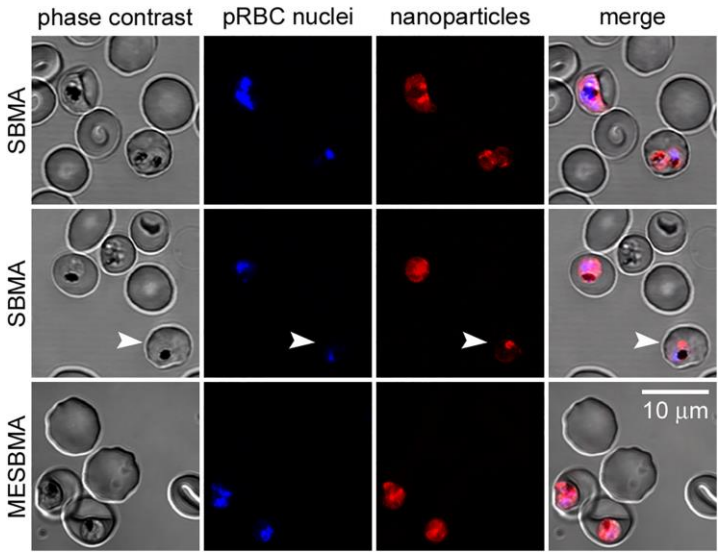

Figure 6. Targeting of (PBMA-MESBMA)-based nanoparticles to *P. falciparum* pRBCs. Selected confocal images showed the intracellular targeting of the nanoparticles in red. Reproduced from (Biosca et al., 2021).

3.2.3. Dendrimers

Dendrimers are highly branched macromolecules with highly controlled structures having a tendency to adopt globular shapes once a certain size is reached (Liu & Frechet, 1999) (Figure 7), whose well-defined structure, globular shape, size monodispersity and controllable attachment of surface moieties make them good candidates for drug delivery.

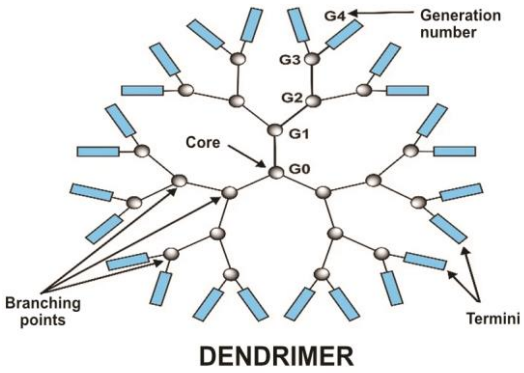

Figure 7. Schematic representation of a dendrimer. Adapted from (Sung & Kim, 2020).

Movellan *et al*. used dendritic derivatives based on 2,2-*bis*(hydroxymethyl)propionic acid (bMPA) and Pluronic® polymers to encapsulate CQ and PQ (Movellan et al., 2014). Out of the four derivatives tested, two of them (C-PQ and D-CQ) exhibited specific targeting to *P. falciparum*-pRBCs and an improved IC_{50} compared to the corresponding free drugs: the IC_{50} for C-PQ was 1.1 µM compared to 4.9 µM for free PQ, and the IC_{50} for D-CQ was 4.0 µM compared to 13.6 µM for free CQ. Nevertheless, preliminary *in vivo* assays in a *P. yoelii* 17XL murine malaria model did not show an improvement in the treatment with the encapsulated drugs relative to the control group treated with the free drugs (Movellan et al., 2014). In a follow-up study Martí Coma-Cros and colleagues explored the use of two dendritic block copolymers based on Pluronic® F127 and amino-terminated 2,2′-*bis*(glycyloxymethyl)propionic acid dendrons with a poly(ester amide) skeleton (HDLDBC-bGMPA), or an amino-terminated dendronized hyperbranched polymer with a polyester skeleton derived from bMPA (DHP-bMPA) (Table 1) (Martí Coma-Cros et al., 2019). *In vitro* assays revealed that DHP-bMPA specifically targeted *P. falciparum*-infected RBCs whereas HDLDBC-bGMPA was incorporated by all RBCs. Dendrimers were then loaded with either CQ, PQ or quinacrine, and tested *in vitro* against *P. falciparum*. The results showed that the *in vitro* IC_{50} of the encapsulated drugs was comparable to that of the free compounds. In a similar manner, when dendrimers loaded with CQ were screened against the *P. yoelii* murine malaria model, no improvement in the *in vivo* activity of the free drug was observed.

3.2.4. Polymeric capsules

Polymer capsules are solid colloidal particles made of polymers that allow high drug encapsulation efficiencies, and relatively higher intracellular uptake than other nanoformulations (Mosqueira et al., 2001; Vauthier & Bouchemal, 2009).

Ismail et al. developed an artesunate-heparin nanocapsule (ART-HEP-NC) with high drug loading efficiency (29.3 w%) and a high release rate in a simulated acidic microenvironment (92.7%), which suggested that drug release will be favoured in the acidic parasite vacuole (Ismail et al., 2019). Data from *in vitro* experiments revealed that the nanoparticles had inhibitory activity in *P. falciparum* with an IC_{50} of 10.2 nM, which was higher than that observed for the free drug (6.3 nM). Neverteless, the

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nanoformulations exhibited specific targeting to pRBCs and improved pharmacokinetic parameteres using a BALB/c mice model by i.v. injection (10 mg/kg) (Table 2), representing a promising platform for ART delivery.

Table 2. Pharmacokinetic parameters of free ART and ART-HEP-NCs. Modified from (Ismail et al., 2019).

*Parameters (unit)	Free ART	ART-HEP-NCs
AUC_{0-t} (mg//L·h)	**25.63**	**56.54**
MRT_{0-t} (h)	**2.44**	**9.39**
Ke	**0.49**	**0.10**
$t_{1/2}$ (h)	**1.39**	**4.51**
V_d	**0.69**	**0.35**
C_L (L/h/kg)	**0.19**	**0.08**
C_{max} (µg/mL)	**14.13**	**18.12**

*Parameters: AUC_{0-t}, area under the curve from zero to time t; MRT_{0-t}, residence time; *Ke*, elimination rate constant; $t_{1/2}$, elimination half-life; V_d, apparent volume of distribuition; C_L, plasma clearance; C_{max}, peak plasma concentration.

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Velasques *et al*. developed nanosized polymer capsules of polysorbate and co-loaded quinine (Q) and curcumin (C) (QC-NC). The generated drug-loaded nanocapsules showed antiplasmodial activity *in vitro* against *P. falciparum* CQ-resistant (W2) and -sensitive (3D7) strains, and although it was not possible to determine the IC_{50}, QC-NC nanoparticles showed greater activity against the parasite in comparison to a mixture of free Q:C (2:1) (Velasques et al., 2018). Moreover, the nanocapsules showed reduced toxicity compared to the free antimalarials in the model of *Caenorhabditis elegans*. In a different study, Souza and colleagues demonstrated that ARM-loaded PCL nanocapules (ARM-PCL) lowered the cardiotoxic effect of the drug in *P.berghei*-infected mice, reducing animal mortality when administered orally even at the highest tested dose (120 mg/kg) (Souza et al., 2018). Similarly, Vidal-Diniz *et al.* encapsulated different amounts of ARM in the biodegradable polyesters poly(D,L-lactide) (ARM-PLA) and PCL (ARM-PCL) (Vidal-Diniz et al., 2022) (Table 1). PCL nanocapsules exhibited the highest drug loading efficiencies and the lowest release rates. Moreover, when tested in

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P. berghei-infected mice using 40 or 80 mg/kg divided in four daily doses delivered by i.v. injection, the antimalarial activities of encapsulated and free drug were similar. However, ARM-containing nanocapsules had a better cardiovascular profile than the free drug at effective antiparasitic doses. These results pointed to a strategy for the production of safer antimalarial treatments, opening the road for new therapeutic approaches.

3.2.5. Polymeric nanoparticles

Biodegradable and bio-absorbable polymers, such as poly(lactic acid), poly(glycolic acid) and their copolymers, have been used as controlled drug delivery systems (Basu et al., 2016; Sung & Kim, 2020). Oyeyemi *et al.* explored the use of poly (D,L-lactic-co-glycolic acid) (PLGA) nanoparticles for co-delivery of curcumin and artesunate (Oyeyemi et al., 2018). The generated nanoformulations were assessed in *P. berghei*-infected mice following the Peter's 4-day suppressive test, showing better parasiticidal properties at low doses (5 mg/kg), with parasite clearance percentages of 79.0% at day 5 and 72.5% at day 8. On the contrary, there was no parasite suppression in the group treated with the free drugs. In a similar approach, Jawahar *et al.* developed PLGA nanoparticles for co-delivery of CQ and azithromycin (Jawahar et al., 2019). *In vitro* analysis in *Plasmodium* cultures demonstrated that encapsulated drugs had synergistic effects against the parasite with an IC_{50} of 1.11 µg/mL and half maximal effective concentration (EC_{50}) of 1.95 µg/mL. Innovative approaches that allow efficient and controlled co-delivery of antimalarials open a new avenue for overcoming drug resistance via intracellular targeting.

Recently, Hamelmann and collaborators synthesized different single-chain nanoparticles (SCNP) with increasing anionic surface charge to target the mosquito ookinete stage of *P. berghei* (Hamelmann et al., 2023), similarly to the effect observed for the highly negatively-charged heparin (Weiss et al., 2017). As expected, *in vitro* targeting assays in *P. falciparum* revealed a positive correlation between anionic surface charge and targeting efficiency. SCNPs were loaded with atovaquone (ATO) and tested in an *ex vivo* ookinete maturation inhibition assay; however, no effect was observed probably due to a decrease in the SCNP anionic charge after ATO conjugation, which may reduce ookinete targeting.

3.3. Metal nanoparticles

Metal-derived nanoparticles have been widely used in biomedicine due to their inherent antimicrobial activity by altering microbial cell integrity and metabolism processes as reviewed in (Nisar et al., 2019). Due to their low cost and amenability to surface modifications, metal nanoparticles represent an attractive and affordable option for malaria treatment.

Kannan *et al.* explored the capacity of surface-coated iron oxide nanoparticles (IONPs) to enhance the efficiency of artesunate (ART-IONPs) (Kannan et al., 2019). Experimental data showed that IONPs increased by 5-fold the efficacy of the free drug *in vitro* in *P. falciparum* cultures, with an IC_{50} of ca. 0.4 nM, compared to 2.0 nM for artesunate alone. On the other hand, the *in vivo* activity in a *P. berghei* ANKA model was also improved by 8- to 10-fold, after a daily i.p. administration of 5 mg/kg during 5 days. Moreover, due to the coating with 2-aminoterphthalic acid, the nanoparticles allowed for a sustained and pH-dependent release of ferrous ions. Hence, internalization of IONPs into the food vacuole provided the ideal environment for a persistent release of ions, activation of artesunate, and generation of free radical species, which contributed to the parasiticidal activity.

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3.3.2. Green nanoparticles

Metal nanoparticles are mainly synthesized using chemical methods which produce environmental pollution, potential health problems, and require a large energy consumption. To deal with these issues, green synthesis, which employs plant extracts instead of chemical agents to reduce metal ions, has been developed (Ying et al., 2022). Titanium oxide nanoparticles were prepared by Gandhi and colleagues (Gandhi et al., 2018) using the *Momordica charantia* leaf aqueous extract as reducing and stabilizing agent. Synthesized nanoparticles exhibited a higher *in vitro* antiplasmodial activity compared to the leaf extracts in both CQ-resistant (3D7) and -sensitive (INDO) *P. falciparum* strains: the IC_{50} values of extracts were 83.6 µg/mL for 3D7 and 88.1 µg/mL for INDO, whereas TiO_2 nanoparticles exhibited values of 53.4 µg/mL (3D7), and 59.7 µg/mL for the INDO strain. Nevertheless, treatments were less effective than CQ (IC_{50} of 0.02 µg/mL for 3D7 and 0.26 µg/mL for INDO). Similarly, Najoom *et al*. used *Rhazya stricta* extracts to prepare zinc oxide nanoparticles that displayed an *in vitro* IC_{50} of 3.4 µg/mL in *P. falciparum* cultures, which was better than the value of 11.6 µg/mL obtained with the leaf extract (Najoom et al., 2021).

Ghazali *et al.* developed silver nitrate nanoparticles using aqueous extracts of *Azadirachta indica* (Ghazali et al., 2022). Nanoparticles were tested in cultures of CQ-resistant (W2) and -sensitive (3D7)

P. falciparum strains, and displayed a 4-fold IC_{50} decrease against both lines when compared to the aqueous extract.

Hawadak and colleagues synthesized silver nanoparticles (AgNPs) using *Azadirachta indica* extracts from either leaves (AIL) or barks (AIB) (Hawadak et al., 2022). The antiplasmodial and hemolytic activity of the generated nanoparticles was then evaluated *in vitro*, and the results showed that AIL- and AIB-AgNPs displayed good antiplasmodial properties with IC_{50} values of 9.3 µg/mL and 8.1 µg/mL in 3D7 *P. falciparum* parasites, and 11.1 µg/mL and 7.9 µg/mL against *P. falciparum* RKL9 (CQ resistant). However, a significant hemolytic activity (>25%) was also detected at higher concentrations of both AgNPs (≥125 µg/mL).

3.4. Targeted nanotherapeutics

Nanoformulations can be properly engineered to deliver an appropriate dose of a drug to specific cells/tissues by following different targeting approaches (Ahmad et al., 2019; Sanna & Sechi, 2020). In this context, nanoparticles can be functionalized with targeting ligands including antibodies, proteins or peptides, nucleic acids (e.g. aptamers), and different small molecules. These ligands can favour a high selectivity towards target cells as well as participate in increasing cellular uptake through internalization processes (Sanna & Sechi, 2020).

In the malaria field, several attempts to generate a “magic bullet” to fight the disease have been made. In 2015, Moles *et al.* analysed different variants of an immunoliposome to provide optimal targeting and antimalarial activity (Moles et al., 2015). As targeting elements, the group tested antibodies raised against a peptide contained in the parasite’s membrane-associated histidine-rich protein ($MAHRP1_{21-40}$), antibodies directed against the surface histidine-rich protein II (HRP2) of *P. falciparum*, and anti-glycophorin A (GPA) monoclonal antibodies, a highly abundant surface protein present in RBCs. Among all, anti-GPA offered the best targeting (100% targeting to RBCs and pRBCs). To crosslink the different antibodies on the liposomal bilayer, three chemical groups were tested: thiol, carbohydrate or primary amines present in the antibodies that could bind to the maleimide-containing lipid DSPE-N-maleimide(polyethyleneglycol)-2000 (DSPE-PEG2000-Mal) included in the liposome formulation. According to *in vitro* studies, the highest binding efficiency was achieved when using the primary amines in antibody molecules. When exposed for only 15 min to *P. falciparum* cultures in early blood stages, free CQ had no significant effect on the viability of the

parasite up to 200 nM, whereas immunoliposomal 50 nM CQ completely arrested its growth. Finally, preliminary *in vivo* studies in a humanized mouse model of *P. falciparum* malaria showed that immunoliposomes cleared the pathogen below detectable levels at a CQ daily dose of 0.50 mg/kg for 4 days, whereas free CQ administered with the same regimen at 1.75 mg/kg was, at most, 40-fold less efficient. Based on this, the authors suggested that their novel immunoliposomes may act as prophylactic and therapeutic agents by the simultaneous deliver of the drug in both RBCs and parasitized RBCs.

Following these encouraging results, Moles and collaborators explored the use of a fully immunocompetent mouse model (contrary to the humanized mice). Hence *P. yoelii* 17XL-infected mice (Fu et al., 2012) were used to analyse the activity of immuno-PEG-liposomes targeted to human GPA (Moles et al., 2017). Different lipophilic aminoalcohol and aminoquinoline drugs were loaded in the liposomes to test their prophylactic and therapeutic effects. Among the different nanoparticles evaluated, GPA-immuno-PEG-liposomes carrying the drug 7c (a CQ analogue) had the best therapeutic effect, with an IC_{50} of 48.6 nM against late-stage CQ-resistant *P. falciparum* parasites. Moreover, the liposomes reached ca. 100% targeting efficiencies to both naïve and *Plasmodium*-infected RBCs. In addition, *in vivo* studies with a dose of 20 mg/kg over 4 days demonstrated a significant parasite clearance (44-55%), and high retention efficacies (more than 95%) in RBCs and pRBCs. In a follow-up study, Fernàndez-Busquets and colleagues used anti-human GPA-functionalized immuno-PEG-liposomes to co-encapsulate both a hydrophilic (pyronaridine) and a lipophilic (atovaquone) drug (Figure 8, Table 1). *In vitro* studies revealed that the generated immunoliposomes had a higher antiplasmodial activity compared to the corresponding free drugs: a 50% growth inhibition was achieved with 43.1 nM of atovaquone and 137.2 nM pyronaridine in the encapsulated form, whereas an equal concentration of the free drugs did not reach 1% inhibition (Biosca et al., 2019).

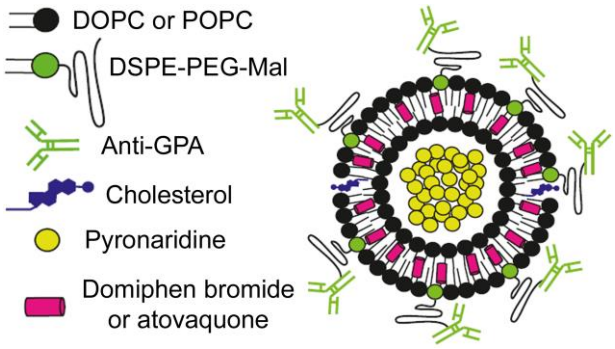

Figure 8. Schematic representation of an immunoliposome designed for targeted co-delivery of antimalarial drugs. Reproduced from (Biosca et al., 2019).

Rosetting in *P. falciparum* is a phenomenon by which the pRBC binds to naïve RBCs through parasite ligands that recognize RBC surface receptors. It has been hypothesized that the parasite uses this system to evade the host immune response by shielding the pRBC and allowing its sequestration within the deep vasculature, a phenomenon often associated to severe malaria (Doumbo et al., 2009; Treutiger et al., 1992). Rosetting also facilitates parasite survival and replication by favouring merozoite reinvasion (Lee et al., 2022; Yam et al., 2017). So far, three *P. falciparum* ligands involved in this process have been identified: the erythrocyte membrane protein1, PfEMP1 (Juillerat et al., 2011); subtelomeric variant open reading frame (STEVOR) proteins (Niang et al., 2014); and repetitive interspersed family protein, RIFIN (Goel et al., 2015). Following their previous studies, Moles *et al.*, explored the use of a polyclonal antibody against the NTS-DBL1α N-terminal domain of a rosetting PfEMP1 variant as a targeting element, and loaded immunoliposomes with lumefantrine (Table 1) (Moles et al., 2016). After 30 min of incubation with encapsulated drug, a 70% growth inhibition for all parasite forms in culture (IC_{50} = 414 nM) and a ~60% reduction of the rosetting phenotype was achieved (IC_{50} = 747 nM). This approach demonstrates the high potential of nanotechnology in combining two mechanisms of action (i.e., antiplasmodial and antirosetting activities) in a single therapeutic agent, which can be exploited in the malaria field.

Despite the promising *in vitro* and *in vivo* results showed by immunoliposomes as antiplasmodial agents, the costs associated with them are higher than those of conventional drugs. Because the available funding for new treatments against infectious diseases that affect low-income countries is

scarce, an important consideration for the development of new antimalarial approaches is their cost-effectiveness, given the preferred target product profiles of US$1 per day (Kirtane et al., 2021). In this context, other strategies to achieve specific targeting towards pRBCs have been explored. Tagami and colleagues developed liposomes conjugated to a phosphatidylserine (PS)-binding peptide (PSP) with the aim of specifically recognizing pRBCs programmed for cell death (eryptosis). This process is characterized by PS exposure on the RBC surface (Wesseling et al., 2016), and has also been associated with *Plasmodium* infection. To partially mimic this, the authors generated an eryptosis-induced RBC model (E-RBC) where they tested their liposomes. *In vitro* results revealed that, 3 h post-incubation, fluorescently-labeled PSP-liposomes bound with higher affinity E-RBCs than RBCs, indicating the potential use of this targeting strategy to deliver antimalarial drugs to pRBCs (Tagami et al., 2015).

Heparin, a glycosaminoglycan with antimalarial properties, has been found to specifically target pRBCs (in comparison to naïve RBCs) (Boyle et al., 2010; Vogt et al., 2003; Vogt et al., 2004). However, due to its high anticoagulant activity, the use of heparin as malaria treatment was largely abandoned. Marques *et al*. explored the potential of heparin as targeting agent to pRBCs (Marques et al., 2017). For this, PQ-loaded liposomes were coated with heparin (Table 1), and *in vitro* experiments in *P. falciparum* cultures indicated that the generated nanoformulations had a higher antiplasmodial activity when compared to the free drug or to plain PQ-loaded liposomes, which suggested an additive effect of heparin as drug and targeting element. Moreover, the anticoagulant activity of heparin bound to liposomes was significantly lower than for the free compound. Heparin-coated liposomes were also explored to encapsulate poupartone B (Ledoux et al., 2020), an antimalarial compound derived from *Poupartia borbonica*, which has an intrinsic high toxicity (Bordignon et al., 2018). The corresponding liposomal formulation had a stronger antiplasmodial activity against a partially artemisinin-resistant *P. falciparum* strain (IPC 3445; IC_{50} of 0.41 µg/mL compared to 0.69 µg/mL for the free drug). Notably, the liposomal formulation was 3 times less toxic than the compound alone in the zebrafish model (Ledoux et al., 2020). Similarly, Muga *et al*. developed heparin-functionalized SLNs containing CQ and tested them in *in vitro* cultures (Muga et al., 2018). The encapsulated drug displayed higher antiplasmodial activities against a CQ-sensitive (D6) strain of *P. falciparum* compared to free CQ (IC_{50} of 2.41 ng/mL and 5.81 ng/mL, respectively). Following the same line, San Anselmo *et al.* recently developed different dendronized hyperbranched polymers

(DHPs) of bMPA and bGMPA coated with heparin to achieve specific targeting to pRBCs (San Anselmo et al., 2023). The generated DHP-heparin complexes exhibited the intrinsic antimalarial activity of free heparin (IC_{50} ~400 nM) with an improved targeting ability. Altogether, these studies demonstrated that nanocarriers exploiting the dual activity of heparin as targeting and antimalarial element, and also capable of carrying other drugs, represent an attractive approach for future antimalarial targeted drug delivery systems.

Finally, glucose-based ultra-small gold nanoparticles (Glc-NPs) that bound to cysteine-rich domains of *P. falciparum* surface proteins were loaded with ciprofloxacin (Varela-Aramburu et al., 2020), a drug with mild antiplasmodial activity (Gaillard et al., 2016). The nanoparticles showed specific targeting to pRBCs and *P. falciparum* gametocytes whereas no binding was observed to naïve RBCs. The IC_{50} for ciprofloxacin-loaded Glc-NPs was significantly lower (27.4 µM) than that observed for the free drug (157.9 µM), which showed the potential usefulness of directed drug delivery.

3.5. Vaccines

Traditional vaccines are made of whole killed or attenuated pathogens, or of components of microorganisms. However, nowadays research is mainly focused on the development of subunit vaccines manufactured on the basis of well-characterized antigens usually in the form of recombinant proteins and peptides (Schwendener, 2014). Considering the often weak immune response obtained with synthetic antigens, nanoformulations are now being employed for vaccine development. The use of nanoparticles as vaccine carriers provides adjuvant activity either by enhancing antigen delivery or by activating innate immune responses (Gregory et al., 2013). In this sense, liposomal formulations stand out for their use as drug carriers due to their versatility and plasticity in several diseases including malaria.

Liposomes have been explored for their use as adjuvants in novel malaria vaccines; for instance, *Pf*s25, a *P. falciparum* antigen expressed on the surface of zygote and ookinete stages, represents a potential vaccine candidate for eliciting transmission-blocking immunity in malaria-endemic regions. However, due to its limited immunogenicity in humans, its clinical application has been hampered. The malaria vaccine branch of the US Walter Reed Army Institute of Research has developed several liposomal adjuvants and formulations for malaria vaccines (Artenstein et al., 2005; Grabenstein et al., 2006; Ratto-Kim et al., 2018). Among them, the army liposome formulation (ALF) and their derivatives

ALF adsorbed to aluminum hydroxide (ALFA), ALF containing QS21 saponin (ALFQ), and ALFQ adsorbed to aluminum hydroxide (ALFQA), have provided a strong and safe immunity (Alving et al., 2020). Among their components, the monosphosphoryl lipid A (MPLA) is thought to be responsible for the adjuvant activity of many vaccine candidates (Alving et al., 2020; Alving et al., 2012), including malaria antigens (Ssemaganda et al., 2019). Indeed, GlaxoSmithKline developed another liposome-based adjuvant system (AS01) which contains a synthetic analog of MPLA (Didierlaurent et al., 2017). The generated RTS,S/AS01 malaria vaccine (Mosquirix™) is the first licensed malaria vaccine, shown to provide partial protection against the disease in young children (Laurens, 2020). RTS,S/AS01 incorporates the central repeat region (R) and the C-terminal region containing the T-cell epitopes (T) of the *P. falciparum* circumsporozoite protein (CSP), and a viral envelope protein of the hepatitis B surface antigen (S) (Figure 9). However, due to its modest efficacy, it might not be sufficient on its own for global malaria eradication (Arora et al., 2021).

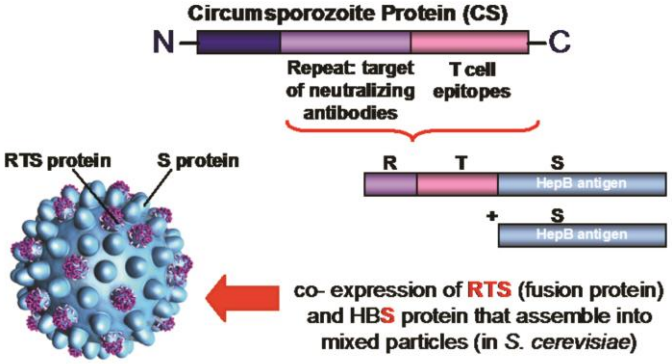

Figure 9. RTS,S recombinant protein virus-like particle. Reproduced from (European Medicines Agency, 2015).

The self-assembling protein nanoparticle (SAPN) approach has also been explored for malaria vaccine candidates. The FMP014 vaccine is constituted by 60 identical monomer protein chains of selected *P. falciparum* CSP (*Pf*CSP) CD4+ and CD8+ epitopes, universal T_H epitopes, portions of the

α-thrombospondin type-I repeat (TSR) domain, and 6 repeats of the NANP motifs of the *Pf*CSP (Figure 10). Moreover, when combined with ALF-based adjuvants, the generated nanoparticle vaccine was highly immunogenic and prevented disease in a murine model (Seth et al., 2017).

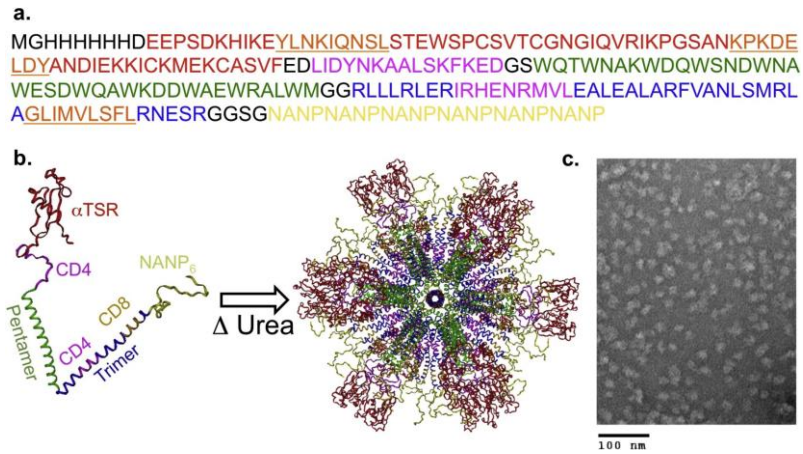

Figure 10. Sequences, formation and structural analysis of the FMP014 vaccine. a: The amino acid sequence of the FMP014 monomer is color-coded to highlight distinct regions of the protein. The pentameric (green) and trimeric (blue) oligomeric domains act as the scaffold of the nanoparticle, presenting B cell epitopes in red (α-TSR) and yellow (NANP-repeat). Additionally, the scaffold includes two CD4 epitopes (pink) and three CD8 epitopes (orange, underscored). b: SAPN is formed by the oligomerization of th coiled-coiled domains within a single nanoparticle. c: Individual particles as visualized using electron microscopy. Reproduced from (Seth et al., 2017).

Comentado [C7]: Parece ser una asociación no covalente entre diferentes polipéptidos más que un único polipéptido.

Otra cosa que habría que mirar es si realmente es un incremento de la concentración de urea lo que induce la asociación, como se indica bajo la flecha de la figura, ya que la urea es un agente desnaturalizante.

In 2018 Huang *et al.* found that, when His-tagged *Pf*s25 was mixed at the time of immunization with pre-formed liposomes containing cobalt porphyrin-phospholipid, spontaneous nanoliposome antigen particleization (SNAP) was obtained *in situ*. The SNAP approach allowed durable antibody responses in mice, whose post-immune sera effectively recognized native *P. falciparum* proteins in different life stages of the parasite (Huang et al., 2018). This approach paved the way for the generation of recombinant vaccines that target different stages of malaria parasites.

One of the main disadvantages of generating vaccines against a particular epitope is the limited success of immunization due to the antigenic polymorphism exhibited by *Plasmodium* parasites (Mendis et al., 1991). To circumvent this problem, Ssemaganda *et al.* designed a clever strategy

using cell membrane-depleted *P. falciparum* parasites, and encapsulated them in mannosylated liposomes (Ssemaganda et al., 2020), which are known to increase antigen cargo targeting to professional antigen presenting cells (Vyas et al., 2010). Following vaccination in mice, liposomes induced a robust parasite-specific immune response whilst avoiding anti-human RBC responses. Moreover, the authors demonstrated the stability of their formulation after lyophilisation, which can facilitate its deployment in endemic regions.

Other routes of vaccine inoculation include transdermal immunization, although the poor immunogenic response elicited by this route has made this strategy little employed in the field of malaria. However, novel nanomaterials like elastic liposomes are now being explored for their use in this type of vaccination. In this context, Tyagi and colleagues developed SPC-based elastic liposomes using Span®80 as surfactant (86:14 mol%), to encapsulate the carboxyl-terminal 19-kDa fragment of the *P. falciparum* merozoite surface protein-1 (*Pf*MSP-1_{19}) (Tyagi et al., 2015). The *in vitro* skin permeation study showed an efficient transdermal delivery when compared to conventional liposomes. Moreover, when tested in albino nude rat models, histopathology analysis demonstrated an enhanced lymphatic accumulation, which is highly desirable for local delivery of antigens. Finally, *in vivo* studies indicated that the elastic liposomes were capable of eliciting a sustained and specific immune response, comparable to intramuscularly-injected liposomal formulations.

Currently the main malaria vaccination approach is focused on the pre-erythrocytic parasite stage to prevent infection after a parasite-carrying mosquito bite. However, transmission-blocking vaccines, which prevent pathogen development in the mosquito vector are an attractive alternative. Recently, Huang *et al*. engineered immunogenic liposomes containing the full-length CSP and a fragment of the sexual-stage *P. falciparum* surface protein *Pf*s230, with the aim of inducing immunity against both infection and transmission (Huang et al., 2022). The authors used a cobalt-porphyrin-phospholipid liposome approach to display antigens on the liposomal surface without the need of covalent attachment.

Conclusion

Given the rapid evolution of parasite resistance to the traditional antimalarials available and the scarce therapeutic options, the unmet medical and patient need of malaria eradication will not be achieved unless new and efficient drugs and/or delivery systems are discovered. Researchers should

focus on multidisciplinary approaches to advance towards the discovery of a cost-efficient and targeted therapeutic strategy contributing to malaria eradication. Moreover, understanding the mechanisms underlying the pathophysiology of malaria can also help us to circumvent the fast emergence of resistance to alternative drug delivery systems. In this sense, nanotechnology holds promise for improving the efficacy and delivery of antimalarial drugs. Similar to the positive outcomes of using nanotechnology to address drug resistance in cancer or bacterial infections, scientists have explored nanotechnology-based approaches to enhance the delivery of existing antimalarial drugs and combat drug resistance. Several examples of antimalarial drug encapsulation in nanoparticles are revised in this work. Among them, we highlight those that have improved the action of the drug, either by lowering side effects or by ameliorating antiplasmodial activity in *in vitro* cultures or in murine models of malaria. These encouraging results demonstrate that nanoparticles constitute an effective drug delivery strategy, especially for compounds with specific challenges like poor solubility or high toxicity. However, their success depends on careful design, appropriate nanomaterial selection, and thorough preclinical evaluation before translation to clinical trials. Nevertheless, it is reassuring that some promising nanomaterials have been tested as antimalarial drug carriers in the last years. For instance, a remarkable effort has been recently done to achieve targeted drug delivery to infected cells. The development of functionalized nanoparticles has considerably improved the activity of the cargo drugs and, in most cases, enhanced their pharmacological features. Moreover, precise delivery of antimalarials to the site of action ensures that the therapeutic agent reaches its intended target in sufficient concentrations, potentially reducing the required dosage to achieve the same effect as when treating with the free drug, which in turn can improve patient compliance. Furthermore, when drugs are delivered to their target with sufficient specificity, they need to be in the circulation for a shorter time, which translates into less opportunities for the parasite to evolve resistance, as opposed to the situation when *Plasmodium* is continuously exposed to the drug. Finally, targeted delivery potentially helps to minimize off-target effects, making the treatment safer for the patient. Overall, targeted drug delivery is a valuable strategy for improving the effectiveness of treatments, especially in situations where resistance is a concern, like for *Plasmodium* infections. It allows for the optimization of drug delivery to the parasitized RBCs, while minimizing the impact on healthy tissues and cells and reducing the potential for resistance emergence.

Whereas nanomedical therapeutic approaches are increasing in the affluent countries, there is an astonishing lack of nanomedicines to treat infectious diseases which are the main cause of death in the low *per capita* income regions. In the case of malaria, in an ideal scenario of unrestricted access to funding and resources, targeted immunoliposomes for i.v. administration of ACTs and future yet to be discovered antimalarial drugs would likely bring us closer to the eradication horizon. However, the current realistic economic landscape of malaria leads us to the need to develop a new generation of less expensive nanocarriers in order to adhere to a cost of the complete treatment course below 3 USD for adults and 1 USD for infants aged less than 2 years (Burrows et al., 2017). In this regard, some type of polymeric nanocarrier with, for instance, heparin targeting to the ookinete stage in the mosquito might comply with this limitation. Imaginative strategies for direct delivery to mosquitoes of such drug-encapsulating nanocarriers (Paaijmans & Fernandez-Busquets, 2014), if the evident hurdles that complicate such an approach can be overcome, would offer the crucial benefit of bypassing the long and costly clinical trials that are nowadays one of the main obstacles in the way of the pharmaceutical development of otherwise promising antimalarials.

Despite the large number of nanotechnology-based formulations with potential applications for malaria, few of them have been tested *in vitro*, and even less have reached *in vivo* studies. The development of nanomedicines for malaria faces several challenges, including safety, scalability, and regulatory approval. Clinical trials and rigorous testing are necessary steps in bringing any new medicine, including nanoformulated drugs, to market. To address this imbalance, international efforts are underway to promote equitable access to nanotechnology research and its benefits. These include capacity-building programs, technology transfer initiatives, collaborations between institutions in high- and low-income countries, and policies that encourage knowledge sharing and open access to research findings. Moreover, it is important to consider the cost-effectiveness of nanotechnology solutions for malaria. While the initial investment may be higher than traditional approaches (especially due to research and development costs), the long-term benefits, such as improved treatment outcomes, reduced drug resistance, and decreased malaria transmission, can outweigh the costs.

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Table 1. Physicochemical properties of the nanoformulations discussed in this review. n/a, not available; PDI, polydispersity index; ζ, zeta potential. In superscript are indicated the references where the corresponding formulations are reported.

Formulation	Encapsulated drug	Size (nm)	ζ (mV)	PDI	Encapsulation efficiency (%)	Stability
SPC-Chol(neutral charge)-LPs[1]	MON	124±10	−11.6±0.7	0.283±0.006	n/a	n/a
SPC-Chol-DSPE-mPEG2000 (0.5 mol%)-LPs[1]	MON	103±14	−14.5±0.7	0.273±0.009	n/a	n/a
SPC-Chol-DSPE-mPEG2000 (2.5 mol%)-LPs[1]	MON	96±11	−12.6±0.5	0.314±0.010	n/a	n/a
SPC-Chol-DSPE-mPEG2000 (5 mol%)-LPs[1]	MON	90±10	−8.8±0.4	0.277±0.004	n/a	n/a
SPC-Chol-PA (negative charge)-LPs[1]	MON	109±15	−23.3±0.5	0.294±0.011	n/a	n/a
SPC-Chol-PA-DSPE-mPEG2000 (0.5 mol %)-LPs[1]	MON	98±18	−29.5±0.9	0.233±0.009	n/a	n/a
SPC-Chol-PA-DSPE-mPEG2000 (2.5 mol%)-LPs[1]	MON	100±9	−18.4±0.8	0.217±0.010	n/a	n/a
SPC-Chol-PA-DSPE-mPEG2000 (5 mol%)-LPs[1]	MON	98±11	−12.7±0.8	0.216±0.005	n/a	n/a
SPC-Chol-SA (positive charge)-LPs[1]	MON	121±20	43.9±0.9	0.247±0.012	n/a	n/a
SPC-Chol-SA-DSPE-mPEG2000 (0.5 mol%)-LPs[1]	MON	107±18	34.7±0.7	0.277±0.008	n/a	n/a
SPC-Chol-SA-DSPE-mPEG2000 (2.5 mol%)-LPs[1]	MON	101±14	18.9±0.3	0.239±0.011	n/a	n/a
SPC-Chol-SA-DSPE-mPEG2000 (5 mol%)-LPs[1]	MON	98±11	15.1±0.7	0.268±0.013	n/a	n/a
SPC-Chol-LPs[2]	DOX	91±11	8.16±0.54	0.23±0.03	n/a	n/a
SPC-Chol-SA-LPs[2]	DOX	105±5	16.46±0.86	0.20±0.02	n/a	n/a
SPC-Chol-DSPE-mPEG2000-LPs[2]	DOX	88±10	−14.13±0.71	0.20±0.01	n/a	n/a
SPC-Chol-SA-DSPE-mPEG2000-LPs[2]	DOX	101±6	11.74±0.35	0.24±0.08	n/a	n/a
SPC-Chol-LPs[3]	MAD	97.0±5.4	−9.3±1.3	0.29±0.03	94±1	n/a
SPC-Chol-DSPE-mPEG2000-LPs[3]	MAD	105.0±10.5	−9.4±0.8	0.27±0.04	96±2	n/a
GPC-LPs[4]	Dimeric ART	190±n/a	−20.35±n/a	0.231±n/a	77.6±n/a	14 days
LPs[4]	ART	147±4	−16±1	0.20±0.02	81.68±3.72	n/a
LPs[5]	Artelinic acid-choline derivative	146.4±1.8	40.87±0.86	0.35±0.03	95.74±0.10	n/a
3-loaded nanoemulsion[6]	9*H*-3-azacarbazole	117.70±1.08	−25.33±1.59	0.186±0.006	n/a	n/a
SLN[7]	PQ	236.4±2.9	23.0±2.8	0.14±0.02	75.2±n/a	90 days
SLN[8]	DHA	240.7±n/a	17.0±n/a	0.16±n/a	62.3±n/a	90 days
SLN[9]	ARM and LFN	150 to 500	n/a	n/a	n/a	n/a
NLC[10]	ARM and LFN	64.4±8.6	−8.0±0.1	0.45±n/a	65.51±6.21 (ARM), 36.14±5.47 (LFN)	n/a
AGMA1 poly(amidoamine)[11]	CQ and PQ	3.4 to 6.8	n/a	1.43 to 1.85	15.1 (PQ), 32.9 (CQ)	n/a
ISA23 poly(amidoamine)[11]	CQ and PQ	2.7 to 6.4	n/a	1.17 to 1.88	29.4 (PQ), 14.2 (CQ)	n/a
Z0 polyaspartamide[12]	PYR and 4-aminosalicylic acid	254.4±68.3	1.6±14.6	0.354±0.040	8% (PYR), 9% (4-aminosalicylic acid)	n/a
Curcumin-compound 6[13]	Curcumin	30 to 90	71.1±7.0	n/a	70±n/a	n/a
PCL-PEG-PCL[14]	Plumbagin	78.4±n/a	−5.37±n/a	0.230±n/a	81.0±0.7	168 days
PBMA-SBMA[15]	Curcumin	ca. 100	n/a	n/a	n/a	n/a
PBMA-MESBMA[15]	Curcumin	ca. 20	n/a	n/a	n/a	n/a
Nanomicelles[16]	PYR	391.1±6.9	−3±1	0.3±n/a	60±3	n/a
bMPA-Pluronic® dendrimer D[17]	CQ	360±131	n/a	n/a	n/a	n/a
bMPA-Pluronic® dendrimer C[17]	PQ	290±78	n/a	n/a	n/a	n/a
bMPA-dendronized hyperbranched polymer[18]	CQ, PQ or quinacrine	11±2	n/a	n/a	37-60 (depending on drug)	n/a
bGMPA-dendritic block copolymer[18]	CQ, PQ or quinacrine	26±6	n/a	n/a	31-48 (depending on drug)	n/a
Artesunate-heparin nanocapsules[19]	ART and heparin	112.1±n/a	−11.2	0.528	29.3±n/a	14 days (4 °C)
Polysorbate nanocapsules[20]	Quinine and curcumin	194±1	−27.2±0.1	0.12±0.00	97±2	n/a
ARM-PCL[21]	ARM	254.5±3.8	−50.2±0.7	0.26±0.03	n/a	3 months (4 °C)
PLA 0.5 (0.5 mg/mL ARM)[22]	ARM	251.9±0.9	−40.8±2.1	0.21±0.07	n/a	n/a

PLA 1 (1 mg/mL ARM)[22]	ARM	301.5±6.5	−56.2±0.6	0.32±0.15	85.8±0.8	n/a
PLA 2 (2 mg/mL ARM)[22]	ARM	328.7±5.7	− 51.9±2.3	0.38±0.41	73.3±0.7	n/a
PCL 2 (2 mg/mL ARM)[22]	ARM	223.1±2.7	−49.3±1.6	0.27±0.03	91.8±0.6	n/a
PCL 4 (4 mg/mL ARM)[22]	ARM	243.2±4.7	−41.9±1.3	0.28±0.04	80.0±0.7	n/a
PLGA[23]	Curcumin and ART	251.1±12.6	−19.1±4.9	0.14±0.06	22.3±0.4	n/a
PLGA[24]	CQ and azithromycin	89.6±n/a	−13.2	0.236±n/a	n/a	n/a
Glycerol ATO-SCNP[25]	ATO	n/a	−11.3±0.62	n/a	15%	n/a
Anionic ATO-SCNP[25]	ATO	8.9±1.98	−18.9±2.13	n/a	4%	n/a
IONPs[26]	ART	10±n/a	n/a	n/a	n/a	n/a
TiO_2 NPs[27]	none	34.6 to 70.4	n/a	n/a	n/a	n/a
ZnO NPs[28]	none	70 to 90	n/a	n/a	n/a	n/a
Azadirachta indica-Ag NPs[29]	none	40.91±22.03	n/a	0.359±n/a	n/a	n/a
Azadirachta indica leaf extract-Ag NPs[30]	none	13.01±2.54	−16.90±n/a	n/a	n/a	n/a
Azadirachta indica bark extract-Ag NPs[30]	none	19.30±3.13	−9.03±n/a	n/a	n/a	n/a
DSPC-LPs[31]	CQ	200±n/a	n/a	n/a	98.3±0.3	14 days
DSPC-LPs[32]	PQ	200±n/a	n/a	n/a	96.7±0.2	14 days
DSPC-LPs[33]	7c (CQ analogue)	200±n/a	n/a	n/a	90.5±0.4	30 days
DOPC-LPs[34]	Pyronaridine and ATO	n/a	n/a	n/a	>90% (Pyronaridine), 50% (ATO)	n/a
DSPC-LPs[35]	LFN	169±5e	n/a	n/a	88%	n/a
PSP-LPs[36]	none	132.6±7.7	33.9±0.3	0.053±0.024	62.0±7.1	n/a
DOTAP-DOPC-LPs[37]	Heparin and PQ	232±8	0.5	0.16±0.01	n/a	n/a
LPs[38]	Poupartone B	183.2±22.0	16.6±1.7	< 0.1	n/a	n/a
Heparin-LPs[39]	Heparin and poupartone B	256±20	11.8±1.4	< 0.1	n/a	n/a
Heparin-SLNs[40]	Heparin and CQ	374.4±7.6	−4.06±0.09	0.272±0.053	78±n/a	n/a
DHP(G4)-MPA[41]	Heparin	40 to 100	n/a	n/a	n/a	n/a
DHP(G4)-GMPA[41]	Heparin	30 to 80	n/a	n/a	n/a	n/a
Glucose-based ultra-small gold NPs[42]	Ciprofloxacin	2 to 5	n/a	n/a	n/a	n/a

ARM, artemether; ART, artesunate; ATO, atovaquone; bGMPA, 2,2′-*bis*(glycyloxymethyl)propionic acid; bMPA, 2,2-*bis*(hydroxymethyl)propionic acid; Chol, cholesterol; CQ, chloroquine; Curcumin-compound 6, curcumin-(3-bromo-N-(4-fluorobenzyl)-benzo[b]thiophene-2-carboxamide) conjugate; DHA, dihydroartemisinin; DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane, DOX, doxycycline; DSPC, distearoylphosphatidylcholine; DSPE, distearoylphosphatidylethanolamine; GPC, glycerophosphorylcholine; IONPs, iron oxide nanoparticles; LFN, lumefantrine; LPs, liposomes; MAD, maduramicin; MON, monensin; mPEG, methoxy-polyethylene glycol; NLC, nanostructured lipid carrier; NPs, nanoparticles; PA, phosphatic acid; PBMA-MESBMA, poly(butyl methacrylate-co-morpholinoethyl sulfobetaine methacrylate); PBMA-SBMA, poly(butyl methacrylate-co-sulfobetaine methacrylate); PCL, poly-ε-caprolactone; PEG, polyethylene glycol; PLA, poly(D,L-lactide); PLGA, poly (D,L-lactic-co-glycolic acid); PQ, primaquine; PSP, phosphatidylserine-binding peptide; PYR, pyrimethamine; QC-NC, quinine and curcumin co-loaded nanocapsule; SA, stearylamine; SCNP, single-chain nanoparticle; SLN, solid-lipid nanoparticle; SPC, soy phosphatidylcholine.

[1]Rajendran et al., 2016
[2]Rajendran et al., 2018
[3]Raza et al., 2018
[4]Ismail et al., 2018
[5]Duan et al., 2020
[6]Jaromin et al., 2021
[7]Omwoyo et al., 2014
[8]Omwoyo et al., 2016
[9]Attama et al., 2016
[10]Prabhu et al., 2016
[11]Urbán et al., 2014
[12]Aderibigbe et al., 2019
[13]Ghosh & Banerjee, 2020
[14]Rashidzadeh et al., 2022
[15]Biosca et al., 2021
[16]Pestehchian et al., 2020
[17]Movellan et al., 2014
[18]Martí Coma-Cros et al., 2019
[19]Ismail et al., 2019
[20]Velasques et al., 2018
[21]Souza et al., 2018
[22]Vidal-Diniz et al., 2022
[23]Oyeyemi et al., 2018
[24]Jawahar et al., 2019
[25]Hamelmann et al., 2023
[26]Kannan et al., 2019
[27]Gandhi et al., 2018
[28]Najoom et al., 2021
[29]Ghazali et al., 2022
[30]Hawadak et al., 2022
[31]Moles et al., 2015
[32]Moles et al., 2016
[33]Moles et al., 2017
[34]Biosca et al., 2019
[35]Moles et al., 2016
[36]Tagami et al., 2015
[37]Marques et al., 2017
[38]Ledoux et al., 2020
[39]Ledoux et al., 2021
[40]Muga et al., 2018
[41]San Anselmo et al., 2023
[42]Varela-Aramburu et al., 2020