Nanomedicines for Malaria Chemotherapy: Encapsulation vs. Polymer Therapeutics

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Abstract

Malaria is one of the oldest infectious diseases that afflict humans and its history extends back for millennia. It was once prevalent throughout the globe but today it is mainly endemic to tropical regions like sub-Saharan Africa and South-east Asia. Ironically, treatment for malaria has existed for centuries yet it still exerts an enormous death toll. This contradiction is attributed in part to the rapid development of resistance by the malaria parasite to chemotherapeutic drugs. In turn, resistance has been fuelled by poor patient compliance to the relatively toxic antimalarial drugs. While drug toxicity and poor pharmacological potentials have been addressed or ameliorated with various nanomedicine drug delivery systems in diseases like cancer, no clinically significant success story has been reported for malaria. There have been several reviews on the application of nanomedicine technologies, especially drug encapsulation, to malaria treatment. Here we extend the scope of the collation of the nanomedicine research literature to polymer therapeutics technology.

We first discuss the history of the disease and how a flurry of scientific breakthroughs in the latter part of the nineteenth century provided scientific understanding of the disease. This is followed by a review of the disease biology and the major antimalarial chemotherapy. The achievements of nanomedicine in cancer and other infectious diseases are discussed to draw parallels with malaria. A review of the current state of the research into malaria nanomedicines, both encapsulation and polymer therapeutics polymer-drug conjugation technologies, is covered and we conclude with a consideration of the opportunities and challenges offered by both technologies.

Keywords: malaria, antimalarial, Plasmodium, nanomedicine, polymer therapeutics

Introduction

Malaria is an infectious disease known to virtually every inhabitant of the Earth's tropical regions like sub-Saharan Africa. It exacts an enormous economic and social impact in the hardest hit countries. It mainly affects children under the age of five years old (1). The incapacitating symptoms of the disease are unmistakeable, whether to the expert observation of the medical professional or an ordinary adult. However, the disease is much less recognized in the generally malaria-free sub-tropical and temperate countries (2). (For an excellent primer on the disease see (3).) This has in part contributed to a lack of significant technological advancement in the clinical management of the disease. Much of the malaria chemotherapy still relies on classes of drugs which have been in use for several centuries. Diagnosis is still primarily by microscopic visualization of the parasite in infected red blood cells (RBCs) (4). Prevention is almost entirely dependent on the use of physical barriers like bed nets (5). While drug toxicity and poor pharmacological potentials have been addressed or ameliorated with various nanomedicine drug delivery systems in diseases like cancer, no clinically significant success story has been reported for malaria. With reports of resistance rising against our latest and most powerful antimalarial treatment—the artemisinin-based combination therapy—there is an imperative in the need for clinically successful antimalarial nanomedicines.

Historical Malaria

Malaria, or at least a disease with closely similar symptoms to it, has been around for over five millennia. From the ancient Chinese, Greek, Indian and Roman civilizations medical experts have described and recorded almost identical symptoms of the disease. Some of these early experts rightly linked the disease to either insect bites or swampy marshlands. Extracts of medicinal plants were the earliest effective treatment and indeed many still rely on these

natural potions for ‘curing’ the disease. The Chinese used the Qinghao plant (*Artemisia annua*) to ‘treat’ the fever. On arrival in the western hemisphere, medieval Europeans learnt that the Peruvian bark from the *Cinchona* tree could be used to the same effect.

In 1880 the French army surgeon Charles Louis Alphonse Laveran was the first to observe in infected RBCs the protozoan that causes malaria. Three years to the end of that century another military physician, the British Ronald Ross, demonstrated that the malaria parasite could be taken up by mosquitoes. As if an ultimatum of the turn of the century for solving the mystery of malaria needed to be met, three Italian scientists convincingly proved that malaria was transmitted by the bite of the *Anopheles* mosquito in 1899. They infected two volunteers in London with the disease using mosquitoes that had fed on an infected patient in Rome. This audacious experiment dropped the curtains on the centuries-old mystery of what caused malaria.

Biology and Pathophysiology of Malaria

Malaria is caused by protozoans of the genus *Plasmodium*. Five *Plasmodium* species cause the disease in humans. *Plasmodium falciparum* is responsible for the most clinically morbid form of malaria and accounts for over 90% of its mortality (6). In sub-Saharan Africa 99% of malaria cases reported in 2016 were caused by *P. falciparum*. *P. vivax* is the most prevalent species outside Africa (7). *P. ovale* (8) and *P. malariae* (9) are even less common. *P. knowlesi* is a zoonotic species that is primarily a simian parasite (10).

The complicated life cycle of the *Plasmodium* parasite occurs in two hosts—the mosquito and the human. Only the female *Anopheles* mosquitoes are able to transmit the parasite because they require regular blood meals to develop their eggs; the males only feed on sugary fluids instead. The *Anopheles* genus has about 430 known species of which 30-40 transmit

malaria. To put these figures into perspective, there are 41 mosquito genera grouping 3500 species.

The human segment of the *Plasmodium* life cycle has three stages which are of significance to the characteristics of the disease (Figure 1). The first is the pre-erythrocytic or exo-erythrocytic liver stage. Upon inoculation of the human by the mosquito during a feeding session the injected parasites—known now as sporozoites—rapidly home in to the liver. Here they undergo morphological changes into schizonts and infect RBCs. However, depending on the *Plasmodium* species, a fraction of the parasites which do not mature immediately into schizonts remains dormant in the liver as hypnozoites. This is observed only in *P. vivax* and *P. ovale* and it is manifested in some patients as a relapse weeks or months after 'successful' treatment of the disease even though there is no re-exposure to the transmitting mosquito. The hypnozoites can only be cleared by a radical cure treatment with a limited selection of drugs [*vide infra*]. Hence, relapse or recrudescence of *P. falciparum* malaria can only be because of failure of treatment or re-exposure to the transmitting vector.

The second intra-human stage of the *Plasmodium* development is the erythrocytic asexual stage in which the parasite lives within RBCs (11). This occurs in all *Plasmodium* species and is responsible for the primary clinical manifestations of the disease like fever, chills, anemia, muscular aches and rigors. As high as 4-5% of the peripheral erythrocytes could be infected; greater than this increases the risk for severe malaria and death (12,13). After further morphological changes and multiplication in the RBCs the parasite, now known as merozoite, emerges with lysis of the host cell. There could be more than 10^{12} parasites circulating in a single patient at this stage. RBC lysis occurs with a circadian consistency that is accompanied by fever and chills. Most antimalarial drugs are most effective against this stage of the parasite.

A divergence also occurs in the erythrocytic stage with a fraction of the parasites differentiating into mature cells called trophozoites. Mature trophozoites further develop into gametocytes to initiate the third intra-human phase contributory to the malaria disease characteristics. *Plasmodium* gametocytes are the transmissible forms of the parasite. All forms of the parasite have this stage and it completes the human-mosquito circuit when the gametocytes are picked up by the insect during a feeding session. *P. falciparum* gametocytes may take up to two weeks to appear in the blood while those of *P. vivax* appear just a few days after infection. A few drugs are able to target the gametocytes and inhibit transmission [*vide infra*].

The three stages, respectively, account for the characteristic relapse, morbidity and infectiousness of malaria. For an excellent review on the disease and the biology of the parasite see (14). Miller LH et al. provide insights into the malaria biology and pathogenesis as it relates to new treatments (15).

Malaria Chemotherapy

Malaria treatment has existed for centuries in traditional folk medicines and many of the most effective 'modern' drugs are derived from this traditional knowledge (16,17). Chemotherapy of malaria is towards three objectives: to cure infection, prevent the development of resistance to the drugs and reduce the risk of further transmission. The multi-stage complicated life cycle of the *Plasmodium* parasite offers multiple sites for chemotherapeutic vulnerability (18) (Figure 1).

Today, malaria treatment is strictly combination drugs-based (4,19,20) (Table I). In the context of malaria, combination therapy "*is the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite*" (20). This definition excludes drug combinations with only one blood

schizontocide or fixed-dose combinations that act synergistically and the individual drug components cannot be given alone. This is why sulfadoxine-pyrimethamine is not a combination therapy but chloroquine-sulfadoxine-pyrimethamine is.

Table I: Antimalarial therapy combination drugs (4,20,21).

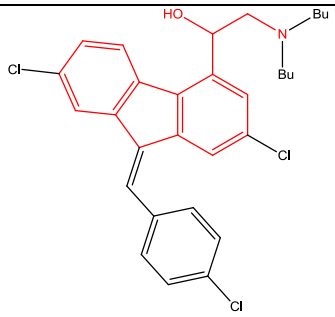
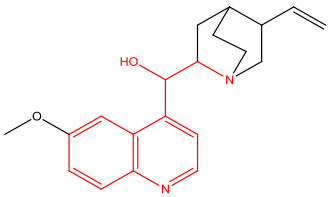
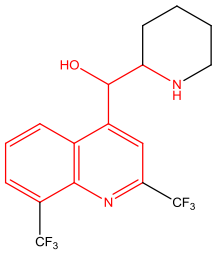
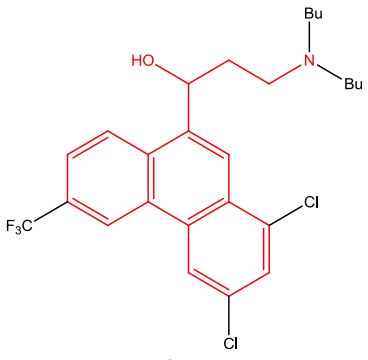
Major artemisinin-based combinations	**Non-artemisinin based combinations**
✓ **Artemether + lumefantrine** ✓ **Artesunate + mefloquine** ✓ **Artesunate + sulfadoxine-pyrimethamine** ✓ **Artesunate + amodiaquine** ✓ **Dihydroartemisinin + piperaquine**	➢ Chloroquine + sulfadoxine-pyrimethamine ➢ Amodiaquine + sulfadoxine-pyrimethamine ➢ Atovaquone-proguanil ➢ Mefloquine-sulfadoxine-pyrimethamine ➢ Quinine + tetracycline or doxycycline ➢ Arterolane + piperaquine
Other artemisinin-based combinations	
➢ Artesunate + pyronaridine ➢ Artemisinin + piperaquine ➢ Artemisinin + napththoquine ➢ Piperaquine-dihydroartemisinin-trimethoprim + primaquine ➢ Chlorproguanil-dapsone + artesunate	

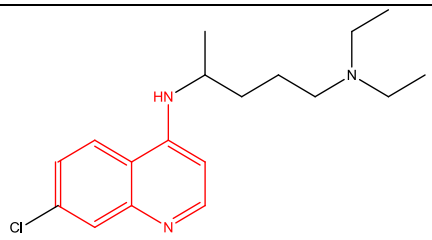
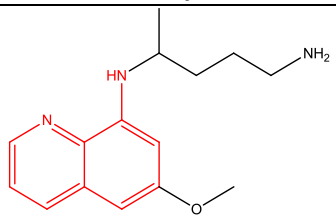
The most significant advantage of antimalarial drug combination is the delay or even prevention of parasite resistance to the chemotherapy. Drug combinations are able to do this because of the synergistic or additive modes of action of the individual components, optimum compatibility in their half-lives and the ability to block transmission of drug resistant strains, i.e. gametocytocidal activity.

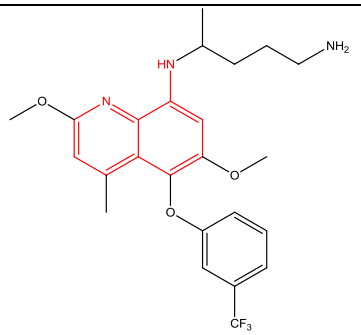
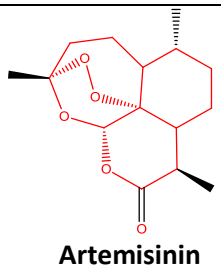
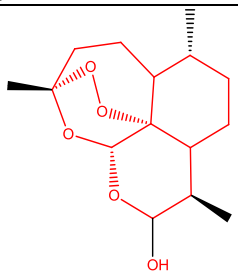
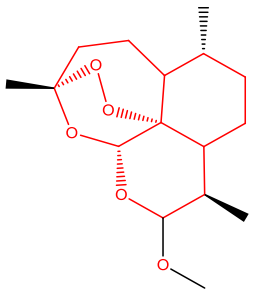
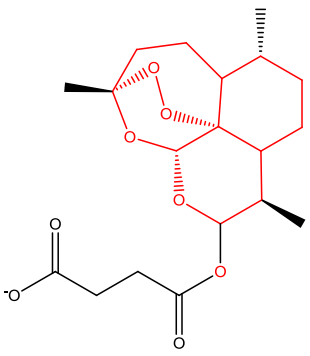
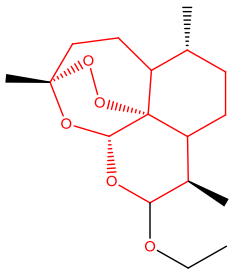
The major antimalarial drugs used to treat various forms of the disease can be grouped into several chemical and functional classes. Of these, four chemical classes—aryl amino alcohols, 4-aminoquinolines, 8-aminoquinolines, and artemisinins—are notable for their historical and clinical impact (Table II). Drugs under each class share a common pharmacophore. We discuss here the most notable members of each group. Some important

antimalarials do not belong to a general class unified by a common pharmacophore but instead share anti-parasite targets. Most notable are the folate synthesis inhibitors, antimicrobials, and iron chelators.

Table II.Classification and structures of common antimalarials and their pharmacophores.

Chemical group	Structure of pharmacophore	Related compounds[a]
Aryl amino alcohols	 **Lumefantrine**  **Quinine**  **Mefloquine**  **Halofantrine**	• Desbutyllumefantrine • Quinidine

4-aminoquinoline	 **Chloroquine**	• Hydroxychloroquine • Amodiaquine
8-aminoquinoline	 **Primaquine**	• Pamaquine

	 **Tafenoquine**	
Artemisinins	 **Artemisinin**  **Dihydroartemisinin**  **Artemether**  **Artesunate**  **Arteether**	• Artemisone • Artelinic acid

[a]The related compounds are either lesser known antimalarials, experimental drugs, or metabolites with significant antimalarial properties.

The Aryl Amino Alcohols

The aryl amino alcohol class of antimalarials which are currently in clinical use include quinine, lumefantrine, mefloquine, and halofantrine. Quinine is the oldest, pure antimalarial compound while lumefantrine is currently the most prescribed member of this class.

Mefloquine is the first choice prophylactic for travellers to high risk malaria areas. Many new compounds being researched as antimalarial drugs are based on the aryl amino alcohol pharmacophore (22).

Quinine

Quinine is an alkaloid that was originally isolated from the bark of the *Cinchona* tree and has been used for the treatment of malaria since 1820 (23,24). It acts against all *Plasmodium spp*. schizonts and the gametocytes of the *P. vivax* and *P. malariae*. It accumulates in the food vacuole of the parasite and inhibits the activity of the parasitic heme polymerase, an enzyme involved in the detoxification of heme by polymerization to the insoluble crystal hemozoin.

Clinically, quinine is administered orally or parenterally with an estimated half-life of 11-18 hrs (25). After oral administration, peak plasma concentration is observed at about 2 hrs in healthy volunteers (26) but at 60 $\pm$ 25 hrs in patients with cerebral malaria (27). In the liver the drug is degraded through metabolism by cytochrome P(450) 3A4 (CYP3A4) enzyme to 3-hydroxyquinine (28) and the by-products are excreted in the urine.

Quinine is relatively cheaper and safer than most other antimalarials and can even be taken by women in the first trimester of pregnancy. Yet, it is not without some side-effects. Patients can experience hearing impairment, rashes, nausea, vomiting, hypotension, hypoglycaemia and abdominal pains (29,30). Overdose can cause renal failure and fatality due to depression of the respiratory system. Parasite resistance to quinine and failure of treatment is largely due to poor patient compliance caused by intolerance to the side-effects and prolonged treatment course.

Lumefantrine

Lumefantrine is an aryl amino alcohol and the most widely administered. Treatment courses go into the hundreds of millions. It is never prescribed alone but as a fixed combination with artemether (4). This combinatorial regimen has staved off any confirmed report of resistance to lumefantrine. Its plasma half-life of 4-6 days ensures complete parasite clearance as artemether only has a half-life of a couple of hours. The seventh-day plasma concentration of lumefantrine is indicative of the combination therapy outcome. A concentration level higher than 500 µg/ml is consistent with a successful treatment while below 200 µg/ml is often a sign of an unsuccessful treatment (31).

Lumefantrine is used to treat multidrug resistant and cerebral malaria. It acts against the blood schizonts and gametocytes by forming complexes in the parasite's food vacuole which interfere with heme polymerisation (32).

The characteristics of lumefantrine which impact majorly on its performance as a drug are its very low aqueous insolubility (3.1×10^{-5} mg/ml) and very high lipophilicity (logP: 8-9). The drug must be taken along with a fatty meal so as to achieve significant gastrointestinal absorption. This simple dietary requirement is problematic as malaria patients have low appetite, nausea and vomiting. Also, due to poverty, which is rampant in malaria-endemic regions, it is difficult for most patients to have access to the appropriate meal for the six-dose regimen of the combination treatment (33).

The primary known liver metabolite of lumefantrine is desbutyllumefantrine. It occurs at about 0.1% of the plasma concentration of the parent drug but has an anti-parasite potency of about seven times that of lumefantrine in laboratory-adapted *P. falciparum* strains (34). It has been suggested that it could potentially be an antimalarial drug in combination with an artemisinin.

Two other important drugs in this class are mefloquine and halofantrine. Mefloquine is both a prophylactic and therapeutic for uncomplicated malaria (35). It is a drug of choice for travellers to malaria endemic regions but because of serious toxicities patients must be screened before administration (36–39). It causes psychological and neurological side effects. Halofantrine is only used for treatment and never as a prophylactic due to a high risk of cardiotoxicity (40). Unlike lumefantrine which must be taken with a fatty diet, halofantrine is taken on an empty stomach even though both drugs are equally lipophilic (logP: 7-8).

The 4-Aminoquinolines

Aminoquinolines have been among the most popular and successful antimalarial drugs. Chloroquine is the best known of the 4-aminoquinoline drugs and for several decades it was the gold standard for malaria treatment. Other important members are hydroxychloroquine and amodiaquine.

Chloroquine

After its introduction in 1940 chloroquine was widely used and for several decades it was reported to be one of the most used and successful drugs for the treatment of malaria (41). Although resistance by the *P. falciparum* parasite began to emerge in the early 1980s, it is still widely used world-wide.

Chloroquine acts rapidly against *P. falciparum* schizonts but it also has gametocytocidal activity against *P. vivax, P. malariae and P. ovale*. Like most antimalarials, its mode of action is not fully understood but there is evidence that the drug concentrates in the food vacuoles of the plasmodia by binding to free heme (42). This prevents heme sequestration through polymerization which results in the parasite's death. However, it is not clear whether this is due to failure of heme detoxification or to an amplified toxicity of the chloroquine-

heme complexes which might result in oxidative damage of the microbe's membranes and digestive proteases (43).

Chloroquine is a cheap and relatively safe compound with good pharmacological properties—indeed one of the most attractive in malaria chemotherapy (44). It is soluble in water and can be absorbed by the gastrointestinal tract. The peak plasma concentration is reached between 4-12 hrs after individual dose and the dosing plasma concentration is reached after 4-6 weeks. It has a half-life between 40-50 days (45)!

Chloroquine and the other drugs of this quinoline class do not pose the same life-threatening toxicity to glucose-6-phosphate dehydrogenase (G6PD) deficiency patients as their 8-amino analogues. It is a safe compound even throughout pregnancy but it has been abandoned as prophylaxis for *P. falciparum* and *P. vivax* due to parasite resistance. Pruritus, severe itching of the skin, is the most common side-effect of chloroquine (46). An antihistamine is often co-administered to ameliorate the itching. In their work, Ademowo O. and Sodeinde O. reported that G6PD deficiency might increase susceptibility to chloroquine-induced pruritus.

The 8-Aminoquinolines

The 8-aminoquinolines are renowned for activity against hepatic infections and also for blocking gametocyte transmission. The only approved member of this group is primaquine and it has been so for over 60 years. Tafenoquine, an analogue of primaquine, is in late stage clinical development.

Primaquine

Primaquine is the most notable 8-aminoquinoline antimalarial compound. It is primarily used as a chemo-preventative drug to avoid relapse of *P. vivax* and *P. ovale* infection because of its ability to target the liver hypnozoites (47). It also targets mature gametocytes of *P.*

falciparum, reducing the risk of transmission (48). Its mode of action and why it is able to act against different stages of the parasite's life cycle is unclear. It has been suggested that this might involve the generation of radical oxygen species that damage the parasite's mitochondria and interfere with the electron transport chain needed for respiration (49). Primaquine has been reported to improve the treatment of chloroquine-resistant plasmodia with chloroquine (50,51).

Oral administration of primaquine results in rapid liver metabolism into the inactive carboxyprimaquine—the major plasma metabolite (52). Primaquine reaches peak plasma concentration at 1-3 hrs and a half-life of 4-9 hrs (53). It is well tolerated by and safe for most patients who are good candidates to receive it (49).

The most significant toxicity of primaquine and other 8-aminoquinolines is hemolysis in patients with G6PD deficiency (See Box 1). Primaquine is not given to pregnant women as it crosses the placenta, which could be dangerous for the unborn baby whose G6PD status is unknown. However, in the over six decades of use, only 14 deaths have been formally reported as been due to primaquine use (54). This equates to an estimated mortality of one in 621,428 and the question is being asked if the available data justifies the restrictive use in the light of the drug's enormous benefits in malaria elimination. Although resistance to primaquine is of less clinical significance than other antimalarials, it is difficult to definitively ascribe failure of treatment singly to either parasite resistance, relapse or re-infection (55).

Box 1. Glucose-6-Phosphate Dehydrogenase Deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a genetic polymorphism of the named enzyme. Present in more than 400 million people worldwide it is the most common

genetic enzyme deficiency. About one in every five Africans is believed to carry this genetic defect, the highest racial population rate. Other populations with significant proportions are the Mediterraneans and Southeast Asians.

G6PD is active in virtually all body cells. In RBCs it is essential for ensuring normal lifespan of the cells. The dehydrogenase activity protects cells against oxidative damage by regenerating the cellular antioxidant glutathione (56).

Phenotypic expression of G6PD deficiency is complex. Firstly, the gene is carried on the X-chromosome. Therefore male hemizygotes and female homozygotes will be deficient while female heterozygotes have variable phenotypes due to the effects of lyonisation (57). Secondly, there are over 140 different genetic variants with a wide spectrum of intrinsic activity (58).

G6PD deficiency is more prevalent in parts of the world where malaria is, or was, previously endemic. This is believed to be the result of a relative survival advantage it confers, especially through resistance to severe forms of malaria (59–61). Several studies have been done regarding this with results indicating advantage for heterozygous females in a balanced polymorphism, but probably not for hemizygous males. Protection was found especially against cerebral malaria but not from severe malaria-induced anemia.

People with G6PD deficiency can suffer sudden hemolytic crises which could lead to life-threatening anemia (2). Most people however are normally asymptomatic when not challenged. These crises are often triggered by certain foods, especially some members of the leguminous class, infections, drugs and some chemicals. Of particular significance to malaria chemotherapy are the 8-aminoquinoline drugs. Primaquine is currently the only WHO-approved drug in this class. There has been reluctance among health personnel to use primaquine and some have advocated that G6PD testing be carried out prior to the drug being

given to patients in the high-risk populations. Several studies have been carried out to investigate the necessity for pre-testing and in general the results have been consistent and suggest that pre-testing is not necessary before prescription of a single low dose of primaquine as severe adverse effects and death were rare (54,62,63). The WHO therefore no longer recommends prior testing for G6PD deficiency in this context. However, pre-testing is still recommended before the relatively higher dose and longer course of primaquine used for radical cure in vivax and ovale malaria is administered.

Tafenoquine

Tafenoquine, which is currently in late clinical development, is the most significant 8-aminoquinoline antimalarial drug after primaquine (64). With an enhanced ability to suppress hypnozoites it is highly effective in the prophylaxis of *P. vivax* and transmission of *P. falciparum* infection (65). It is 4-100 times more potent than primaquine and has a plasma half-life of two weeks compared to the latter's 6-8 hrs. It is more lipophilic with a logP of 5 compared to primaquine's logP of 2.8. Unlike primaquine it has activity against the blood stage parasites although with reduced gametocytocidal activity (66). However, like other compounds of this class it causes hemolytic anemia in individuals with G6PD deficiency (See Box 1) (67). The mechanism of action is not well understood although it has been speculated to act similarly to quinine and primaquine.

The Artemisinins

This class of antimalarial drugs is also referred to as peroxides because of the presence of an endoperoxide in the pharmacophore (68,69). Artemisinin is the parent compound of this group of sesquiterpene endoperoxide drugs originally isolated from the herb *Artemisia annua*

in 1972 (70). The medicinal use of this herb in treating malaria has been known in traditional Chinese medicine for several centuries. At the beginning of the 21st century the WHO recommended artemisinin-based combination therapies (ACTs) as frontline treatment for multidrug resistant *P. falciparum* (19). Today, five ACTs form the cornerstone of antimalarial chemotherapy (4) and they have been pivotal to recent successes achieved in the fight against the disease (71,72). Mortality is down 60% while morbidity has fallen about 40% since the adoption of these drugs.

The artemisinins are currently the most powerful and effective antimalarial therapeutic agents in clinical use. They have the highest parasitemia clearance rate, achieving two of the three treatment objectives—prevention of clinical morbidity and transmission. Artemisinin, the parent compound, is mainly used for complicated malaria (21). Instead, the derivatives artemether, arteether, dihydroartemisinin and artesunate are used to treat uncomplicated, severe, complicated and multidrug resistant cases of the disease. They act rapidly against the asexual erythrocytic stage and inhibit the development of the gametocytes. As with many other antimalarials their exact mode of action is not understood but it is believed that they are activated by interaction with heme (73,74). Artesunate inhibits the *P. falciparum* antigen EXP1, a membrane glutathione S-transferase that degrades the iron-bound heme cytotoxin hematin (75). Several reviews and original research articles have been published on the contentious issue of the mode of action of artemisinins (73,76–78). It is however almost universally accepted that the endoperoxide bridge is crucial to the antimalarial activities of these compounds (68).

Research is still limited on the metabolism of artemisinins. The major metabolite so far reported of cytochrome P450 enzyme metabolism of artemether, arteether and artesunate in the liver is dihydroartemisinin (79,80) (Figure 2). Dihydroartemisinin is further metabolized to α-dihydroartemisinin-β-glucoronide. Artemisinin undergoes a different metabolic pathway

to four metabolites: 9,10-dihydrodeoxyartemisinin, deoxyartemisinin, deoxydihydroartemisinin and crystal-7. Unlike dihydroartemisinin, none of these metabolites are active as they have lost the endoperoxide bridge. This is a strong indication of the indispensability of this functionality to antimalarial activity and why artemisinin is a less effective antimalarial than its derivative.

Oral artemisinins are rapidly absorbed and metabolized after administration and reach a maximum plasma concentration after 2 - 3 hrs (73,76,81). This rapid disappearance may be the reason why resistance is slow and recrudescence is common. The WHO has therefore recommended that these drugs be used only in combination therapy and as first line treatment of uncomplicated *P. falciparum* (4).

Depending on the route of administration of artemisinins, especially the highly lipophilic artemether and arteether, have been shown to be neurotoxic in laboratory animal models at high doses (82–84). This toxicity has not been observed in humans; in fact, the artemisinins have a remarkably low toxicity profile in humans.

Apart from the short plasma half-life and high recrudescence rate — when used in monotherapy — artemisinin and most of its derivatives are poorly soluble in water and oil. Artesunate is the only water-soluble artemisinin derivative currently approved for clinical use. It is a highly effective antimalarial which has made significant impact on the treatment of severe malaria, providing rapid regain of consciousness and is recommended preferentially to quinine for these cases. It has lower toxicity than the hydrophobic analogues. Artesunate is the salt form of artesunic acid which in turn is derived from the succinoylation of dihydroartemisinin. A 5% sodium bicarbonate solution is used to dissolve the acid. This preparation is very unstable as the sodium artesunate has a half-life in solution of less than 15 minutes.

New Antimalarials

Other synthetic derivatives of artemisinin have been made to circumvent issues of possible toxicity and stability. Haynes R. et al. reported the synthesis of artemisone, a 10-alkylamino derivative, with an improved logP of 2.49 compared to artemether's logP of 3.98 (85). Artemisone also showed significantly less neurotoxicity than other artemisinins in fetal rat brain stem cells assays. Artelinic acid was synthesized to resist rapid *in vivo* degradation (86). In spite of these attractive properties, neither these nor any new artemisinins have made it to clinical development (87).

New medicines for malaria are slow in coming through even though improvements are being seen (87,88). A significant recent highlight is the compound MMV390048 which was developed by a team led by Kelly Chibale of the University of Cape Town, South Africa (89). An aminopyridine, MMV390048 represents a novel class of antimalarial drugs capable of blocking the three human life stages of the *Plasmodium* parasite and has no cross resistance with current drugs (90). Many other compounds have been investigated but very few have made it significantly far enough in development to hold promise of actually reaching the clinic (87). Even those compounds that show potentials as powerful antimalarial agents still have traditional hurdles to skill and challenges to duck. Over 70% of newly discovered drug candidates suffer from solubility issues which require high doses to achieve a therapeutic effect that increase the risk of toxicity and patient non-compliance. With the historical resilience of the *Plasmodium* parasite and *Anopheles* vector as a reference, coupled with our poor understanding of their resistance mechanisms (91), resistance to our current anti-malaria drugs is anticipated (92–95) and the only question is how much time do we have to buy.

Nanotechnology offers an opportunity to save the current chemotherapy (96–99), reclaim some of the lost ones (100) and strengthen what might be developed next (21,89). It is very expensive to develop new pharmaceutical compounds, even if it is just to solve a physico-

chemical problem with an existing compound. Even worse is that the new compound might not provide the same therapeutic benefits as the original drug. Hence, delivery systems offer a cheaper and less R&D-intensive alternative.

Antimalarial Nanomedicines

Nanomedicines have had a remarkable impact on the chemotherapeutic management of diseases like cancer where many of the drug agents used for treatment have significant pharmacological inadequacies and toxicities (101). They have reduced toxicity, increased efficacy, extended drug release and exposure, and improved stability and bioavailability. Nanomedicines have even reversed drug resistance (102). This impact is yet to be seen in malaria — indeed in most infectious diseases (103) but the therapeutic and pharmacological potentials offered by nanomedicines are increasingly being recognized (104,105).

Encapsulated Nanomedicines

Most nanomedicines use encapsulation techniques to physically entrap drugs (Figure 3). Encapsulation in a carrier system has, for decades, played a major role in the development of nanomedicines to modulate the pharmacokinetics and pharmacological potentials of drugs. The first nanomedicine to be given FDA approval, Doxil®, is a liposomal encapsulation of the anticancer drug doxorubicin (Dox) (101). Doxorubicin is an antitumor anthracycline antibiotic known to exhibit severe toxicity. Doxil® demonstrated the potential of nanomedicines by providing prolonged circulation of the drug, evasion of the reticuloendothelial system, and tumor-specific delivery.

Liposomes

Lipid-based drug delivery systems are a key technology for improving the pharmacological potentials of hydrophobic drugs (106,107). In particular, liposomes have been favored and are seen as the most successful drug delivery system (102,108–112). Liposomes are spherical assemblies of amphiphilic natural or synthetic phospholipids which were first described in the 1960s. These systems mimic the structure of biological cells with an aqueous core that is suitable for carrying hydrophilic drugs and a lipid lamellar that can entrap hydrophobic drugs (113). They can therefore deliver both drug classes simultaneously. The structure, size and chemical composition of liposomes can be controlled by the preparation method but the drug encapsulation efficiency, release rate, and particle size are affected by the lipid composition and content.

Liposomes can be tailored to effect rapid or slow drug release which would be ideal for drugs like the ACT combination artemether and lumefantrine that have different pharmacokinetic profiles and therapeutic requirements. However, application of liposomal delivery systems to malaria treatment is limited because these constructs are not amenable to oral administration, require temperature-controlled storage and must possess specific fluidity for fusion with the RBC plasma membrane. A balance must be struck between fluid RBC plasma membrane-binding liposomes which are leaky to small drugs and would have lost most of their payload and less fluid saturated lipids liposomes which retain high drug loading but with significantly reduced membrane fusing potentials. Marques et al. reported on their attempts to adapt liposomes as cost effective nanocarriers of antimalarial drugs to *Plasmodium*-infected RBCs (114). PQ was encapsulated in a liposomal system which included the antimalarial lipid 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(4-(p-maleimidophenyl)butyramide (MPB-PE) and it was observed that this system could transport the drug with minimal loss. The use of liposomes in antimalarial

Other more stable encapsulation systems have also been investigated for antimalarial drugs. Several reviews on the application of nanotechnology to malaria treatment have been published (96,115). These reviews explored in details the variety of delivery systems that have been designed for antimalarials. We focus on some of the recent formulations of the main antimalarials and the therapeutic enhancements they offer.

Dendrimers

Movellan J. et al. recently investigated the use of amphiphilic dendritic derivatives for the encapsulation of chloroquine and primaquine (116). The dendrimers were able to discriminate between *Plasmodium*-infected RBCs and non-infected cells. This resulted in up to four times reduction in the amount of drugs required to effect parasite death, vis-à-vis IC_{50}.

Solid lipid nanoparticles (SLN)

Lipid-based systems are more suitable for the delivery of highly hydrophobic drugs. However, the low mechanical stability and the requirement for special storage conditions of liposomes have made them less attractive for delivery of drugs used to treat tropical diseases. Solid lipid nanoparticles (SLN) offer an alternative encapsulation system for both hydrophilic (117,118) and hydrophobic drugs (119). Dwivedi et al. produced SLN of arteether through a high pressure homogenization technique (120). They obtained particles with sizes of about 100 nm and encapsulation efficiency as high as 69%. The bioavailability of the SLN-encapsulated drug was 170% greater than when the free drug was taken with groundnut oil. This is significant as the artemisinins are often administered with a fatty meal. The drug release rate from the SLN was slow. This is favourable for survival of the entrapped drug while transiting through the acidic gastric environment but it is not clear how this affects the clinical performance of the formulation as rapid clinical relieve is one of the significant advantages of the artemisinins.

Pheroids

Pheroids are lipid-based delivery vesicles which have been demonstrated to improve the pharmacological properties of several antimalarials (121,122). Pheroid-encapsulated mefloquine showed enhanced antimalarial activity while reducing toxicity (123). Lumefantrine was also encapsulated into a Pro-Pheroid delivery system by du Plessis et al. (122). The formulation improved the solubility, absorption, and bioavailability of the drug. Bioavailability was 3.5 times better and antimalarial efficacy was improved 46.8%.

Nanostructured lipid carriers (NLCs)

The low solubility and poor bioavailability of the most popular WHO-approved ACT, the artemether-lumefantrine combination, has also been tackled by formulating into nanostructured lipid carriers (NLCs) for both oral and intravenous delivery (124,125). NLCs also belong to the class of lipid-based drug delivery systems. The formulation showed 100% clearance of the parasite in a *P. berghei*-infected mice model without recrudescence at just 10% of the normal daily dose. The formulation performed equally well in fasted and fed animals. At a 600 mg/kg daily dose of the artemether-lumefantrine NLC no adverse effect was observed in the test animals compared to the controls.

The same formulation was investigated as an intravenously delivered system for the treatment of cerebral malaria, an often fatal complication of the disease (125). The pharmacological performance of the formulation mirrored that of the oral experiments. Further, it was found that this formulation was stable when sterilized by autoclaving. This work is similar to that of Parashar et al. (126) but distinguishes itself because the intravenous combination was used to treat cerebral malaria. There currently exists no intravenous formulation of an ACT. Apart from complicated malaria which must first be treated intravenously with a monotherapy solution of artesunate, many patients with uncomplicated malaria—usually paediatrics—

suffer severe nausea and vomiting that limits their ability to take anything orally and intravenous administration is the most viable option.

Unlike SLNs, NLCs are made from a mixture of lipids in different phases, often including saturated and unsaturated fatty acids (127,128). They improve the oral bioavailability of poorly aqueous-soluble drugs.

The experimental drug tafenoquine was formulated into a microemulsion with particle sizes less than 20 nm (129). This lipid-based delivery system increased bioavailability of the drug, after oral administration, from 55% to 99% in healthy mice and more than doubled the area under the curve. Probably most remarkable is the significant reduction in hemolysis observed in humanized G6PD-deficiency mouse model treated with the microemulsion.

Encapsulation in lipid-based systems has improved gastrointestinal absorption and bioavailability, reduced toxicity, enhanced stability and solubility, reduced therapeutic dosages etc. of antimalarial drugs. It is not however clear if the release kinetics from these lipidic systems, especially for the artemisinin drugs, is sufficiently rapid to offer relief from life-threatening clinical symptoms. The artemisinins have been very successful and widely accepted in part due to their ability to alleviate the critical presentations of patients in timelines of minutes. Dwivedi P. et al. showed less than 10% of arteether released in the first two hours from all four SLNs reported (120). Prabhu P. et al. and Parashar D. et al. achieved approximately 20% release in the same period for both the oral and the intravenous formulations (124–126). This relatively slow release may be intrinsic in the use of a hydrophobic matrix where the thermodynamics of diffusion into the aqueous solution is not favourable to the hydrophobic drug (122). We will next discuss polymer therapeutics, a nanomedicine delivery platform that is distinguishable by the use of a hydrophilic carrier and non-diffusion, stimulus-responsive drug release kinetics.

Polymer Therapeutics

In 1975 the German polymer chemist Helmut Ringsdorf described a model of a pharmacologically active polymer (130). This was actually a polymer-based delivery system in which a low molecular weight, water insoluble bioactive agent or drug is covalently conjugated to a hydrophilic polymeric carrier (Figure 3). He predicted that the system would have several significant advantages over small drugs such as increased solubility and reduced toxicity. This macromolecular conjugated construct would affect the cellular pharmacokinetics of the drug such that its therapeutic potential is enhanced. Conjugation to a polymer can prevent uptake of the drug through the conventional route which is susceptible to small molecule efflux-mediated resistance. Cellular uptake is instead by endocytosis (131,132).

Ringsdorf's pharmacologically active polymer included a physiologically labile but chemically stable covalent linker between the drug and polymer which is responsive to a specific pre-determined intracellular trigger like enzymolysis or pH-dependent hydrolysis. Many conjugates are made with an ester or other simple chemical linkages but some constructs have linkers that are recognized by specific enzymes which may be upregulated in diseased tissues (133–135).

A fourth aspect of the original model is a targeting ligand for active delivery to specific tissues (136–138). Antibodies have been investigated as targeting ligands (139,140) but so have small molecules like the amino sugars galactosamine (141) and mannosamine (142,143) and peptides (144). One of such actively targeted conjugates made it to clinical trials (145,146). Targeted conjugates are taken up by receptor-mediated endocytosis (132) but this has not always been shown to present a distinct advantage over non-targeted conjugates (147) and might actually be dysfunctional in certain disease states (148). Most anti-cancer polymer

therapeutics passively localize in tumors through the enhanced permeability and retention (EPR) effect of cancer vasculature which was first described by Matsumura Y. and Maeda H. (149) (Figure 5A) (See Box 2).

Today polymer therapeutics is a collective term used to describe any polymeric drug, polymer-drug conjugates, polymer-protein conjugates and polymeric micelles where the drug is covalently bonded to the carrier polymer (131). Conjugation is reversed with drug release *in vivo* by normal metabolic processes (Figure 4). Polymer therapeutics are used for disease treatment, diagnostics and theranostics, i.e. both treatment and diagnosis in one conjugate. Polymer therapeutics offer similar advantages like other drug delivery systems that entrap the drug by solubilizing and controlling drug release.

N-(2-Hydroxypropyl)methacrylamide (HPMA)–doxorubicin conjugates were the first synthetic polymers to make significant clinical progress (150). HPMA-doxorubicin conjugates were designed for use in treating cancer patients who were refractory to conventional chemotherapy. The safety seen with the polymer encouraged investigation of other conjugates of 20(*S*)-camptothecin (CPT), paclitaxel (PTX), and platinates for cancer treatment albeit with varying success — reviewed by (151). These conjugates met with mixed success and no HPMA conjugate has made it to the market.

CPT is a plant-derived potent antitumor agent which acts by inhibiting topoisomerase I. Apart from very poor aqueous solubility, it has a pH-sensitive equilibrium between active lactone and the inactive carboxylate forms (152). At the physiological pH of 7.4, the carboxylate form predominates and possesses systemic toxicity while the lactone is favored at acidic pH. The equilibrium is further shifted towards the inactive form by its preferential binding by serum albumin. These problems were solved by the development of CPT analogues with better solubility and stability (153,154); (155,156). CPT was also conjugated to polymeric

carriers (157–159). Conjugating CPT to a cyclodextrin-polethyleneglycol (CD-PEG) polymeric carrier provided a solution without altering the original drug. The lactone was conjugated via an ester bond which prevented conversion to the acid form while transiting through the blood. Release of the free drug is possible in the low pH milieu of the endolysosomal compartment where the equilibrium is shifted towards the lactone. Conjugation also offered the advantage of passively accumulating and entrapping the drug in the tumor by the extravasation of the local vasculature, i.e. the EPR effect. The CPT-CD-PEG conjugate showed better antitumor activity than either the free drug or the derivative irinotecan (158).

A PTX conjugate of the polymer polyglutamic acid (PGA) has gone furthest in clinical development of polymer-drug conjugates for cancer (160). It is currently approved as an orphan drug by the Food and Drug Administration (FDA) of the United States for the treatment of the malignant brain cancer glioblastoma multiforme. It is in clinical trials for non-small cell lung, ovarian and head and neck cancers. The use of PTX as an anticancer drug is limited by low tumor but high systemic exposures and organic solvents like polyethoxylated castor oil and ethanol are used to administer it and other taxane drugs. Conjugation to PGA afforded a water-soluble prodrug with significantly reduced toxicity. Drug release is facilitated by Cathepsin B, an enzyme which is upregulated in cancer tissues.

The taxanes, CPT and other anticancer agents have been investigated as conjugates of many polymers and polymeric designs. A detailed discussion is outside the scope of this review but there are several excellent reviews (132,161–163).

Combination therapy is a common strategy used in many treatment regimens today in an attempt to reproduce the success achieved from tuberculosis treatment. The WHO strictly recommends and approves combination drug regimens for HIV/AIDS and malaria.

Combining multiple drugs in treatment has provided significant reduction in the risk for drug resistance. Cancer chemotherapy also involves combination drugs but there have been difficulties in achieving clinical success for some drug combinations. CPT-Dox combination has failed to proceed beyond phase II clinical trials either because no significant difference in therapeutic efficacy is observed or there might be even heightened toxicity. In his original 'polymeric drug' model Ringsdorf predicted the possibility of combining drugs on the carrier polymer (130). A combination polymer therapeutic of CPT and Dox to the biopolymer hyaluronic acid was reported by Camacho K. et al. (164). The synergy offered by combining both drugs allowed for low doses of each drug to be administered for tumor shrinkage in mice while avoiding toxicity observed with free drugs. Conjugating both drugs to a single polymeric carrier also ensured simultaneous delivery, and in the synergistic ratio initially administered. This would be impossible for free drugs. These results are similar to those obtained by Markovsky et al. for the drugs PTX and Dox conjugated in combination to PGA and Noh et al. for the hydrophilic gemcitabine conjugated to hyaluronic acid and PTX conjugated to poly (L-lysine) (165,166). Combination polymer-drug conjugates offer superiority in synergistic drug delivery and are seen as the next generation of polymer therapeutics (167–170).

Box 2. Windows of Opportunities: The Enhanced Permeability and Retention Effect and New Permeability Pathways

EPR is an abnormal physiological phenomenon of solid cancer tissues (171,172) (Figure 5A). It is characterized by increased permeation of local blood vessels due to the presence of pores of sizes varying from 10 nm to 1 μm. Increased permeation is accompanied by retarded lymphatic drainage which results in retention of substances in the interstitial tissue.

Macromolecules and nanoparticles with sizes 20 and 200 nm are favourably extravasated through the blood vessels compared to small molecular drugs. The net effect is a high tumor concentration and low systemic distribution of the macromolecular drug without the need for an active tumor targeting ligand. It is however disputed whether the amount of administered nanomedicine entering the tumor is significant (173). EPR has not been exploited or characterized in infectious diseases as much as it has in cancer even though EPR-like effects have been identified in other, non-cancerous, diseases (174,175). A major difference is that most non-tumoric infections retain normal or significant lymphatic drainage which prevents passive accumulation of the drug. Malaria being primarily an infection of RBCs, EPR-dependent delivery of polymer therapeutics into infected cells is precluded. However, the new permeability pathways (NPPs) which are characteristic of *Plasmodium*-infected cells are an attractive alternative for passive concentration of conjugates into these cells (176) (Figure 5B). NPPs are new transmembrane channels that develop within 12-16 hrs after infection in the plasma membrane of an infected RBC. It is believed that these channels are crucial for the parasite to exchange materials with its environment given that mature RBCs lack a eukaryotic organelle system. Waste materials like lactic acid are excreted while nutrients including proteins are brought in. These channels have been estimated to be in the range of 50-80 nm in diameter (177) and permeable to bio-macromolecules like antibodies and albumin (178). With this size range most polymer therapeutics as well as some encapsulated particles like microemulsions are well within the limits to be internalized via these routes (97) (Table III). Considering that RBCs do not have an endocytotic system, NPPs could provide a means of passive selectivity in discriminating between infected and non-infected RBCs.

Table III. Size distributions of encapsulated and polymer-conjugated nanomedicines.

Encapsulated Nanocarriers		**Polymer Therapeutics**	
Block copolymer micelles	50-200 nm	Polymeric drugs	2-20 nm

Polymeric nanoparticles	20-1000 nm	Polymer-drug conjugates	5-20 nm
Liposomes	80-200 nm	Polymer-protein conjugates	10-20 nm
Nanosuspensions	100-1000 nm	Polymer-DNA complexes	40-100 nm
Solid lipid nanoparticles	10-1000 nm	Polymeric micelles	60-100 nm

Polymer-Drug Conjugates in Infectious Diseases

To the best of our knowledge and research, no polymer-drug conjugates for the major infectious diseases, malaria and tuberculosis, are currently in any stage of pre-clinical or clinical trials. Most of the research on the application of this technology to infectious diseases has focussed on anti-retroviral drugs (179–183). The anti-retroviral drug saquinavir was conjugated to PEG (184). SGV is a protease inhibitor characterized by poor bioavailability. Conjugation to PEG resulted in improved solubility and plasma half-life. Another anti-HIV drug, the nucleoside reverse transcriptase inhibitor zidovudine (AZT), has also been conjugated to several different polymers including k-carrageenan and dextrin. Conjugated AZT showed lower anticoagulant effect and a potentiated therapeutic activity (180,185). A novel conjugation of AZT to dextrin resulted in an extended half-life from 1.3 hrs, as observed for the free drug, to 19.3 hrs (186).

A limited selection of other anti-infectious disease therapeutics has also been conjugated to polymers. Lamivudine was conjugated to the polysaccharide dextran for the treatment of hepatitis B (187). This work was in part remarkable because the polymeric carrier, dextran, served the dual role of also targeting the conjugate to the liver. In animal studies, lamivudine was only released in the presence of the rat liver tritosomes. More recently, Wohl et al. investigated synthetic polymers-based macromolecular prodrug conjugates of the antiviral ribavirin for the treatment of hepatitis C (182). The conjugates elicited better therapeutic

response and significantly lower drug toxicity. They highlighted that the polymeric carrier was the most significant determinant of conjugate potency.

The antimicrobial Amphotericin B (AmB) has also been developed into nanomedicines for the treatment of leishmaniasis. Leishmaniasis is a parasitic disease caused by infection with protozoans belonging to the genus *Leishmania*. It has the second largest mortality rate owing to a parasitic infection; *malaria is first*! The parasite harbors in a vacuole in human tissue macrophages. AmB is the first-line treatment for leishmaniasis. The main pharmacological challenges with using AmB are poor aqueous solubility and high toxicity. Clinically, AmB is sometimes informally referred to as 'ampho-terrible' because of its notorious and potentially lethal side-effects. Even with this notoriety, it is the only member of its class of about 200 polyenes with a low enough toxicity profile to be used as an intravenously administered drug (188).

In an attempt to ameliorate the toxic side-effects of AmB a liposomal formulation, AmBisome, was produced (189). AmBisome was much less toxic than the free drug but its use was limited by its high cost. AmB has also been conjugated to the cheaper polymeric carriers arabinogalactan and HPMA copolymer (147,190). These conjugates demonstrated similar anti-parasitic activity compared to AmBisome and lower toxicity even at higher LD_{50}.

Nan et al. synthesized a multicomponent lysosomally-targeted HPMA conjugate of an 8-aminoquinoline for the treatment of visceral leishmaniasis (143). They used the tetrapeptide linker glycylphenylalanylleucylglycine (GFLG) which is only hydrolysable in lysosomes, effectively targeting this organelle for drug release. A glycine-glycine linker which is not labile in the lysosomes was investigated as control. The lysosomotropic conjugates showed superior anti-leishmanial activities than the free drug or the non-hydrolysable conjugates.

The successful development of polymer-drug conjugates against leishmaniasis is significant because the disease has several similarities to malaria. Both diseases, like leishmaniasis, are caused by intracellular parasites. The malaria *Plasmodium* also resides in an intracellular vacuole similar to the vacuole of *Leishmania*. Like in leishmaniasis the malaria parasite infects liver cells. These parallels strongly support the premise that polymer-drug conjugates can be designed to effectively and safely treat malaria infection.

Polymer-Drug Conjugates for Malaria

In 2010 the team of Maria Vicent of the Polymer Therapeutics laboratory—the world's first such dedicated facility—at the CIPF, Valencia, Spain, published a review of the research literature on the application of the polymer therapeutic 'platform technology' to diseases other than cancer (191). Malaria did not make the list of infectious diseases to which polymer therapeutics had been applied. (The four diseases were HIV/AIDS, hepatitis, fungal infections and leishmaniasis.) In a 2017 commentary Natfji A. et al. reviewed non-cancer applications of polymer therapeutics (192). Polymer-drug conjugates for malaria treatment were reviewed, an indication that the possible application of polymer therapeutics to the disease was emerging.

Primaquine appears to have attracted the most interest for conjugation (193–195). Rajic and co-workers synthesized primaquine-polymer conjugates of two polymers — poly[a,b-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) and poly[a,b-(*N*-3-hydroxypropyl-DL-aspartamide)] (PHPA) (194). The conjugates were investigated for differences in the type of covalent bonding, the length of the linker, drug loading and molecular weights of the polymers. A small molecule conjugate of primaquine-glucosamine was also synthesized to investigate the effect of using a macromolecular carrier. All the polymeric conjugates were better than the

primaquine-glucosamine. They showed improved aqueous solubility, extended activities, and better anti-plasmodial activities in experimental Swiss mice.

To ensure that there is maximum delivery of primaquine to the hepatocytes targeting ligands have been included as co-pendants on carrier polymers (196,197). Tomiya N. et al. reported the synthesis of a liver-targeted primaquine-polyglutamic acid conjugate (196). Using a trivalent glycoside ligand, N^{ε}-Z-N^{α}-dicarboxymethyl lysine conjugated with 6-aminohexyl glycoside of GalNAc, conjugated also to the PGA-primaquine the researchers were able to achieve rapid concentration of the PGA-primaquine conjugate in the liver. These ligands have high binding specificity for the asialo-glycoprotein receptor (ASGP-R) (198,199) and result in significant reduction in required therapeutic doses. ASGP-R, also known as the Ashwell-Morell receptor, binding sites exist in the order of 10^5 per liver hepatocyte and have selective affinity for galactosyl-derived ligands (200,201).

While there have been significant recent attempts at developing an effective polymer-conjugated delivery system for primaquine these efforts go back almost 40 years. Probably due to the field of polymer therapeutics being in its infancy, or that synthetic polymer chemistry was not as developed as today, early conjugates were mainly of short peptide or protein carriers. Those early days progenitors of present-day polymer therapeutics followed very similar criteria as present day polymer-drug conjugates. The carrier should be internalizable and the linker should be stable in the bloodstream but scissile in lysosomotropic intracellular vesicles. The Belgian researcher A. Trouet and his colleagues conjugated primaquine to the dipeptide Ala-Leu and the tetrapeptide Ala-Leu-Ala-Leu (202). The conjugates showed superior therapeutic indices than the free drug or liposome-encapsulated primaquine. However, a primaquine-Leu conjugate showed similar toxicity and chemotherapeutic profiles to free primaquine; an indication that conjugation to a single amino acid might not have deactivated the primaquine to a pro-drug. Andrè Trouet and his team

went on to show the advantage of conjugation by encapsulating primaquine in liposomes. While the toxicity was reduced about three times compared to the free drug the efficacy was unaffected as the amount of drug required to achieve therapeutic effect remained almost unchanged. Hofsteenge et al. in 1986 went further by conjugating primaquine to unmodified and lactosinylated bovine serum albumin (203). Conjugation to unmodified and modified BSA improved the therapeutic index of the drug. The conjugate showed as high as a 12 times better therapeutic index than the free primaquine. This allowed for enough of the drug to be administered so as to achieve a 100% cure rate in *P. berghei*-infected mice. Glycosylation conferred further superiority beyond just pro-drug formation by preferentially targeting the conjugates to the liver and improving their uptake by the liver hepatocytes. As in cancer, albumin has continued to attract interest as a carrier in conjugated drug delivery systems (204,205). Recently, Ibrahim N. et al. reported the synthesis of a human serum albumin-bound artemisinin nanoformulation suitable for intravenous treatment of malaria (206). They reported a 96% parasitemia inhibition rate at just 10 mg/kg/day with no recrudescence. Apart from solubilizing artemisinin, human serum albumin also selectively targets *Plasmodium*-infected RBCs (207).

Dihydroartemisinin has also been conjugated to polymeric carriers but most of these have been investigated for anticancer therapy (208–210). Artemisinins are able to induce apoptosis in cancer cells while maintaining low toxicity towards healthy cells. One mechanism of this anti-neoplastic activity is believed to involve iron ions which are known to be trafficked at a high flux via a transferrin-mediated mechanism. By conjugating dihydroartemisinin or other derivatives to transferrin the over-expressed transferrin receptors on cancer cells can be used for targeted delivery of the drug. This strategy is reviewed by Nakase I. et al. (211).

Multi-arm PEG conjugates of dihydroartemisinin of different molecular weights were synthesized by Dai L. et al. (209). They showed remarkable stability at the physiologically

significant pH of 6.1, 7.4 and 8.1 and 37 °C. At pH 7.4 as much as 50% of the conjugates were still intact after incubation for over 24 hrs. Although these experiments were conducted to show the improvements in the circulating half-lives offered by polymeric conjugation, it also serves as a positive outcome for overcoming the very low stability of artesunate in solution.

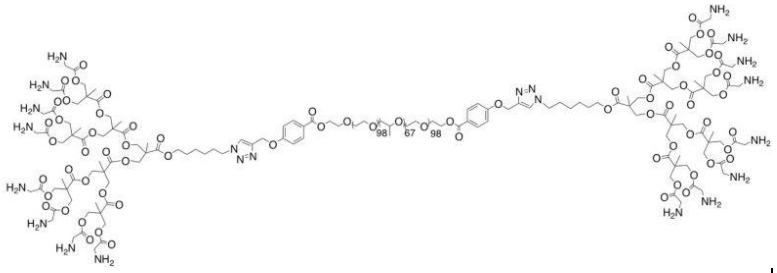
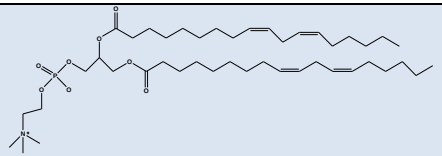
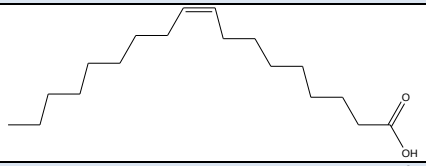
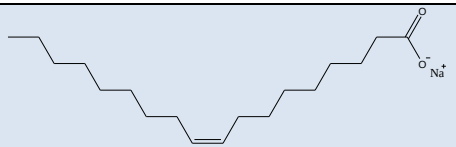
If this technology is to have a significant clinical impact in malaria, the development of polymer-combination drug conjugates of antimalarial drugs is essential as the WHO has strictly prohibited monotherapy (4). Polymer therapeutics for combination therapy is still in its early stages even in the treatment of cancer (148,168). Kumar S. et al. ventured into conjugating multiple antimalarial drugs onto a single polymer (212,213). They conjugated the antimalarial drugs primaquine and DHA to a polyphosphazene, an inorganic polymer. The use of the hepatic-tissue schizonticidal primaquine and the blood schizonticidal DHA together would seriously limit the ability of the parasite to raise resistance against the therapy. The team observed that the release kinetics of the drug involved a burst release phase followed by a more lengthy sustained release phase. Such kinetics will, respectively, provide significant immediate relieve from the debilitating clinical symptoms of malaria and ensure total clearance of all parasites.

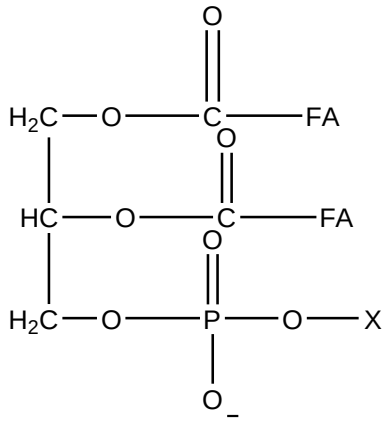
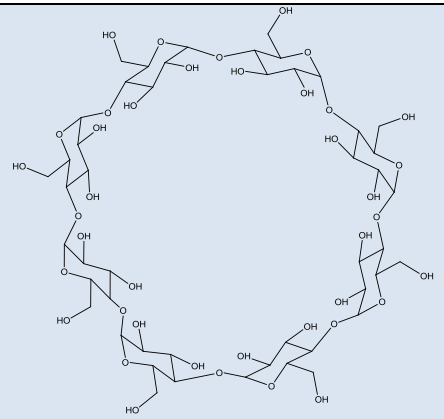
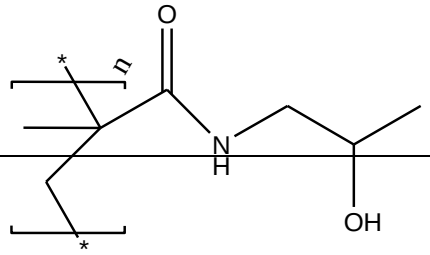
Non-covalent conjugates of antimalarials have also been researched (214,215). Many antimalarial drugs do not have chemical functional groups which can serve as conjugation handles. This thus precludes these drugs from the traditional hydrolysable covalent polymer-drug conjugation. For example, of the major artemisinin derivatives only DHA and artesunate can be covalently conjugated. However, many of the antimalarials have acidic and/or basic functional groups which allow them to be ‘conjugated’ via salt formation with polymeric carriers. Tripathy S. et al. synthesized chitosan-tripolyphosphate (TPP)-conjugated chloroquine as a delivery system to augment the antioxidant and free-radical scavenging

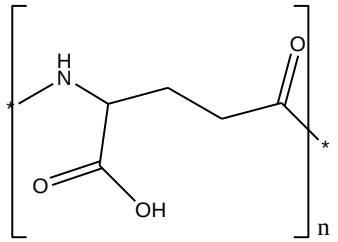
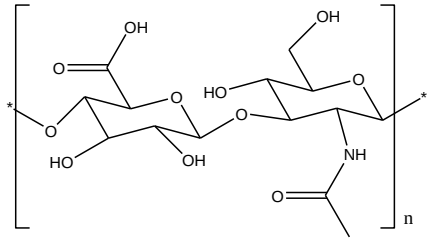
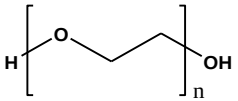
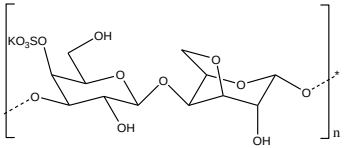
properties of the drug (216). Chitosan-TPP was synthesized by ionic gelation while the chloroquine was conjugated by mere physical shaking. TPP served as the linker between the polymer and the drug, thereby presenting a basic architectural frame analogous to a polymer therapeutics polymer-drug conjugate. The hydrodynamic size of the particles ranged between 100-150 nm which are significantly larger as would be obtained from normal covalent polymer-drug conjugates (217). This was tested for antimalarial efficacy against the *P. berghei NK65* but more importantly the focus was to prevent or reverse malaria-associated liver necrosis. They observed reduced reactive oxygen species formation, lipid oxidation and protein damage. Free chloroquine reduced liver cells apoptosis by 25.31% while the conjugate reduced apoptosis by 61.56%.

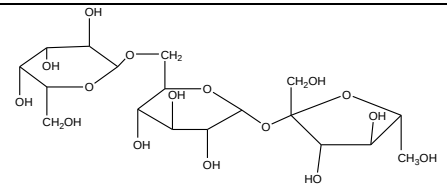
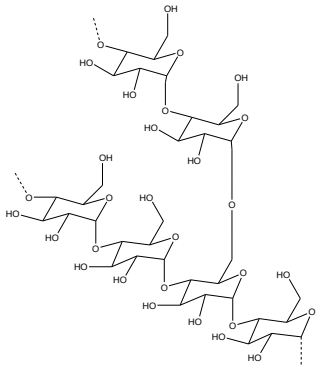
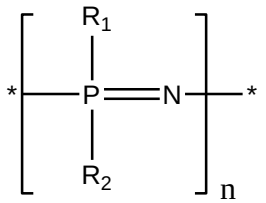
Urban P. et al. also synthesized salt conjugates of poly(amidoamine) carriers and primaquine and chloroquine bases (218). These non-covalent-polymer therapeutics were remarkable because they combined drug carriage with intrinsic antimalarial activities of the polymers and selectivity for infected RBCs. The hydrodynamic diameters of the conjugates were in the single digit nanometer range. It is however noteworthy that these conjugates did not display a clear significant advantage in *in vitro* antimalarial capacity assays compared to the free drugs.

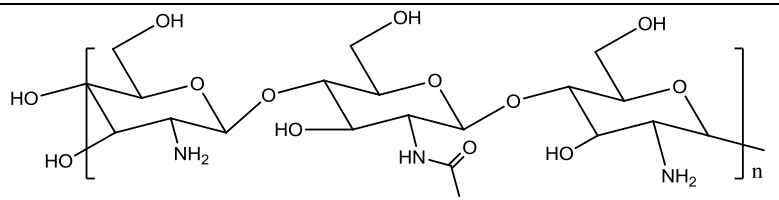
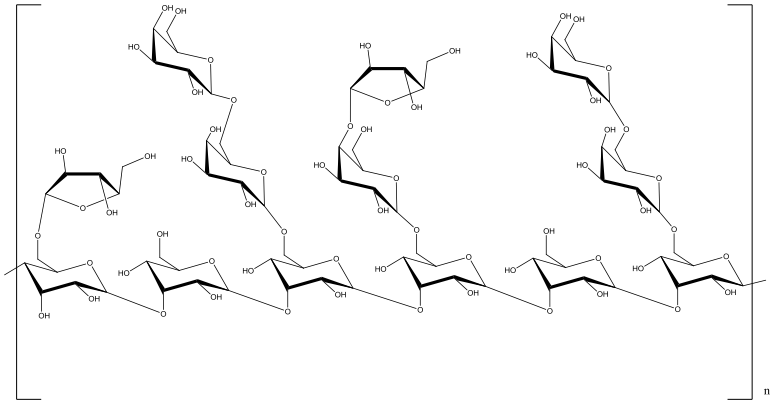
Table IV. Nanomedicines and their main component carriers.

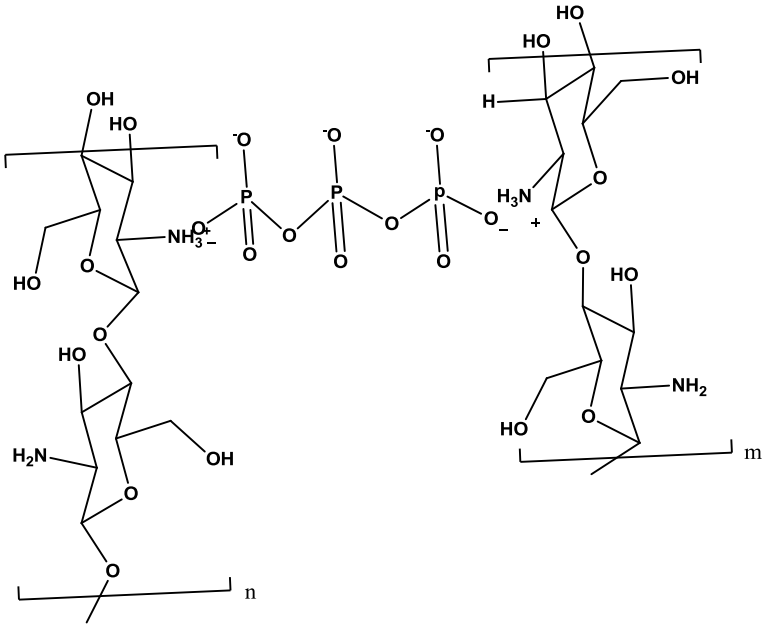
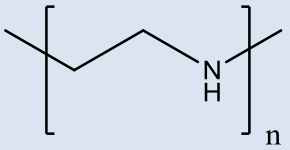
Nano-construct	Drug	Carriers	Disease	Representative structures of main carriers	Ref
Dendrimers	Chloroquine Primaquine	Hybrid glycine-terminated dendritic-linear-dendritic block copolymers	Malaria		(116)
Solid lipid nanoparticle	Arteether	Soya lecithin	Malaria		(120)
Nanostructured lipid nanoparticles	Artemether-lumefantrine	Oleic acid	Malaria		(124,125)
Microemulsions	Tefanoquine	Sodium oleate	Malaria		(129)

Liposomes	Amphotericin B 8-aminoquinoline analogs Primaquine	Phospholipids	Leishmaniasis Leishmaniasis Cancer	 Phospholipids *(X= ethanolamine, choline; FA = fatty acids)*	(143,189,195,202)
Cyclodextrin/ dextrin	Lamivudine Camptothecin	Polysaccharide-dextran CD-PEG	Hepatitis B Cancer	 α-cyclodextrin	(158,187)
Conjugates	Doxorubicin, camptothecin, paclitaxel, platinates	HPMA	Cancer		(151)

	8-aminoquinoline		Leishmaniasis	HPMA	(143)
	Amphotericin B		Leishmaniasis		(147)
	Paclitaxel-Poliglumex	PGA	Cancer		(160)
	Paclitaxel-doxorubin		Cancer		(165)
	Primaquine		Malaria	α-PGA	(196)
	Camptothecin-Doxorubin	Hyaluronic acid	Cancer		(164)
	Paclitaxel, Gemicitabine	Poly(L-lysine), Hyaluronic acid	Cancer		(166)
	Saquinavir	PEG	AIDS		(184)
	Zidovudine	k-Carrageenan	AIDS	Hyaluronic acid	(180)
	Azidothymide	Sulfated-alkyl-oligosaccharide	AIDS		(185)
	Zidovudine	Dextrin	AIDS		(186)
	Primaquine-dihydroartemisinin	Polyphosphazene	Malaria	Polyethylene glycol	(213)
	Chloroquine	Chitosan	Malaria		(215)
	Paclitaxel		Cancer		(219)
	Docetaxel		Cancer	K- Carrageenan	(220)
	Amphotericin B	Arabinogalactan	Leishmaniasis		(190)

				 Oligosaccharide  Dextrin  R= organic, inorganic, organometallic Polyphosphazene	

				 Chitosan  Arabinogalactan	

	Chloroquine	Chitosan-tripolyphosphate	Malaria	 Chitosan-tripolyphosphate	(216)
Non-covalent conjugates	Nucleic acids (DNA)	Polyethylenimine	Gene therapy	 Polyethylenimine	(221)

1

This starkly contrasts with the dendrimeric encapsulation system developed by the same group (116).

It is interesting, but not unprecedented, that salt complexes are being investigated for conjugation of drugs to polymers. Anionic nucleic acids have been complexed to polycationic carrier polymers in what are known as polyplexes or interpolyelectrolyte complex (131). These polymer therapeutics are molecular mimics of viruses and have been researched as gene delivery vehicles (221,222). While these constructs are non-covalently conjugated they could be seen as occupying the penumbra of conventional covalent polymer therapeutics.

Due to the harsh hydrolytic environment of the gastrointestinal tract most polymer therapeutics can only be administered parenterally. The ADME of polymer therapeutics is reviewed in (223). This raises the question of whether polymer therapeutics will be clinically acceptable for malaria treatment, especially for uncomplicated cases which are normally treated with oral drug formulations. Some researchers have attempted to extend the boundaries by developing oral polymer therapeutics. Lee, E. and colleagues synthesized polymer-drug conjugates of chitosan and the anticancer drugs paclitaxel and docetaxel (219,220). In both instances the drugs were linked to the polymer via the homo-bifunctional linker succinic acid to form an ester with the drug and an amide with the polymer. They investigated the stability of the conjugate in simulated gastric and intestinal fluids. In the transit time typically observed for materials going through the stomach, i.e. less than 3 to 4 hrs, only 9.3% and 24% of paclitaxel and docetaxel were lost, respectively. Improved pharmacokinetic data were obtained for both orally administered conjugates compared to the conventional intravenously administered free drugs. The use of chitosan as the polymeric carrier increased the retention of the conjugates in the gastrointestinal tract through its renowned mucoadhesive property (224,225).

Box 3. In vitro and in vivo models for antimalarials: The Goldilocks Conundrum

Several cellular and animal models have been developed for various aspects of the disease because the complexity of the *Plasmodium spp*. lifecycle means that no single biological model can adequately or completely represent the disease's pathophysiology (226,227). For example, Theron et al. recently reported that *P. falciparum* preferentially invades blood type O erythrocytes, an observation that contradicts the previous belief that this blood group offers protection against severe malaria (228). Further, since most antimalarials are life-stage specific, selection of a testing model must be appropriate for the drug target. Ringwald et al. reported wide variations in the IC_{50}s of common antimalarials depending on the serum or serum substitute used in *in vitro* parasite cultures of *P. falciparum* (229).

The most ideal model for assessing transmission blockage is the membrane-feed assay in which the test agent is incubated with mature *Plasmodium* gametocytes either before or at the time of the mosquito taking a blood meal. After one week the fed mosquitoes are dissected and the insect's midgut is examined for oocysts (230). Gametocyte inhibition assays have been developed as less demanding alternatives to membrane-feed assays for the investigation of the parasite transmission-blocking abilities of antimalarials. These assays are less ideal compared to the membrane-feed methods as a trade-off for greater simplicity. In a recent comparative study, Reader et al. highlighted the difficulty and probably inappropriateness of adopting a single assay to test different anti-gametocyte pharmacophores (227). This reasoning could also be extended to *in vitro* antimalarial testing for the hepatic and asexual erythrocytic stages.

In vivo animal models present less variability challenges as most of these models exhibit the full life cycle stages of the specific infecting parasite. An ideal *in vivo* model remains elusive

partly because *Plasmodium* species are extremely host species restricted. This has resulted in the adoption of highly approximated models for investigating the efficacies of antimalarials.

The most often reported *in vivo* model is of the murine rodent *P. berghei*, a non-human infecting *Plasmodium* species. This model is a convenient and simple animal model because the *P. berghei* naturally causes malaria disease in mice with very close similarities to the human disease such as a hepatic infection stage and even cerebral malaria complications. However, concerns still exist as to the extent of the similarity between this model and the human pathophysiology (226). Some researchers have used genetically-modified or humanized severe combined immunodeficient (SCID) mice infected with *P. falciparum* as alternative models (231). This less natural model allows for mice to be infected with human erythrocytes parasitized with *P. falciparum*. The lack of an immune system and a non-natural infection clearly indicate the limitations of this model. In some large research projects, the two models have been adopted to provide a better picture of antimalarial activity before progressing to higher organisms (89). The recent publication of Paquet et al. on the development of the revolutionary new antimalarial drug MMV390048 is an excellent reference for the use of *in vitro* and *in vivo* models in the development of an antimalarial (89). They used *P. cynomolgi*-infected monkeys to evaluate the prophylactic efficacy of MMV390048. *P. cynomolgi* is a non-human simian parasite with close similarities to *P. vivax* (232). It has been favored for testing anti-hypnozoite agents. Another non-human primate model, *P. knowlesi*-infected primates, which has been established for several decades, is beginning to attract interest since the description of full clinical malaria disease caused by the parasite in humans (233,234). The ability to cause severe disease in both humans and non-human primates might provide an *in vivo* model for true translational research into the pathophysiology of malaria and pharmacodynamics of antimalarial agents.

The use of *in vitro* cellular models to investigate the efficacy of antimalarial nanomedicines must be carefully considered for what information they can actually provide. As delivery systems, nanomedicines are not analogous to small drugs—in fact they are, in most cases, at best inactive pro-drugs that require special physiological stimuli for release of the active drugs or uptake. These stimuli may be absent in microbial culture media. For example, lysosomotropic polymer conjugates require the low pH and/or hydrolytic enzymes of endolysosomes for drug release. The observed IC_{50}s will therefore not be representative of the actual antiparasite activity possible *in vivo*.

The special role of G6PD deficiency in malaria, especially with reference to treatment with 8-aminoquinolines (discussed in Box 1), necessitates that an appropriate attention be paid to the *in vitro* and *in vivo* biological systems used to assay for hemolytic activities. Up to about a decade no animal model of the deficiency existed and researchers relied on donated blood from G6PD-deficient persons to test the hemolytic properties of chemical compounds. This *in vitro* system had many limitations including the lack of metabolic system to determine the effect of metabolites and degradation byproducts of the test compound. This weakness is even more critical when the test material is a nanomedicine which depends on metabolic processes for drug release and trafficking. Ko et al. reported a novel mouse model developed by introducing a mutant allele which resulted in the expression of the enzymopathy (235). Rochford and colleagues reported a humanized mouse model developed by grafting nonobese diabetic/SCID mice with human G6PD-deficient blood through daily transfusions for two weeks (236). These animal models offer great testing platforms for testing the efficiency of nanomedicine delivery systems to reduce the G6PD deficiency-dependent hemolytic toxicity of the 8-aminoquinolines and other antimalarials (129).

Conclusions and the Future of Nanomedicines for Malaria

Malaria is a curable acute infectious disease that could be fatal if not treated urgently. Even uncomplicated malaria presents with life-threatening symptoms. The arsenal of antimalarial chemotherapeutic drugs share pharmacological challenges with other drugs used to treat other diseases including poor aqueous solubility and bioavailability, systemic toxicity, molecular instability, high lipophilicity (logP) etc. Nanotechnology, in the form of nanomedicine, has provided improved therapeutics for treating cancer, diabetes, and infectious diseases like leishmaniasis. Both encapsulation and conjugation technologies have offered nanomedicines with enhanced pharmacological potentials. Indeed, some of the drugs like the artemisinins and 8-aminoquinolines, which have been formulated as anti-cancer and anti-leishmaniasis nanomedicines, are also essential antimalarials. However, apart from pharmacological challenges with antimalarials, arguably the greatest challenge and threat to these drugs is the rapid development of resistance by the *Plasmodium* spp. parasites. While both encapsulation and polymer therapeutics technologies offer improved pharmacokinetics, the latter holds an edge in the fight against drug resistance. The adoption of combination therapy as the accepted treatment strategy means that multiple drugs must be administered in a fixed ratio and there must be synergism in their delivery. This is more efficiently accomplished by conjugating both drugs to a single carrier in an appropriate ratio. Conjugation to hydrophilic polymers can readily and even tuneably attenuate the astronomical logP of drugs like lumefantrine and halofantrine. This can provide truly water-soluble forms of these drugs. The size range of 5-20 nm of most polymer-therapeutics means that conjugates could take advantage of the NPPs of infected RBCs for efficient selective passive concentration in these cells. These channels could direct the conjugates straight into the acidic belly of the parasite which is perfect for cleavage of acid-labile linkers. This compartment has a pH range of 5.2-5.8, analogous to the endolysosomal compartment targeted for release of lysosomotropic conjugates developed for

other diseases. It is in this vacuole that most antimalarials are believed to work by either inhibiting hemazoin synthesis or, as in the case of the artemisinins, being activated to a form toxic to the parasite. The other intracellular compartments of an infected RBC have a pH of 7.2-7.4; a range at which the linkers are stable. The low pH which is potent for drug release intracellularly is also a powerful limitation for oral administration of most conjugates as the acidic gastric juice could hydrolyse linkage bonds. Further, the ability to also incorporate a liver-targeting molecule on a polymer-drug conjugate could rapidly sequester the 8-aminoquinolines into the hepatocytes thereby limiting exposure to G6PD-deficient RBCs.

Encapsulated drugs still hold advantages like being more amenable to oral administration. Also, a wider range of drugs can be encapsulated while only those drugs with appropriate chemical functional groups for physiologically scissile linkers can be conjugated.

Author Contributions

All authors listed on this manuscript have contributed to its writing and compilation. They have all consented to being included in the list of authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

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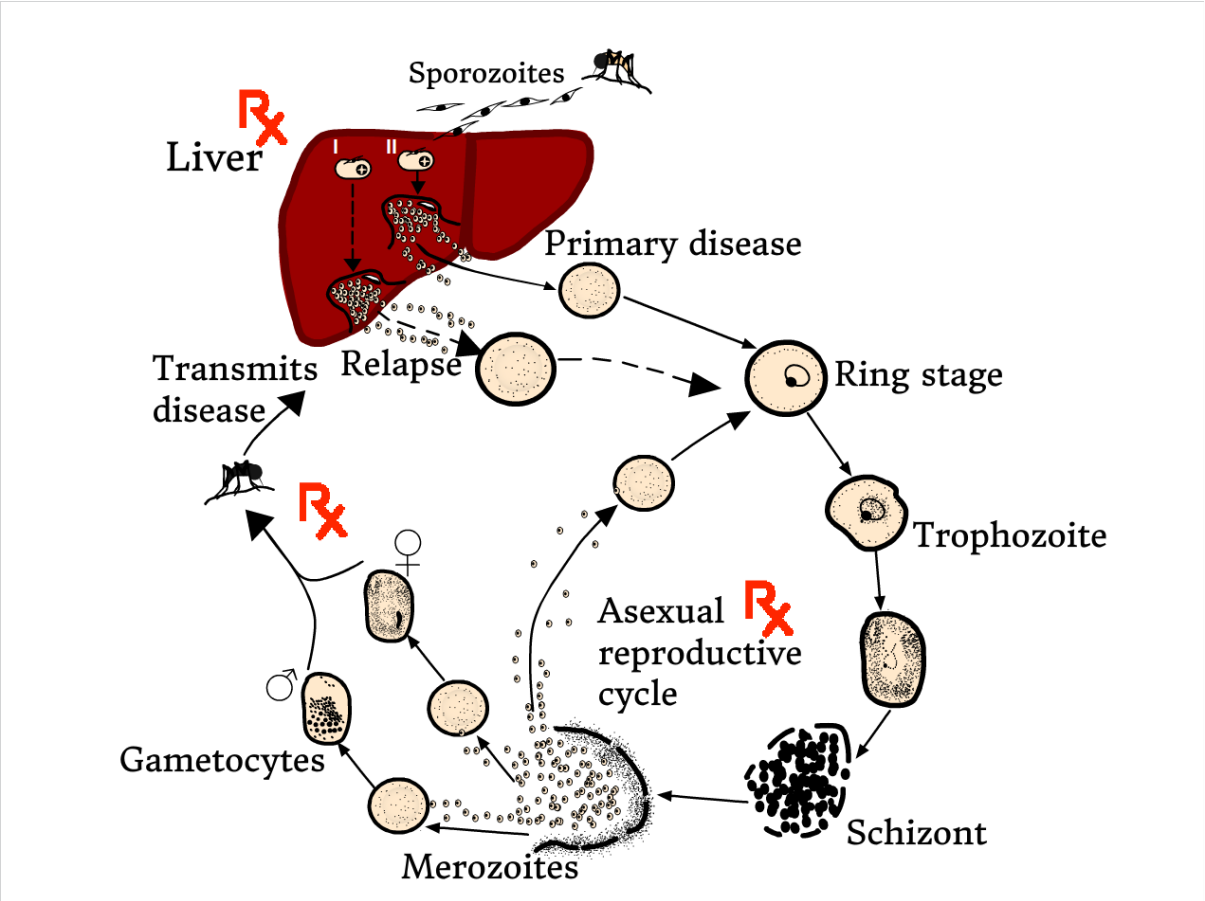


Figure 1. A schematic of the human segment of the *Plasmodium spp*. life cycle. **Rx** indicates stages targeted by antimalarial chemotherapy. Hypnozoites of *P. vivax* and *P. ovale* take the (**I**) life cycle path while all species causing immediate illness take the (**II**) path. The symbols ♂ and ♀ represent male and female gametocytes, respectively.

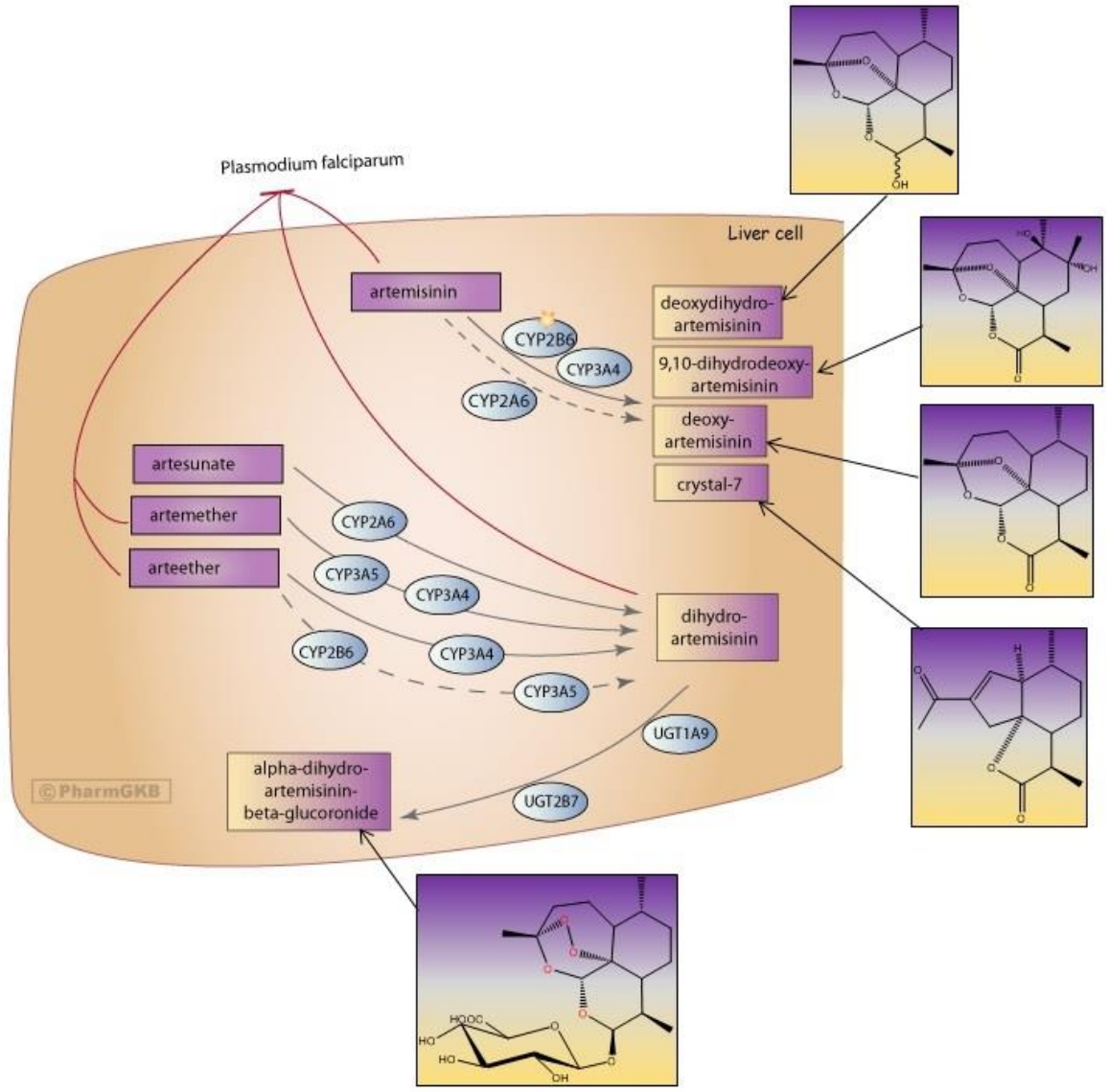


Figure 2: Major known liver metabolic products of artemisinins and their chemical chemical structures. ©PharmGKB. Permission to reproduce granted by PharmGKB and Stanford University. https://www.pharmgkb.org/pathway/PA165378192

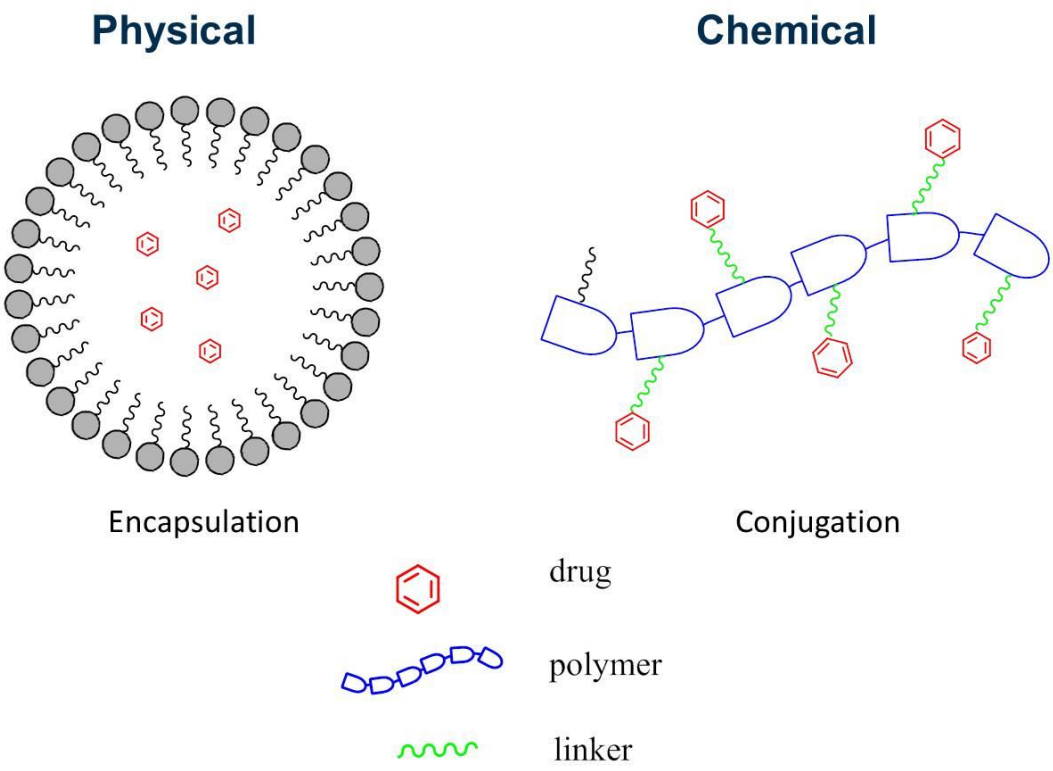


Figure 3. A schematic comparison of physical encapsulation vs. chemical conjugation of drugs.

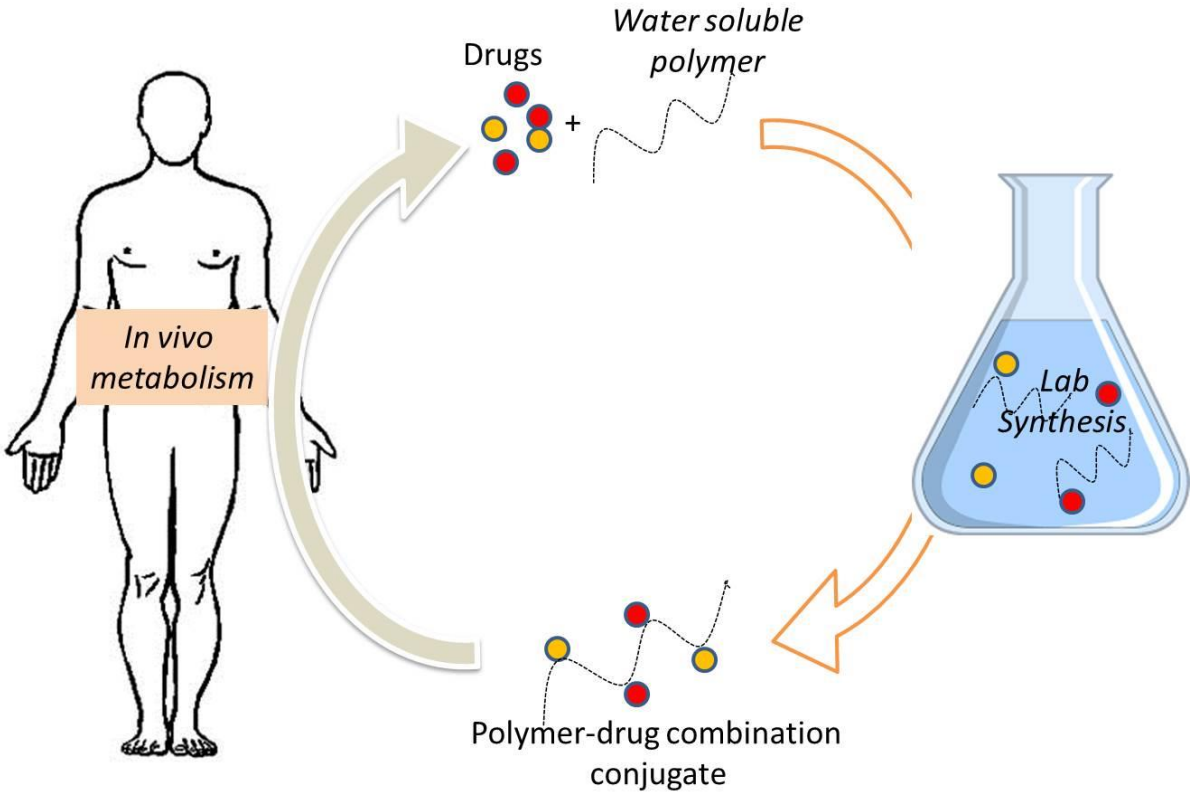
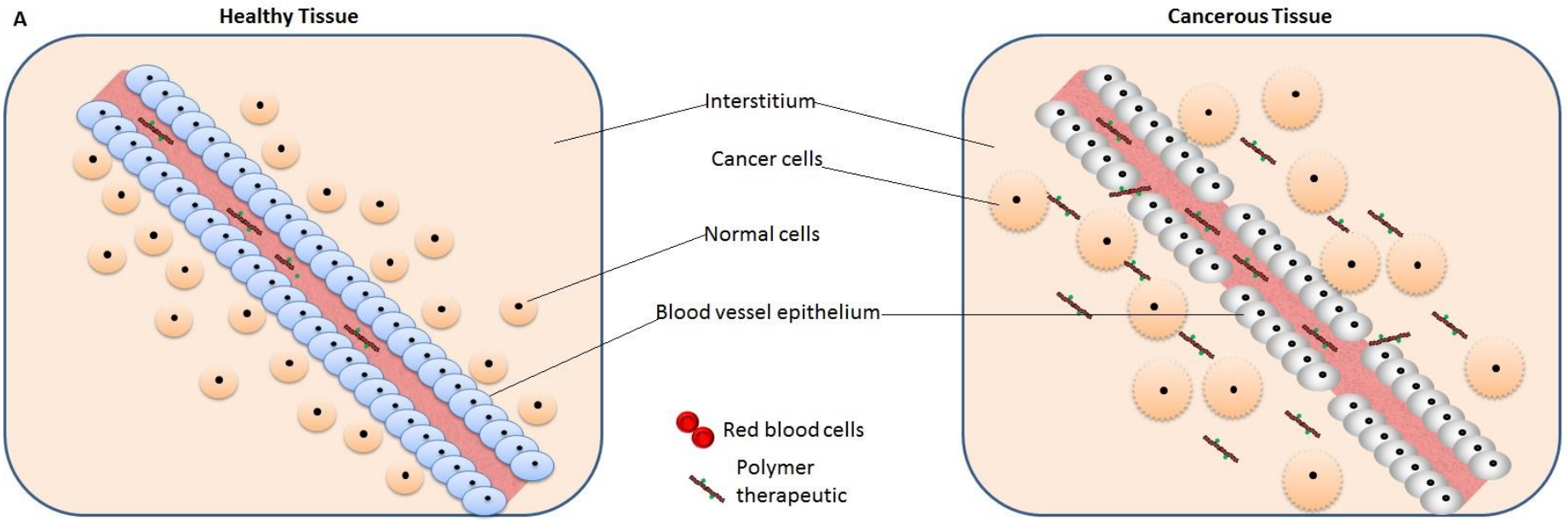


Figure 4. Schematic representation of reversibility of polymer-drug conjugation.


A
Healthy Tissue
Cancerous Tissue
Interstitium
Cancer cells
Normal cells
Blood vessel epithelium
Red blood cells
Polymer therapeutic

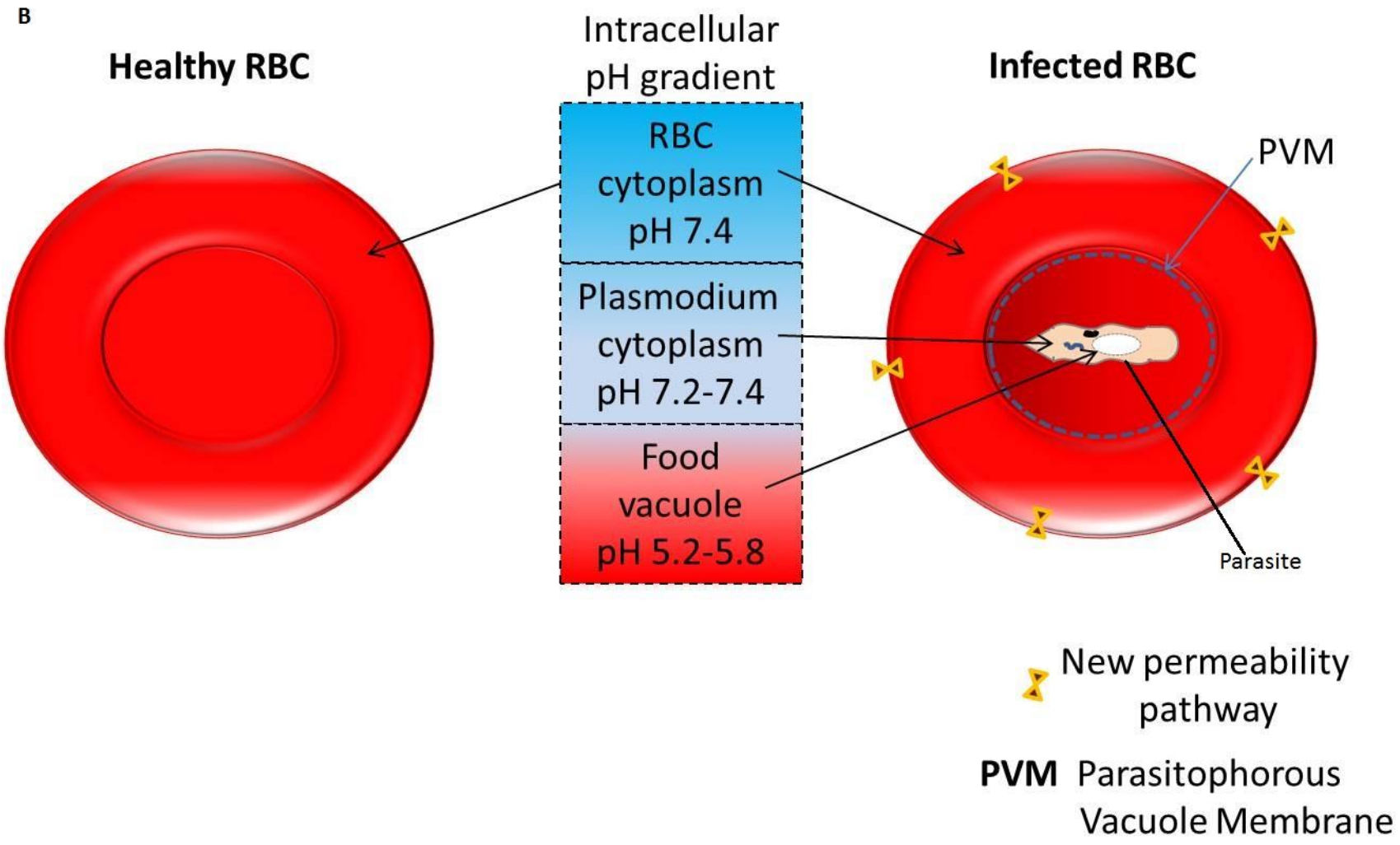


Figure 5. Schematic representation of the pathophysiological nano-fenestrations of tumoric tissues and *Plasmodium*-infected RBCs. **A.** The enhanced permeability and retention (EPR) effect. **B.** The new permeability pathways (NPPs) and pH gradation of membrane-bound compartments of infected RBCs.