Acta Pharmaceutica Sinica B 2021;11(8):2585–2604

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Acta Pharmaceutica Sinica B

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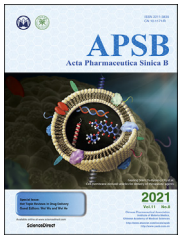

REVIEW

Delivery strategies of amphotericin B for invasive fungal infections

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Received 22 December 2020; received in revised form 18 February 2021; accepted 15 March 2021

Abbreviations: γ-CD, γ-cyclodextrin; γ-PGA, γ-poly(gamma-glutamic acid; ABCD, AmB colloidal dispersion; AIDS, acquired immunodeficiency syndrome; AmB, amphotericin B; *Aspergillus fumigatus*, *A. fumigatus*; AmB-GCPQ, AmB-encapsulated *N*-palmitoyl-*N*-methyl-*N*,*N*-dimethyl-*N*,*N*,*N*-trimethyl-6-*O*-glycol-chitosan nanoparticles; AmB-IONP, AmB-loaded iron oxide nanoparticles; AmB-PM, AmB-polymeric micelles; AmB-SD, AmB sodium deoxycholate; AmBd, AmB deoxycholate; AP, antisolvent precipitation; ARDS, acute respiratory distress syndrome; BBB, blood–brain barrier; BCS, biopharmaceutics classification system; BDDE, butanediol diglycidyl ether; BUN, blood urea nitrogen; BSA, bovine serum albumin; *C. Albicans*, *Candida Albicans*; CFU, colony-forming unit; CLSM, confocal laser scanning microscope; CMC, carboxymethylated L-carrageenan; CP, chitosan-polyethylenimine; CS, chitosan; DDS, drug delivery systems; DMPC, dimyristoyl phosphatidyl choline; DMPG, dimyristoyl phosphatidylglycerole; DMSA, dimercaptosuccinic acid; GNPs, gelatin nanoparticles; HPH, high-pressure homogenization; HPMC, hydroxypropyl methylcellulose; ICV, intensive care unit; IFIs, invasive fungal infections; L-AmB, liposomal AmB; LNA, linolenic acid; MAA, methacrylic acid; MFC, minimum fungicidal concentrations; MIC, minimum inhibitory concentration; MN, microneedles; MOP, microneedle ocular patch; MPEG-PCL, monomethoxy poly(ethylene glycol)-poly(epsilon-caprolactone); NEs, nanoemulsions; NLC, nanostructured lipid carriers; NPs, nanoparticles; P-407, poloxamer-407; PAM, polyacrylamide; PCL, polycaprolactone; PDA, poly(glycolic acid); PDLLA, poly(D,L-lactic acid); PDLLGA, poly(D,L-lactic-*co*-glycolic acid); PEG, poly(ethylene glycol); PEG-DSPE, PEG-lipid poly(ethylene glycol)-distearoylphosphatidylethanolamine; PEG-PBC, phenylboronic acid-functionalized polycarbonate/PEG; PEG-PUC, urea-functionalized polycarbonate/PEG; PGA-PPA, poly(L-lysine-*b*-L-phenylalanine) and poly(L-glutamic acid-*b*-L-phenylalanine); PLA, poly(lactic acid); PLGA, polyvinyl alcohol poly(lactic-*co*-glycolic acid); PLGA-PLH-PEG, PLGA-*b*-poly(L-histidine)-*b*-poly(ethylene glycol); PMMA, poly(methyl methacrylate); POR, porphyran; PVA, poly(vinyl alcohol); PVP, polyvinylpyrrolidone; RBCs, red blood cells; RES, reticuloendothelial system; ROS, reactive oxygen species; SEM, scanning electron microscope; SL-AmB, sophorolipid-AmB; SLNs, solid lipid nanoparticles.

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Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2021.04.010

KEY WORDS

Invasive fungal infections;
Amphotericin B;
Poor water-solubility;
Drug delivery;
Topical administration;
Nanoparticles;
Conjugates;
Toxicity

Abstract Invasive fungal infections (IFIs) represent a growing public concern for clinicians to manage in many medical settings, with substantial associated morbidities and mortalities. Among many current therapeutic options for the treatment of IFIs, amphotericin B (AmB) is the most frequently used drug. AmB is considered as a first-line drug in the clinic that has strong antifungal activity and less resistance. In this review, we summarized the most promising research efforts on nanocarriers for AmB delivery and highlighted their efficacy and safety for treating IFIs. We have also discussed the mechanism of actions of AmB, rationale for treating IFIs, and recent advances in formulating AmB for clinical use. Finally, this review discusses some practical considerations and provides recommendations for future studies in applying AmB for combating IFIs.

1. Introduction

The invasive fungal infections (IFIs) are a growing public health concern. The increase in IFIs has been attributed to various factors including HIV, immunosuppressive therapy, cancer, neutropenia, peritoneal dialysis, promiscuous use of antibiotics and people in the intensive care unit (ICU)[1,2]. Due to compromised immune function, IFIs patients become more vulnerable for systemic fungal infections. Generally, deep fungus does not invade the skin and nails like shallow fungus, but intrude the subcutaneous tissue, internal organs, and can spread through blood and lymph[3,4]. Consequently, the high morbidity rate of IFIs patients further increased due to the limited antifungal treatments as well as development of resistant fungi[2,5]. In this respect, *Candida albicans* (*C. albicans*), *Aspergillus*, and *Cryptococcus* are the predominant pathogens that cause systemic fungal infections[6,7], besides few rare and new opportunistic species such as *Fusarium*, *Histoplasma capsulatum*, *Paecilomyces*, *Curvularia*, and *Coccidioidomycosis*[8,9]. Table 1 summarizes the clinical presentations and host reactions produced by the mycoses.

Currently, the treatments for IFIs depend mainly on certain classes of antifungal drugs, summarized in Table 2. Based on their pharmacological targets, these antifungal drugs can be classified as, (1) inhibitors of cell wall synthesis that works *via* inhibition of β1,3-glucan synthase or chitin, including caspofungin, micafungin, anidulafungin, nikkomycins, and polyoxins[10], (2) agents targeting the cell membrane components-phospholipid, sphingolipid or sterol, such as aureobasidin A and fluconazole[11], (3) nucleic acids and protein synthesis inhibitors, such as 5-fluorocytosine and tavaborole, and (4) microtubules inhibitors, as griseofulvin and vinblastine[12,13]. However, poor water-solubility and membrane-permeability limit the use of most of those drugs for IFIs treatment. Besides, the emergence of drug-resistant fungi due to upregulated drug transporters, activated efflux pumps and mutations in resistant genes leads to treatment failure[14,15]. Moreover, drug-associated adverse effect and lack of adequate diagnostic strategies are additional factors that limit the success of IFIs treatment[16].

Amphotericin B (AmB) is a polyene antibiotic naturally produced by *actinomycete Streptomyces nodosus*. It was initially isolated in 1955[17]. It was originally launched by Bristol–Myers Squibb in 1958 and was approved by US Food and Drug Administration (FDA) as the first antifungal agent in 1965. Due to its broad-spectrum antifungal effect and low incidence of clinical resistances, it is of a particular importance in clinical practice for treating IFIs[18]. The most outstanding feature of AmB is its

Table 1 Clinical presentation and host reaction to the common mycoses.

Fungus	Clinical presentation	Main host response
Cryptococcus spp.	Pneumonia	Granulomatous inflammation
	Pleural effusion	Inflammatory responses
	Disseminated	Inflammatory responses, tissue necrosis
Candida spp.	Cutaneous	Mixed suppurative, granulomatous inflammation
	Superficial infections	Minimal to suppurative inflammation
	Invasive disease	Invasion of blood vessels, necrotizing vasculitis
Aspergillus spp.	Allergic bronchopulmonary aspergillosis	Mucosa with suppurative and granulomatous inflammation, vasculitis, and fibrosis
	Allergic fungal rhinosinusitis	Similar to that for allergic bronchopulmonary aspergillosis
	Chronic pulmonary aspergillosis	The wall surrounding the fungus ball consists of fibrosis
Histoplasma capsulatum	Acute pneumonia	Vascular necrosis, vasculitis and rare granulomatous inflammation
	Acute respiratory distress syndrome (ARDS)	Diffuse alveolar damage
	Mediastinitis	Granulomatous inflammation
	Chronic pneumonia	Granulomas
	Disseminated	Similar to *Cryptococcus* spp.

Table 2 Drugs used in clinic for the treatment of IFIs.

Name	Dosage form/administration	Strength	Target
Caspofungin			*b*-1,3-Glucan synthase or chitin
Cancidas	Powder/intravenous	50/70 mg/vial	
Caspofungin acetate	Powder/intravenous	50/70 mg/vial	
Micafungin			
Mycanine	Injectable/injection	50 mg	
	Injectable/intravenous	50/100 mg base/vial	
Anidulafungin			
Eraxis	Powder/intravenous	50/100 mg/vial	
	Injectable/injection	50 mg	
Fluconazole			Cell membrane components
Diflucan	Tablet/oral	50/100/150/200 mg	
	Suspension/oral	50/200 mg/5 mL	
Fluconazole	Tablet/oral	50/100/150/200 mg	
Diflucan in sodium chloride 0.9%	Injectable/injection	2 mg/mL	
Diflucan In dextrose 5% In plastic container	Injectable/injection	2 mg/mL	
Diflucan In sodium chloride 0.9% in plastic container	Injectable/injection	2 mg/mL	
Fluconazole	Tablet/oral	50/100/150/200 mg	
Tavaborole			Protein
Kerydin	Solution/topical	5%	
Giseofulvin			Microtubules
GRIS-PEG	Tablet/oral	125/250 mg	
Grisactin	Capsule/oral	125/250 mg	
Grifulvin V	suspension/oral	125 mg/mL	
	Tablet/oral	125/250/500 mg	
Fulvicin P/G	Tablet/oral	125/165/250/330 mg	
Fulvicin-U/F	Tablet/oral	250/500 mg	
Grisactin ultra	Tablet/oral	125/250 mg	
Vinblastine			Microtubules
Vinbl	Injectable/injection	10 mg/vial	
Vinbl astine sulfate	Injectable/injection	10 mg/vial	
	Injectable/injection	1 mg/mL	
Itraconazole			Cell membrane components
Sporanox	Capsule/oral	100 mg	
	Solution/oral	10 mg/mL	
	Injectable/injection	10 mg/mL	
Onmel	Tablet/oral	200 mg	

amphiphilic and amphoteric behavior, arising from its apolar and polar sides of the lactone ring and ionizable carboxyl and amine groups. It forms soluble salts in both acidic and alkaline media. Due to its low solubility and permeability, it is placed in BCS class IV. The physicochemical properties of AmB are described in Table 3. The toxicity, spectrum, and available marketed formulations of AmB are described in Fig. 1.

AmB interacts with ergosterol of the cell membrane of susceptible fungi and alters its structure and permeability. Moreover, inhibitory effect on *Aspergillus*, *Cryptococcus neoformans*, *C. albicans* and *Coccidioides* has also been observed[13]. It has high affinity to bind with fungal cell membrane sterols particularly the ergosterol (up to 30%, mol/mol)[19]; and this effect is responsible for its serious nephrotoxicity due to interaction with cholesterol rich membrane of kidney cells. The chemical structure and possible mechanism of actions of AmB on fungal cell are described in Fig. 2A and B, respectively. Moreover, AmB can also alter the cellular ionic homeostasis as described in Fig. 2B.

In the recent years, the application of nanotechnology in the development of drug delivery systems (DDS) to treat various diseases showed promising results[20–22]. These nanosized carrier systems offer multiple advantages over traditional delivery systems, such as protection of encapsulated drugs from degradation and metabolism, increased residence time, and enhanced targetability to specific cell or organs[23–27]. In line with this, the nanoparticle-based AmB formulations significantly reduced side effects as well as increased its therapeutic index. The nanoparticles (NPs) including polymeric NPs, conjugates, lipid-based delivery systems, nanoemulsions, nanosuspensions, metal-based NPs and microneedles are considered as the most promising DDSs for AmB. The aim of this review is to demonstrate the potential impact of the above mentioned AmB mediated delivery system intended to treat IFIs.

2. NP-based DDS for AmB

NPs possess several advantages for drug delivery due to their small size that allow their penetration into small capillaries and result in efficient accumulation at the target sites[28–30]. After intravenous administration, the conventional NPs are taken-up by the monocytes and macrophages of the reticuloendothelial system (RES) and rapidly cleared from the bloodstream, thereby limiting

Table 3 Some major physicochemical properties of AmB.

Chemical property	Value	Condition
Mass intrinsic solubility	Soluble (10 g/L)	pH 1 Temp: 25 °C
	Slightly soluble (1.0 g/L)	pH 2 Temp: 25 °C
	Sparingly soluble (0.12 g/L)	pH 3–9 Temp: 25 °C
	Slightly soluble (1.5 g/L)	pH 10 Temp: 25 °C
p*Ka*	3.72 ± 0.70	Most acidic Temp: 25 °C
	8.12 ± 0.70	Most basic Temp: 25 °C
Lipophilicity (log*P*)	2.296 ± 0.894	Temp: 25 °C
Distribution coefficient (log*D*)	–0.80	pH 1 Temp: 25 °C
	–0.78	pH 2 Temp: 25 °C
	–0.64	pH 3 Temp: 25 °C
	–0.98	pH 9 Temp: 25 °C
	–1.37	pH 10 Temp: 25 °C
Koc	1.0	pH 1–4 Temp: 25 °C
	1.29	pH 5 Temp: 25 °C
	1.32	pH 6 Temp: 25 °C
	1.25	pH 7 Temp: 25 °C
	1.0	pH 8–10 Temp: 25 °C

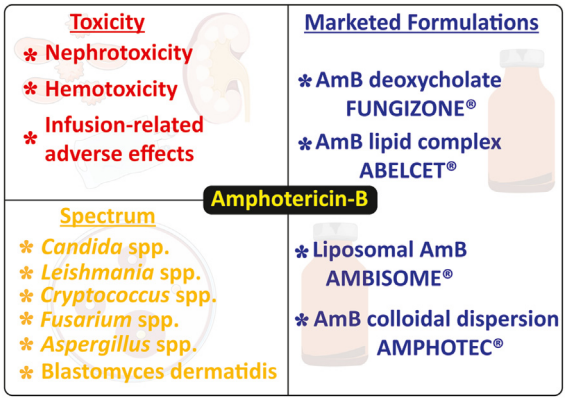

Figure 1 Toxicity, spectrum, and available marketed AmB formulations.

therapeutic effects[31,32]. Mainly, the NPs' clearance from the blood circulation occurs through their recognition by cellular receptors bound to the carrier rather than recognition of the carrier themselves[33]. The nanoparticle recognition by macrophages is generally mediated by opsonization and depends on their interaction[34]. When the distance between nanoparticle and opsonin is sufficiently closed, the opsonin bound to the surface of NPs, leads to the macrophage recognition and then phagocytosis occurs[35,36]. Importantly, this recognition becomes critical when macrophages are considered as the therapeutic target as in case of the macrophage-associated acquired immunodeficiency syndrome (AIDS), leishmaniasis as well as IFIs. In this respect, the development of NP-based DDS for AmB to treat IFIs is promising, given the NPs' ability to target the infected cells and provide a sustained release allowing longer contact between the drug and the fungi/parasite. NPs are being considered extensively to improve

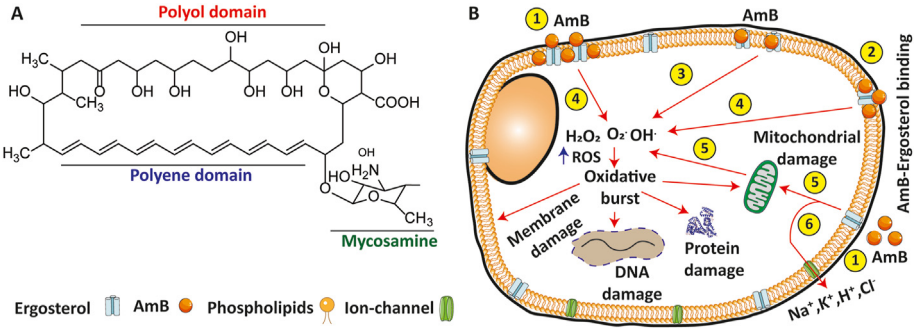

Figure 2 Chemical structure (A), and mechanism of AmB on fungal cell (B). AmB induces its action by various means. On cell membrane: (1) It binds with membrane ergosterol and prompt ergosterol sequestration and (2) disrupt membrane stability. Inside the cell: It induces an oxidative burst as a production of prooxidant and (3) increases reactive oxygen species inside the cell. (4) The production of oxygen species (ROS) is produced during respiratory chain reaction, suggested that AmB influences the mitochondrial function, and (5) encourages oxidative burst. Also, (5) the AmB can alter the cellular ionic homeostasis and permit leakage of monovalent Na^+, K^+, H^+, and Cl^- ions. Thus, the increased level of these free-radicals and imbalance of ions produces multiple deleterious effects on the vital components of cell (membrane, mitochondria, proteins, and DNA) resulting in the cell death.

AmB delivery (Table 4)[37–48]. The NP-based DDS employed for AmB delivery for the treatment of IFIs are detailed bellow.

2.1. *Polymeric NPs*

The polymeric NPs can be prepared from various natural materials such as chitosan, alginate, gelatin or synthetic polymers including poly (lactic acid, PLA), poly (glycolic acid, PGA), poly-caprolactone (PCL) poly (lactide-*co*-glycolide acid, PLGA) among others. The ideal polymer employed for nanoparticle formulation should be non-toxic, non-antigenic, biodegradable, biocompatible, protects drug payload from premature degradation and provides drug delivery in a sustained manner[27].

Polymeric NPs can be engineered to enhance accumulation of the therapeutic molecule to the target tissues upon *in vivo* adaption and to ministrrelease the entrapped drug by diffusion through the polymeric matrix, polymer erosion or from the polymeric coat and to efficiently accumulate at the site of action to induce the desired therapeutic effects[49]. Specifically, the AmB have been successfully encapsulated in the biocompatible polymers by simple preparation method resulting in stability in biological fluids and a higher shelf-life.

Among the natural polymers, chitosan is one of the most widely studied polymers, owing to its biocompatibility, biodegradability, film-forming ability, mucoadhesive properties, ease of chemical modification besides its antimicrobial properties. The chitosan-based NPs have attracted great attention as drug carriers[50] due to their high drug-loading ability, protection of drug from degradation and providing sustained drug release. However, there are few reports describing AmB delivery using chitosan NPs.

Table 4 A summary of nanocarriers mediated AmB delivery system studied in recent years.

Formulation	Main materials	Result and conclusion	Ref.
Carboxymethylated L-carrageenan Conjugated AmB-loaded gelatin NPs (CMC-AmB-GNPs)	Gelatin A 240 AmB L-Carrageenan C	Enhanced antifungal activity *in vitro* and uptake by RAW 264.7 cells	38
AmB-PLGA-NPs	AmB Polyvinyl alcohol poly(lactic-*co*-glycolic acid) (PLGA)	Significantly reduced the survival rate of fungal	39
Chitosan-coated PLGA NPs	AmB Chitosan PLGA	Lower MIC and cytotoxic	40
AmB-PEGylated polylactic-polyglycolic acid copolymer NPs	AmB Clopidogrel Glycyrrhizic acid PEGylated poly (D,L-lactide-*co*-glycolide) copolymer	Low toxicity and improved efficacy over Fungizone®	41
AmB-loaded PLGA-PLGA-PEG NPs	AmB PLGA-PEG PLGA	2-Fold increase of MIC against *C. albicans*, no hepatic cellular alteration or kidney alteration, inhibition of the AmB-induced hemolysis	42
AmB-loaded and chitosan-coated lipid NPs	AmB Chitosan ligosaccharides Injectable soya lecithin Poloxamer 188	Improved penetration into the cornea with no obvious irritation to the rabbits' eyes	43
AmB/Diblock copolymer MPEG-PCL micelles	AmB Polyethylenimine Milk protein concentrate Diblock copolymer MPEG-PCL	Significantly enhanced the antifungal activity against the biofilm state of *C. albicans*	44
AmB-micelles (aerosol)	AmB Sodium borohydride Deoxycholic acid Anhydrous sodium sulfate Sodium deoxycholate	Nontoxicity even up to 8 μg/mL, significantly reduced MIC and minimum fungicidal concentrations (MFC) against *C. neoformans*, and *C. albicans*	45
Poly (gamma-glutamic acid, γ-PGA)-AmB complexes	γ-PGA Dimethyl sulfoxide *N*,*N*′-Diisopropylcarbodiimide	Improved efficacy against experimental murine *candidiasis* over Fungizone® and AmBisome®	46
AmB-Cu(II) complex	AmB 2-Propanol $CuCl_2$	Promoted fungicidal activity	47
Nanoemulsions	AmB Propylene glycol Tween® 80	Sustained release and no toxicity	48
Polyglucose−AmB conjugate	AmB Glucose Sodium borohydride Sodium periodate	High dose administrated with negligible hemolysis	49

The mucoadhesive properties of chitosan can be employed to enhance the transport ability across the intestinal barrier and improve AmB oral bioavailability. In this regards, chitosan-coated poly (ε-caprolactone) NPs of size of 318 nm and encapsulation efficiency of 69% were prepared for oral delivery of AmB[51]. The *in vitro* release in simulated gastrointestinal fluids, demonstrated a good stability during the entire evaluation period and very low molecular aggregation was observed, after the AmB release from NPs. Most importantly, the *in vitro* antifungal activity of those NPs against *Candida parapsilosis* strain was 5-fold higher than free AmB. This study further affirms the possibility of oral AmB delivery at reduced toxicity[51]. Similarly, Bhatia et al.[52] proposed that packing of AmB in between oppositely charged polymers by polyelectrolyte complexation could be promising to reduce AmB toxicity and increase its antifungal activity. They synthesized a polyelectrolyte complex of AmB by using chitosan and porphyran (CS-POR-AmB NPs, ≥200 nm). The optimized CS-POR-AmB NPs not only showed enhanced stability over a wide range of pH but also did not induce hemolytic activity. Moreover, compared with marked formulations the CS-POR-AmB NPs showed promising antifungal potential against *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus* (Fig. 3A)[52]. In another report, Chhonker et al.[53] prepared AmB-encapsulated chitosan/lecithin NPs using ionic-gelation method for ophthalmic delivery. Compared with marked formulation, the *in vivo* pharmacokinetics revealed improved bioavailability and residence time of up to 2.04- and 3.36-fold in the New Zealand albino rabbit eyes by low molecular-weight chitosan NPs. Furthermore, the AmB-NPs showed higher susceptibility against *C. albicans* and *A. fumigatus* than marketed formulation, Fungizone® (Fig. 3B)[53].

For site-specific targeting, Serrano et al.[54] synthesized AmB-encapsulated *N*-palmitoyl-*N*-methyl-*N,N*-dimethyl-*N,N,N*-trimethyl-6-*O*-glycol-chitosan NPs (AmB-GCPQ) to enhance AmB bioavailability in the targeted organs and reduce nephrotoxicity (Fig. 3C). The therapeutic efficacy of AmB-GCPQ was tested in three industrial standard disease models (candidiasis, aspergillosis and leishmaniasis) in small animals. The interaction between AmB and GCPQ resulted in the formation of highly stable 216- and 35-nm NPs with 90% of AmB encapsulation efficiency. The pharmacokinetic profile following single oral dose of AmB-GCPQ to mice showed initially low AmB concentration and then observed sustainability for 8 h. Importantly, after being taken up by intestinal epithelia the AmB-GCPQ were mainly transported to the lung, liver and spleen. Furthermore, the AmB-GCPQ also delivered AmB to the brain and bone marrow, an important issue for systemic fungal infection and clearance of *Leishmania*, respectively[54].

Other natural materials were also considered to prepare formulation for AmB delivery. Nahar et al.[55] prepared AmB-loaded gelatin NPs (AmB-GNPs) to reduce nephrotoxicity. Such NPs had a diameter of 213 nm and entrapment efficiency of 49.0%[55]. Interestingly, the AmB-GNPs showed biphasic release kinetics by an initial burst followed by controlled release of up to 196 h[56]. Furthermore, compared with plain AmB, the AmB-GNPs and AmBisome® did not result in increased blood urea nitrogen (BUN) nor serum creatinine levels, indicating that a better safety

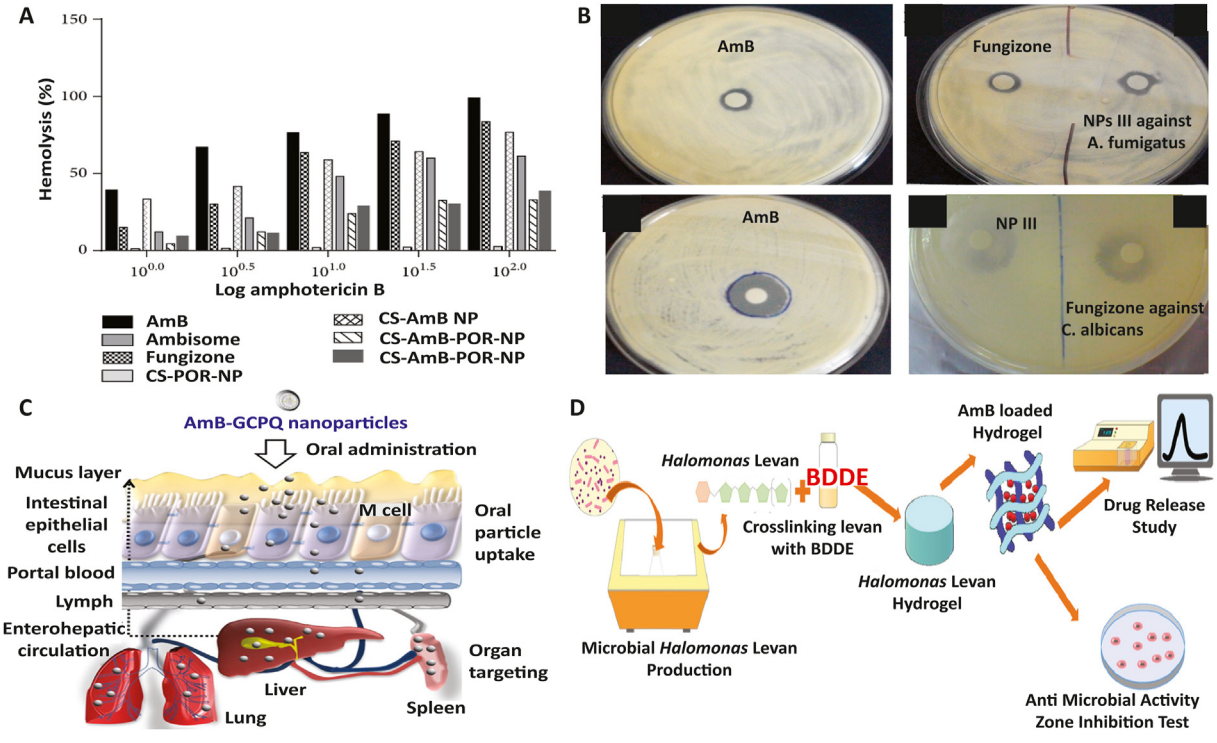

Figure 3 (A) *In vitro* hemolytic activity of AmB, Fungizone, Ambisome, CS blank, CS-AmB, and various CS-POR based NPs. The data is represented as mean ± SD ($n = 3$). Adapted with permission from Ref. 53. Copyright © 2014 Saurabh Bhatia et al. (B) Inhibition zone of AmB, Fungizone and NPs III against *A. fumigatus* and AmB, NPs III and Fungizone against *C. albicans*. Adapted with permission from Ref. 54. Copyright © 2015 Elsevier. (C) Biodistribution and liver, lung, spleen targeting of orally administered AmB-NPs. Adapted with permission from Ref. 55. Copyright © 2015 American Chemical Society. (D) Preparation characterization, biocompatibility and antifungal efficacy of Levan-based hydrogels for dermal delivery. Adapted with permission from Ref. 67. Copyright © 2020 Elsevier.

profile of AmB-GNPs for kidneys[55]. In another report, AmB nanoassemblies (AmB-NAs) were synthesized with Aloe-vera leaf extract *via* biomimetic synthesis method[56]. Compared with AmBisome, the AmB nanoaggregates showed significantly low hemolysis and renal toxicity. Compared to free drug, the AmB-NAs were more effective in killing various fungal pathogens including *Candida* spp. and evoked less drug related toxic manifestations in the host. This study suggested that the biomimetically synthesized AmB-NAs circumvented toxicity issues and offered a promising approach to eliminate systemic fungal infections. Thought, it is unknown why AmB-NAs show greater antifungal efficacy, it could be related to macrophages, which were involved in the pathogenesis of *Candida* spp. It was reported that macrophages could phagocytose NPs, thus AmB-NAs could act as "secondary depot", or "cellular drug reservoirs" be engulfed, and released the drug into the pathogenic site eventually[56].

However, natural polymers differs in purity and always need crosslinking to prepare NPs, that can probably cause degradation of the loaded drugs[57]. Conversely, the synthetic polymers offer great advantages over the natural ones as they can be easily modified and imparted with various properties. The synthetic polymers PLA or its co-polymer PLGA are the most applied polymers in drug delivery. They have been approved by FDA and by European regulatory authorities for clinical application as they are highly biocompatible and are biodegraded into nontoxic byproducts, reducing the material-associated systemic toxicity Moreover, both lipophilic and hydrophilic drugs can be efficiently encapsulated in these polymer matrices.

The AmB-loaded PLGA NPs were first prepared by Venier-Julienne et al.[58] in 1996 by using solvent evaporation technique. However, due to PLGA and AmB non-miscibility, the AmB loading was very low (0.7%−1.3%)[58]. Later, Italia et al.[59] prepared PLGA NPs for AmB by two different processes, nanoprecipitation or emulsion diffusion-evaporation using vitamin E-TPGS as stabilizer. They selected nanoprecipitation method as it produced small NPs with narrow size distribution. Compared with Fungizone®, the AmB-loaded NPs exhibited lower hemolytic effect and less renal toxicity with oral bioavailability of 800%, conferring the potential of orally administered AmB-NPs to treat systemic fungal infections. They further examined the efficacy of AmB-loaded NPs for oral delivery in murine disseminated and invasive pulmonary aspergillosis models. Their results suggested that orally administered AmB-NPs had superior efficacy compared to that of parenterally administered Ambisome or Fungizone and demonstrated the potential of PLGA NPs for oral delivery of AmB[60].

In another report, Tang et al.[61] prepared AmB-loaded pH-responsive charge-reversal PLGA-*b*-poly (L-histidine)-*b*-poly (ethylene glycol) (PLGA-PLH-PEG) NPs by emulsion-solvent evaporation method to increase the antifungal efficacy of AmB. To reduce toxicity and increase targetability, they modified the surface of the NPs with anti-*C. albicans* antibody. Compared with free AmB, the NPs showed less toxicity in RBCs and human renal tubular epithelial cells and in BALB/c mice at the dose of 2 mg/kg/day for 3 days[61]. Importantly, the synthesized NPs showed significant antifungal activity both *in vitro* and *in vivo* experiments[61]. Accordingly, Souza et al.[62] encapsulated AmB in PLGA-DMSA NPs and examined their biocompatibility, pharmacokinetics and antifungal efficacy. Interestingly, notable antifungal effects were produced even at low drug dose of 4.5 mg/kg when the NPs were administrated. In addition, no significant toxicity was observed when compared with Fungizone[62]. Similarly, Carraro et al.[41] synthesized PLGA-PEG NPs to minimize AmB-associated nephrotoxicity, hemolytic anemia, and hepatotoxicity. The histopathological examination indicated no sign of hepatic toxicity or hemolysis in the NP-group, while the marketed AmB deoxycholate induced liver damage.

The synergistic antifungal effects of ultrasound and AmB-encapsulated PLGA NPs (AmB-NPs) prepared by double-emulsion method against *C. albicans* biofilm was investigated by Yang et al.[63] The AmB-NPs showed lower toxicity compared to free AmB and in contrast to AmB alone, ultrasound treatment alone, the biomass, proteinase and phospholipase activities of biofilms were significantly decreased with combined treatment of AmB-NPs and 42 kHz of ultrasound irradiation at an intensity of 0.30 W/cm^2 for 15 min. In addition, CLSM examination revealed that biofilm thickness and structure were remarkably altered after combination treatment. The synergistic anti-biofilm efficacy was observed in subcutaneous catheter biofilm rate model, indicating that this treatment strategy might provide novel safe, effective and non-invasive therapy for *C. albicans* infection[63].

Based on the aforementioned research efforts, we can conclude that natural NPs based on chitosan have shown promising outcomes for oral delivery of AmB mainly due to their mucoadhesive properties. Further functionalization of chitosan with targeting moieties can help in achieving site-specific targetability and reducing nephrotoxicity. Gelatin NPs also showed improved pharmacokinetic properties and improved safety profile. Those natural based NPs are also suitable for ocular and topical AmB delivery. Synthetic biodegradable polymers-based NPs have also shown enhanced antifungal activities and they pose better reproducibility compared to natural polymers which can facilitate the industrial scale up of AmB-NP formulation for commercial use as.

2.2. *Hydrogels*

Hydrogel is a cross-linked water-swollen 3D network predominantly prepared from polymers or biomaterials[64]. The water in the hydrogels provides a condition comparable to the natural tissues, an adjustable mechanical nature coordinating both soft and hard tissues, an ability to encapsulate active compounds, protect them from decomposition and sustain drug release over time controlled by diffusion, degradation of the network by external or endogenous stimulation. Thus, hydrogel-mediated AmB delivery provides benefits including sustained and targeted drug release, increased efficacy and reduced side effects. Furthermore, unlike conventional drug delivery the AmB-hydrogel formulations improves patient compliance due to noninvasive administration. In this respect, Demirci et al.[65] prepared AmB-entrapped hydrogel by covalent cross-linking of *Halomonas* levan with butanediol diglycidyl ether (BDDE). The resulted hydrogel showed pH-dependent rather than temperature-dependent swelling at a ratio of 9.1 ± 0.1 with 51% AmB-loading efficiency. The AmB-loaded hydrogel displayed extended drug release over 120 h in PBS buffer and demonstrated potent antifungal activity against *C. albicans* (Fig. 3D).

Poly-aggregated AmB formulations have been reported to have potential to reduce the toxicity and enhance the efficacy due to their biodegradability, biocompatibility, and functionality[66]. Commonly used polymers include chitosan, PLGA, PLA, PEG, PVA, alginate (Alg), and xanthan Gum[67]. To prepare AmB-conjugated hydrogels, anti-inflammatory drugs, 2-naphthalene

acetic acid, dexamethasone, or naproxen, were used to promote self-assembly of AmB-conjugated polypeptide sequence (Phe-Phe-Asp-Lys-Tyr, FFDKY)[68]. The hydrogels facilitate sustained drug release, enhanced antifungal activity; and importantly, the formulation of the anti-inflammatory drugs enabled reduction in side effects such as allergic reactions and cell damage caused by AmB[68,69]. The polypeptide-hydrogels represent a promising delivery strategy for combined antifungal therapy.

2.3. *Polymeric conjugates*

There has been much interest in the polymeric conjugates, particularly natural polymer-based ones, in the recent years[70]. AmB conjugates have been prepared with proteins or polysaccharides to reduce AmB toxicity by attenuating its aggregation state and providing some additional properties as improved penetration through membranes and macrophage uptake. In this respect, protein-based conjugates have significantly captured attraction as drug carrier due to their intrinsic features such as biodegradability, nontoxicity, and excellent binding capacity with various drugs. In addition, the protein-based conjugates are also easily amenable for surface decoration and covalent attachment of targeting ligands and drugs, respectively[71].

Albumin is a kind of protein that possesses the aforementioned advantages besides its easy availability, and non-immunogenicity[72]. Worth of mentioning that even some products based on albumin were commercially marketed, including Levemir®, Tresiba®, Victoza® and Abraxane®[76]. Gurudevan et al.[73] used bovine serum albumin (BSA) as a carrier to synthesize AmB−albumin conjugates through amide linkage. The developed conjugate exhibited similar release profile in plasma as compared to AmBisome®. In addition, the conjugate demonstrated excellent anti-fungal activity against yeast strains such as *C. albicans*, *C. neoformans*, and *C. parapsilosis*. The conjugate approach efficiently addressed the limitations of AmB, such as poor-water solubility, toxicity and hemolytic potential as well[73].

Also, Kothandaramanet al.[74] synthesized a pectin−AmB conjugate using citrus pectin by Schiff's linkages in unreduced imine and reduced amine. Compared to pure AmB, the pectin−AmB conjugates exhibited lower toxicity. Additionally, the anti-fungal activity of AmB conjugates showed reduced resistance against *C. albicans* and *A. fumigatus*. In another report, polysaccharide−AmB conjugate was developed using oxidized galactomannan by reductive amination method[75]. This conjugate allowed for 40% increase in antifungal activity with little hemolysis and no influence on the viability of VERO kidney cells[75]. In another attempt to improve the solubility of AmB, it was further linked with polyethylene glycol (PEG), a highly biocompatible polymer[76]. The AmB-PEG formulations exhibited enhanced antifungal efficiency with 2-fold reduction of toxicity to mammalian cells as compared to free AmB (Fig. 4A)[76]. Nishi et al.[77] studied whether oxidized gum arabic conjugated-AmB still retained its anti-fungal activity and evaluated its bioavailability and toxicity in animal model. Results revealed that the AmB-conjugates were stable, non-toxic and non-hemolytic to the internal body organs along with promising anti-fungal activity[77].

2.4. *Polymeric micelles*

Polymeric micelles show great superiority in terms of solubilizing ability, permeability, and lower toxicity[78]. Zhang et al.[79] prepared AmB-loaded MPEG-PCL micelles that were formulated into buccal tablets for topical applications. The micelles remarkably improved AmB solubility and demonstrated sustained release behavior in both normal oral conditions (pH 6.8) and *C. albicans* infection conditions (pH 5.8). The results revealed that the micelles reduced the overall toxicity of AmB compared with other standard preparations, while the buccal tablet allowed to suppress *C. albicans* biofilm formation due to the enhanced penetration ability of MPEG-PCL micelles in biofilm[79]. In another study, Rodriguez et al.[80] developed micelles for AmB using PEG and conjugated retinol by ring opening polymerization with subsequent Steglich esterification. This synthesis method increased the encapsulation efficiency and antifungal activity was significantly improved without any hemolytic effects. Wang et al.[81] developed a cost-effective micellar formulation to minimize AmB associated toxicities (Fig. 4B). The AmB was loaded in polymeric micelles self-assembled by phenyl-boronic acid functionalized polycarbonate-PEG and urea-functionalized polycarbonate-PEG deblock copolymers by hydrogen bonding, boronate ester bond, and/or ionic interactions between boronic acid group in the micellar core and AmB amine group[80]. The particle size of the micelles was in the range of 54−84 nm with a narrow size distribution and almost neutral surface charge. The micelle formulation demonstrated sustained drug release profiles and showed enhanced antifungal activity compared to free AmB or Fungizone. Importantly, 2 days post injection in mice, the histological examination of mice-kidney tissues revealed that the micelles did not display significant apoptotic cells. Thus, the polycarbonate micelles offered a promising AmB delivery carrier to treat systemic fungal infections[81]. For precise and controlled delivery, Patel et al.[82] developed a micelle-hydrogel composite based on amphiphilic polypeptides for switchable and controlled release of dual drugs (Fig. 4E). They synthesized wo di-block polypeptides, poly (L-lysine-*b*-L-phenylalanine) and poly (L-glutamic acid-*b*-L-phenylalanine) (PGA-PPA), to prepare micelles loaded with curcumin and AmB. The micelles were cross-linked by the pendant amino groups of L-lysine side chains *via* genipin to yield a micelle-hydrogel composite with PGA-PPA micelles. This system allowed controlled multiphasic drug release kinetics, and a sustained release profile of up to 168 h (Fig. 4F). Interestingly, this system could be tuned to control drug release by modulating factors including pH, cross-linking density or composition[82].

Song et al.[83] introduced linolenic acid (LNA) and mPEG to the backbone of oligochitosan (CS) and prepared LNA-modified mPEG-CS conjugate (mPEG−CS−LNA) into which AmB was loaded by dialysis method to fabricate AmB-loaded micelles (Fig. 4C). The micelles had drug encapsulation efficiency of up to 82.27% and increased the water solubility by 1640-fold. Furthermore, those micelles exhibited enhanced pharmacokinetics and promoted the antifungal efficacy against *C. albicans* over marketed formulation while causing little hemolysis and renal toxicity.

Commercially available Fungizone (AmB-SD) containing AmB solubilized by sodium deoxycholate often shows aggregation of AmB that results in dose-dependent renal toxicity. To overcome this limitation, PEG-DSPE micelles were prepared (Fig. 4D). The PEG-DSPE micelles had little hemolytic effect on bovine RBCs. Administration *via* jugular vein cannulation infusion at 2 mg/kg/day to mice did not drastically rise the blood urea nitrogen and creatinine level. Additionally, the micelles demonstrated thorough absence of colony-forming unit (CFU), whereas free AmB and marked AmB-SD induced complete absence of CFU after 4 h of administration. The micelles not only increased AmB solubility but also reduced its toxicity with no effect on its antifungal activity[84]. Interestingly, the conjugation of cholesterol

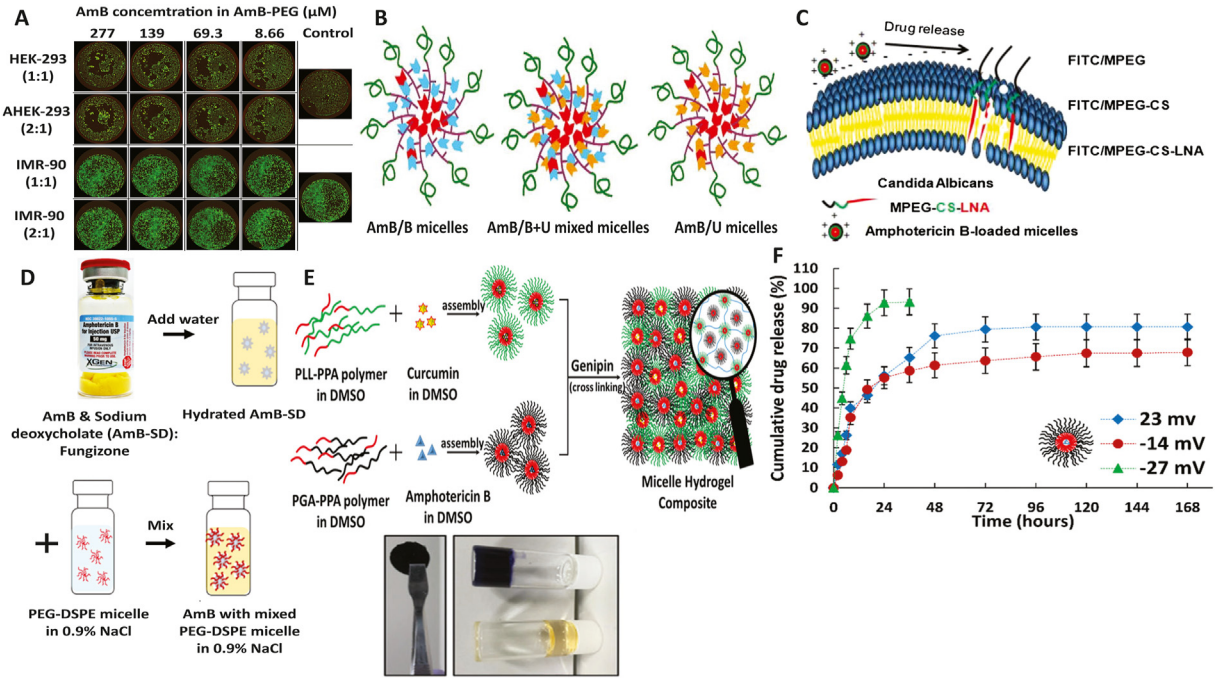

Figure 4 (A) LIVE/DEAD staining of HEK293 and IMR-90 cells after exposure to AmB-PEG and free AmB for 24 h (Live cells stained green and dead cells stained red). The AmB-PEG did not induce cell death at 139 and 277 μmol/L concentration in HEK293 and IMR-90 cells, respectively. While the molar ratio of AmB to PEG did not have any visible effect on cell toxicity. The experiment was performed twice with three independently prepared AmB-PEG formulations. Adapted with permission from Ref. 79. Copyright © 2016 Tan et al. (B) Preparation of AmB-loaded micelles using PEG-PBC and PEG-PUC. Adapted with permission from Ref. 84. Copyright © 2016 Elsevier. (C) Schematic presentation of preparation and action of AmB loaded linolenic acid-modified methoxy poly (ethylene glycol)−oligochitosan conjugate micelles on *C. albicans* membrane. Adapted with permission from Ref. 86. Copyright © 2019 Elsevier. (D) Reformulation of AmB-SD with PEG-DSPE micelles in saline and a proposed fungus−membrane interaction of monomeric AmB. Adapted with permission from Ref. 87. Copyright © 2016 Springer Nature. (E) Preparation of AmB loaded micelle-hydrogel composite by genipin crosslinking method and photograph of AmB loaded micelle-hydrogel composite before and after gelation and as a self-standing gel. (F) AmB release kinetics from micelle-hydrogel at different zeta-potentials. Adapted with permission from Ref. 90. Copyright © 2017 Royal Society of Chemistry.

to mPEG-*b*-PCL further enhanced the micellar encapsulation efficiency of AmB and showed a controlled release profile[85]. Although those micelles showed a lower *in vitro* efficacy against *C. albicans* SC5314 compared with Fungizone, they showed a comparable antifungal activity in a study on invertebrate Galleria-mellonella model for invasive candidiasis. Upon encapsulation of the AmB, hemotoxicity was also attenuated[85]. Similarly, in order to reduce toxicity and enhance antifungal activity, Xu et al.[86] employed α-linolenic acid (ALA) modified monomethoxy poly-ethylene glycol-*g*-PEI-*g*-ALA conjugate to prepare AmB-encapsulated micelles. The micelles enhanced AmB's water solubility of up to 1.2 mg/mL and showed good storage stability. Notably, compared with commercial AmB formulation, the micelles showed similar antifungal activity and biofilm inhibition against *C. albicans* as free-AmB injection and demonstrated low hemolytic and little renal toxicity.

For oral delivery of AmB, Nimtrakul et al.[87] examined potential of AmB-polymeric micelles (AmB-PM) for enhance intestinal absorption. The micelles assembled from polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol copolymer by a modified solvent diffusion and microfluidics method were 80 nm in size with 95% encapsulation efficacy and 20% drug-loading efficiency. Importantly, the micelles allowed for drug protection from degradation in acidic condition. Moreover, micelles indicated 6-fold increase in uptake over free AmB in Caco-2 cells within 30 min and enabled 2-fold increase in permeability of AmB across Caco-2 cells monolayers than the free drug.

From the aforementioned research efforts, we can conclude that polymeric micelles offer a promising approach for enhancing solubility, improving bioavailability and antifungal activity of AmB, while reducing its nephrotoxicity. They can be designed to provide a tailored release profile, as well as to impart characteristics that fit the route of administration as mucoadhesive properties and inhibition of biofilm formation. The composition can be tailored using a variety of starting materials, types of chemical bond and conjugation of functional ligands.

2.5. *Lipid-based carriers*

2.5.1. *Niosomes*

Niosomes, are vesicular drug delivery system, have been used to encapsulate both hydrophobic and hydrophilic drugs. They were first described by Verma et al.[88] in 1979. Niosomes are formed from self-assembly of hydrated surfactant monomers. Compared to liposomes, niosomes are made up of single hydrophobic chains and also possess amphiphilic nature with the advantages of increased absorption, permeability and solubility of the drug and decreased toxicity[89]. They can serve as drug reservoirs and tuning of vesicular composition as well as their surface modification allow adjustment in drug release rate and affinity for target site.

Niosomes have been widely employed as carriers for a variety of drugs including cancer chemotherapeutics, antiviral and antibacterial agents intended for various routes of administration, such as oral, parenteral, ocular and topical. Recently reported studies demonstrated that delivery of antifungal agents with niosomal system has superiority over other delivery systems. Haque et al.[90] prepared sophorolipid-AmB (SL-AmB) noisome using a thin film hydration method. The biofilm assay confirmed that SL-AmB noisome could act on mature biofilms of *C. albicans*, and showed similar efficacy with phosome (marketed liposomal formulation of AmB)[90]. Salerno et al.[91] prepared niosomes using different surfactants and AmB as an amphiphilic model drug. The results revealed that formulations with Span 60® and Span 80® at 30 mmol/L exhibited higher stability and entrapment efficiency. Then, Alsaadi et al.[92] investigated effects of aerosolized niosomes loaded with AmB on lungs of Sprague−Dawley rats (200−250 g) for invasive pulmonary aspergillosis. They prepared aerosolized niosomes loaded with inclusion of hydroxypropyl-γ-cyclodextrin/AmB using cholesterol/Tween 80. These formulations considerably improved drug delivery to the lungs and liver with minimal systemic toxicity and significantly reduced fungal lung burdens and enhanced pharmacological efficacy as well. Most recently, a triazole based non-ionic surfactant (TBNIS) was synthesized and then was examined for biocompatibility using NIH 3T3 cell line, blood hemolysis assay and systemic toxicity assessment in adult Swiss albino mice (20−24 g). The TBNIS was biocompatible and had little cytotoxicity, negligible hemolysis and systemic toxicity. The AmB-loaded niosomes were well stabilized by TBNIS and possessed a diameter of 180 nm with 89% entrapment efficiency, sustained drug release profile and excellent stability. Importantly, compared with free drug solution, the niosomes demonstrated enhanced oral bioavailability of the drug, along with approximately 9-fold increase (Fig. 5A−C)[93].

2.5.2. *Solid lipid NPs*

The solid lipid NPs (SLNs) was first prepared in 1990s as potential substitute drug carriers of polymeric NPs, emulsions and liposomes. Their composition which is devoid of organic solvents make them attractive candidate as a drug delivery system[94−96]. They are mostly spherical particles with size up to 1000 nm. The supramolecular SLNs are mainly composed of lipid matrix produced by solid lipids and coated by surfactant wall for structure stabilization. Lipids used for the development of SLNs include steroids, waxes, fatty acids, mono/di/tri-glycerides[97,98] Bile salts, polyvinyl alcohol, polyoxyethylene ethers and polyethoxylated Sorbitan esters are utilized as biocompatible emulsifier. Due to ease of preparation, higher stability, ability to encapsulate both hydrophilic and lipophilic drugs, good biocompatibility, less bio-

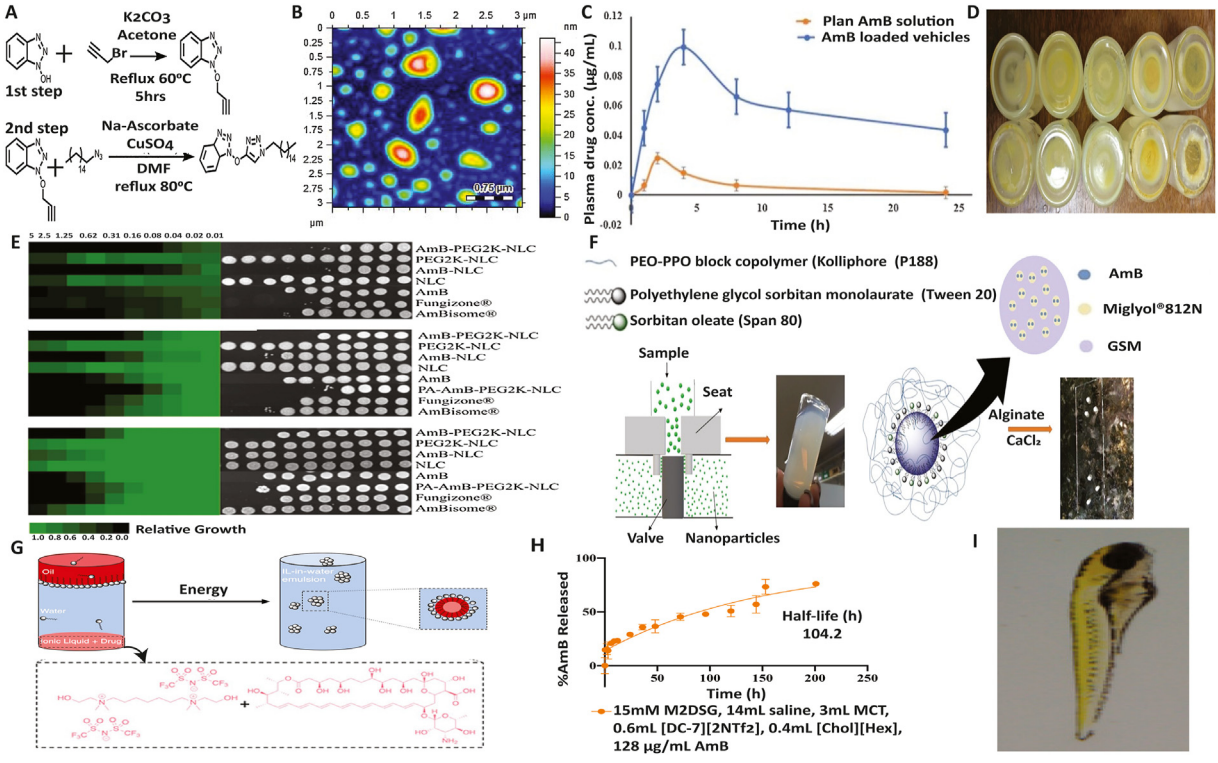

Figure 5 (A) Synthesis of triazole based nonionic surfactant (TBNIS). (B) AFM image of AB-loaded TBNIS based vesicles. (C) Plasma drug concentration of AmB-loaded vesicles and plan AmB solution after oral administration at the dose of 6 mg/kg body weight ($n = 6$, mean ± SE). Adapted with permission from Ref. 96. Copyright © 2020 Elsevier. (D) Photographic examination of AmB-PEG-NLC prepared by using different molecular weight PEG (from left to right: 1 K, 2 K, 5 K, 10 K, 20 K and the first row shows Day 1 and second row indicates Day 30) and (E) Concentration of AmB (µg/g) in rabbit ocular tissues after hourly instillation of 50 µL (150 µg AmB) AmB-PEG2K-NLC and AmBisome over 6 h, indicates that the AmB concentration for AmBisome and PEGylated NLC were statistically insignificant ($P < 0.05$). Adapted with permission from Ref. 110. Copyright © 2019 Elsevier. (F) Schematic presentation of dual alginate-lipid nanocarriers for oral delivery of AmB. Adapted with permission from Ref. 108. Copyright © 2020 Elsevier. (G) Formulation of ionic-liquid-in-water nanoemulsions for systemic delivery of AmB. (H) Microscope image of zebrafish exposed to the solution of ionic liquid ([DC-7][2NTf2]) indicate complete biocompatibility, and (I) *in vitro* AmB release from ionic liquid nanoemulsion over 201 h. Adapted with permission from Ref. 115. Copyright © 2020 American Chemical Society.

toxicity, low cost and high scale production, SLNs are superior to other drug delivery system. Furthermore, smaller size and lipid solubility allow SLNs to penetrate across biological barriers such as blood–brain barrier (BBB) and enable reduced uptake by reticuloendothelial system (RES)[99]. However, SLNs have their intrinsic disadvantages such as lipid crystallinity, particle growth and gelation affinity. To overcome these problems, a second generation of SLNs contained both solid and liquid lipids was developed, named as nanostructured lipid carriers (NLC), demonstrating advantages including reducing polymorphic transition, improving stability and increasing drug-loading capacity[100]. Lipid NPs are always well taken up by macrophages, making them good candidates as carriers for antifungal drugs[101]. In general, the SLNs developed for AmB aimed to increase solubility[102,103], improve oral bioavailability[104,105], promote permeability[106], and reduce toxicity[104,107].

To optimize formulation, various surfactants including Cremophore RH40, Tween-20, Poloxamer-407 (P-407) and Myrj-52 were employed to stabilize the SLNs and NLCs. It was found that only P-407 could stabilize the AmB lipid dispersion[103]. PEGylation may benefit the loading of AmB in NLCs. Recently, the AmB-encapsulated PEGylated NLCs was formulated by hot-melt emulsification followed by high-pressure homogenization[107]. The mPEG-DSPE of different molecular weights (PEG 1 K, 2 K, 5 K, 10 K, 20 K) was screened for NLC stability. The results showed that AmB-PEG2K-NLC prevented AmB leaching and significantly increased drug loading and entrapment efficiency up to 4.6% (*w*/*w*) and 92.7%, respectively[107]. Furthermore, the optimized AmB-PEG2K-NLCs showed comparable or enhanced antifungal activity against wild type and AmB resistant *C. albicans* than Fungizone and AmBisom with negligible toxicity in human retinal-pigmented epithelium cells (Fig. 5D and E)[107].

To improve oral bioavailability of AmB, Chaudhari et al.[104] prepared SLNs of AmB (AmbiOnp) by nanoprecipitation method. The *in vivo* pharmacokinetics of AmbiOnp in rats revealed 1-fold increase of relative bioavailability with C_{max} of 1109 ng/mL at 24 h that was comparable to the C_{max} of 1417 ng/mL achieved with Fungizone given intravenously. Moreover, the AmbiOnp were stable at varied gastrointestinal conditions (pH 1.2 to 7.4) and showed excellent storage stability for 3 months under refrigeration condition[104]. In another study, Senna et al.[105] prepared alginate hydrogel-loaded NLCs for targeted intestinal AmB delivery (Fig. 5F). The results uncovered that the release of AmB-loaded NLCs from the hydrogel was pH-dependent and was governed by the polymer swelling rate. The drug release was limited under acidic conditions, whereas a large number of intact NLCs showed release under intestinal circumstance[105].

2.5.3. *Nanoemulsions*

Nanoemulsions (NEs) of AmB have been designed to minimize side effects, reduce toxic potential and improve therapeutic efficacy because of their increased stability and safety as compared to liposomal formulations[108]. They hold a great potential of enhanced and sustained topical delivery of AmB. A novel cost-effective topical emulsion of AmB was developed, consisting of canola oil, hydroxypropyl methylcellulose (HPMC) Carbopol and Tween 80,[109]. *In vitro* antifungal activity showed the novel AmB emulsion had a great potential against *A. fumigatus*, *A. flavus, A. niger and Fusarium solani*. Sosa et al.[110] designed a NE formulation of AmB for the treatment of skin *candidiasis* and *aspergillosis*. They demonstrated that the NE was well tolerated by the skin and possessed an effective local antifungal effect without systemic absorption, assuring safe localized action. In addition, this novel AmB-loaded emulsion had the potential for large scale production for the treatment of topical fungal infections (Table 7). Hussain et al.[111] prepared various batches of AmB-loaded NEs comprised of sefsol-218 oil to evaluate the stability and permeation at varying pH and temperatures. Compared to AmB solution and Fungizone, the NEs showed enhanced permeation across rat skin with minimum nephrotoxicity, and the formulation was stable for up to 90 days at different temperatures regarding the globule size, PDI, zeta potential, optical density and pH[111].

Most recently, Esson et al.[112] proposed that ionic-liquid-in-water NE could allow intravenous delivery of various insoluble drugs. To address the hydrophobicity and self-aggregation associated severe side effects of AmB, a novel AmB-loaded ionic-liquid-in-water NE was formulated[112]. The absorption spectrum of AmB in ionic mixture and NE indicated its solubilization in a monomeric form[112]. The AmB-NE displayed high biocompatibility with zebrafish, little hemolytic activity and enhanced antifungal efficacy against *C. albicans in vitro* (Fig. 5G, H and I)[112]. It was argued that such NE could be used for intravenous injection; however, no results in terms of pharmacokinetics, biodistribution, and penetration into the infected site in animal model was demonstrated.

2.6. *Nanosuspensions*

Nanosuspensions are sub-micron colloidal dispersions of pure drug particles ranging from 1 to 1000 nm[113–115]. Nanosuspensions are widely studied due to their high drug loading, low toxicity and enhanced stability[20,116]. Compared with other delivery approaches nanosuspensions significantly improved drug's solubility, biodistribution, attenuate pharmacokinetics and enhance treatment efficacy[20,117]. In addition, nanosuspension formulation had lower cost over lipid-based formulations due to the absence of expensive phospholipids[118]. Previously, nanosuspension has been employed for drugs used for various diseases including cancer, inflammatory diseases, ophthalmic diseases, prevention of preterm birth, anti-bacterial actions, anthelmintic treatment, anti-viral and liver diseases[119,120]. Due to its promising drug loading ability (as high as 100%), nanosuspensions allow efficient drug delivery to the cells or tissues and induce potent therapeutic effects.

Various nanosuspension-based AmB formulations have been developed and administrated *via* intravenous or oral route[121,122]. In order to improve AmB solubility for oral administration, Zu et al.[123] prepared optimized 135-nm amorphous nanosuspensions of AmB by antisolvent precipitation and freeze-drying methods. The screened formulation demonstrated 13-fold enhancement in solubility and 2-fold increase in dissolution rate. Due to AmB instability, the oral bioavailability of nanosuspension was critically related on the preparation methods. Zhou et al.[124] prepared AmB-nanosuspension using high-pressure homogenization (HPH) and antisolvent precipitation (AP) methods. They studied the effect of preparation techniques on the solubility and bioavailability. Interestingly, the HPH-based nanosuspensions exhibited irregular-shape particles with a diameter of 200 nm and had a crystalline state, whereas those prepared using AP displayed sphere-like amorphous particles of size 60 nm. As a result of amorphous state and reduced diameter, the dissolution and saturation solubility of AP-based formulation were higher than those from HPH-

based one. Nonetheless, the HPH-based formulation demonstrated improved oral bioavailability over the AP-based one, whereas they both exhibited higher bioavailability than the marketed product (Fig. 6A).

For improved antifungal activity, van de Ven et al.[125] prepared a polyvinyl alcohol (PVA)-stabilized AmB nanosuspension with a diameter of approximately 150 nm *via* solvent-antisolvent precipitation. This nanosuspension demonstrated a profound reduction in the burden of *A. fumigatus* with 50% decrement of dose compared with Fungizone or AmBisome. The improved efficacy might be ascribed to the potential of the nanosuspension to deliver the drug specifically to the tissue compartment, and to be efficiently taken up by RES macrophages in the infected site, which consequently, acted as secondary reservoirs for sustained drug release over time[125]. Recently, to prevent the toxicity of AmB on the phagocytes and obtain effective drug loading, AmB nanosuspensions were encapsulated in erythrocytes. Interestingly, 4 h incubation with peripheral blood disclosed that over 98% of the peripheral phagocytes, granulocytes and monocytes, engulfed the nanosuspension-loaded RBCs. Moreover, the internalized drug-loaded leukocytes continually released AmB over 10 days with 1000-fold reduced toxicity. Efficacy studies demonstrated an immediate and durable intra and extracellular antifungal activity[126]. The cell-mediated delivery opened an avenue to fight fungal infections and might inspire an improved antifungal treatment approach.

Considering the extremely high loading ability, AmB nanosuspensions were prepared to combat local fungal infection[127,128]. For instance, Jansook et al.[127] developed a γCD-AmB complex-coated 400-nm nanosuspensions of AmB. To offer long-term extraocular exposure, Das et al.[128] fabricated cationic AmB nanosuspension stabilized with Eudragit by solvent-displacement method to deliver the drug to the ocular mucosa. The prepared nanosuspensions had a size of 150−290 nm and 6-month stability. Furthermore, the sustained drug release from the nanosuspensions in a 24 h period was obtained and it showed a comparable antifungal efficacy to free-drug formulation with little irritation to the rabbit eyes.

To fight against vaginal mucosal infection, a 247-nm AmB-nanosuspension with a rod-like structure was prepared by high-pressure homogenization and was loaded in a thermogel based on Poloxamer P407 for vaginal administration (Fig. 6B)[129]. The gel enabled sustained drug release over a 12 h period and prolonged the drug retention in the vaginal tissue for over 12 h. The gel demonstrated reduced vaginal inflammation and improved anti-candida activity in a candidiasis-induced model when administered at a reduced dose in comparison with marketed effervescent formulation[129].

According to the reviewed literature, we can conclude that nanosuspensions offer a promising alternative for AmB delivery *via* different routes including oral, parenteral and mucosal. The properties of the nanosuspension and the subsequent *in vivo* performance are dependent on the preparation technique which in terms can be tailored to achieve the desired characteristics including particle size, surface charge, and surface coating that best fit the intended route of administration.

2.7. *Metal-based NPs*

Several metal-based DDSs such as silver[130] and magnetic NPs[131] were utilized to improve AmB delivery and achieve synergistic anti-fungal activities. AmB and silver hybrid NPs with a size of

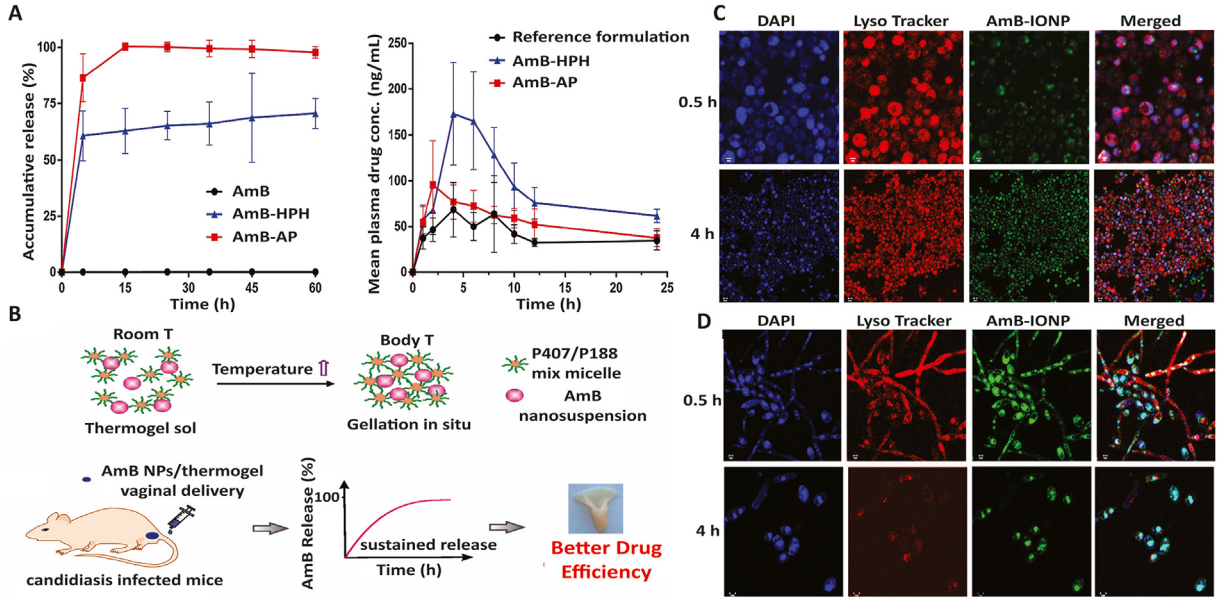

Figure 6 (A) The AmB-loaded nanosuspension prepared by high-pressure homogenization method and an antisolvent precipitation method. The relative bioavailability of AmB-HPH was higher than AmB-AP in male SD rats. Adapted with permission from Ref. 127. Copyright © 2018 Elsevier. (B) Preparation of AmB nanosuspension loaded thermogel for vaginal delivery. The AmB was released in a sustained manner, leading to improved antifungal activity. Adapted with permission from Ref. 132. Copyright © 2018 Taylor & Francis. (C) CLSM images of cellular uptake of BSA coated AmB loaded iron oxide NPs (AmB-IONP) in *C. glabrata.* (D) CLSM images of cellular uptake of BSA coated AmB loaded iron oxide NPs (AmB-IONP) in *C. albicans*. Both (C) and (D) indicated the endolysosomal localization with colocalization of AMB-IONP (green) and lysotracker in red and time-dependent uptake was maximum at 4 h (X = 60 and scale bar was 1 μm for 0.5 h and 2 μm for 4 h. Adapted with permission from Ref. 134. Copyright © 2020 MDPI.

7 nm were prepared by coating the silver NPs with AmB. Such a coating process helped prevent the silver NPs aggregating and inhibited AmB's aggregation, thereby facilitating its binding to the fungal ergosterol and increasing its antifungal activity[130]. Due to the antibacterial features, the usage of hybrid NPs conferred synergistic effects against *C. albicans* specie[130]. Another report indicated the synergistic effect of AmB and silver NPs after their coadministration[132]. Besides, it assumed that AmB acted as a surfactant to stabilize the NPs and had a robust association with ergosterol in the fungal membrane, consequently allowing the NPs to penetrate inside the infected sites and release the drug over time[131].

BSA-coated AmB-loaded iron oxide NPs were prepared through layer-by-layer approach for targeted delivery of AmB to treat life threatening fungal infection such as cryptococcal meningitis. The NPs exhibited a size smaller than 36 nm and had a drug loading capacity of up to 13.6 ± 6.9 μg of AMB/mg of NPs. The *in vitro* release study indicated initial burst release at the first 3 h, followed by sustained release over 72 h period. Importantly, compared with marketed AmB-deoxycholate formulation the NPs displayed time-dependent and 16−25-fold increase of cell uptake in *Candida glabrata* and *C. albicans* clinical isolates, and achieved promoted treatment efficacy (Fig. 6C and D)[131]. The targeted and controlled release of AmB can be achieved as well by coating of biocompatible and biodegradable polymers such as PLGA, PEG and PVP on AmB-loaded silver NPs[133].

2.8. *Microneedles*

Microneedle technology is becoming a robust technique to improve transdermal drug delivery by overcoming the skin barrier *via* creating microscale pores in the stratum corneum layer of the skin[134,135]. Zan et al.[136] developed polymeric microneedles by using biodegradable chitosan-polyethylenimine copolymer for treating subcutaneous fungal infection. The microneedles showed antimicrobial effects and sustained drug release kinetics. Importantly, the AmB-encapsulated microneedles demonstrated excellent antifungal effectiveness and synergistic effects of antifungal polymer and AmB (Fig. 7A−D). Similarly, Givi et al.[137] fabricated AmB-encapsulated polymeric microneedle system by using biodegradable PVP (polyvinylpyrrolidone) and MAA (methacrylic acid) for skin fungal infections.

Besides the skin applications, microneedles technology has also been implemented for ocular delivery. Roy et al.[138] synthesized AmB-loaded microneedles ocular patch (MOP) using a dissolvable polymeric matrix, composed of PVA and PVP for treating fungal keratitis. The corneal permeation studies revealed that MOP possessed prolonged AmB = corneal retention compared with AmB solution and liposomal AmB formulation. After insertion in cornea, the microneedles got softened within 15 s and completely dissolved within 60 s, and the baseplate of MOP also become softer as the time progresses (Fig. 7E). Moreover, the MOP significantly decreased the *C. albicans* loaded in the cornea as well as in rabbit infection model. The

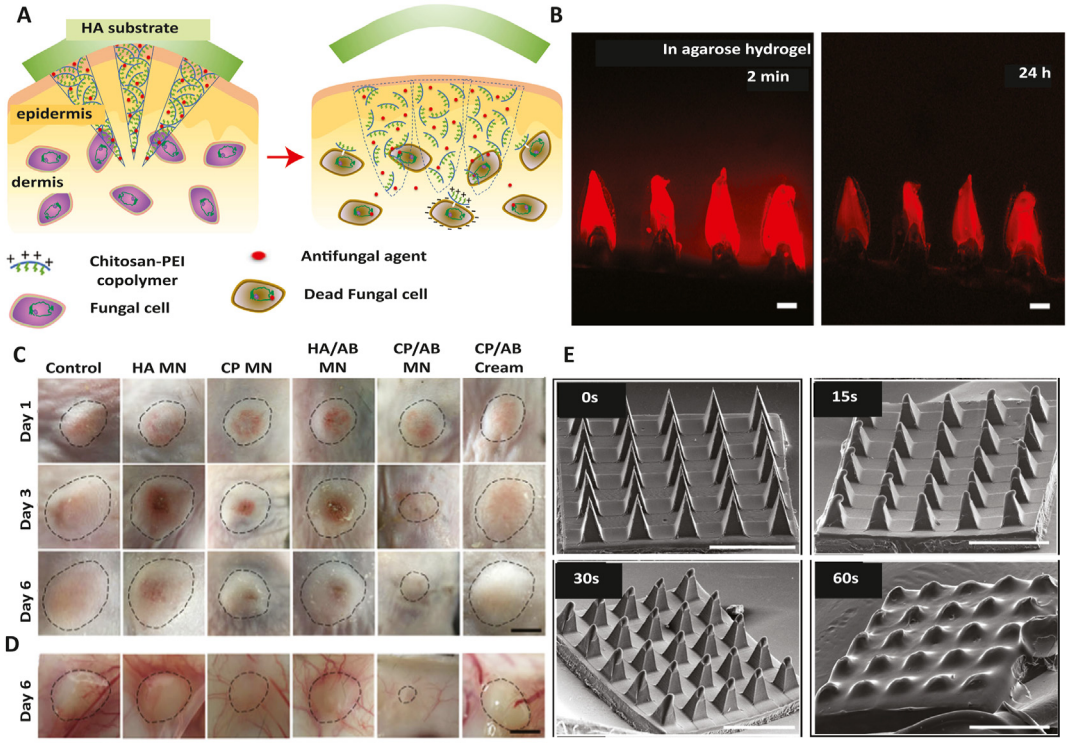

Figure 7 (A) Schematic description of AB loaded antimicrobial microneedle patch (CP/AB MN) prepared by chitosan-polyethylenimine copolymer (CP) for subcutaneous fungal infection. HA, hyaluronic acid. (B) CLSM images of Cy5 released from CP microneedles in agarose gel at 2 min and after 24 h, respectively (scale bar = 200 μm). (C) Photograph of fungal infection sites after applying different microneedle patches on Days 1, 3, 6, and (D) images taken to observe skin underneath infection on Day 6. Adapted with permission from Ref. 139. Copyright © 2019 John Wiley & Sons. (E) The SEM images showed dissolution of AmB loaded microneedle ocular patch prepared by dissolvable polymeric matrix (polyvinyl alcohol and polyvinyl pyrrolidone) after insertion in excised cornea at 0, 15, 30 60 s (scale bar = 1 mm). Adapted with permission from Ref. 141. Copyright © 2019 Elsevier.

histopathological evaluation revealed improved epithelial and stromal differentiation of corneal membrane.

3. Marked formulations

3.1. AmB deoxycholate (AmBd)

The micellar dispersion of AmB and sodium deoxycholate (deoxycholate AmB) is the first marketed AmB formulation specifically intended to treat life-threatening fungal infections induced by *Aspergillus*, *Candida*, *Cryptococcus neomorfans*[139]. The representative marketed product is FUNGIZONE®[140], in which sodium deoxycholate is employed as a solubilizer to overcome the poor aqueous solubility of AmB. However, despite its values for treating IFIs, adverse effects including, headache, fever, hypotension, dyspepsia and pain at the injection site are reported[141]. Particularly, nephrotoxicity is the most serious adverse effect that could lead patients to dialysis or even death[142].

3.2. AmB lipidic formulations

AmB has a tendency to bind with cholesterol of mammalian cells and induce toxicity particularly in kidney cells, rich in cholesterol. Owing to the increased toxicity with conventional AmBd, less toxic alternative delivery system, such as liposomes (AmBisome®), and lipidic complexes (Amphotin LIP®, Abelect®) have been developed[143–145]. These new AmB-delivery systems are designed to achieve low AmB serum concentration, while increasing the concentrations in targeted tissues, to reduce the nephrotoxicity.

3.2.1. AmB lipid complex (ABLC)

ABELCET® is an AmB lipid complex containing AmB conjugated with two phospholipids (DMPC and DMPG, 7:3) in a 1:1 drug-to-lipid molar ratio. ABELCET® is a second-line treatment agent for severe systemic mycosis, particularly for refractory and resistant patients.

ABELCET® is a sterile suspension for i.v. infusion. Compared to AmBd, ABELCET® is more tolerable, allowing administration at high doses (0.6–5 mg/kg/day)[146]. Transient chills and fever during infusion are common side effects with ABELCET® and could require hospitalization and medical supervision. Moreover, attention should be paid that anaphylaxis has been reported with AmB-containing formulations. In this case, the infusion should be immediately discontinued.

3.2.2. Liposomal AmB (L-AmB)

Liposome is a class of DDS that has opened several new possibilities in improving target to fungal treatment, increasing the affinity between AmB and ergosterol and reduction of body damage[147]. In L-AmB, AmB is intercalated into liposomes consisting of hydrogenated soy phosphatidylcholine and cholesterol.

L-AmB is usually used in febrile or neutropenic patients due to fungal, *Aspergillus* infections, *Candida* infections, *Cryptococcus* infections and refractory to AmBd[148–150]. L-AmB is less toxic than ABELCET® and AmBd[146,151]. In addition, L-AmB induces comparatively less adverse effects to the ABLC[8]. However, liver function should be closely monitored during the medication because the drug concentration in the liver is higher than the spleen and kidney, which could induce higher liver damage compared to common formulations[152,153].

3.3. AmB colloidal dispersion (ABCD)

AMPHOTEC® is one of the colloidal dispersions prepared based on the affinity between AmB and sterol. It consists of AmB and cholesteryl sodium sulfate at 1:1 M ratio. It also includes monohydrate lactose, hydrochloric acid, tromethamine and disodium edetate as excipients. In spite of its nephrotoxicity, ABCD is still better than AmBd, considering its stable blood concentration and higher security[154]. It is usually used for treating IFIs especially for patients who cannot receive conventional AmBd because of the severe kidney damage. Similar to ABELCET® and FUNGIZONE®, AMPHOTEC® may initiate anaphylactoid acute reactions, however, can be compromised by administering antihistamines and adrenal corticosteroids[155]. Additionally, some AmB-listed products or products being clinically developed are summarized in Tables 5–7.

4. Outlook and perspectives

The number of immunocompromised patients is significantly increasing as a consequence of increased cancer patients treated with the cytotoxic chemotherapeutic drugs that result in immunodeficiency. Moreover, patients subjected to organ transplant, catheterization, dialysis, complex surgeries, HIV positive and under intensive care treatment are at high risk of fatal fungal infection[156,157]. In this side, the most frequent yeasts isolated from IFIs patient are *Candida*, *Aspergillus* and *Cryptococcus* spp., along with increased prevalence of *Fusarium*, *Scedosporium*, *Penicillium* and *Zygomycetes* spp[158,159]. A huge burden of fungal infections and occurrence of antimicrobial resistance calls for the development of more efficacious and cost-effective antifungal therapy. The therapeutic arsenal to tackle IFIs is limited to a few antifungal drugs, mainly polyenes and azoles (triazoles)[160]. Of those, AmB represents a broad-spectrum membrane-acting polyene antibiotic. There is no doubt that the value of AmB for combating fungal infections is unparalleled to the other available drugs in market. However, the biggest constrain for its clinical use is the intrinsic host toxicity accompanying its systemic administration. Intravenous AmB administration of the conventional parenteral AmB-deoxycholate formulation is accompanied by acute side effects including nausea and fever in addition to dose related renal toxicity occurring in up to 50%–90% of the patients. This necessitates patient hospitalization to facilitate critically monitoring the drug plasma level. Accordingly, there is a critical need for the design of delivery systems of AmB that improve the therapeutic index and reduce the toxicity. Looking at the clinical landscape revealed that there are several formulations that have been designed for that purpose. Lipid-based formulation as AmB lipid complex (Abelcet) and liposomal AmB (AmBisome), were developed and approved for clinical use as effective and less toxic options compared to Fungizone[5]. They show different pharmacokinetic parameters depending on particle size. The lipid complex being of larger size is taken up rapidly by macrophages in the liver, spleen and lungs showing less nephrotoxicity while liposomal AmB is being of smaller size and negative charged, shows higher peak plasma levels and less infusion related reactions. However, still the low drug loading and the i.v. administration compromise patients' compliance. For that reason, a lot of research efforts were directed toward the development of safer and more effective formulations for AmB harnessing the power of nanocarriers at that regards. This includes polymeric NPs, poly-

Table 5 List of the approved AmB formulations.

Country	Brand name	Formulation/dosage form	Dose	Note
France	Fungizone	Suspension/oral	10%	IFI and dermatomycoses
		Capsules/oral	250 mg	
		Topical	3%	
		Injectable/injection	50 mg	
Germany	Ampho-Moronal	Tablets/oral	100 mg	IFI and dermatomycoses
		Suspension/oral	10%	
		Topical	3%	
Italy	Fungizone	Injectable/injection	50 mg/15 mL	IFI
USA	Fungizone	Injectable/injection	50 mg	IFI, cutaneous and mucocutaneous *candida* infection
		Cream/topical	3%	
		Lotion/topical	3%	
		Suspension/oral	100 mg/mL	
	AmB	Injectable/injection	50 mg/vial	IFI, American mucocutaneous leishmaniasis
	Abelcet	Lipid complex/injection	5 mg/mL	IFI
	Amphotec	Lipid complex/injection	50 mg/mL 100 mg/mL	IFI
	Ambisome	Injectable, liposomal/injection	50 mg/vial	IFI, presumed fungal infection, visceral leishmaniasis (VL)

Table 6 The development status of AmB-active.

Country	Phase	Formulation/dosage form	Brand name	Note
Switzerland	Phase II	Cream/topical	Anfoleish	Cutaneous leishmaniasis
	Preclinical	Oral	AmB, polytherics	Leishmaniasis
	Registered	Unspecified	AmB + meglumine antimoniate	Visceral leishmaniasis
	Preclinical	Oral	AmB, polytherics	Leishmaniasis
Canada	Phase I	Capsule/oral	iCo-019	HIV, VL
USA	Preclinical	Tablet/oral	NM147	Leishmaniasis
	Phase II	Suspension/oral	MAT-2203	VL, IFI

Table 7 The development status of AmB-No development reported.

Country	Phase	Description	Brand name	Note
Israel	–	A topical ethanolic formulation	Hadasit	Cutaneous leishmaniasis
Argentina	–	A lyophilized reformulation	Anfonax	IFI and leishmaniasis
USA	Phase I	Powder for inhalation	NKTR-024	Pulmonary fungal infections
	Phase III	An intranasal, encochleated formulation	AmB, BDSI	Chronic rhinosinusitis
	Preclinical	A targeted formulation using magnetic targeted carrier	MTC-AmB	IFI
	Preclinical	Glycopolymer	AP-1110	IFI

aggregated formulations, conjugates, micelles, niosomes, emulsions, nanosuspensions, metal-based NPs and microneedles. As discussed in this review, those were designed with different purposes as increasing solubility and improving pharmacokinetics, and thereby enhancing therapeutic efficacy. Others were designed to improve bioavailability facilitating oral administration. However, in designing the AmB-oral formulation, few points must be considered. Firstly, prior to intestinal absorption, the NPs must diffuse across the mucus barrier[161]. The negative electrostatic mucin interacts with positively charged NPs to pass the mucus layer and encourage internalization by epithelial cells[161]. Consequently, the positively-charged NPs demonstrated better tendency to cross the mucus more effectively. In line with this, positively charged chitosan is used as building block for nanoparticle preparation or to coat NPs intended for oral administration. Secondly, NPs can cross the intestinal-barrier by transcellular transport *via* M-cells or enterocytes or by paracellular transport. During enterocyte, depending on the size of NPs, they can be phagocytized by different endocytic pathways including caveolae-mediated endocytosis or clathrin-mediated endocytosis or caveolae and clathrin-independent endocytosis[162]. So, surface charge and size of NPs are critical for their transport mechanism, which directly influence the drug bioavailability after oral administration[163,164]. For localized therapy and improved antifungal activity, various nanocarriers have been proposed for ocular, vaginal and dermal delivery[107,165,166]. These delivery systems allowed noninvasive administration of AmB with active targeting and immunomodulation to obtain excellent efficacy in IFIs.

A promising strategy already validated for cancer treatment is combining different drugs in the same carrier at a synergistic ratio,

with the aim to maintain a precise synergistic drug concentrations of combined drug, *in vivo*[21,160]. For instance, a multi-drug combination of tamoxifen with AmB is used for the treatment of *C. neoformans* infection. A combination of bacillomycin-D with AmB displayed anti-biofilm and wound-healing activities against *Candida*-associated infections showed promising antifungal results[167,168]. However, the development of optimized nanocarrier for the codelivery of these combinations still needs to be figured out.

5. Conclusions

AmB is the drug of choice for more than 50 years and is considered as the gold standard for treating systemic fungal infections without emergence of significant resistance. Its clinical use is hampered by its poor solubility, poor pharmacokinetics and severe nephrotoxicity that arises from its binding to the to the cholesterol rich cell membrane of mammalian cells particularly in the kidney. Accordingly, research efforts have been directed towards the development of nanocarrier-based DDS that improve the targetability to macrophages in order to reduce the side effects and provide a reservoir for the drug release at the site of infection. Other approaches proved success for localized treatment of skin, ocular or vaginal fungal infections eliminating the need for systemic administration and improving the antifungal efficacy. While lipid-based formulations are the first to be developed for improving the pharmacokinetic properties of AmB, they still face some limitations. Other nanocarriers as nanosuspensions and polymer-based systems can be tailored to provide improved efficacy while limiting the drug associated toxicity. Importantly, the scaling up of these formulations should be carefully investigated and their efficacy and safety associated clinical data must be monitored.

For the oral route, despite the promising research efforts, the AmB-oral formulation has not been translated from bench to bedside, perhaps due to the complexity of new formulation in preclinical stage as well as a comprehensive requirement that must be fulfilled. These requirements may include complete preparatory method, efficacy, biodistribution, safety profile, cost-effectiveness and setting up a public-private partnership to permit access to the diseased population in developing countries. Finally, other antifungal drug arsenal must be achieved by discovering new compounds, novel targets, optimization of azole structure and developing novel traditional Chinese medicine.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Nos. 81872823, 81871477 and 82073782), the Double First-Class (CPU2018PZQ13, China) of the China Pharmaceutical University, the Shanghai Science and Technology Committee (No. 19430741500), the Key Laboratory of Modern Chinese Medicine Preparation of Ministry of Education of Jiangxi University of Traditional Chinese Medicine (TCM-201905, China), the Guangdong Basic and Applied Basic Research Foundation, China (No.2020A1515010593), and the Fundamental Research Funds for Central Universities (No.20ykpy111, China).

Author contributions

Wei He conceived and designed the work. Xiaochun Wang, Imran Shair Mohammad, Lifang Fan, Marwa A. Sallam, Jun Wu, Zhongjian Chen, and Wei He co-wrote the paper. Wei He, Lifang Yin, Md Nurunnabi, and Marwa A. Sallam commented and corrected the paper. All of the authors have read and approved the final manuscript.

Conflict of interest

All the authors declare that this article content has no conflict of interest.

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