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Micro-nanoemulsion and nanoparticle-assisted drug delivery against drug-resistant tuberculosis: recent developments

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SUMMARY Tuberculosis (TB) is a major global health problem and the second most prevalent infectious killer after COVID-19. It is caused by *Mycobacterium tuberculosis* (*Mtb*) and has become increasingly challenging to treat due to drug resistance. The World Health Organization declared TB a global health emergency in 1993. Drug resistance in TB is driven by mutations in the bacterial genome that can be influenced by prolonged drug exposure and poor patient adherence. The development of drug-resistant forms of TB, such as multidrug resistant, extensively drug resistant, and totally drug resistant, poses significant therapeutic challenges. Researchers are exploring new drugs and novel drug delivery systems, such as nanotechnology-based therapies, to combat drug resistance. Nanodrug delivery offers targeted and precise drug delivery, improves treatment efficacy, and reduces adverse effects. Along with nanoscale drug delivery, a new generation of antibiotics with potent therapeutic efficacy, drug repurposing, and new treatment regimens (combinations) that can tackle the problem of drug resistance in a shorter duration could be promising therapies in clinical settings. However, the clinical translation of nanomedicines faces challenges such as safety, large-scale

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production, regulatory frameworks, and intellectual property issues. In this review, we present the current status, most recent findings, challenges, and limiting barriers to the use of emulsions and nanoparticles against drug-resistant TB.

KEYWORDS *Mycobacterium tuberculosis*, TB, nanotechnology, drug resistance, MDR, XDR, antibiotics, nano-carriers

INTRODUCTION

Tuberculosis (TB) is a serious global problem that continues to pose a challenge as an epidemiological disease because of its drug-resistant form. After COVID-19, TB is the second most prevalent infectious killer worldwide and the 13th leading cause of mortality (1). It is a potentially life-threatening infectious disease caused by the airborne bacterium *Mycobacterium tuberculosis* (*Mtb*) (2–5). *Mtb*, the most well-known mycobacterium, infects one-third of the population across the globe (6). The morbidity of this mycobacterium continues and has become increasingly challenging to treat because of drug-resistant mechanisms (2, 7–9).

In 1993, the World Health Organization (WHO) declared TB a global health emergency (10). A 6-month standard drug regimen can successfully treat around 85% of patients infected with TB (11). In a phase III clinical trial (ClinicalTrials.gov Identifier: NCT02410772), a new 4-month regimen [rifapentine (RPT)–moxifloxacin regimen] was developed against drug-resistant TB. A 4-month course of daily 1,200 mg rifapentine and 400 mg moxifloxacin was as effective as the standard 6-month treatment regimen for curing tuberculosis (12).

Between 2019 and 2021, the expected number of TB deaths worldwide increased. Among HIV-negative and HIV-positive TB patients, an estimated death increment of 1.4, 1.5, and 1.6 million was observed in 2019, 2020, and 2021, respectively. According to an estimated report for 2021, multidrug-resistant/rifampicin-resistant (MDR/RR)-TB incidents increased from 2015 to 2020 (1). According to the AMR Review (2016), drug resistance will cause 10 million deaths annually by 2050, but approximately 25% will be from drug-resistant TB forms (13).

Continuous drug exposure leads to mutational changes in the *Mtb* genome. Mutation is a natural and evolutionary process, but it can be optimized and accumulate in the antibiotic-exposed *Mtb* genome due to prolonged drug exposure, poor patient compliance, and improper use of antibiotics (14–17). The four backbone drugs isoniazid (INH), rifampicin, ethambutol, and pyrazinamide are categorized as first-line drugs (18) which are highly effective against drug-susceptible TB, and the clinical treatment is categorized as first-line TB chemotherapy (19).

The treatment of mono-resistant or RR, MDR, extensively drug-resistant (XDR), and totally drug-resistant (TDR) TB is therapeutically challenging (2, 20, 21). TB chemotherapy is still in use, but the failure of first- and second-line drugs such as fluoroquinolones has pushed researchers to develop more powerful drugs and novel drug delivery systems to combat drug resistance (22, 23).

Combating the current drug-resistant TB forms with newly discovered drugs is insufficient; however, at the clinical level, novel drug delivery is a strategic and challenging task. Scientific revolutions in drug delivery have become a hot topic because they can improve the therapeutic efficacy of drugs loaded into nanocarriers. In this regard, the discipline of nanomedicine, using the concept of nanotechnology, has proven novel and robust. Nanotechnology shows great promise for the diagnosis, treatment, and prevention of infectious diseases such as TB (24). Targeted, site-specific, and precise drug delivery using nanotechnology techniques and principles has made it a valuable drug delivery system (25, 26). Safety, potency, biocompatibility, biodegradability, non-biotoxicity, and non-immunogenicity are the considerable advantages of nanodrug delivery (27). Since the emergence of critical cases of drug-resistant TB in several countries, scientists have become inclined toward nanotechnology-based therapies. Nanotechnology and its implementation offer great opportunities for treating infectious diseases

(such as TB), developing new nanomedicines, and unlocking their potential (28, 29). The importance of nanomedicines in improving TB treatment includes short treatment duration, multiple drug delivery, reduced drug side effects, and improved therapeutic efficacy (30, 31).

This review discusses the current advancements in micro (nano) emulsions and nanoparticle (NP)-assisted drug nanodelivery and covers some basic concepts related to TB and nanotechnological interventions. Drug discovery and regimen improvement are critical tasks for treating drug-resistant TB. Most of our attention has been focused on the current state, most recent discoveries, and challenges against drug-resistant TB.

BIOLOGY OF MYCOBACTERIUM TB

Mtb is an acid-fast tubercle bacillus. It is an intracellular mycobacterium and a slow-growing pathogen, and its width and length are between 0.2–0.5 and 2–4 µm, respectively (32). It is a Gram-positive bacterium with a high percentage of guanine-cytosine content. Tubercle bacilli are characterized by slow growth, dormancy, complex cell architecture, intracellular pathogenicity, and genetic homogeneity (33). Its cell wall contains three types of biopolymers—arabinogalactan-mycolate, peptidoglycan, and trehalose dimycolate—associated with pathogenicity (34, 35).

CO-INFECTION OF TB WITH OTHER DISEASES

Few reports or case studies have been published on the link of TB with infectious diseases, including HIV (36, 37), malaria (38, 39), and COVID-19 (40–42), and noninfectious diseases, including chronic obstructive pulmonary disease (43, 44), diabetes mellitus (45, 46), head-neck cancer (47–49), and lung cancer (50, 51). Owing to the COVID-19 pandemic, global efforts to tackle diseases such as HIV, TB, and malaria have derailed care with devastating impacts (52, 53).

DISEASE TRANSMISSION AND PATHOGENESIS

From pathogenesis to transmission, all events can be divided into major events: infected droplet inhalation, innate immune response, adaptive immune response, immune evasion-provocation mechanisms, granuloma formation, and infected droplet (airborne particles or bio-aerosols) exhalation for disease transmission from infected to healthy individuals. A person inhales infectious droplet nuclei (1–5 µm), and to reach the lung alveoli, they pass through the mouth or nasal cavity, the upper respiratory system, and the bronchi (54). Once tubercle bacilli enter the alveolar region, immune responses are initiated by alveolar macrophages (AMs), which internalize mycobacterial cells via receptor-mediated phagocytosis by pattern recognition receptors and various other receptors (55). Host defense against mycobacteria is assisted by innate immune mechanisms that play crucial roles (56).

A murine model suggested that AMs are major targets of early infection with *Mtb* (57). Macrophages and dendritic cells are mainly involved in both the pathogenesis and host defense mechanisms of TB; however, Chuquimia et al. (58) identified alveolar epithelial cells (AECs) as another key player and confirmed their role in *Mtb* internalization. They also play crucial roles in communication between various types of lung cells during innate and adaptive immunity (58). Delayed $CD4^+$ T-cell priming precedes bacilli infection and multiplication in the pulmonary lymph nodes before the adaptive immune system mounts an effective response (59). AECs, which act as direct barriers and first responders to bacilli, are sensed by the pattern recognition receptors (60). Infected AMs (IAMs) migrate to the lung interstitium, subsequently enabling the early dissemination of bacteria to the lymph nodes and initiating Th1 priming in an IL-1R-dependent manner. These responses promote the development of Th17 immunity, fostering neutrophilic inflammation and increasing bacterial replication, suggesting greater transmissibility by bacterial escape into the airways. These mechanisms can lead to lung infections, granuloma formation, and *Mtb* infection (61). These granulomas provide a niche for

intracellular growth, replication, and persistence of the latent form of TB (Fig. 1). A granuloma cannot control the infection if the bacterial load increases excessively, and the infection will then disseminate to other organs. Bacteria can enter the bloodstream and return to the respiratory tract, which can be transmitted to an uninfected person. This form of TB is both active and symptomatic (62, 63); therefore, the outcome of *Mtb* infection in the lungs depends on the balance between host immune response and bacterial evasion strategies. Understanding the complex interactions between *Mtb*, the epithelium, and immune cells is important for further exploration (60). The complete mechanisms of transmissibility, pathogenicity, and immunogenicity remain poorly understood (64, 65).

DRUG-RESISTANT FORMS OF TB

Drug-susceptible TB (DS-TB) and drug-resistant TB (DR-TB) are two epidemic forms of TB in several countries (66). DS-TB can turn into DR-TB due to antibiotic stress on the bacilli for longer during patient treatment. Global TB patient cohorts can be categorized into these two TB forms. During treatment, proper patient compliance is essential. When the drugs are lipophilic, their solubility, bioavailability, and distribution determine their therapeutic efficacy. Body weight and drug dose are related to patient compliance. For example, an inadequate amount of rifampicin (<9 mg body weight/day) can lead to treatment failure and cause drug-resistant TB (67). According to the WHO annual reports (1) and other literature, various forms of drug resistance have been reported: monoresistant TB (Hr-TB, RR-TB), MDR-TB, XDR-TB, and TDR-TB with their severity increasing in that order (2, 68–70). Recently, the WHO updated the definitions of pre-XDR-TB (MDR/RR-TB + resistant to any fluoroquinolone) and XDR-TB (MDR/RR-TB + resistant to any fluoroquinolone + at least one additional group A drug). The most potent second-line drugs were

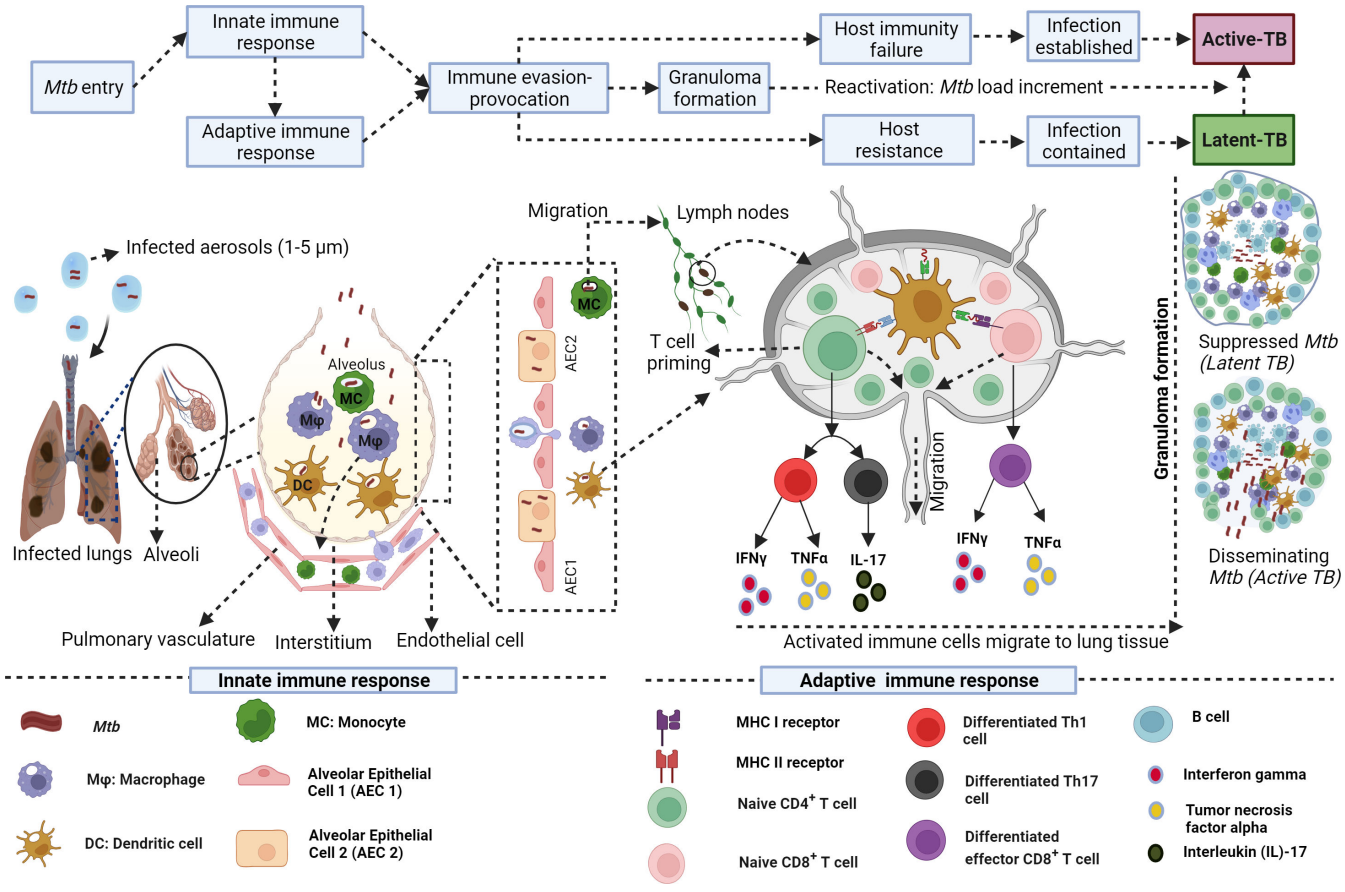

FIG 1 Tuberculosis transmission and pathogenesis.

categorized into group A (71). Mutations in the drug target genes of drug-susceptible bacilli are responsible for the evolution of drug-resistant TB (2, 20, 72, 73). The first case of extremely drug-resistant tuberculosis (XXDR-TB) was reported in two patients in Italy. Iranian researchers coined the name "TDR-TB" to describe the "XXDR-TB" strain (69). These extreme forms of TB were later found in India and South Africa (68, 74, 75). Treating DR-TB is complex and strategic because disease diagnosis and treatment strategies remain challenging (76). Both the mode of action of drugs and an improved understanding of drug resistance mechanisms could play crucial roles in the fight against drug-resistant forms of TB (77). Table 1 represents first-line drug resistance reports.

BEHAVIOR OF MDR/XDR/TDR-TB WITH MODERN DRUGS

The treatment of MDR/XDR/TDR-TB at the clinical level is challenging and requires advanced management and facilities. Patients with HIV infection are more likely to acquire drug-resistant TB that is challenging or difficult to treat. A systematic review and meta-analysis (88) recently reported an increase in the risk of MDR-TB among HIV-infected individuals. Currently, WHO guidelines are used to diagnose and treat drug-resistant forms of tuberculosis (89), where chemotherapy (combination therapy) based on the patient's condition and severity is still relied on, using first-line, second-line, and newly developed and repurposed drugs (Table 2). Modern anti-TB drugs (ATDs) and repurposed drugs are especially M/XDR-TB centric. Mutations in clinical strains of *Mtb* could play a crucial role in the evolution of various types of DR-TB (20). Drug resistance has been reported in modern drugs against clinical isolates of both M/XDR-TB. In a clinical study, a repurposed clofazimine (CFZ) resistance was detected in clinical isolates from patients with M/XDR-TB. This study did not link clofazimine resistance to bedaquiline or clofazimine therapy (90). Many research articles and reviews have been published on clofazimine and bedaquiline resistance over the past 10 years in countries such as China, Taiwan, Bangladesh, Brazil, India, Zambia, South Africa, Lithuania, Pakistan, South Korea, the Philippines, Thailand, Turkey, Vietnam, and the United States (91–98). Ismail et al. (93) reported that bedaquiline resistance is linked to poor patient compliance (93). Du et al. (99) observed a low rate of linezolid resistance in patients with MDR-TB receiving bedaquiline-linezolid (99). A randomized multicenter clinical study on MDR-TB was designed using a novel treatment regimen that included delamanid, linezolid, levofloxacin, and pyrazinamide. This study revealed that a shorter regimen could effectively combat MDR-TB (100). At the clinical level, most modern drugs approved by the FDA and WHO are recommended for drug resistance. In particular, the clinical history of TB patients can be used before initiating the drug regimen for a better cure; in the case of DR-TB forms, it could be a safeguard for patients without delaying treatment.

NANODRUG DELIVERY OF OLDER, NEW, AND REPURPOSED DRUGS

To increase the therapeutic efficacy of a drug, many researchers have chosen to use nanodrug delivery carriers. Anti-TB medications can be classified into three categories based on the drug(s) to be nanodelivered: existing drugs (first and second line), newly approved drugs (pretomanid, bedaquiline, and delamanid), and repurposed drugs (linezolid, clofazamine, and meropenem/clavulanate) (96, 101). After the nanodelivery of existing drugs, a few scientists explored the novel nanodelivery of repurposed drugs,

TABLE 1 First-line drug resistance report in *Mtb*

Anti-TB drugs	Discovery year	References	Resistance reported year	References
p-Aminosalicylic acid	1943	(78)	1950	(79)
Streptomycin	1944	(2)	1950	(79)
Isoniazid	1952	(2)	1993	(80)
Rifampin	1965	(81)	1970	(82, 83)
Ethambutol	1961	(84)	1978	(85)
Pyrazinamide	1952	(86)	1967	(87)

TABLE 2 Treatment options for non-resistant TB, XDR-TB, MDR-TB, and TDR-TB based on WHO guidelines (2022)[a]

TB forms	ATD regimens	Duration	Remarks
Non-resistant TB	2HRZE/4HR	6 months	A strong recommendation, also applicable for extrapulmonary TB, except CNS, bone, or joint TB, because expert groups suggested longer therapy.
	2HPMZ/2HPM	4 months	Conditional recommendation (new), people aged ≥12 years.
	2HRZ(E)/2 HR	4 months	A strong recommendation (new) for non-severe TB between 3 months and 16 years of children and adolescents.
Hr-TB	6(H)REZ	6 months	Conditional recommendation
MDR/RR-TB/ pre-XDR-TB	Bedaquiline, pretomanid, linezolid, and moxifloxacin (BPaLM)	6 months	Conditional recommendation (new), except for the CNS, osteoarticular, and disseminated (miliary) TB, for people aged ≥14 years and regardless of HIV status. BPaL without moxifloxacin would be initiated or continued.
	All-oral bedaquiline-containing regimens	9 months	Conditional recommendation (new): patient with MDR/RR-TB and without resistance to fluoroquinolones.
XDR-TB	No standard regimen	Longer treatment	Serious clinical conditions need more clinical trials and a proper treatment regimen not defined by WHO. Clinical trials are ongoing.
TDR-TB	No standard regimen	Longer treatment	Serious clinical conditions need more clinical trials and a proper treatment regimen not defined by WHO.

[a]Isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E), rifapentine (P), and moxifloxacin (M).

which is critical for improving therapeutic efficacy and new drug discovery (102) (Table 3).

NANOFORMULATION DRUG DELIVERY SYSTEMS

Nanomedicines facilitate various compartmentalized drug delivery methods at the micron and nanoscales. Advances in nanomedicine have been common in recent years. The following sections describe the improvements and new achievements in emulsion and nanoparticle-assisted drug delivery (Fig. 2).

TABLE 3 Various drug regimens and mechanisms

Drugs	Associated genes	Mechanisms	References
First-line drugs			
Isoniazid	*katG*, *inhA*, *kasA*	Inhibits synthesis of cell wall mycolic acid	(103, 104)
Rifampicin	*rpoB*	Inhibits RNA synthesis	(105, 106)
Pyrazinamide	*pncA*, *rpsA* (need more investigation), *panD* (need more investigation)	There are several targets, including preventing the synthesis of coenzyme A and pantothenate, decreasing membrane energy, and inhibiting trans-translation.	(105)
Ethambutol	*embCAB operon* (*embC*, *embA*, *embB*), *ubiA*	Inhibits cell wall biosynthesis (arabinogalactan)	(107, 108)
Streptomycin	*rpsL*, *rrs*, *gidB* (poorly understood)	Inhibits translation/protein synthesis	(105)
Second-line drugs			
Moxifloxacin (repurposed)	*gyrA* (major target), *gyrB*	Inhibits DNA synthesis	(105)
Gatifloxacin			
Amikacin (injectables)	*rrs*, *eis*, *tlyA*	Inhibits protein synthesis	(105)
Capreomycin (injectables)			
Kanamycin (injectables)			
New anti-TB drugs			
Delamanid, pretomanid	*fgd1*, *fbiA*, *fbiB*, *fbiC*, *ddn*	Inhibits mycolic acid biosynthesis	(109, 110)
Bedaquiline	*atpE*, *rv0678*, *pepQ*	Mycobacterial ATP synthesis inhibition	(111, 112)
Repurposed drug			
Clofazimine	*rv0678*, *rv1979c*, *rv2535c*, *ndh*, *pepQ*	Action mechanism poorly understood; multiple effects on *Mtb*.	(105, 113–115)
Meropenem/clavulanate	*blaC* (*blaA*)	Synergistic effect; clavulanate improves meropenem, possibly by inhibiting mycobacterial beta-lactamase	(116–118)
Linezolid	*rrl*, *rplC*	Inhibition of protein synthesis	(99)

MICRO/NANOEMULSION-BASED DRUG DELIVERY SYSTEMS

Microemulsions

Microemulsions can be described as a mixture of water, oil, or one or more colloidal surfactants. They are isotropic, clear, transparent, and thermodynamically stable (119) and can have a variety of structural shapes, including spherical, ellipsoidal, and cylindrical (120, 121). According to the International Union of Pure and Applied Chemistry, their size varies depending on the ingredients used, from 1–100 nm but usually 10–50 nm (122). Microemulsions are a successful drug delivery carrier for lipophilic, hydrophilic, and amphiphilic drugs (123) and can be classified as oil in water (o/w) and water in oil (w/o) (124) (Fig. 3A and B). Microemulsion-based drug delivery has numerous advantages over conventional drug delivery because it can enhance drug solubility and bioavailability (125), absorption, and permeability, enabling controlled drug release over an extended period, and avoidance of first-pass metabolism (126). Bedaquiline fumarate-loaded microemulsions were recently developed to enhance oral bioavailability. This could be a promising remedy for MDR-TB (127). Some scientists have previously encapsulated ATDs such as rifampicin, isoniazid, and pyrazinamide with combinations of various lipids, emulsifiers, and surfactants for drug delivery (Table 4).

Nanoemulsions

This is a nanoscale or submicron-sized colloidal dispersion system (130, 131). It is an isotropic, thermodynamically unstable, and biphasic system in which stabilizers combine two immiscible liquids in a single phase (132, 133). According to Aswathanarayan and

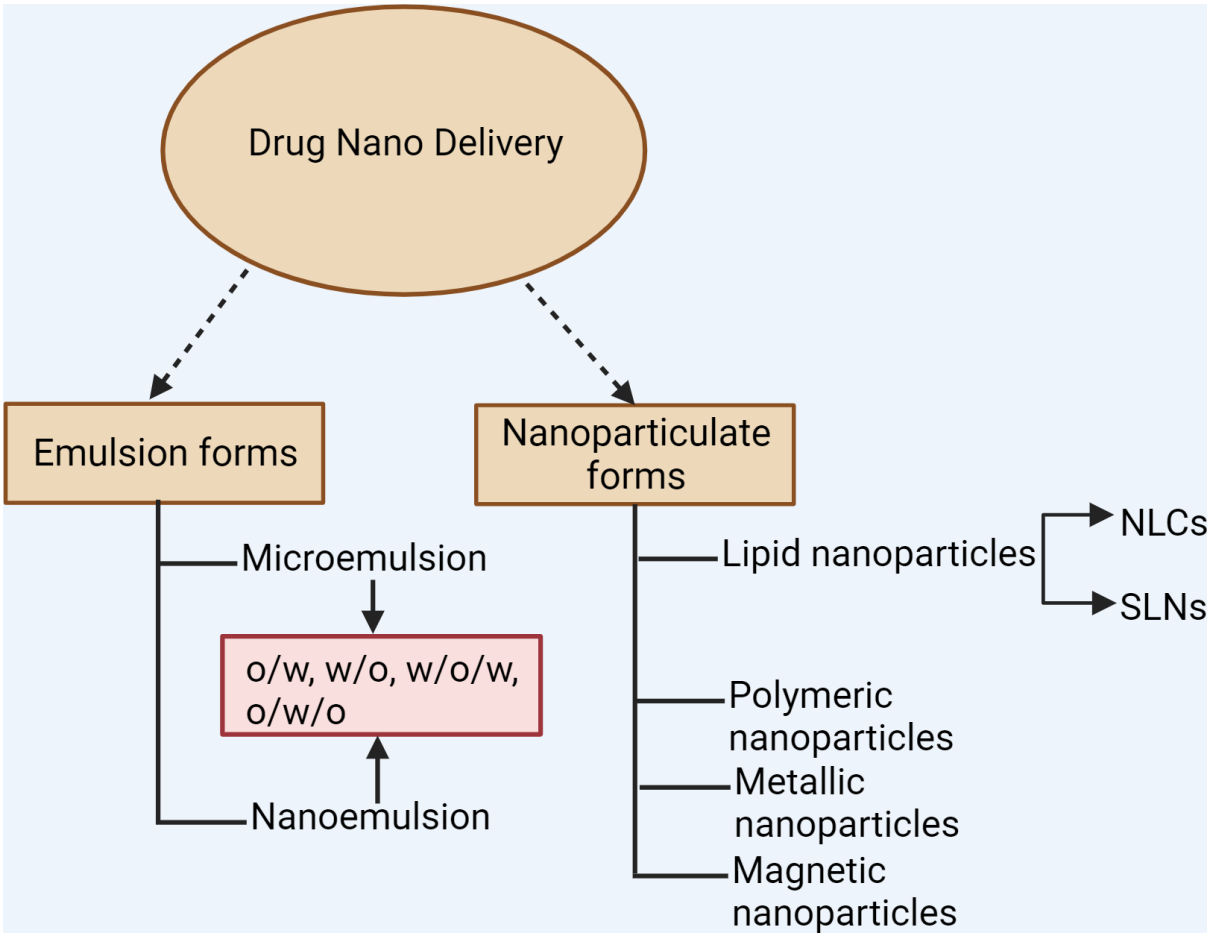

FIG 2 Emulsions and nanoparticle-based drug delivery systems.

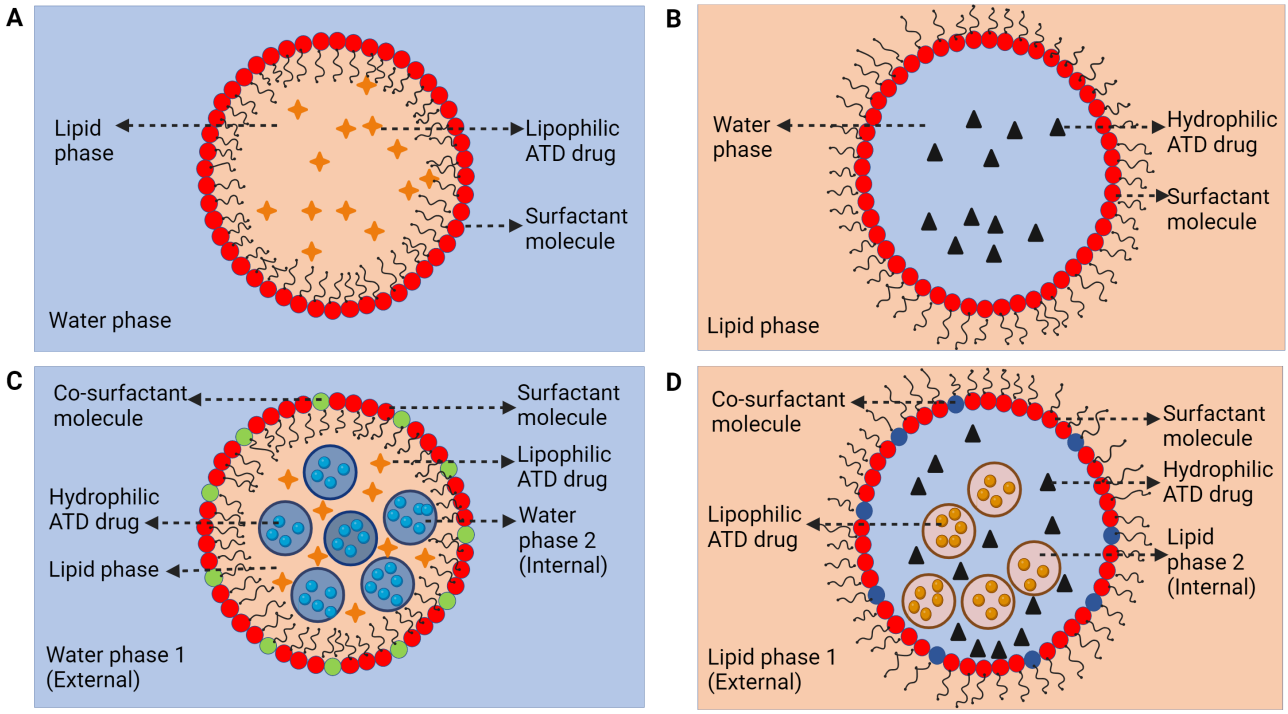

FIG 3 Types of micro (nano) emulsions; (A) oil in water (o/w); (B) water in oil (w/o); (C) water in oil in water (w/o/w); (D) oil in water in oil (o/w/o).

Vittal (134), it is thermodynamically metastable and kinetically stable. The controlled and sustained drug release, targeted delivery, remarkable pharmacokinetic value, avoidance of first-pass metabolism, oral bioavailability, and biocompatibility of the formulated materials make them novel drug delivery nanocarriers (135–138). Particulate size ranges are 20–200 (139), 10–1,000 (132), 10–100 (140, 141), and <100 nm (142, 143).

Based on the number of phases, nanoemulsions can be categorized into two domains: single-phase nanoemulsions (o/w and w/o types) (134) and multiphase nanoemulsions [water in oil in water (w/o/w) and oil in water in oil (o/w/o)] (144) (Fig. 3A through D). Nanoemulsions of first- and second-line ATDs have been reported within the last decade. Table 5 describes recent developments in nanoemulsions against mycobacterium strains using various ATDs.

NANOPARTICLE-BASED DR-TB DRUG DELIVERY

Nanoparticle technology offers considerable promise in combating drug resistance in TB through advanced drug delivery systems. The term nanoparticle may be considered as colloidal particles with a size (diameter) ranging from 1 to 1,000 nm (154). According to Poste and Kirsh (155), ideal nanoparticle carriers should be biodegradable, stable, non-immunogenic, easily surface engineered, affordable, and able to discharge their payloads onto the target (155). Lipids, polymers, and metals can also be used to synthesize NPs. Based on the nature of the components used, NPs can be classified into four types: lipid, polymeric microparticle, metallic, and magnetic NPs.

Lipid nanoparticles (LNPs)

LNPs have gained attention in the pharmaceutical sector as potential carriers of drugs and nucleic acids (156). The adherence of drug molecules to LNPs for targeted drug delivery has been scientifically observed and proven to be promising and valuable. LNPs are well-suited candidates for combating three main challenges: controlled drug

TABLE 4 Recent developments of microemulsions against *Mtb*[a]

Lipids and surfactant	ATD used	Used experimental A/M/CL	Highlights	References
Oleic acid and Tween 80	Rifampicin, isoniazid, pyrazinamide	NA/NA/NA	Tween 80 microemulsion was used in this study to reveal microstructure changes. The data indicated how probes were partitioned into several microenvironments inside the microemulsion system. Nile Red occupied the oil-surfactant interface in the direction of the apolar side, while tris(2,2′-bipyridine) ruthenium (II) dichloride occupied the water-surfactant interface in the direction of the polar side. This Tween 80 system could be used to study the location of anti-TB drugs based on the solubilities of the probe.	(128)
Ethyl oleate and Brij 96	Rifampicin, isoniazid, pyrazinamide	NA/*E. coli*, *S. aureus*, *A. niger*, *A. fumigatus*, *C. herbarium*, *C. lunata*, *H. oryzae*/Vero cells	In this study, three pharmaceuticals, rifampicin, isoniazid, and pyrazinamide, were co-encapsulated in single, binary, or ternary mixtures. In multiple drug-loaded microemulsion systems, they occupied the same solubilization sites. RIF demonstrated non-Fickian release, but isoniazid and pyrazinamide followed the Fickian release mechanism.	(123)
$BmimPF_6$ (as lipid phase), Brij35	Rifampicin	NA/NA/NA	Here, researchers formulated rifampicin-loaded, biocompatible 1-butyl-3-methylimidazolium hexafluorophosphate ($BmimPF_6$) in water (IL/w) microemulsions. The drug in MEs causes noticeable changes, indicating drug accumulation in MEs palisade layers. This study clarifies MEs characteristics and opens up possibilities for its use in pharma. Data from the spectroscopic study revealed good stability.	(129)

[a]A/M/CL: A, animals; M, microorganisms; CL, cell lines. NA, not available.

release, high drug-carrying capacity, and specific drug targets. The concept of LNPs was proposed by German scientist R.H. Müller and Italian scientist Professor M. Gascon (157). LNPs can be classified as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs).

LNPs have typical sizes of around 200–400 nm (158). Both SLNs and NLCs deliver potent ATDs. Both the repurposed and existing ATDs can be delivered via SLNs and NLCs. These LNPs can overcome the antibiotic resistance of TB drugs. Banerjee et al. (159) have developed LNPs (SLNs and NLCs) for isoniazid and rifampicin. Based on these findings, both drug carriers can potentially deliver ATDs. No significant differences were observed between SLNs and NLCs (159).

Solid lipid nanoparticles

According to their structural makeup, lipids are typically categorized as fatty acids, fatty esters, fatty alcohols, triglycerides, and partial glycerides (160) (Fig. 4A). They are generally spherical with mean diameters between 50 and 1,000 nm (161). Many scientists are interested in SLN-based drug delivery; some have tested it against DR-TB. NLCs, lipid drug conjugates, polymeric lipid hybrid nanoparticles, and long-circulating SLNs are the new-generation SLNs (160). SLNs can be employed as tunable drug delivery systems for different chemotherapies and for treating parasitic infections, including TB (162–164). Nanosized SLNs offer multiple advantages, including regulated drug release, a larger surface area, drug solubility, minimal nanotoxicity, remarkable biocompatibility, high drug-loading capacity, precise molecular-level interactions with target sites, and enhanced drug bioavailability (164–166).

To develop therapeutic SLNs that can deliver drugs to treat mycobacterial infections, many researchers have synthesized drug-loaded SLNs and evaluated their anti-mycobacterial efficacy in preclinical studies using experimental organisms and mycobacteria. Pandey et al. (167) developed SLNs for rifampicin, isoniazid, and pyrazinamide by using an emulsion solvent diffusion method. The encapsulation efficiency (EE) of developed SLNs of rifampicin, isoniazid, and pyrazinamide were 51% ± 5%, 45% ± 4%, and 41% ±

TABLE 5 Recent developments of nanoemulsions against *Mtb*[a]

Lipids and surfactant/ emulsifier	ATD used	Used experimental A/M/CL	Highlights	References
Phospholipid Phosal 53 medium-chain triglycerides & α-tocopheryl polyethylene glycol 1000 succinate	Ethambutol, Rifampicin, Isoniazid, and Pyrazinamide	C57BL/6 female mice / *Mtb* (H_{37}Rv-pSMT1 & H_{37}Rv)/ J77A.1 mouse macrophage	Here, researchers developed phospholipid-based SQ641-NE. It was found to be effective against *Mtb* in J774A.1 mouse macrophage and significantly reduced the tubercle count by 1.73 $\log_{10}$ CFU in a murine TB model. Additionally, it was found to be bacteriostatic in the lungs.	(145)
Oleic acid & Tween 80	Rifampicin	Sprague–Dawley rats/NA/ NR8383 cell lines	Here, first-, second-, and third-generation nanoemulsions were synthesized. All generations showed better than 95% aerosol output and > 75% inhalation efficiency. The third-generation nanoemulsion demonstrated a lower plasma drug concentration, higher lung drug content, and greater cell internalization capacity. The average size of the nanoemulsions was 40 to 60 nm.	(146)
Soybean oil & Tween 80	BCG	C57BL/6J (B6) mice/ *Mycobacterium bovis*, *Mtb* HN878/NA	Here, scientists developed an NE-TB vaccine with a combination of NE adjuvant and Mtb immunodominant antigens (ESAT-6 and Ag85B). This formulation potentially induced mucosal IL-17 T-cell responses. Disease severity was reduced when the NE-TB formulation was delivered with BCG.	(147)
Safflower, olive oil & Tween 80, Span 60	Clofazimine, Artemisone, Decoquinate, Isoniazid	NA/*Mtb* / HaCaT cell line, J774 macrophage cell line	The purpose of the study was topical drug delivery for cutaneous tuberculosis (CTB). In this experiment, scientists used two different oils for the development of eight different drug-loaded nanoemulsions. INH was used as a positive control. Safflower oil-based nanoemulsions showed higher percentages of inhibition compared to olive oil-based nanoemulsions.	(148)
Oleic acid & polysorbate 80(Tween 80)	Rifampicin	NA/ *Mtb* (H_{37}Rv)/NA	Here, scientists developed the first ophthalmic cationic nanoemulsion against ocular tuberculosis. Chitosan and polymyxin B were used for the surface modification of the RIF-loaded nanoemulsion. The antimicrobial efficacy of Rif was not affected by the high-pressure homogenization method or the surface modification process under in-vitro settings. This finding might be helpful for the treatment of ocular TB, allowing for longer intervals between doses.	(149)

(*Continued on next page*)

TABLE 5 Recent developments of nanoemulsions against *Mtb*[a] (*Continued*)

Lipids and surfactant/ emulsifier	ATD used	Used experimental A/M/CL	Highlights	References
Capmul PG8 (CPG8) & Labrasol (LAB)	Rifampicin	Sprague Dawley rats/ *Mtb* ($H_{37}Rv$)/NA	The emphasis of this study was increased effectiveness, facilitated intestinal permeability, and Gastro-Plus™-based prediction of cationic RIF-NE. GastroPlus™ had a significant impact on globular size, permeability, and nanonization on pharmacokinetic parameters. It was revealed that using this novel formulation will significantly improve therapeutic efficacy.	(150)
Capmul PG8 NF, Transcutol-HP and labrasol (LAB), Tween 80	Rifampicin	Sprague Dawley rats/ *Mycobacterium smegmatis* (MS-995, MS- 942), *Mtb* H37 Rv (ATCC 25618)/ NA	The results showed that transdermal rifampicin could be an alternative to conventional methods for treating both local and systemic TB, as well as other bacterial infections.	(151)
Sunflower oil, Span 80, Span 85, Tween 80	Linezolid	Wistar rats/ *Mycobacterium smegmatis*/ NA	Here, primary water-in-oil (w/o) nanoemulsions were formulated, followed by water-in-oil-in-water (w/o/w) emulsions, which were then optimized. The drug-loaded optimized emulsions were tested against *Mycobacterium smegmatis* to evaluate their antibacterial killing efficiency and potential for dose reduction. The results support the application of these emulsions in treating lymph node TB.	(152)
Essential oils (Eugenol, cinnamon leaf oil, and clove essential oil), Tween 20, Tween 80	Rifampicin, ethambutol, pyrazinamide, and isoniazid	NA/Hospital strains of *Mtb*/NA	Here, researchers formulated multi-drug-loaded nanoemulsions (o/w) using plant-based essential oils that have antimicrobial properties. They optimized the nanoformulations using a central composite design. The optimized formulations were stable at a 1:5 (oil : surfactant) ratio and showed stability for more than three months. The formulations exhibited high antimicrobial activity against hospital strains of *Mtb*. The results were promising and suggested the potential of this formulation to combat MDR/XDR-TB forms. However, further *in vivo* studies are required to assess the toxicity of the formulation.	(153)

[a]NA, not available.

4%, respectively. When a single oral dose was administered to Laca mice, the therapeutic drug concentration remained in the plasma and organs for 8 and 10 days, respectively, whereas the free forms of the drugs were degraded after 1–2 days. Oral drug-loaded SLNs (all formulations) cleared *Mtb* $H_{37}Rv$ from the lungs and spleen of infected mice at five doses administered every 10 days (167). Oral-free drugs achieved equivalent results at 46 daily doses. No toxicity study was performed using this methodology; however, a

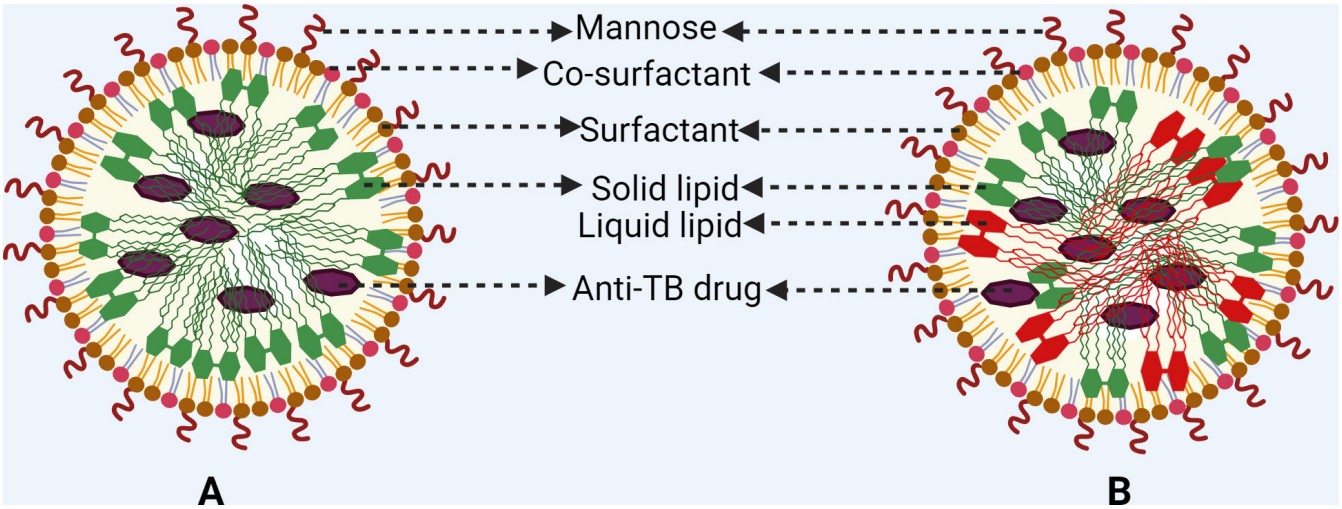

FIG 4 Lipid nanoparticulate systems: (A) solid lipid nanoparticles and (B) nanostructured lipid carriers.

pharmacokinetic study was used to determine the relative bioavailability of all formulations. This study demonstrated improved therapeutic efficacy and reduced drug dose frequency using drug-loaded SLNs (167).

Aboutaleb et al. (168) examined the anti-mycobacterial effects of rifampin HCL on *Mycobacterium fortuitum*. They used a modified microemulsion-based method with cetyl palmitate as the lipid phase. Eight formulations were synthesized, and their stability was assessed. Two formulations (F7 and F8) using poloxamer 188 (surfactant) showed 6 days of stability, the highest among the other formulations. This indicates that the surfactant played an important role in stability but was insignificant for its application. The preparatory parameters for both formulations were the same, except for the amount of surfactant. The amount of Tween 80 in F7 (4 g) was higher than in F8 (2 g). The criteria for selecting the formulation for further characterization were not clearly explained, but the F8 formulation was possibly chosen because of concerns regarding the lower toxicity of the surfactant. The size of the spherical SLNs was approximately 100 nm, with a low zeta potential and a drug EE of 82%. The minimum inhibitory concentration (MIC) of drug-loaded SLNs against bacteria was eightfold lower than that of free rifampicin. This formulation could be a useful drug carrier, but a preclinical test in animals is needed for further studies (168). Singh et al. (67) developed SLNs using two drugs (rifampicin and isoniazid) and a modified microemulsion-based method. In this formulation, Compritol ATO 888 was used as the lipid phase. In this study, researchers examined the degradation of rifampicin with and without isoniazid in SLNs at pH 1.2. At acidic pH, rifampicin SLNs without isoniazid-induced degradation provided 60% protection, whereas rifampicin and isoniazid individually incorporated into the SLNs provided 74.7% protection. This nanoparticle-based study could be used to examine drug-drug interactions and the co-encapsulation of multiple drugs within SLNs (67).

Chuan et al. (169) synthesized SLNs containing rifampicin using soybean lecithin as the lipid phase and a modified lipid-film hydration method. They were round and approximately 800 nm in size. Rifampicin SLNs were tested for their toxicity on lung cells using (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) MTT assays. Both AMs and AECs had more than 80% viability after rifampicin SLN exposure, indicating low toxicity to these cells. Comparatively low cytotoxicity was observed when the concentration of the formulation was below 20 µg/mL. *In vitro* cellular uptake of rifampicin SLNs in both cell types showed that AMs had a greater ability to selectively internalize these SLNs than AECs, which was validated by an *in vivo* study using Sprague–Dawley rats. This formulated drug delivery system can target active pulmonary TB but should also be extended to latent pulmonary TB (169). Bhandari and Kaur (170) used a novel

method (Indian patent application: 127/DEL/2012; international patent application: PCT/IN2012/000154) for isoniazid-SLN formulation, and Compritol 888 ATO was used as a lipid excipient. The relative bioavailability of isoniazid SLNs in both the plasma and brain increased significantly ($P < 0.001$) compared to that of the free drug solution at the same dose. Plasma bioavailability was six times higher, and brain bioavailability was four times higher with isoniazid SLNs. The formulation showed a threefold higher lethal dose (LD_{50}) in female Wistar rats. In this study, researchers compared the isoniazid entrapment efficiency of 69% with that of Pandey et al. (167), who reported an isoniazid EE of 45%. At this point, the formulation is more advantageous than that proposed by Pandey et al. (167). However, dose-dependent side effects were reported in this study, indicating a low incidence of hepatotoxicity (170). In one experiment, sonication was used to formulate solid lipid microparticles (SLMs) using stearic acid as a lipid excipient. In this study, inhalable SLMs were developed to deliver rifampicin (lipid soluble) to AMs. They are non-cytotoxic and capable of inducing endocytosis by AMs. For the cytotoxicity and internalization studies, the murine macrophage J774 cell lines were used. The drug-loaded and unloaded SLN sizes were about 1 µm, and no significant difference was found between them. This size is suitable for deposition onto the alveolar epithelium and internalization into AMs. The antimicrobial activity of the formulation was studied on *Bacillus subtilis* (highly rifampicin susceptible) strain ATCC 6633, which demonstrated based on the zone of inhibition that SLM showed the same rifampicin activity as the drug standard solutions, proving that the drug activity was not altered by sonication. Overall, the biological activities of the SLMs in water (likely o/w) were less effective than that of the SLMs in methanol. This indicates an incomplete drug delivery system and its unsuitability for solving real-world challenges (171). Kumar et al. (172) used the nanocolloidal aqueous dispersion method (Indian patent application: 3093/DEL/2012) to synthesize streptomycin sulfate (STRS)-SLNs for the noninvasive delivery of STRS. Also, in this study, Compritol 888 ATO is used as a lipid component in this formulation. The particle size and entrapment efficiency were 140.1 ± 7.0 nm and 54.83% ± 2.1%, respectively. The stability was maintained for 2 years at 5°C ± 3°C, and no significant changes were found in its size and EE. Male BALB/c mice were used for the intranasal delivery of STRS-SLNs to the brain and blood. An *in vivo* biodistribution study, which was conducted using radio-labeled ^{99m}Tc (technetium-99m), revealed that compared to free STRS, STRS-SLNs had a much higher area under the curve ($AUC_{0-\infty}$) and area under the first moment curve ($AUMC_{0-\infty}$) in blood by 7.7 and 10.31 times, respectively, and in the brain by 3.5 and 5.8 times, respectively. The presence of Tween 80 in the formulation improved the cytoplasmic permeability of the drug by blocking or inhibiting the P-gp efflux pump on the intranasal membrane and blood–brain barrier and accounted for the improvement in the brain bioavailability of the drug. This formulation showed promising results in preclinical trials and may be suitable for intranasal delivery. It lowers toxicity and enhances drug penetration at lower doses (172). Singh et al. (173) formulated rifampicin-loaded SLNs using a novel micro-emulsification method (Indian patent application: 3356/DEL/2013) and Compritol 888 ATO used as a lipid phase. The average diameter of the nanoparticles was 130 nm, and the entrapment efficiency was approximately 67%. After 18 months, rifampicin SLNs remained stable. A single oral dose showed a significant increase of 8.14 times in plasma bioavailability in Wistar rats compared with free rifampicin, with sustained levels over 5 days. Rifampicin SLNs had better pharmacokinetic parameters and a relative bioavailability of 8.16 compared to drug-unloaded SLNs. The rats (male and female) underwent a 28-day repeated-dose oral toxicity study (OECD TG 407), and none of them died or suffered any adverse effects from the low (125 mg/kg) or median doses (250 mg/kg) which were 12.5 times and 25 times higher than the human therapeutic dose, respectively. This formulation is suitable for clinical trials and can potentially be used to treat human diseases (173). Pooja et al. (174) used a solvent emulsification/evaporation method to formulate wheatgerm agglutinin-conjugated rifampicin-loaded SLNs. The drug can be protected from enzymatic degradation and has improved bioavailability in the body because

wheatgerm agglutinin is not affected by enzymatic degradation. Rifampicin release from the designed nanoparticles followed diffusion-controlled, non-Fickian, and supercase-II mechanisms. This study did not assess the cytotoxicity of the SLNs, which may be important for their therapeutic potential (174). Wheatgerm agglutinin has both toxic and therapeutic effects on human cells depending on the dose, cell type, and delivery method used (175). For example, one study reported that wheatgerm agglutinin induced cytotoxicity in AML cells (176) and human pancreatic cancer cells *in vitro*, with different dose-response curves (177). Thus, wheatgerm agglutinin may exert cytotoxic effects on normal cells. Gaspar et al. (178) used a modified hot high-shear homogenization method to synthesize stable rifabutin-loaded SLNs using two different lipids (glyceryl dibehenate and glyceryl tristearate). Rifampicin showed an EE of 89.9% for glyceryl dibehenate SLNs and 81.0% for glyceryl tristearate SLNs. Both types of SLNs were targeted to THP-1-derived macrophages, and the nanoparticle uptake study showed higher uptake of glyceryl dibehenate SLNs (46.3%) than glyceryl tristearate SLNs (25.6%) after 24 h. Smaller-sized nanoparticles (glyceryl dibehenate SLNs: ≈100 nm) were more phagocytized than larger-sized nanoparticles (glyceryl tristearate: ≈200 nm). The drug release study showed that the drug was almost completely released from both types of SLNs over 24 h. The lung cell lines (A549 and Calu-3) viability study showed low cytotoxicity, and this formulation could be a potential drug delivery system for lung TB (178). Maretti et al. (179) developed mannosylated SLNs using a melt-emulsifying technique with sonication to deliver rifampicin and used them to treat TB. They developed two sets of SLNs using three lipids: palmitic acid, tripalmitin, and cholesteryl myristate. The palmitic acid + cholesteryl myristate (PA) and tripalmitin + cholesteryl myristate (TP) sets were subjected to cytotoxicity studies. Compared with the PA1 and NF-PA1 samples, TP2E and NF (non-functionalized)-TP2E were significantly ($P < 0.05$) more cytotoxic to cells at all doses and durations of exposure. J774A.1 murine macrophages increased their uptake rate when exposed to SLNs coated with mannose. The highest EE (44.32%) was found in the PA1 (175 mg palmitic acid + 75 mg cholesteryl myristate + 75 mg rifampicin), followed by the TP2E (43.60%) sample (175 mg tripalmitin + 75 mg cholesteryl myristate + 125 mg rifampicin). The average amount of rifampicin inhaled into AMs was 113 times more than that obtained from oral intake, which can improve bioavailability. Overall, the performance of the PA set, particularly that of PA1, was superior to that of the TP set. This formulation was suitable for passive targeting of AMs and could be used to target macrophages via powdered inhalation therapy (179). Using a w/o/w double emulsion method and three types of solid lipids (Witepsol E85, stearic acid, and Compritol 888 ATO), Costa et al. (180) synthesized INH-SLNs, and the optimized SLNs were further functionalized with mannose. Witepsol E85 was chosen as the main lipid for the SLN formulation because of its physical-chemical properties. To improve the attachment of mannose to INH-SLNs, the lipid matrix (Witepsol E85 + stearylamine) of the NPs was also optimized by varying the concentration of stearylamine. Both mannose-functionalized and non-functionalized SLNs were non-toxic to human lung cells (NCI-H441) and dTHP-1 cells and reduced the intrinsic cytotoxicity of INH when incorporated into SLNs. A cellular uptake study revealed that mannose-anchored SLNs enhanced the uptake of isoniazid-SLNs by macrophages compared with non-functionalized SLNs. Synthesizing SLNs based on double emulsions is challenging because they may not be stable in the long term and may require multiple parameter optimizations. In this study, the duration of stability of the optimized and mannosylated SLNs was not mentioned, but it is an important parameter for future applications. This nanocarrier targeted *Mtb*-infected macrophages (180). Vieira et al. (181) developed SLN-rifampicin and mannose-SLN-rifampicin formulations by hot ultrasonication. The therapeutic efficacy was increased owing to the protection provided by mannose-SLNs from pulmonary fluids, and the uptake rate of THP1-derived macrophages was improved by the mannosylation of drug-loaded NPs. Overall, these results suggest that the mannose-SLN-rifampicin formulation is suitable for inhalation (181). A dry powder inhaler was developed using the ethambutol hydrochloride-SLN formulation by Nemati

et al. (182), and the overall results suggest that it has high potential for treating TB. Two methods were used in this study: hot homogenization and ultrasonication (182). Khatak et al. (162) synthesized SLNs using a modified microemulsion technique and a central composite design to optimize the entrapment efficiency of drugs. SLN_8 NPs had a mean size of 187.9 nm and a zeta potential of −47.4 mV. The optimized SLN_8 had an entrapment efficiency of 86.40%, 83.84%, and 81.43% for rifampicin, isoniazid, and pyrazinamide, respectively. Drug release kinetics showed a slower release of ATDs from SLN_8 than from marketed formulations and pure ATDs. The drug-loaded SLNs had an MIC value of 3.125 µg/mL against *Mycobacterium marinum*. The cytotoxicity study used the IC_{50} value of drug-loaded SLN_8 (0.94), indicating that SLN_8 was more toxic than the individual pure drugs and their physical mixtures. As shown by the modified MTT assay, ATDs-SLN_8 inhibited the growth of murine macrophage cells (RAW 264.7) to twice that of the standard ATD. These results suggest that ATDs-SLN_8 could be a potential drug delivery carrier for treating TB (162). Obinu et al. (183) synthesized two different formulations of SLNs loaded with SS13 (a new compound), SLN-W, and SLN-G using a modified solvent emulsification–evaporation method. The SS13-loaded SLN-G formulation was more effective than drug-loaded SLN-W against various strains of *Mtb*. This could be an effective approach for countering MDR TB. Both formulations were subjected to antibiotic sensitivity tests against an *Mtb* strain ($H_{37}Rv$) and nine clinically isolated strains. Four first-line drugs (streptomycin, isoniazid, rifampicin, and ethambutol) and one fluoroquinolone (ciprofloxacin) were tested for their antitubercular activity. In this study, four clinical strains were identified as MDR. A resazurin microtiter assay was performed based on the drug sensitivity results, and all therapeutic antibiotics individually combined with SS13 were used to assess MIC values. Overall, the results suggested that combining SS13 with therapeutic drugs increased antitubercular activity and that oral administration of SLN-G could enhance SS13 permeation in MDR-*Mtb*, making it a good candidate against resistant *Mtb* (183).

Recently, Chokshi et al. (184) developed mannose-rifampicin SLNs against TB. The cytotoxicity results demonstrated that the fabricated SLNs were safe and non-toxic compared to the free drugs using the J774A.1 cell line. Pharmacokinetic studies *in vivo* were conducted in Sprague–Dawley rats, and the relative bioavailability of mannose-rifampicin SLNs was remarkably increased around 17 times compared to the drug solution when administered orally. The lung accumulation of mannose-rifampicin SLNs was 1.8 times higher than that of Un-RIF-SLNs, as shown in studies on drug biodistribution in rats. Mannose-rifampicin SLNs may be useful for delivering drugs to *Mtb*-infected lungs (184). Singh et al. (185) developed STRS-SLNs by a cold, high-pressure homogenization technique against *Mtb* $H_{37}Rv$ and *M. bovis* Bacillus Calmette-Guerin (BCG). The entrapment efficiency of drug-loaded NPs was 51.17%. The MIC of STRS-SLNs against *Mtb* $H_{37}Rv$ (256182) was three times lower than that of free STRS. STRS-SLNs also exhibited enhanced anti-mycobacterial activity against both *M. bovis* BCG and *Mtb* $H_{37}Rv$ (272994) compared with free STRS. The effects of STRS-SLNs were tested *in vitro* and *in vivo* following the OECD 425 guidelines for acute toxicity studies, and their safety was demonstrated (185). Ma et al. (186) developed a pH-sensitive inhalable MAN-IC-SLN (mannose modified) using both emulsification and ultrasonication, a formulation that targets macrophages to reach intracellular bacilli. The formulation showed higher intracellular antibiotic efficacy than the isoniazid solution in *in vivo* antibiotic tests and an *in vitro* latent TB infection model. The *in vivo* intracellular antibiotic efficacy and macrophage uptake of MAN-IC-SLNs were four times higher and increased due to the INH-CHO (isonicotinic acid octylidene-hydrazide) and MAN-SA (stearylamine) in SLNs at different pH levels. The SLN formulation had an 83% reduction of the bacterial count, while the free isoniazid solution had only a 60% reduction. The SLNs with isoniazid-CHO released more at pH 5.5 (82.63 %) than at pH 7.4 (58.83 %). The experimental organisms used *in vivo* and *in vitro* were Wistar rats and *Mycobacterium smegmatis* (MSG, ATCC 700044), respectively. This study showed that SLNs that target macrophages and release drugs in response to pH could be a potential strategy for treating latent TB infections

(186). The WHO praises levofloxacin as a better drug for the treatment of drug-resistant TB. Shah et al. (187) synthesized levofloxacin-SLNs using a single emulsification method, followed by solvent evaporation and lyophilization. The average particle size was 79.70 nm, and the Weibull model best described the drug release kinetics. The mass median aerodynamic diameter was 3.71 µm, indicating that the optimized formulation lodged deeply into the lungs. The minimum inhibitory concentration of levofloxacin-SLN was 0.7 µg/mL, whereas the pure medicinal product had a minimum inhibitory value of 1.0 µg/mL. The pharmacokinetic/pharmacodynamic parameters of levofloxacin-SLNs must be established in preclinical tests to improve our understanding of real dosing regimens (187).

Nanostructured lipid carriers

NLCs are an improved version of second-generation SLNs. NLCs develop because of the instability of SLNs and drug expulsion issues (188). In 1999, a German company (PharmaSol GmbH Berlin) patented NLCs. Its typical size is also in the range of 200 to 400 nm. NLCs can be classified as classical or ultra-small (158). In addition to oral, ocular, pulmonary, and intravenous routes of administration, dermal and transdermal delivery routes are also possible (158, 189). In SLNs, the starting material is a solid lipid; however, in NLCs, a blend of solid and liquid lipids (oil) is used (158). Techniques such as microemulsion, high-pressure homogenization, and ultrasonication are generally employed for NLC formulations. Recently, most first-line drugs have been used to formulate NLCs against *Mtb* (Fig. 4B).

Song et al. (190) used a thin-film homogenization method to synthesize rifampicin-NLCs and RFP (rifampicin)-Man-NLCs (mannose coated). This method uses ovolecithin and medium-chain triglycerides in the lipid phase. The size of both NPs was approximately 160 nm, with a polydispersity index (PDI) value <0.30. The EE of rifampicin was higher in non-mannosylated NPs (96.6 ± 0.3) than in mannosylated NPs (91.6 ± 2.3). Both RFP-Man-NLCs and RFP-NLCs displayed superior lung targeting compared to commercially available rifampicin. *In vitro* (procured cell line: NR8383 AMs) and *in vivo* tests (Wistar rat AMs) were conducted to determine the cellular uptake of RFP-Man-NLCs, which showed better performance than RFP-NLCs. Intracellular uptake of mannosylated NPs in Wistar rat AMs showed three times more uptake than in procured AMs at 37°C; however, a concentration-dependent cytotoxicity was observed. Overall, the minimum cytotoxicity and lack of an inflammatory response revealed the safety of mannosylated NPs based on concentration dependence. The published data could be used to design experiments, especially for cytotoxicity study purposes, but further studies are needed for safer formulation designs (190). Pinheiro et al. (191) synthesized rifabutin-NLCs (NLC-RFBs) and M-NLC-RFBs (mannosylated) using high-shear homogenization followed by ultrasonication. The formulation consisted of Precirol ATO 5 (solid lipid: 58%, wt/wt), miglyol-812 (liquid lipid: 25%, wt/wt), polysorbate 60 (16%, wt/wt), and the drug (1%, wt/wt). In this study, the particle size was 175–213 nm. The EE of M-NLC-RFB was 90% ± 4% higher than that of NLC-RFB. The M-NLC-RFB had an IC_{50} of 238.9, 185.7, and 108.7 µg mL^{-1} for Calu-3, A549, and RAW cells, respectively. This suggested the drug could reach therapeutic levels with concentrations between 100 and 1,000 µg mL^{-1}, below the IC_{50} values. Drug-unloaded M-NLCs were more toxic to these cell lines, possibly because of the positive charge on the NP surface. These findings suggest that the synthesized NPs could be effective drug delivery systems for the pulmonary administration of RFB to AMs (191).

Vieira et al. (192) synthesized NLC-rifampicin and M-NLC-rifampicin (mannosylated) using high-shear homogenization and ultrasonication techniques. In this study, Precirol ATO 5 and miglyol-812 were used as the lipid phase. The average size of both NLC-rifampicin and M-NLC-rifampicin was approximately 300 nm, with only a small difference between them. They also had similar drug-loading efficiencies of over 90%. A preclinical study was carried out on female C57Bl/6 (B6) mice, and an antimicrobial assessment of the formulation was performed on *Mycobacterium avium* strain

2447-infected bone marrow-derived macrophages. M-NLC-rifampicin showed higher cytotoxicity than NLC-rifampicin, which varied with the dose and duration of treatment. The rifampicin-loaded formulations were less cytotoxic than the M-NLCs in a concentration-dependent manner for the same reason (positive charge) previously mentioned by Pinheiro et al. (191) (192). Sato et al. (193) synthesized NLCs based on $[CuCl_2(INH)_2]\cdot H_2O$, $[Cu(NCS)_2(INH)_2]\cdot 5H_2O$, and $[Cu(NCO)_2(INH)_2]\cdot 4H_2O$ using the melt emulsification method, while caprylic/capric triglyceride and polyoxyl 40 hydrogenated castor oil were used as the lipid phase. The results of *in vitro* and *in vivo* studies in bacteria and Swiss mice suggested that the synthesized NLCs could optimize the activity of copper (II) complexes against *Mtb* H_{37}Rv. The biological activity revealed that NLCs with copper (II) complexes boosted the antibacterial effect on *Mtb* by 27 times. Swiss mouse toxicity tests showed that NLCs containing copper (II) complexes exhibited lower toxicity. Copper (II) complex-based NLCs can also be used against *Mtb* (193). Kanwar et al. (194) reported for the first time that rifampicin-NLCs were coated with Tween (20, 40, 60, and 80) using a hot dispersion method. In this study, sophorolipid (a biosurfactant) was used to prepare the NLCs. *In vitro* and *in vivo* behaviors of bovine serum albumin-NLCs interactions were conducted to understand the fate of nanodrug carriers for application in targeted drug delivery systems (194).

Carneiro et al. (195) using microemulsion technique, synthesized a tuftsin-modified peptide (pTUF-OA), and effectively bound to the interface of nanoparticles, resulting in NP-prifampicin. Simultaneously, nanoparticles without peptide, referred to as NP-rifampicin, were also produced for the purpose of comparison. The particle sizes of NP-rifampicin and NP-prifampicin were 210 ± 8 and 285 ± 11 nm, respectively, while the EEs were 90% ± 6% and 81% ± 8%, respectively. The results showed that macrophages internalized NP-prifampicin much better than the NP-rifampicin. However, the MIC of NP-rifampicin and NP-prifampicin against *Mtb* was the same (0.48 µg/mL), showing that the pTUF-OA did not affect the ability of NP-rifampicin to kill the bacteria. NP-prifampicin exhibited a twofold greater killing effectiveness against the *Mtb* H_{37}Rv strain than free rifampicin. A cytotoxicity study on J774 A.1 cells (macrophages) indicated that the both formulations were non-toxic even after the addition of peptide. This approach could have a significant impact on improving the effectiveness of TB treatment and requires *in vivo* studies (195).

Magalhaes et al. (196) developed RPT-loaded NLCs by hot ultrasonication. PrecirolATO 5 and Miglyol812 were used as the lipid phase. The average diameter of the optimized RPT-NLCs was 242 ± 9 nm (PDI <0.2). The RPT EE was 86% ± 4%, optimized by the Box–Behnken design. The viability of primary human macrophages was not affected by RPT-NLCs at a concentration of up to 1,000 µg mL^{-1}, indicating that they were safe to use. However, more *in vitro* and *in vivo* studies are needed to obtain clinically relevant data for future applications (196). Patil and Deshpande (197) synthesized clofazimine-loaded NLCs with and without the fabrication of mannose using a Quality by Design methodology to target AMs. In this study, a precipitation-hot microemulsification-probe sonication method was used. To the best of our knowledge, this is the first systematic study on mannose fabrication on clofazimine-loaded NLCs. The current study successfully applied Quality by Design principles to formulate clofazimine-NLCs and enhance the performance of clofazimine (197).

Polymeric micro (nano) particles

Polymeric NPs (PNPs) are natural or synthetic polymers used for precise nanodrug delivery. PNPs are small particles with diameters (size) of 1–1,000 nm and are colloidal and solid (154, 198). It includes two nanoforms: nanocapsules and nanospheres (Fig. 5). Both nanoforms offered controlled drug release, bioavailability, and therapeutic index improvement (198, 199). Polymers such as chitosan, gelatin, alginate, poly (lactic-co-glycolic acid) (PLGA), polycaprolactone, poly lactic acid, and albumen are used frequently (200, 201). Scientists have developed PNPs against TB by using first- and second-line and repurposed drugs. Previously, some researchers developed polymeric microparticles/NPs

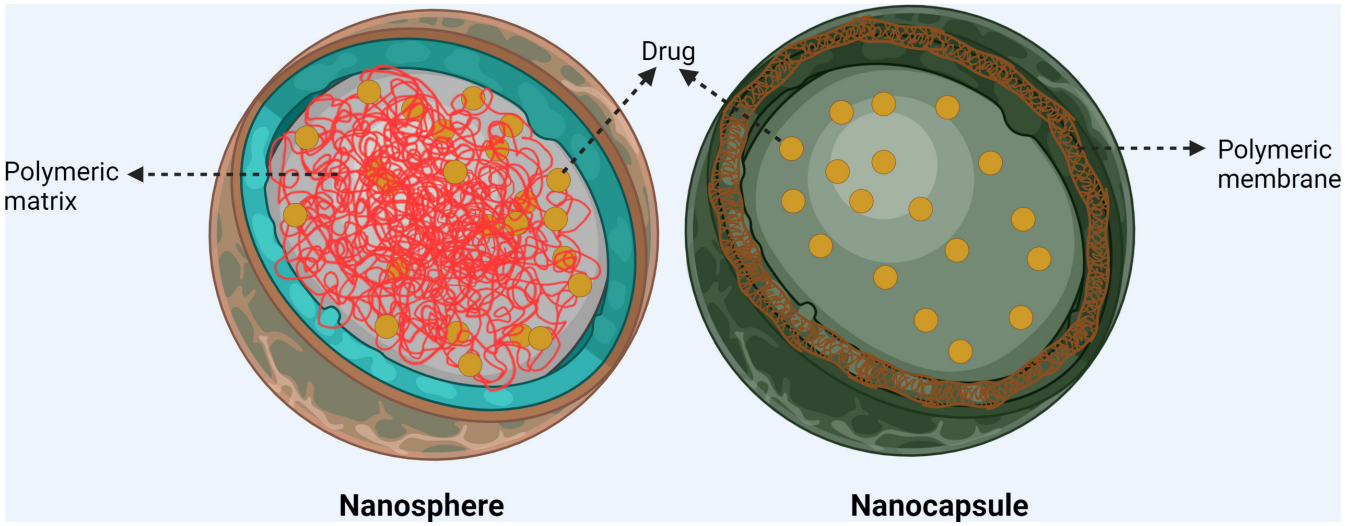

FIG 5 Polymeric particulate systems: nanospheres and nanocapsules.

such as gelatin NPs (202), chitosan NPs (203), PLGA NPs (204–206), poly(lactide-co-glycolide) (PLG) NPs (207), PLG microparticles (208), and alginate NPs (209, 210). Jahagirdar et al. (211) developed dual-loaded rifampicin-curcumin NPs of approximately 400 nm in size using a nanoprecipitation method. They observed a high killing efficacy against *Mtb*-infected macrophages at 25× MIC; however, they achieved complete clearance at 50 times MIC. The NPs exhibited no cytotoxicity to RAW 264.7 and facilitated 1.5-fold higher drug uptake than free drugs. Dual-loaded NPs showed potential as novel nanosystems for combating TB (211). Castro et al. (212) developed clofazimine-loaded PLGA-PEG NPs using nanoprecipitation. Peptide conjugation to NP-CFZ facilitated the delivery of the drug to the brain to target the central nervous system. NPs containing CFZ were less toxic to brain endothelial hCMEC/D3 cells *in vitro* than the free drugs. NPs with the transferrin receptor-binding peptide showed better cell interactions and higher CFZ transport across the hCMEC/D3 cell layer than NPs without the peptide. This demonstrates the functionalization strategy's effectiveness in enhancing CFZ delivery through the blood-brain barrier *in vitro* (212). Pawde et al. (213) synthesized mannose-fabricated CFZ chitosan NPs (CFZ-CS-MNS-NPs) and tested their anti-mycobacterial activity. A modified solvent evaporation method was used for the formulation. The drug entrapment efficiency and CFZ-CS-MNS-NP particle sizes in this study were 73.45% ± 1.47% and 184.7 ± 2.37 nm, respectively. The Luciferase reporter phase assay against *Mtb* H_{37}Rv indicated that CFZ NPs inhibited the bacteria 49.5 times more effectively than the free drug. However, following regulatory approval, preclinical *in vivo* studies are required to obtain clinically relevant data for future clinical trials (213). Shah et al. (214) developed inhalable linezolid-PLGA NPs (LZ NPs) by using a modified emulsion-solvent evaporation method against *Mtb*. The optimized (full factorial design) formulation had a mean particle size of 45.2 nm, entrapment efficiency of 85.33%, and drug release percentage of 89.84%. The LZ NPs had a lower MIC of 0.6 µg/mL than the free drug solution. These results demonstrate that LZ NPs can effectively reach the lungs for efficient TB treatment; however, an *in vivo* model experiment could provide more information (214). Thomas et al. (215) synthesized rifampicin-loaded alginate NPs using a green method. This study demonstrated that a significant amount of rifampicin is released from the formulation at pH 7.4, suggesting that it could serve as an effective drug carrier for controlled drug release (215). Parikh and Dalwadi (216) synthesized isoniazid-loaded poly-ε-caprolactone microparticles using a w/o/w emulsion spray drying method, which exhibited controlled drug release deep in the lungs where tubercular bacilli are located. This nanoformulation reduced the frequency of drug dosing (216).

Gajendiran et al. (217) synthesized di- and tri-block copolymeric nanoparticles using sonication, followed by double emulsification. The experiments revealed that tri-block copolymeric NPs (CSNPs) had higher loading efficiency and isoniazid content than di-block copolymeric (PLGA) NPs. The drug-loaded CSNPs showed sustained drug release for up to 124 h and had a relative bioavailability 11–28 times higher than that of free isoniazid. The CSNPs also improved the C_{max}, T_{max}, and $AUC_{0-\infty}$ of isoniazid compared to the PLGA NPs. These results suggest that PLGA–PEG–PLGA triblock copolymeric CSNPs are a potential oral drug delivery system for isoniazid (217). Kumar et al. (204) synthesized levofloxacin-loaded NPs using a modified emulsion–diffusion–evaporation method. The drug-loaded formulation showed sustained drug release for 4 days in the plasma after a single dose, compared with 24 h for free levofloxacin in mice. The drug-loaded NPs remained in the liver, spleen, and lungs for up to 4–6 days, whereas free levofloxacin was excreted within 24 h. The new formulation did not significantly affect the body weight or health of the mice. Levofloxacin can be incorporated into this safe oral formulation and has long-lasting effects (204). Kumar et al. (218) synthesized ethionamide-loaded PLGA oral NPs using a modified emulsion–diffusion–evaporation method. The size, zeta potential, and drug EE of the developed NPs were 286 nm, −13 mV, and 35.2% (wt/wt), respectively. The pharmacokinetic study in Swiss albino mice showed that the pharmacokinetic parameters from plasma, such as C_{max}, T_{max}, $AUC_{0-\infty}$, and $AUMC_{0-\infty}$, were also significantly higher for ethionamide-loaded PLGA NPs in comparison to free ethionamide. Encapsulated ethionamide remained in the organs (lungs, liver, and spleen) for 5 to 7 days, whereas free ethionamide was eliminated within 12 h. Ethionamide in PLGA can reduce the frequency of ethionamide doses in MDR-TB treatments (218). Pourshahab et al. (203) synthesized chitosan NPs by ionic gelation. An *in vitro* study showed that NP drug release decreased as the amount of chitosan increased. As shown by the lower MIC values, isoniazid became more effective against *Mycobacterium avium intracellulare* when incorporated into chitosan/tripolyphosphate NPs. *In vitro* data showed that spray-dried isoniazid NPs with lactose and leucine produced inhalable powders with a high fine particle fraction (45%). This formulation can target pulmonary TB, and an *in vivo* preclinical test is required to obtain clinically relevant data for future applications (203).

Saraogi et al. (202) synthesized mannosylated gelatin NPs and isoniazid-loaded nanoparticles by a modified two-step desolvation method. This study demonstrated that the formulation significantly reduced drug hepatotoxicity and the bacterial load in the lungs and spleens of TB-infected mice. This study suggested that mannose-containing gelatin nanoparticles (GNPs) could deliver isoniazid more safely and effectively for TB treatment than plain GNPs or free drugs (202). Lawlor et al. (219) synthesized PLGA microparticles via double emulsion, solvent evaporation method. This study revealed that PLGA MPs, even without carrying any drug, were capable of inhibiting the replication of *Mtb* within the cells, without causing any changes in the cytokine profile of the Mtb-infected macrophages. When treated uninfected cells with MPs, no induction of pro-inflammatory cytokines or significant changes in cell viability was observed. Interestingly, MPs triggered the increased activation of the NFκB pathway and induction of autophagy in macrophages that were not infected. However, the activation of NFκB by MPs was found to be dependent on the size of the MPs. Specifically, MPs that were 2.2 µm in size showed a significant increase in NFκB activation (219).

Metallic nanoparticles

Metallic NPs have huge potential to target a broad range of microorganisms (220, 221). Metal NPs, such as silver (AgNPs), gallium (GaNPs), selenium (SeNPs), and NPs of metal oxides like copper and zinc oxide [Cu(II)ONPs and ZnONPs], as well as bimetallic silver-gold (Au-AgNPs), zinc oxide (ZnONPs), and titanium dioxide (TiO_2NPs), have been developed by researchers against mycobacteria (29, 222, 223). Silver is still used to prevent diseases because of its antimicrobial properties. In addition to AgNPs, many researchers across the globe have tested other metal NPs against virulent and avirulent

MDR/XDR-TB strains. A review by Simões et al. (223) mentioned the various sizes and killing kinetics of AgNPs against mycobacteria, including both TB and non-TB strains (223). Various strategies have been used to increase the efficacy of metallic NPs against tubercle bacilli. The combination of two metallic NPs can increase anti-TB activity. Singh et al. (126) showed that bimetallic Au-AgNPs have the highest anti-TB activity among AuNPs and AgNPs (224). Some scientists have reported the antitubercular optimization of AgNPs with other metal NPs (225, 226), while fewer studied NPs with conjugation of antibiotics (227, 228). Heidary et al. (225) used Ag, ZnO, and Ag-Zn NPs against MDR and XDR strains of *Mtb*. The overall result suggested that neither single NPs (Ag and ZnO) nor bimetallic NPs (Ag-Zn NPs) can kill these deadly strains (225). Recently, Chen et al. (229) developed a smartphone-assisted highly fluorescent analysis test for detecting TB DNA using a polymerase chain reaction (PCR)-padlock probe (PLP)-rolling circle amplification (RCA) polyT-CuNPs sensing platform. This research used copper nanoprobes (CuNPs) and exhibited a detection limit at 5 fg/µL DNA concentration. Fluorescent DNA-templated CuNPs provide greater biosensing capability to detect DNA in biological samples. First, the nucleic acid amplification test process was carried out using PCR-PLP-RCA methods in a sequential manner and amplified the TB DNA from the samples. The RCA products were added to copper ions and sodium ascorbate to form fluorescent polyT-CuNPs, and PCR was used to amplify the TB DNA samples. A smartphone was used to record the data, and ImageJ software (National Institutes of Health, United States) was used for image analysis. A PLP and RCA were used to amplify the signal approximately 10^3–10^4 times and enhance the accuracy, specificity, and precision of the test. This technique does not require gel electrophoresis, complex fluorescent primers, or fluorescence spectrophotometry for confirming the amplified DNA (229) (Fig. 6).

Hwang et al. (230) developed beta-glucan-conjugated silica NPs (SiNPs) for isoniazid delivery. The nanocomplexes are suitable for drug delivery and immunomodulation (230). Zhu et al. (231) recently developed α-linolenic acid nanoemulsion-templated silica NPs against drug-resistant and drug-susceptible *Mtb* using an ultrasonication technique. These results suggest the potential anti-mycobacterial efficiency of silica NPs conjugated with isoniazid and rifampicin (231). Priya et al. (232) biosynthesized gold NPs and their nanoconjugates against *Mtb* H_{37}Rv to determine their antimicrobial activities. Both nanoformulations showed 99% inhibition (232).

Magnetic nanoparticles

Moving NPs exhibit magnetic effects owing to their masses and electric charges. An NP with unique magnetic properties can be used in industrial, biotechnology, biomedical, therapeutic (*in vitro* and *in vivo*), environmental, analytical, and engineering applications (233) (Fig. 7). They are mostly used for the development of nanobiosensors for biomedical and pharmaceutical applications (234). Few studies have been published on magnetic NP (MNP) applications for the biorecognition and detection of mycobacteria or their surface biomolecules using immunoassay techniques.

León-Janampa et al. (235) developed amine-silanized magnetic NPs (MNP@Si@NH_2) for a sandwich ELISA-MNP assay. This assay detected the recombinant *Mtb* antigen Hsp16.3 and fixed and concentrated using biofunctionalized MNPs (235). Gordillo-Marroquín et al. (236) developed glycan-coated magnetic NPs for the rapid and cost-effective detection of acid-fast bacilli. This study compared sputum smear microscopy (SSM) and a nanoparticle-based colorimetric biosensing assay (NCBA) to identify tubercle bacilli. The results indicated that compared to SSM, NCBA demonstrated an enhanced count of acid-fast bacilli (236). Costa et al. (237) developed a simple and sensitive biosensor made of MBA-Fe_3O_4NPs to detect the genomic DNA of *Mtb*. The charge transfer resistance changed owing to the interaction of the sensor with different concentrations of genomic DNA, indicating a possible application for detecting TB at low concentrations (6 ng μL^{-1}) (237). Barroso et al. (238) developed a novel magnetoresistive nanobiosensor (MNP@Abs@BCG) for point-of-care TB diagnosis. This paper presents a proof-of-concept magnetoresistive (MR) biosensor for tuberculosis diagnosis. The

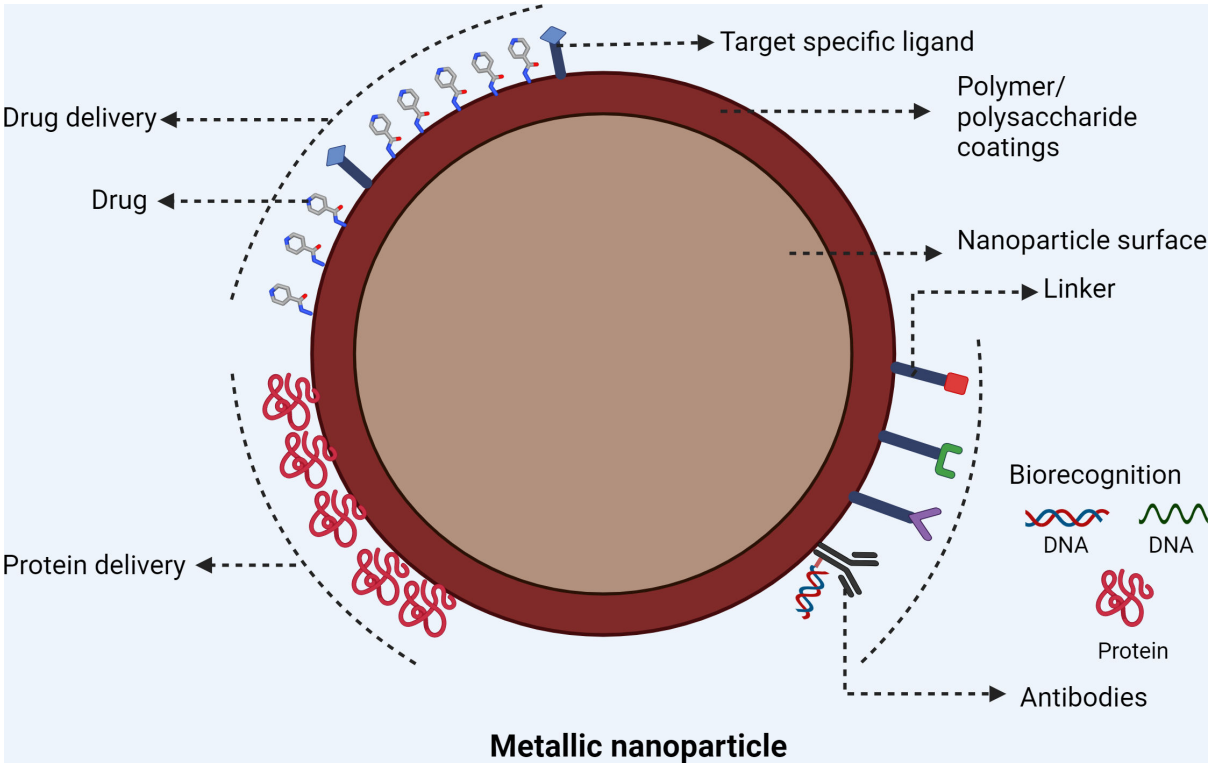

FIG 6 A schematic representation of metallic NPs for biorecognition, protein, and drug delivery systems.

findings demonstrate that while BCG binding was not 100% specific, there was always statistical significance in the difference between the MR voltages of the positive sample and negative control or zero-level background. The limit of detection of the MR-low biosensor indicates significant potential for diagnosing TB (238). Saifullah et al. (239) developed a formulation loaded with ethambutol, based on the fabrication of graphene oxide with iron oxide magnetite nanoparticles, to test its biocompatibility and therapeutic efficacy. According to the study, the designed nanoformulation exhibited sustained release of ethambutol at two physiological pH levels (7.4 and 4.8) and demonstrated potent anti-TB activity (239). Zhao et al. (240) developed magnetic Fe_3O_4/CS/INH-MNPs for the targeted delivery of isoniazid. The Fe_3O_4/CS/INH-MNCs demonstrated pH responsiveness and sustained drug release capabilities, as confirmed by evaluating their loading efficiency, stability, and release profile. Cytotoxicity assays revealed that the formulation was nontoxic and biocompatible (240). Poh et al. (241) developed Q203-bedaquiline-super paramagnetic iron oxide (SPIOs)-MNPs using a nanofabrication method for drug delivery against active *Mtb*. The nanofabrication method employed to synthesize the formulation and its subsequent encapsulation into an inhalable poly (D, l-lactide-co-glycolide) (PDLG) carrier represents a promising approach for combating active TB (241). Bhusal et al. (242) developed glycan-coated magnetic nanoparticles, an NCBA, for the rapid and cost-effective detection of acid-fast bacilli. The NCBA method demonstrated excellent sensitivity and specificity with Xpert MTB/rifampicin for 500 samples. The process requires 10–20 min to complete and costs approximately $0.10 per test (242). Smit and Lutz (243) synthesized chitosan-coated superparamagnetic magnetite nanoparticles (SPMNs) and poly (styrene-*alt*-maleic anhydride) (SMA)-coated SPMNs against *Mtb*. The results showed that SMI-qC12 (Quaternary SMA)-coated SPMNs could efficiently trap and concentrate bacilli in a sample. The trapped bacilli were extracted using an external magnet. Consequently, this finding has enormous potential as a tool for the rapid and precise diagnosis of TB using microscopic techniques such as fluorescence, scanning, and transmission electron microscopy (243).

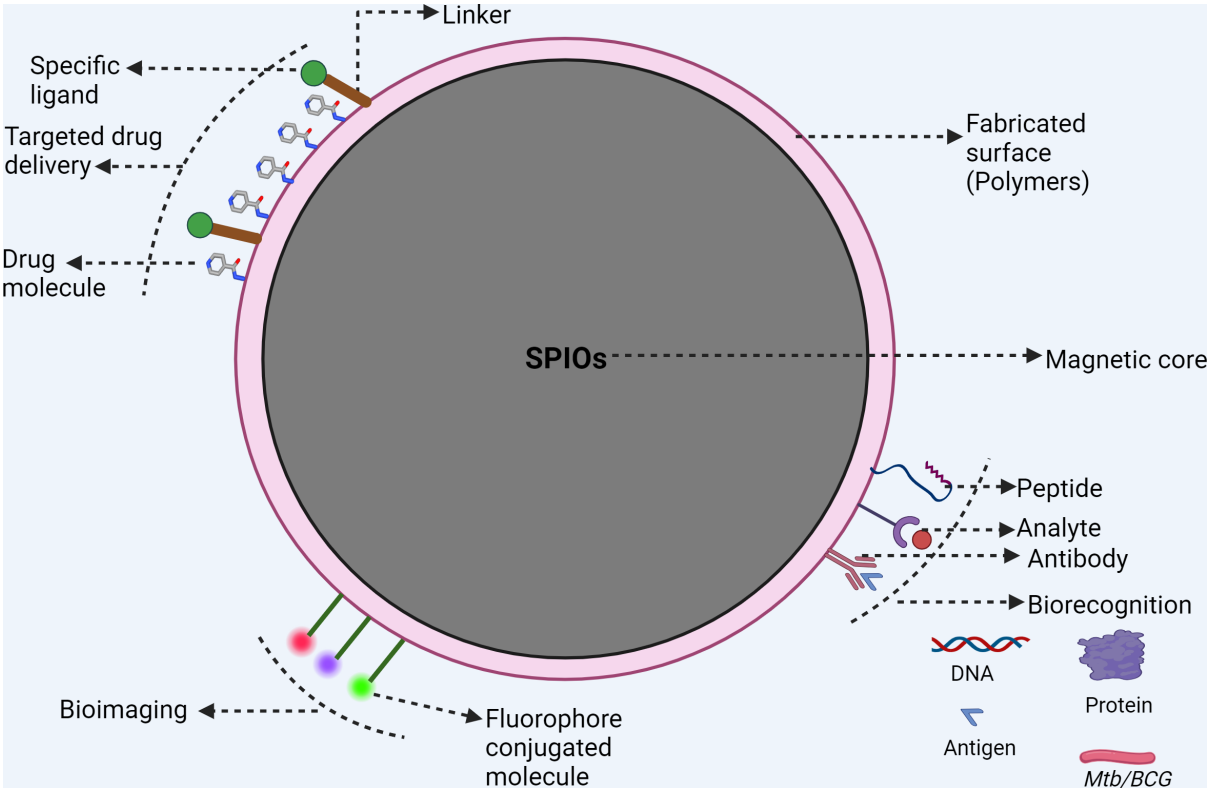

FIG 7 A representative diagram of magnetic NPs for drug delivery, imaging, and nanobiosensor development.

Zargarnezhad et al. (244) synthesized isoniazid@MNP and isoniazid@surface modified MNP (isoniazid@smMNP) and compared the anti-mycobacterial and anti-non-mycobacterial activities of isoniazid with those of its nanoconjugates. According to the findings of this study, the MIC of isoniazid-conjugated MNPs decreased by 44.8% and 16.7 when using isoniazid@MNP and isoniazid@smMNP, respectively, against *Mtb* (244). El-Zowalaty et al. (227) developed streptomycin-chitosan-MNPs and evaluated the potential anti-TB activity of the developed NPs. The formulated NPs exhibited antimicrobial activity, demonstrating a more potent effect against Gram-negative than Gram-positive bacteria (227). Lee et al. (245) synthesized SPIO-MtbsAb-NPs (innovative probe) for the ultrasensitive imaging of biomarkers involved in extrapulmonary *Mtb* infection. The results showed that *Mtb* infection can be detected and targeted by SPIO-MtbsAb NPs and indicated that it can be used as a magnetic resonance imaging contrast agents for detecting extrapulmonary *Mtb* (245).

NEW TREATMENT REGIMENS BASED ON CLINICAL TRIALS

At a clinical level, drug combinations and regimens are crucial for full therapy. Currently, XDR-TB and TDR-TB are categorized as highly resistant forms. Currently, no standard drug regimen is available for XDR-TB treatment. A few clinical trials have been completed, and some are ongoing. This section describes the three most important clinical trials on drug regimens for treating severe TB.

The Nix-TB Trial (ClinicalTrials. gov Identifier: NCT02333799) team conducted a single-group study against XDR-TB in South Africa and investigated three modern oral drugs (BPaL regimens): bedaquiline, PA-824 (pretomanid), and linezolid. The treatment success rate in this phase 3 study was 90% (101). Developing a new treatment regimen is necessary to cure the disease and reduce side effects and treatment duration.

The endTB trial (ClinicalTrials.gov Identifier: NCT02754765) was designed against MDR-TB and used existing, newly developed, and repurposed drugs, including

bedaquiline, delamanid, clofazimine, linezolid, moxifloxacin, levofloxacin, and pyrazinamide. The endTB phase 3 trials aimed to identify several new combinations of drugs against TB that respond to fluoroquinolone antibiotics in the shortest possible time (246).

The ZeNix phase three trial (ClinicalTrials.gov Identifier: NCT03086486) was initiated in 2017 and has served as a successor to the Nix-TB trial. The ZeNix trial, a randomized study, aimed to determine the optimal dose of linezolid within the BPaL regimen for patients from heterogeneous populations. This study was designed for XDR- and pre-XDR-TB. This outcome was published in September 2022 in the *New England Journal of Medicine* (247).

CURRENT STATUS AND CHALLENGES OF TB VACCINE DEVELOPMENT

To combat the aforementioned resistant forms of TB, developing an effective TB vaccine has become a key research priority. A novel TB vaccine is needed to combat drug resistance in addition to new drug discovery, drug delivery, drug repurposing, and treatment regimens. To achieve the WHO End TB Strategy target of 90% reduction in mortality and 80% reduction in incidence worldwide by 2030, a new vaccine must be effective in all age groups. Vaccination is also the most effective way to prevent the rapid spread of MDR-TB (248). Shifting the treatment regimen from oral to injectable at the clinical level may be a promising solution for the treatment of drug-resistant TB. However, BCG is the only approved and licensed TB vaccine used primarily for newborns worldwide, although its effectiveness is varied. Although this vaccine completed 100 years of discovery, many more test vaccines are currently in clinical stages (249); however, many of these fail to demonstrate efficacy and safety during the final stages of testing. The specific immune mechanisms that confer protection against TB are not fully understood, making it difficult to design and evaluate vaccine candidates. Kagina et al. (250) revealed that the protection against TB provided by the BCG vaccination given to newborns was not correlated with the cytokine expression profile and frequency of T cells specific for mycobacteria (250). However, current research has not clarified these immunological correlates. There is a lack of surrogate biomarkers or endpoints that can predict vaccine efficacy in humans, and they need to be validated with strong scientific evidence (251). The immune correlations of host defense mechanisms against *Mtb* infection remain a gray area of research. The identification and validation of biomarkers at various clinical stages are critical steps; however, TB biomarkers can be used to develop diagnostic tools for the early diagnosis of active TB. The induction of immune responses after TB vaccination, either from T cells (cellular response) or antibodies, depends on the vaccine type. The key players in adaptive immunity are $CD4^+$ and $CD8^+$ T cells, with $CD4^+$ cells producing cytokines such as interferon-gamma, tumor necrosis factor-alpha, and interleukin-2 that activate macrophages to kill intracellular *Mtb*. The specific and unclear points regarding mechanisms behind the multifunctionality of $CD4^+$ T cells could be the target of the TB vaccine (252). Various preclinical studies have shown that the multifunctionality of cells play an important role in vaccine-induced TB protection (253). Orr et al. (254) attempted to develop an antigenic vaccine based on a stable squalene-in-oil emulsion, ID93/GLA-SE, which showed protective efficacy and a strong Th1 response (254). Th1 cytokines have been used to measure the protective efficacy of TB vaccines. However, human trials have shown that vaccines that strongly induce Th1 cytokine production do not fully protect against TB (251). Test vaccines can be in the form of immunogenic antigens (adjuvanted protein subunits), live/recombinant whole cells, inactivated whole cells, or viral vectors (255–257; Table 6). Clinical development of TB vaccines is a lengthy and expensive process (258) that requires large-scale trials in high-burden countries. However, many barriers and limitations hamper the conduct and quality of these trials, such as the lack of adequate infrastructure, funding, coordination, and regulatory harmonization (259). Several promising vaccine candidates with different modes of action, target populations, and delivery platforms are currently in various stages of clinical testing (Table 6).

TABLE 6 Current progress on the TB vaccine pipeline[a]

Whole cell killed/inactivated vaccine	Whole cell live vaccine	Protein/adjuvant subunit vaccine	Viral vector vaccine	References
MIP/Immuvac Phase: III Inactivated *M. indicus pranii*; Sponsor: ICMR; Development partner(s): Cadila Pharmaceuticals; Target population(s): adolescents, children; Registry number: CTRI/2019/01/017026	**VPM1002** Phase: III Attenuated r-BCG; Sponsor: SIIPL; Collaborator: VPM; Target population(s): newborn infants (primary), adolescents, children, active TB-cured adults; Identifier: NCT04351685, NCT03152903	**GamTBvac** Phase: III Recombinant antigens vaccine; Sponsor: Ministry of Health, Russian Federation; Target population(s): HIV-negative, BCG-vaccinated, MTB-uninfected adults; Identifier: NCT04975737	**ChAdOx1 85A-MVA85A aerosol/IM (Intramuscular)** Phase: IIA Inactivated non-tuberculous mycobacteria; sponsor: University of Oxford; collaborator: MRC/UVRI and LSHTM Uganda Research Unit; target population(s): adolescents, adults, children, infants, people cured of active TB, people living with HIV, people with Mtb infection, and people without Mtb infection; identifier: NCT03681860*	255–257
DAR-901 booster Phase: IIB Inactivated non-tuberculous mycobacteria; sponsor: Dartmouth-Hitchcock Medical Center; target population(s): BCG-vaccinated HIV-negative adolescents; identifier: NCT02712424*	**MTBVAC** Phase: III Attenuated *M. tuberculosis* (genetically engineered); sponsor/lead developer: Biofabri; development partner(s): University of Zaragoza, IAVI, TBVI; target population(s): HIV-uninfected infants from HIV-infected and HIV-uninfected mothers; identifier: NCT04975178	**M72/AS01E** Phase: IIB M72 fusion protein + AS01E adjuvant system; sponsor: Bill & Melinda Gates Medical Research Institute; development partner(s): GSK; Collaborator:Wellcome Trust; target population(s): adolescents and adults; identifier: NCT04556981*	**AdHu5Ag85A (formerly Ad5Ag85A) aerosol /IM** Phase: I Recombinant human adenovirus vector (type V); sponsor: McMaster University; development partner(s): CanSino Biologics Collaborator: Canadian Institutes of Health Research (CIHR); target population(s): adults; identifier: NCT02337270*	
RUTI Phase: IIB Fragmented *Mtb*; sponsor: Archivel Farma, S.L; target population(s): adult, older Adult; identifier: NCT04919239	**BCG (Tokyo 172) vaccine** Phase: III Attenuated *M. bovis* BCG; sponsor: Henry M. Jackson Foundation (HJF); collaborators: US Department of Defense, Uniformed Services University of the Health Sciences; target population(s): Mtb-uninfected adults; identifier: NCT04453293	**H56:IC31:** Phase: IIB Mtb antigens (Ag85B, ESAT-6, Rv2660c) + IC31 adjuvant; sponsor: International AIDS Vaccine Initiative; development partner(s): Valneva, IAVI; target population(s): HIV-negative adults with active-TB; identifier: NCT03512249	**TB/FLU-01L and TB/FLU-04L** Phase: I Live recombinant influenza vector; sponsor: Research Institute for Biological Safety Problems; collaborators: Ministry of Health (Kazakhstan); Research Institute of Influenza (Russia); target population(s): adults; identifier: NCT03017378, NCT02501421*	
BNT164a1 and BNT164b1 Phase: I mRNA-based vaccines; sponsor: BioNTech SE; target population(s): adults; identifier: NCT05537038	**BCG vaccine** Phase: III *M. bovis* BCG; sponsor: Tuberculosis Research Centre (India); target population(s): BCG-vaccinated, HIV-negative child and adolescents; identifier: NCT05330884	**ID93 + GLA-SE (QTP101)** Phase: IIA ID93 recombinant protein + GLA-SE adjuvant; sponsor/lead developer: Quratis (QTP101), NIAID/NIH (ID93 + GLA-SE); target population(s): adolescents and adults; identifier: NCT03806686	**TB/FLU-05E** (liquid nasal spray) Phase: I mRNA-based vaccines; sponsor/lead developer: Smorodintsev Research Institute of Influenza; target population(s): adolescents, adults, and children; identifier: NCT05945498	
	BCG vaccine Phase: IIB *M. bovis* BCG; sponsor: Bill & Melinda Gates Medical Research Institute; target population(s): Mtb-uninfected adolescents; identifier: NCT04152161	**AEC/BC02** Phase: IIA AEC (Ag85b + ESAT6-CFP10 r-protein) + B.C.0202 complex adjuvant system; sponsor: Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd.; target population(s): adult, older adult; identifier: NCT05284812		

[a]*Completed.

CHALLENGES OF CLINICAL TRANSLATION OF ANTI-TB NANOMEDICINES

Despite the numerous advantages of nanotherapeutics and nanomedicine over conventional therapeutics, their clinical translation is progressing slowly. Compared to conventional therapeutics, nanomedicines can transport drugs with diverse physicochemical characteristics (260). They can increase therapeutic efficiency, decrease dosing intervals and adverse effects, and expand the choice of chemotherapy routes and infection-targeting strategies (261). By providing regulated and prolonged drug release, they can circumvent the low bioavailability, rapid clearance, and toxicity of first- and second-line ATDs (262). Ensuring the safety and quality of nanoformulations is an urgent requirement in the battle against various serious diseases, including drug resistance in TB. The major obstacles to the clinical translation of nanomedicines include biological compatibility issues, large-scale production challenges, high research and development costs, long-term product stability, the need for a governmental regulatory framework, and intellectual property concerns (263–265). Various anti-TB nanoformulations have been developed and tested preclinically in small animals and cell lines; however, only a few have been tested at the clinical level. Preclinical trials have demonstrated the efficacy of anti-TB DNA vaccines in mice. However, only a limited number of vaccines are currently undergoing various stages of clinical trial (266–268).

CONCLUSIONS

Drug resistance in TB results from prolonged drug exposure, poor patient adherence, and mutations in the *Mtb* genome. To address this problem, researchers are exploring new drugs and innovative drug delivery systems, such as nanotechnology-based therapies, to improve treatment efficacy and reduce side effects. Nanoformulations mostly consist of drug-loaded lipidic nanoparticles (SLNs and NLCs) and polymeric micro/nanoparticles fabricated using mannose to target *Mtb*-infected macrophages in pulmonary TB. Most routes of administration for nanoformulations are oral, but the nasal route is also possible via inhalation. However, no anti-TB nanomedicines have been approved by the FDA or are currently available on the market. Most TB nanomedicines have been tested preclinically *in vitro* and *in vivo*, but more clinical tests are needed. However, addressing the nanotoxicity of nanoformulations remains challenging.

The synthesis of nanoformulations also depends on the targeted sites (affected cells, tissues, and organs) and the route of administration. Among the nanoformulations mentioned above, it is difficult to say which one is the "most important," but the "most appropriate" should be chosen based on the severity of TB forms. A safer novel design of nanocarriers could reduce the conversion of drug-susceptible to drug-resistant forms and further reduce the transmissibility of drug-resistant *Mtb*. However, the clinical translation of nanomedicines faces various obstacles including safety concerns, large-scale production, regulatory frameworks, and intellectual property issues. Co-infections with HIV, malaria, and COVID-19 complicate TB diagnosis and treatment. Efforts are underway to improve diagnostic techniques, develop new drugs, and advance treatment strategies to combat TB and its drug-resistant forms. Formulating a new TB vaccine is crucial for the fight against TB. However, no TB vaccine is available on the market, except for the BCG vaccine. Moreover, only a few clinical trials are currently underway. Although TB remains a challenging problem, advancements in its diagnosis, treatment, and novel and safer drug delivery systems offer hope for improved outcomes in the fight against this deadly disease.

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