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Clinically Relevant Metallic Nanoparticles in Tuberculosis Diagnosis and Therapy

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Globally a significant burden of tuberculosis (TB) is faced, which is difficult to eradicate due to patients' non-adherence, and drug-resistant strains that are spreading at an alarming rate. Novel approaches are required to improve diagnosis and treatment. Metallic nanoparticles (MNPs) have demonstrated potential as sensor probes and in combination therapy, which combines MNPs with antimycobacterial drugs to develop new treatment and theranostic approaches. To strengthen the theoretical foundation toward the clinical application of TB nanomedicine, this review focuses on the properties and effectiveness of therapeutically relevant MNPs. It also elaborates on their antimycobacterial mechanisms. This review aims to analyze the body of literature on the topic, pinpoint important empirical findings, and identify knowledge gaps that can provide a basis for future research endeavors and translation of the technologies. Current data suggest that MNPs are potential systems for efficient diagnosis and treatment although additional pre-clinical and clinical research is needed to bring these technologies to the clinic.

1. Introduction

Tuberculosis (TB) is a major public health concern worldwide, with an estimated 10.6 million individuals becoming ill with TB and 1.3 million dying from the disease in 2022.[1] Despite a slow decline in incidence, prevalence, and mortality, global elimination of TB is still a distant goal. USAID has also launched the Global Tuberculosis (TB) Strategy 2023–2030, emphasizing the importance of addressing TB as a global health priority.[2] The WHO's End TB Strategy aims to address the challenges of low-burden countries, where TB tends to concentrate in selected marginalized groups.[3] The strategy includes a targeted 90% reduction in TB deaths and an 80% reduction in TB incidence by 2030 compared to 2015.[3] The strategy emphasizes the importance of addressing TB as a global health priority, strengthening health systems, engaging communities, and promoting research and innovation.[3]

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TB mainly affects the most vulnerable populations in the poorest countries;[4] 30 000 cases were reported in 2022 due to undernutrition due to poverty. The disease is also associated with HIV, with 12.8% of TB cases in 2022 being HIV related.[1] The WHO's Global TB Report 2023 highlights the importance of addressing the co-epidemic of TB and HIV, including the need for integrated service delivery and the use of antiretroviral therapy to prevent TB in people living with HIV. The report notes a slow decline in TB incidence, with a 2.3% decline from 2018 and a 9% decline from 2015. However, eliminating the disease globally is still out of reach. The WHO European and African regions have experienced the largest declines in TB incidence (19% and 16%, respectively) and mortality (31% and 19%, respectively) since 2015.[5] The rate of decline of TB incidence at the country level varies greatly. Between 2015 and 2022, 83 countries – mostly in the WHO's African and European regions – achieved anticipated reductions of at least 20%, meeting or exceeding the End TB Strategy's first milestone in 2020. An emphasis is placed on strengthening health systems, engaging communities, and promoting research and innovation to achieve the goal of a world free of TB.[6]

Any organ can be impacted by *Mycobacterium tuberculosis (M. tb)*, the primary causative organism of TB. Although the lung is the main organ affected; termed pulmonary TB (PTB), TB can be found in organs other than the lungs; termed extra-pulmonary TB (EPTB). The central nervous system, lymph nodes, pleura, abdomen, skin, genitourinary tract, joints, and bones are the most often sites of EPTB.[7] Lung bacilli infect one or more extra-pulmonary locations by spreading through the lymphatic system. Effective diagnosis and management are hampered in EPTB because the disease affects almost any organ, generates a wide range of clinical symptoms, involves difficult-to-access areas for drugs, and affects bodily fluids (mostly pleural and peritoneal) that are paucibacillary.[7,8]

The persistence of pathogen strains resistant to antibacterial drugs[9] resulting from the high rate of treatment regimen, and non-compliance due to undesirable side effects of the drugs,[10] has made the eradication of TB challenging. Therefore, it is crucial to develop novel tubercular therapeutic and diagnostic agents that are both safe and effective. The use of nano-based approaches offers unparalleled advantages in enhancing drug efficacy, minimizing toxicity, and circumventing the mechanisms of microbial resistance.[11]

Nanoparticles (NPs) have been applied to overcome the limitations of therapeutics due to their unique properties and ability to interact with biological systems at the nanoscale level.[12] The use of NPs has shown promise in various treatments ranging from bacterial infections to viral diseases, through their ability to enhance drug delivery, combat pathogens directly, and improve diagnostic and preventive measures.[12–14] The surface of NPs can be engineered to selectively target cells or tissues, thereby increasing efficacy and reducing the side effects of the drugs. Additionally, NPs can be designed to release their cargo in a controlled manner, allowing for sustained drug delivery over time.[15,16] Thus, leveraging the high surface area-to-volume ratios characteristic of metallic nanoparticles (MNPs) (metal/metal oxide NPs) presents a compelling and transformative strategy for combating TB. In addition, MNPs have been reported to show interesting remedial activities due to their intrinsic properties such as high atomic number, high radiation sensitivity, surface charge, and magnetic and electric field responsivity.[17,18] These properties enable MNPs to find applications in stimuli-responsive drug release,[19] radiation-induced cell damage,[17] and hyperthermia[20,21] treatment of various cancer cells, thereby reducing the side effects of conventional drugs.[22]

Herein, we focus on MNPs because of their additive or synergistic effect, allowing for lower doses of drugs with reduced side effects while maintaining high efficacy against TB and multidrug-resistant tuberculosis (MDR-TB). MNPs have been reported to increase the antitubercular efficacy of first and second-line TB drugs.[23] Additionally, metal/metal oxide NPs used in combination with other TB drugs have demonstrated increased effectiveness. The incorporation of MNPs into drug formulations presents a promising approach to enhance drug delivery to the affected site, potentially increasing potency.[23]

This review is important as it reveals the potential of MNPs to eradicate pathogen strains that are sensitive and resistant to TB treatment and provides new concepts for designing biocompatible MNPs. The relevance of MNPs and recent developments in their theranostic applications are covered. This review therefore offers insights for the discovery and development of MNPs with enhanced *M. tb* selectivity, thus advancing their clinical usefulness.

2. Pathology of TB

Understanding the pathology of TB alongside the applications of MNPs in its treatment provides a comprehensive framework for developing effective and targeted theranostics and drug delivery strategies while considering the complexities of the disease. Upon inhaling infectious aerosols (3–5 mm in size) containing the bacteria, *M. tb* primarily enters the alveolar macrophages, evades the host immune response, and establishes a persistent infection. This intracellular residency within macrophages is a key aspect of TB pathology, contributing to the difficulty in eradicating the bacteria. The bacilli enter the lungs through the bronchi and upper respiratory tract and reach the alveoli.[24] As the bacilli can use a variety of strategies to evade the host's immune defense and interfere with the immune system component involved, phagocytic cell lines that phagocytize *M. tb* once it reaches the lung tissue may show different immunopathologic patterns after breathing in as few as 10 *M. tb* cells.[25] Bacilli may persist intracellularly for a considerable amount of time as a source of post-primary infection into the alveolar macrophage under the circumstances of weak host defense.[24]

Chemo-attractants such as chemokines are released by macrophages that cannot eliminate the mycobacterial invaders. The resident alveolar macrophages and pneumocytes release chemokines, which attract neutrophils, monocyte-derived macrophages, NK cells, and T cells, all of which exacerbate inflammation. Subsequently, the cells form granulomas, under this circumstance, the tubercle bacilli can survive for several years inside the granulomas in a state known as "dormancy".[26] In the active type of TB, bacilli proliferate inside the host macrophage, burst through the macrophage cell membrane, and get discharged outside of it.[27] The freed bacilli from the damaged macrophage spread through the hematogenous or lymphatic route, affecting both pulmonary and extrapulmonary locations. Accurate and timely diagnosis of the freed bacilli is fundamental in managing TB disease effectively, reducing transmission, and improving health outcomes for affected individuals.

Detection of *M. tb* often involves techniques such as sputum smear microscopy, culture, nucleic acid amplification tests (NAATs), and chest imaging. Sputum smear microscopy, which stains sputum samples with specific dyes, and culture, which involves the incubation of clinical samples on specialized media, are commonly used diagnostic methods. NAATs, particularly polymerase chain reaction (PCR) assays, provide rapid and sensitive detection by amplifying the genetic material of the bacteria. Imaging techniques such as chest X-rays and computed tomography (CT) scans aid in diagnosing and monitoring TB-related lung abnormalities. Furthermore, EPTB manifestations necessitate considering other target sites for drug delivery. Given the intracellular nature of *M. tb* within macrophages and its potential dissemination to the bloodstream, these become crucial target sites for drug delivery. MNPs, with their antimicrobial properties, can be engineered to specifically target and penetrate macrophages, enhancing the efficacy of treatment against intracellular bacteria. Targeting the bloodstream with MNPs-based drug delivery systems may also be beneficial in addressing disseminated or EPTB infections. By understanding the residency of *M. tb* and current detection methods, researchers can develop innovative approaches to drug delivery, aiming to improve treatment outcomes and combat TB effectively.

2.1. Diagnosis of TB

The diagnosis of TB involves a combination of conventional methods such as sputum culture, advanced molecular tests recommended by WHO, and clinical procedures such as bronchoscopy and urine analysis. These diagnostic tools and techniques are crucial for accurately confirming TB infection, identifying drug resistance, and initiating appropriate treatment promptly. Diagnosing TB poses significant challenges due to limitations in current diagnostic methods. These challenges include long turnaround times,[28] low sensitivity, high costs,[29] and the inability to distinguish active TB disease from latent infection.[30,31] Traditional diagnostic tools such as sputum smear microscopy are being replaced by molecular techniques such as Xpert MTB/RIF, which provide faster and more accurate diagnoses. However, despite advancements, there is still a need

for simple, sensitive, and rapid point-of-care (POC) tests for TB screening in resource-limited settings.[32] A POC test that could quickly identify active TB would close many of the present worldwide TB control gaps, shorten diagnostic delays, and interrupt transmission with the right treatment.

Immunologically based TB detection tests have not yet received WHO approval due to their limitations in effectively diagnosing active pulmonary TB disease.[33] Molecular diagnostic tests such as GeneXpert offer rapid results within 2 h and are widely used in resource-limited settings for TB screening.[33] Challenges in TB diagnosis contribute to delays in seeking health services and poor health service delivery, leading to missed diagnoses and increased TB incidence rates.[33,34] To address these shortcomings, ongoing research is focused on developing innovative diagnostic tools that can provide accurate and timely detection of TB, especially in high-burden settings where the disease is prevalent.[29,34] Despite progress, investments and further technological developments are essential to optimize and validate these new diagnostic methods for effective TB management globally.[29] The potential of MNPs to improve TB diagnostic accuracy is highlighted by their integration with optical probes or sensors.[35]

MNPs have shown significant promise in the imaging and diagnosis of TB, offering innovative approaches to enhance visualization, early detection, and accurate diagnosis. MNPs with sizes <100 nm possess unique properties that make them valuable tools in TB diagnostics. Recently, Patnaik and Dey developed a label-free citrate-stabilized silver nanoparticles (AgNPs) aggregation assay as an innovative molecular-biosensing strategy that offers sensitive, cost-effective, and swift detection of *M. tb*.[36] Furthermore, the diagnosing efficiency of silica-supported gold nanoparticles (MSNs@GNPs) was evaluated by calorimetric analysis, which showed that MSNs@GNPs are useful for rapid diagnosis of TB when applied to an in vitro culture of the *M. tb*.[37] However, their report needs further verification on human clinical samples from TB patients.

Also, integrating MNPs with imaging techniques has opened new avenues for improving the accuracy and efficiency of TB diagnosis. One key aspect where MNPs excel is as contrast agents in medical imaging, enhancing tissue visualization and aiding in the early detection of TB. Leveraging the distinctive properties of MNPs, such as surface plasmon resonance (SPR) and enhanced photoluminescence, researchers have developed advanced imaging modalities that can detect TB-related changes at a molecular level. This capability is crucial for identifying TB lesions, monitoring disease progression, and assessing treatment efficacy. Moreover, MNPs have been utilized in developing novel diagnostic systems for TB. For instance, Tsai's group developed an SPR method of colorimetric analysis based on unmodified gold NPs (AuNPs)-paper to detect *M. tb*.[38] In this instance, a single-stranded DNA probe hybridizes with a double-stranded target *M. tb* DNA. Variations in the colloid's surface charge density brought about by the hybridization process would manifest as a color shift. After that, it is easily quantified using the DNA analyte. Before optimization, a LOD of 1.95×10^{-2} ng mL^{-1} of *M. tb* DNA could be found with a 1-hour turnaround time.[38] These NPs can be functionalized with specific biomolecules, such as antibodies or nucleic acids, to target TB-specific markers, facilitating precise and sensitive disease detection. By harnessing the high surface area-to-volume ratio and catalytic activity of MNPs, researchers have created diagnostic platforms that offer rapid, accurate, and cost-effective TB screening methods.

2.1.1. Challenges in Imaging and Diagnosis of Pulmonary and Extra-Pulmonary TB

Diagnosis of TB, particularly extrapulmonary TB (EPTB), remains a challenge due to the paucibacillary nature of the disease,[39] the variable clinical presentation, the need for invasive procedures to secure appropriate samples, and the lack of laboratory facilities in resource-limited settings.[40] The diagnosis of EPTB usually depends on clinical symptoms, radiologic imaging such as a computerized tomography (CT) scan, and the presence of extra-neural TB. The role of radiologic imaging is crucial in the EPTB diagnosis, and knowledge of its pathophysiology and imaging features can help the radiologist make an accurate diagnosis.[41] However, the imaging findings of EPTB are often non-specific and can mimic other diseases, making the diagnosis challenging.[42]

Histopathology is another diagnostic tool for EPTB, and it is often used when other diagnostic methods are inconclusive.[7] In a study conducted in Ethiopia, histopathology findings consistent with TB were found in 59.1% of EPTB cases, while only 1.5% had a documented acid-fast bacilli-positive result.[7] The study also found that all EPTB patients were started on anti-TB therapy without definitive microbiology results, indicating the diagnostic challenge of EPTB faced in their setting.[7] In addition to histopathology and radiologic imaging, other diagnostic methods for EPTB include endoscopic ultrasound-guided fine-needle aspiration, which has been used for diagnosing extrapulmonary TB lymphadenitis.[43] The diagnosis of EPTB remains challenging, and a combination of clinical, radiological, and microbiological methods is often used.[44]

2.2. Treatment of TB

The main antibiotics used in TB treatment include isoniazid (INH), rifampin, pyrazinamide, and ethambutol. In cases of drug-resistant TB, additional drugs including fluoroquinolones, amikacin, bedaquiline, ethionamide, and para-aminosalicylic acid may be prescribed. The treatment regimen for TB typically involves a combination of these antibiotics taken for 6 to 9 months. Antibiotics have played a crucial role in the reduction in the incidence of TB globally as evidenced by the fact that before the mid-20th century, the mortality rate within five years of the onset of the disease was 50%. However, antibiotic therapy faces significant challenges due to *M. tb* adaptive mechanisms toward available drugs, which result in drug resistance.[18]. Antibiotic resistance in *M. tb* is developed via several intrinsic and acquired mechanisms.[45] Mycobacteria's unique cell wall, which contains the intricate structure of mycolic acid, contributes to its limited permeability for the absorption of drugs.[45] Low drug concentrations may cause the transporter protein to become overexpressed after prolonged exposure to the bacteria. This overexpression results in the irreversible development of phenotypic resistance.[46] The bacteria's β-lactamase enzymes can block β-lactam antibiotics, leading to drug resistance. Additionally, Rv1698 and Rv1973

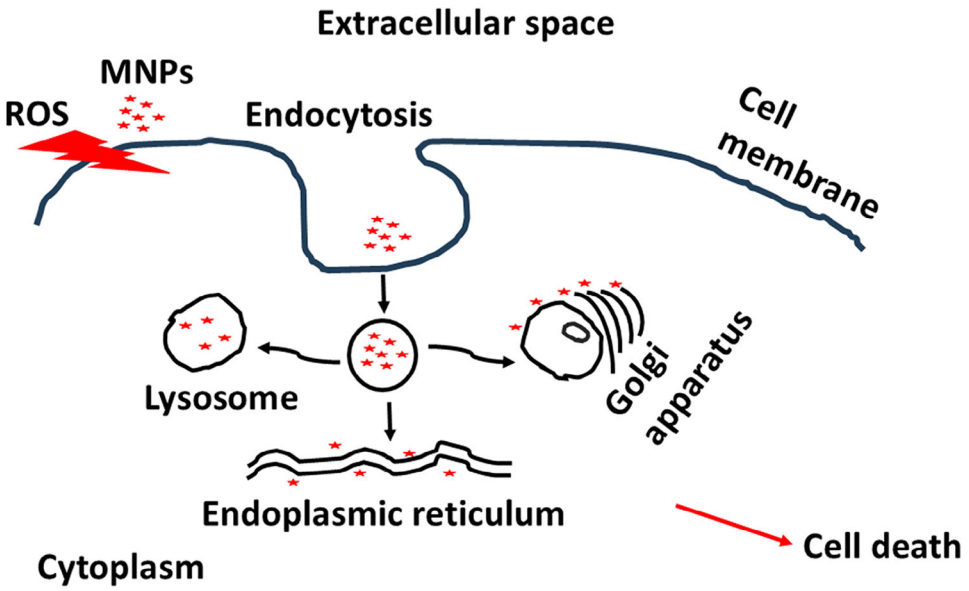


Figure 1. Cellular interaction and intracellular trafficking of MNPs resulting in *M. tb* cell death.

are crucial to the mechanism of intrinsic resistance in mycobacterium and are found in the membrane of the mycobacteria.[46] Apart from the intrinsic resistance mechanisms, chromosomal mutations confer the most clinically relevant drug resistance in *M. tb*.[47] Chromosome mutation of the drug-target genes causes mycobacterium to develop acquired resistance during treatment. Anti-TB drugs that are ineffective or administered at inadequate dosages can provide the bacteria an extra edge during therapy, allowing the resistant forms of the bacteria to proliferate. This may ultimately lead to mutagenesis, which may then encourage further drug resistance and ultimately multidrug resistance.[46]

Treatment of EPTB is much like that of PTB, and the standard regimen consists of a combination of four drugs: INH, rifampicin, pyrazinamide, and ethambutol. However, the duration of treatment for EPTB is longer than that for PTB, and it varies depending on the site of infection. A study observed extended treatment duration by 6 months in 23% of 251 EPTB patients.[48] The WHO is actively working to accelerate rapid diagnostics and treatments for drug-resistant TB and expand access to better and more effective treatments.[3] The management of MDR-TB and XDR-TB often demands 6–18 months of uninterrupted therapy, with multidrug administration, further complicating the treatment process.[49] In a recent drug-susceptible pulmonary TB regime development, a 4-month regimen of rifapentine and moxifloxacin was found to be non-inferior to the usual 6-month regimen consisting of rifampin, INH, pyrazinamide, and ethambutol at the 12-month follow-up,[50] marking the first such finding in four decades.[51] However, there is a need for shorter, safer, and simpler regimens to cure all patients with TB and their expansion to low-resource settings is crucial in addressing the challenges associated with drug-resistant TB.[49,51] Accelerating efforts to combat TB, especially in the face of drug resistance underscores the importance of exploring alternative treatments, such as the use of MNPs, to address the challenges posed by drug-resistant tuberculosis.[52]

3. Theranostic Applications of Metallic Nanoparticles

MNPs have garnered significant attention for their diverse medical applications, particularly in antimicrobial strategies, drug delivery systems, and theranostic applications.[53] These NPs offer high antimicrobial activity and biocompatibility, making them promising tools to combat antimicrobial resistance. Various MNPs including gold, silver, zinc oxide, and iron-oxide have shown potential in enhancing drug efficacy against resistant microbes.[54] These can target microbial cell membranes, block efflux pumps, release ions to induce oxidative damage, improve drug stability and pharmacokinetics. Additionally, MNPs serve as carriers for antimicrobial agents, enhancing drug efficacy by increasing cell permeability and weakening bacterial cell envelopes.[55] Their unique properties make them effective in delivering drugs with increased specificity and reduced side effects.

As shown in **Figure 1**, MNPs can penetrate directly through the membrane of *M. tb* and increase the permeability by forming nano-sized pores because of electrostatic interaction between negatively charged molecules of the cell wall of the microorganism and positively charged NPs resulting in a leakage of cytoplasmic contents,[56] as well as through causing membrane potential disorder. Abdel-Aziz et al. observed lysed *M. tb* cells devoid or near-devoid of cytoplasm at the end of the incubation period (7 days) of chitosan/Ag nanocomposite with *M. tb* cells. The physical interactions caused structural alterations and damage to the cell membrane, making the mycobacterium more permeable.[57] Also, the anti-mycobacterial action of alginate-capped Ag NPs is mediated by increasing cell wall permeability.[58] Zinc oxide NPs can interact with the membrane of *M. tb*,[59] while gallium NPs can deliver a drug to macrophages, inhibit *M. tb* and nontuberculous mycobacterial growth,[24] and reduce the inhibition of phagosome maturation. Iron NPs can act as nanocarriers of anti-tuberculosis drugs. Mixed metal oxide NPs have also been found

to inhibit the growth of *M. tb* into macrophages.[24,60] Utilizing NPs could help reduce drug dose and treatment duration.[52]

Another mechanism comprises the generation of toxic reactive oxygen species (ROS). The excessive production of ROS causes disturbances in redox homeostasis, which results in oxidative stress, consequently, affecting membrane lipids, and altering DNA and the protein structure.[55] Oxidative stress leads to glutathione oxidation, disrupting the antioxidant defense mechanisms of bacteria against ROS. A further mechanism involves MNPs binding to intracellular components, causing damaged DNA and proteins, and inhibiting the enzymatic activity.[61] The interaction of MNPs with DNA denatures or shears the DNA and disrupts cell division. In addition, MNPs inhibit protein synthesis by denaturing ribosomes. The above factors ultimately cause apoptotic cell death.[55,62]

Magnetic metal oxide NPs have shown promise in theranostic applications; from diagnosis and therapeutics to bioseparation due to their tunable stability and functionality.[63] Several studies have explored their potential applications, particularly in improving the sensitivity and specificity of diagnostic techniques. These theranostic NPs are utilized in nuclear, optical, magnetic resonance, ultrasound, and computed tomography encompassing positron emission tomography and single-photon computed tomography.[64] Iron oxide nanoparticles (IONPs) have been investigated for their use in magnetic resonance imaging (MRI) for detecting TB. These NPs have been shown to accumulate in detectable amounts in TB granulomatous lesions, supporting their potential for TB diagnosis.[65]

In addition to MRI, magnetic nanoparticle-based biosensing assays have been developed to enhance the detection of *M. tb* and improve the sensitivity of TB diagnosis. For instance, a study reported functional nanobiosensors based on magnetic NPs for rapid and accurate TB diagnosis at a low cost.[66] Furthermore, the efficacy of magnetic IONPs has been explored for the molecular-level diagnosis of TB, offering a potential improvement over conventional diagnostic methods such as the Tuberculin Skin Test (TST) and Interferon-Gamma Release Assay (IGRA).[67]

In addition, IONPs have been explored for their superparamagnetic properties and low toxicity.[68] Alloy magnetic NPs, such as FeCo NPs, have also been assessed as possible replacements for IONPs in theranostic applications.[63,69] Furthermore, polymer-coated magnetic NPs have been developed for the efficient capture of *M. tb* to improve the diagnosis of TB.[70,71] These coated MNPs have the potential to capture the bacilli and remove them, making them a promising tool for the diagnosis of TB.[71]

The use of magnetic metal oxide NPs in TB diagnosis holds promise for addressing the limitations of current diagnostic strategies. Conventional methods, such as smear microscopy and culture, have constraints such as being time-consuming and requiring specialized laboratory facilities, which can hinder prompt TB diagnosis, particularly in resource-limited settings.[72] Therefore, the development of novel nanodiagnostic approaches, including those based on magnetic metal oxide NPs, could offer a more accessible and efficient means of TB diagnosis. Magnetic metal oxide NPs, particularly super-paramagnetic iron oxide nanoparticles (SPIONs), show potential for enhancing the sensitivity, specificity, and accessibility of TB diagnosis. Further research and development in this area could lead to integrating these NPs into advanced diagnostic techniques for TB, addressing the current limitations of conventional methods. However, it is important to continue investigating the efficacy and safety of these NPs to ensure their reliable and accurate application in TB diagnosis.[73]

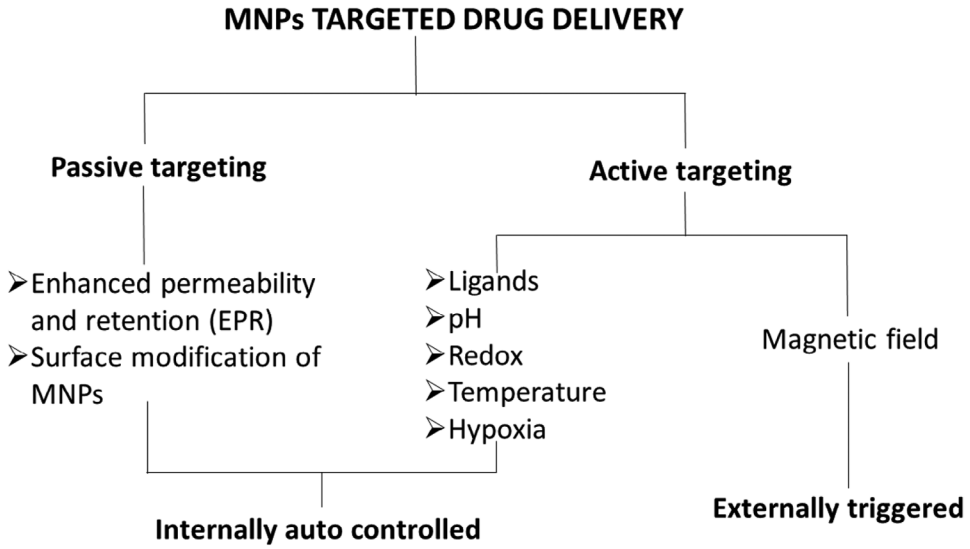
On the other hand, magnetic metal oxides, particularly IONPs, have shown great potential in various bacterial infection therapies, including drug delivery, antimicrobial activity, and hyperthermia.[74] Recent research has focused on developing smart magnetic drug delivery systems for the treatment of TB, where magnetic NPs are used to enable targeted drug delivery and trigger drug release in response to external stimuli[75] such as magnetic fields or near-infrared (NIR) radiation. One of the most used magnetic particles for drug delivery is SPIONs. These NPs have been investigated for their potential to trigger drug release and improve lesion targeting either by passive or active targeting (**Scheme 1**). SPIONs can be magnetized in an external magnetic field, allowing for externally triggered targeted drug delivery to specific sites, such as TB granulomatous lesions. Furthermore, SPIONs have been integrated into smart drug delivery systems, where the release of antibiotic drugs is triggered by magnetic fields or NIR radiation, enabling controlled and targeted drug release.[70,76]

The unique magnetic properties of IONPs make them suitable for crossing the blood-brain barrier opening new possibilities for treating neurological diseases[15,77] including TB of the central nervous system. Moreover, recent developments have aimed to develop smart magnetic drug delivery systems that increase the spatial specificity of drugs, trigger drug release in response to external stimuli, and improve magnetic mediated targeting to deep tissues[76–78] These strategies improve spatial control and drug delivery targeting, which is particularly important for treating diseases such as TB.[15]

Furthermore, the use of magnetic IONPs where the heat generated by the NPs is used to treat TB, has been a subject of recent research. These NPs can ensure magnetic targeting and produce hyperthermia, leading to altered physiology of *M. tb* infected cells and ultimately their apoptosis/necrosis. This approach, known as magnetic hyperthermia therapy, holds promise for the treatment of TB and has been the focus of ongoing research efforts. A review article on multifunctional magnetic IONPs for biomedical applications also mentioned the potential of these NPs for hyperthermia therapy.[79] The review highlighted the importance of developing composite nanoplatforms based on integrating magnetic IONPs with organic dyes, biomolecules, and other materials for various biomedical applications, including hyperthermia therapy. One study reported the fabrication of magnetite (Fe_3O_4) NPs coated with various biocompatible surfactants, such as glutamic acid (GA), carboxymethyl cellulose (CA), polyvinylpyrrolidone (PVP), and polyethylene glycol (PEG).[80] These NPs were investigated for their magnetic and inductive thermal properties critical to magnetic hyperthermia therapy.

3.1. Metallic Nanoparticles for Targeted Drug Delivery in TB Treatment

Drug delivery nanomaterials and approaches have advanced to a point where they can enable the modulation of a drug's pharmacokinetics, stability, absorption, and exposure to tumors and



Scheme 1. MNPs in drug delivery. Stimuli-responsive MNPs directed therapy. Drug release is either controlled internally or externally.

healthy tissues, and facilitate the administration of synergistic drug combinations.[81,82] Targeted drug delivery has the potential to greatly improve drug-delivery efficacy, reduce side effects, and lower treatment costs[83] hence, the development and application of novel multiple drug delivery systems (DDSs).

Traditional drug delivery systems suffer from poor bioavailability and fluctuations in plasma drug levels and are unable to achieve sustained release. The whole therapeutic process can be rendered ineffective without an efficient delivery mechanism.[84] Recent developments in DDSs have been focused primarily on smart drug delivery, which enhances the therapeutic effectiveness of new and existing drugs with targeted and sustained delivery while meeting real and appropriate drug demand.[85] Drug delivery is a growing field in pharmaceutical science, with smart targeted drug delivery (STDD) offering several advantages over traditional drug delivery methods[82,86] including improved pharmaceutical activity, reduced side effects and fluctuation in circulating drug levels, avoidance of the first-pass effect, simpler drug administration, decreased toxicity, and reduced treatment costs.

MNP DDSs (Scheme 1) utilize the unique properties of MNPs for drug delivery and bring an opportunity for controlled release of drugs, allowing sufficient time for drugs to act with enhanced therapeutic action and respond to specific stimuli, such as pH, light, heat, or enzymes.[87] A study carried out on the delivery of INH revealed the increased rate of drug release from iron oxide nanocarriers when the pH was lowered from pH 7.4 to pH 5.7 after 7 h,[75] as shown in **Figure 2**.

MNPs have several additional advantages for drug delivery, including increased stability and half-life of the drug and the ability to overcome the problem of first-pass metabolism. The key properties of MNPs that make them suitable for drug delivery include well-characterized structure,[88] tunable pore size, and porosity which allows for the encapsulation of drugs and other therapeutic agents, ultrahigh surface area which increases their potential for drug loading and targeting, chemical functionalization permits the attachment of drugs and active or passive targeting moieties, for the delivery of drugs specifically to target cells and tissues, while multifunctionalization permits combining drug delivery, imaging, and other therapeutic functions.[89]

In treating TB, MNPs can be a helpful tactic for two different reasons: (i) their inherent antimycobacterial activity[90] and (ii) their capacity to serve as carriers for antitubercular drugs to enable administration through pulmonary or oral routes[91] that are more user-friendly. The drug delivery systems exploring MNPs as nanocarriers lead to significant benefits including enhanced drug carrier stability and half-life in circulation, sufficient biodistribution, passive or active targeting into the desired target site, and reduced dose- and drug-associated side effects.[87] However, MNPs are still in the early stages of development, and further research is needed to generate several novel and more effective agents with varying potential for progression into optimization, preclinical development,[87] and clinical trials.

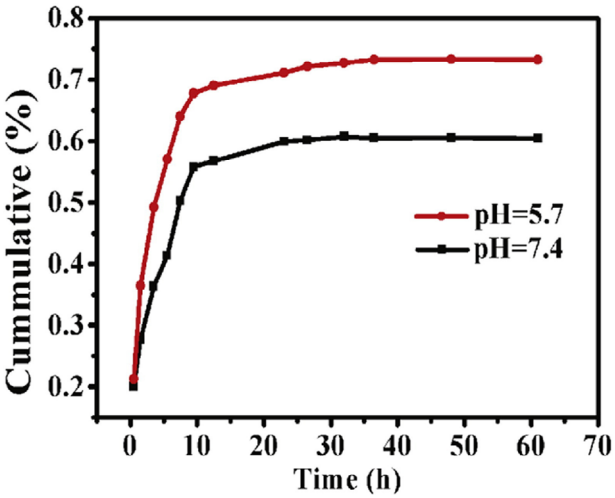


Figure 2. In vitro release profile for INH showing the influence of pH on INH release from iron oxide NPs. Reproduced with permission.[75] Copyright 2017, Elsevier.

3.2. Clinically Relevant Metallic Nanoparticles in TB Treatment

MNPs have shown potential in the management of TB.[34] The effectiveness of MNPs, particularly Ag and zinc oxide (ZnO), in inhibiting *M. tb* and enhancing phagosome maturation into the infected macrophage was reported.[24,92] MNPs remain an underexplored weapon to fight TB and other mycobacterial infections despite their benefits.[52] Hence, further research is needed to optimize their properties and achieve the ability to deliver therapeutic molecules effectively.[93] The overview study of Tăbăran et al. underscores the potential of AgNPs as a future therapeutic option for TB management, shedding light on their advantages, limitations, and the need for further research to optimize their efficacy and safety in combating TB.[62] The viability of this complementary strategy depends on overcoming several critical therapeutic issues such as poor delivery, variable intramacrophagic antimycobacterial efficiency, and residual toxicity.[62] In this section, we highlight the recent clinically relevant metal nanoparticles in the last decade for combating TB in terms of diagnosis, and therapeutics (as free or cargo agent) (**Table 1**).

3.2.1. Selenium Nanoparticles

Estevez et al. reported that selenium nanoparticles (SeNPs) exhibited intrinsic antimicrobial activities, inhibiting the growth of two types of mycobacteria, i.e., *Mycobacterium smegmatis* (*M. sm*) and *M. tb*. The results showed that SeNPs inhibited the growth of mycobacteria by damaging cell wall integrity.[94] In another study performed by Pi et al., SeNPs were found to enter the macrophages preferentially, accumulate in lysosomes, release loaded isoniazid drug, and induce the autophagic sequestration of *M. tb* evolving into lysosome-associated autophagosomal *M. tb* degradation.[95]

3.2.2. Silver Nanoparticles

AgNPs have been extensively studied for their sole efficacy in TB treatment including the drug-resistant *M. tb* strains.[58] These particles have been used for direct antimycobacterial therapy and as a carrier for therapeutic agents,[96] and have shown significant potential. The increased antibacterial activity of AgNPs could be attributed to their permeability across cell membranes.[91] A green biosynthesized spherical Ag nanocomposite exhibited in vitro antimycobacterial activity with a minimum inhibitory concentration (MIC) of 1.95 µg mL^{-1} using Alamar blue assay.[57] Similarly, spherical AgNPs synthesized using garlic extract were utilized as cargo for the anti-TB INH drug, the in vitro and molecular docking studies performed showed their potential activity against *M. tb* and the protein anthranilate phosphoribosyltransferase (trpD) from *M. tb*, respectively; however, further studies are required to confirm the computational results.[96] In another study, the immune system of mice was restored to balance when polyvinylpyrrolidone stabilized AgNPs were delivered via inhalation and exhibited a pronounced bactericidal action against the strain *M. tb* H37Rv.[97] Therefore, AgNPs can be considered promising in TB prevention and treatment. Similarly, a recent study successfully combined maltol-capped AgNPs (McAgNPs) with streptomycin sulfate ($STR.SO_4$) and INH against *M. tb* H37Rv. The obtained data demonstrated that McAgNPs efficiently and simultaneously detected $STR.SO_4$ and INH. The McAgNPs-drug complexes substantially suppressed *M. tb*, well below the critical concentrations compared to each drug alone. Therefore, McAgNPs have considerable dual potential as sensors and TB therapeutics through their complexes with $STR.SO_4$ and INH.[98]

3.2.3. Gold Nanoparticles

AuNPs have shown significant antimycobacterial activity[99] due to their intrinsic properties such as conductivity, catalytic nature, enhanced solubility efficiency, chemical stability, half-life, and mechanism in binding with cell membranes to induce cell death (Figure 1). AuNPs have shown potential for targeted drug delivery due to their ability to accommodate drugs by conjugation via physical adsorption, and ionic or covalent bonding.[100,101] Moreso, AuNPs not only infiltrate blood vessels to reach the site of the cell membrane but also enter organelles.[100] All these properties are uniquely dependent on the particle size of the AuNPs. Biosynthesized AuNPs and nanoconjugates using plant extracts showed exceptional activity against *M. tb* H37Rv, the MIC demonstrated 99% inhibition and MIC_{99} was found to be 6.42 µg mL^{-1}.[99] Ali et al. identified the efficacy of Au in the form of nanorods (AuNRs) in the treatment of TB both as a free anti-TB agent and carrier for rifampicin, which is released after uptake into macrophage cells (RAW264.7).[102] Sun et al. designed and employed silica-supported gold nanoparticles (MSNs@GNPs) in TB treatment and reported that the designed MSNs@GNPs retained significantly lower minimal inhibitory concentration (MIC) values and could effectively eradicate *M. tb*.[37]

3.2.4. Palladium Nanoparticles

Recently, Nandan et al. designed calix[4]pyrrole tetrabenzohydrazide capped-palladium nanoparticles (CPTBH-PdNPs) through a one-pot reaction, the CPTBH-PdNPs demonstrated exceptional efficacy against *M. tb*, with an MIC value of 0.8 µg mL^{-1}.[103]

3.2.5. Zinc Oxide Nanoparticles

Studies have highlighted the biphasic roles of ZnONPs in inducing bacteriostasis in *M. tb* and regulating the pathogenic processes associated with TB.[104] Additionally, ZnONPs have been synthesized and evaluated for inhibitory effects on MDR and XDR strains of *M. tb*, demonstrating bacteriostatic effects against these strains.[105] Furthermore, an overview of ZnONPs synthesized using plant extracts for anti-TB treatment has been described, emphasizing the potential of ZnONPs in combating drug-resistant TB and offering a novel approach to TB management.[106] The synergistic effects of ZnONPs with rifampicin have been investigated, showing damage to the membrane of mycobacteria, which indicates a potential mechanism for overcoming mycobacterial drug resistance.[59]

Table 1. Summary of clinically relevant metallic nanoparticles in TB treatment as free antimycobacterial agent or drug carrier.

NPs	Mode of synthesis	Physicochemical properties	Drug Agent loaded	Observations	Ref.
SNP-PVP	Polyvinylpyrrolidone stabilized single dispersion	Particle size = 43.6 ± 10.7 nm	-	In vitro anti-TB determination via suspension method using H37Rv. The number of mycobacterial CFUs decreased by a factor of two after exposure to SNP-PVP at a concentration of 50 µg/ml. In vivo determination in TB-infected mice. Before the introduction of SNP-PVP, the average number of mycobacteria was 4.2 × 102 CFUs in the spleen and 4.4 × 102 CFUs in the lungs. The mice demonstrated a significant reduction in organ contamination ten days following SNP-PVP inhalation (the average number of mycobacteria was 10 CFUs in the lungs and 21 CFUs in the spleen), which continued for thirty days.	[97]
TMC/Ag	Green biosynthesis	Well-distributed spheres and their diameter ranged from 11 to17.5 nm	-	In vitro antimycobacterial activity with minimum inhibitory concentration (MIC) of 1.95 µg/ml.	[57]
AgNPs	Green synthesis using garlic extract	Zeta particle size = 145.3 ± 2.1 nm, Zeta potential = −33.1 mV	Isoniazid (INH)	In vitro anti-TB determination and docking method. The drug was released sustainably. Also, Sativoside R2, Degalactotigonin, Proto-desgalactotigonin, Eruboside B, and Sativoside R1 showed a better docking score than anthranilate phosphoribosyltransferase (trpD) from *M. tb*.	[96]
MSNs-AgBr	One-pot sol-gel co-condensation using CTAB as structure directing agent	12 ± 6 nm Ag particles supported in mesostructured silica network with ≈2.5 nm pore size.	-	In vitro *test in M. tb* using cryo-electron microscopy. Inhibit the growth of *M. tb* by damaging the *M. tb* envelope integrity at a concentration of 15 µg mL^{-1}.	[112]
ALG-AgNPs	Chemical reduction using natural biopolymer alginate as a reducing and/or stabilizing agent.	Round shaped with Zeta particle size = 70 nm, Zeta potential = −47 mV	-	Effective anti-mycobacterium activity against various pathogenic strains of *M. tb* in vitro. Safe and effective therapeutic in zebrafish and mouse tuberculosis animal models in vivo.	[58]
Ch-Se	Redox system in the presence of a biomacromolecule as a soft template	Particle size = 105.7 ± 2.5 nm, Zeta potential = 66.6 ± 4.7 mV.	-	Effective in inhibiting dose-dependent mycobacterial growth, MIC values of 0.400 µg mL^{-1} for *M. sm* and 0.195 µg mL^{-1} for *M. tb*.	[94]
AgNPs	Seaweed-mediated reduction process	Cluster and spherical in shape, particle size = < 100 nm.	Nil	Anti-TB activity screening using Luciferase reporter phage (LRP) assay. Showed potent activity against *M. tb* H37Rv.	[113]
ZnO	Biosynthesis method using *Capparis zeylanica* extract	Spherical, hexagonal/Wurtzite structure, particle size = ≈34 nm	Nil	Anti-TB activity screening using standard agar well diffusion assay. Maximum diameter of inhibition zone = 35 ± 1.86. Better resistance than commercial antibiotics such as *Pyrazinamide*, *Ciprofloxacin*, and *Streptomycin*.	[114]
ZnO	Commercial powder	Partcle size = < 50 nm	Nil	Inhibitory effect against various strains of *M. tb* (BCG, H37Rv, and clinically susceptible MDR and XDR strains). ZnONPs decreased the *M. tb* load in vitro, ex vivo, and in vivo. Co-treatment with ferroptosis inhibitor enhanced the anti-*M. tb* activity of ZnONPs.	[104]
$MSNs\text{-}COOH_{ext}$	Post-synthetic grafting method	Particle size = 200 nm	Immunomodulatory proteins	Utilized as immunomodulatory nanocarriers for proteins. The created nanosystems function similarly to the natural *M. tb* EVs due to their ability to produce pro- and anti-inflammatory cytokines.	[115]

(*Continued*)

Table 1. (Continued)

NPs	Mode of synthesis	Physicochemical properties	Drug Agent loaded	Observations	Ref.
MCM-41	Sol-gel method. Functionalized via post-synthetic grafting method	Zeta potential = −20 mV, Zeta particle size = 188 ± 2.12 nm, pore size = 2.04 nm, surface area = 503.4 m^2 g^{-1}	Pretomanid and MCC7433	Inhibitory effect against the *M. tb* H37Rv strain using a resazurin-based microtiter assay. Enhanced solubility of pretomanid and MCC7433 with retained anti-*M. tb* in vitro. Amino-functionalized MCM-41 NPs enhanced the systemic exposure of MCC7433 in mice (1.3-fold higher C_{max}) compared to MCC7433 alone.	[116]
DMSN	Chemical method: Oil-water biphase stratification reaction system	Particle size = 163 ± 7 nm, zeta potential = −7.5 ± 0.4 mV, specific surface area = 554 m^2 g^{-1}, pore diameter = 4.7–10 nm	NapFab	Antimycobacterial activity against intracellular *M. tb* is notably increased because of cellular absorption.	[117]
ZnO-Se	Chemical method	Spherical core-shell, average diameter = 90 nm, zeta potential = – 60 mV	Nil	Inhibitory effect against extracellular *M. tb including BCG and* H37Rv strain using colony-forming units (CFU) counting, bacterial ATP analysis, bacterial membrane potential analysis, and scanning electron microscopy imaging. Also, against intracellular *M. tb* in THP-1 cells by colony-forming units (CFU) counting. The ZnO-Se NPs combined *M. tb* killing and host cell immunological inhibitory effects for synergistic anti-TB efficacy.	[118]
MgO-ZnO		Mono dispersed with particle size < 50 nm	Nil	The anti-tubercular activity against MDR-*M. tb* was evaluated using microplate alamar blue assay (MABA) and the proportion method. The hybrid NPs showed synergistic activity against *M. tb* with MIC values of 1.328 µg mL^{-1}. Additionally, the dose-response curves demonstrated that the anti-tubercular effect of NPs depends on the concentration of MDR-TB present.	[60]
Cu-MIONS	MIONS synthesized by chemical co-precipitation and Cu deposited by chemical reduction using $NaBH_4$	Spherical NPs, particle size = 25.21 ± 13.24		Extracellular antimycobacterial assays against *Mycobacterium smegmatis* $mc^2$155. Exhibited total growth inhibition at concentrations of 15.63 µg mL^{-1}	[110]

3.2.6. Metal Nanocomposites

Recent studies have demonstrated that transition metal nanocomposites comprising more than one metal can have synergistic antimicrobial effects.[107,108] Harnessing the interactions between metal groups and the organic complex structures present in various mycobacterial targets, it is worthwhile to investigate the design and synthesis of metal nanocomposites. This will help develop more effective combinatorial antimycobacterial made of synergistic metals. Metallic nanocomposites have demonstrated enhanced theranostic applications compared to their respective monometallic counterparts in TB treatment, due to synergistic interactions of different metals.[109] For instance, the activity of magnetic iron oxide NPs against *M. sm* $mc^2$155 was enhanced when doped with Cu.[110] Still, further research is needed to explore the intrinsic properties and synergistic advantages of existing and new integrations of transition metals to achieve increased theranostic efficiency.

3.3. Mechanisms of Anti-Mycobacterial Activity

The general mechanisms of anti-mycobacterial drug activity primarily consist of two main approaches: inhibiting cell wall synthesis and disrupting essential cellular processes. Inhibition of cell wall synthesis is a common mechanism of action for many antimycobacterial agents. These drugs target the mycobacterial cell wall needed for the bacterium's survival, as they disrupt the synthesis of components such as peptidoglycans, arabinogalactans, and mycolic acids, consequently weakening cell walls and ultimately leading to bacterial death. Secondly, activity involves the disruption of essential intracellular processes. For example, the bedaquiline (BDQ) targets the mycobacterial adenosine triphosphate (ATP) synthase, a key enzyme involved in energy production. BDQ inhibits ATP synthesis by stalling the rotation of the mycobacterial F-ATP synthase c-ring and uncoupling electron transport from ATP synthesis, leading to mycobacterial death.[111] Compared to human mitochondrial ATP synthase,

BDQ binds to mycobacterial ATP synthase with an affinity that is over 20000 times greater and as a result there is limited host cell damage.

4. Cytotoxicity Concerns for MNPs' Biomedical Applications

Despite the interesting features and efficiency of MNPs in biomedical applications, concerns on MNPs' bioaccumulation and toxicity to healthy cells have impacted their wide acceptance and clinical relevance. Hence, addressing this issue and considering the possibility of attaining a balance is critical. The production of ROS and subsequent ROS-induced oxidative stress are predominant mechanisms of MNP-driven toxicity.[119] The cellular interaction of MNPs depends on various factors, including size, shape, metal components, surface charge, capping agent, exposure dose, and duration,[120,121] and can also depend on the different organisms' genetic makeup and DNA coverage.[122] MNP size influences cytotoxicity, for example, 20 nm-sized AgNPs and bovine serum albumin (BSA)-coated (negatively charged) AgNPs showed greater toxicity compared to 50 nm-sized AgNPs in macrophage RAW 264.7 cells.[123] The smaller size allows greater cellular uptake and interaction, leading to increased oxidative stress, tumor necrosis factor (TNF)-α induction, and cell death in macrophages. Modifying size and composition of MNPs can help optimize their antimycobacterial efficacy while minimizing cytotoxicity in macrophages, which are critical for TB treatment.[124] Conflicting submissions are found in the literature; hence, concluding on a "rule of thumb" is difficult due to various factors. For instance, the Co/Cr/Ti nanocomposite was more toxic than the individual Co, Cr, or Ti NPs at low doses.[125] However, another review study concluded that metal nanocomposites can also be a good way to increase antibacterial properties and, in turn, reduce the toxicity of MNPs to macrophage cell lines specifically the mixture of colloidal Ag:ZnO NPs with a 1:1 ratio with an average size of 12 nm.[24] By understanding the mechanisms of MNPs toxicity and the factors influencing their behavior in biological environments, researchers can engineer and design MNPs with reduced toxicity and improved performance for biomedical applications. Strategies to reduce the toxicity of MNPs include green synthesis techniques, meticulous physicochemical characterization,[119] responsive surface modification, and localized delivery approaches.[126]

5. Conclusion

The "End TB Strategy" established by the WHO, aims to reduce TB deaths by 95% and new cases by 90% by 2035.[127–129] Achieving this goal involves applying improved diagnostics and therapeutics against TB. In addition to PTB, it is imperative to address the impact of EPTB and rising drug resistance. Early and accurate diagnosis and drug susceptibility testing are essential to initiate the correct treatment regimen without delay. Further research is needed to develop more precise and efficient diagnostic methods for EPTB.

MNPs offer versatile theranostics and diagnostic applications in TB management. Their ability to deliver drugs to specific sites of infection, provide real-time imaging of lesions, and enable rapid and sensitive detection of TB biomarkers make them valuable tools for combating the disease. Further research and development of MNP-based technologies hold the potential to revolutionize TB management, facilitating early diagnosis, improving treatment outcomes, and reducing the burden of the disease globally.

Modifying the surface of MNPs through functionalization with appropriate organic molecules or constructing a core of metals that work synergistically with one another can accomplish this. Combination therapy, which combines MNPs with antimycobacterial drugs, is another option for developing new antimycobacterial agents. While MNPs such as those synthesized from Ag, iron oxide, Ag, copper oxide, show promise, challenges such as poor absorption, variable intramacrophagic efficiency, and residual toxicity must be addressed. In addition, addressing challenges of stability, targeting, size control, and toxicity is crucial. Understanding the risks and benefits, along with further in vitro and in vivo research, is essential to harness the full potential of MNPs as safe and effective drug carriers for TB treatment. Future work could further investigate the effect of the MNPs physicochemical properties on biocompatibility and to devise novel MNPs. Biocompatibility can be determined through extensive in vitro and in vivo toxicity studies and computational approaches to predict toxicity based on intrinsic properties at the molecular level. This will inform insightful tailoring of their properties to modulate their cytotoxicity and efficacy. Monitoring and adjusting the dose of MNPs to mitigate potential toxicity risks is also essential. This approach ensures the safe use of metal-based formulations in clinical studies and minimizes adverse effects on human subjects. Leveraging the potential of metal nano-carriers can enhance the delivery of TB drugs to the affected site. Optimized delivery of formulations can maximize the efficacy of existing drugs and those in the pipeline, improving treatment outcomes in clinical settings. In conclusion, although further pre-clinical and clinical research is needed to bring this strategy to the clinic, MNPs are potential systems for efficient TB diagnosis, medication delivery, and treatment.

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Conflict of Interest

The authors declare no conflict of interest.

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