



## Review

### Nanotechnology: A focus on Treatment of Tuberculosis

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#### Abstract

Despite the fact that we live in an era of advanced technology and innovation, infectious diseases, like Tuberculosis (TB), continue to be one of the greatest health challenges worldwide. The main drawbacks of conventional TB treatment are the development of multiple drug resistance, resulting in high dose requirements and subsequent intolerable toxicity. Therefore there is a need of a new system have been receiving special attention with the aim of minimizing the side effects of drug therapy, such as poor bioavailability and the selectivity of drugs. Nanoparticle-based drug delivery systems have considerable potential for treatment of TB. The important technological advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation. Nanoparticles can also be designed to allow controlled (sustained) drug release from the matrix. These properties of nanoparticles enable improvement of drug bioavailability and reduction of the dosing frequency, and may resolve the problem of nonadherence to prescribed therapy, which is one of the major obstacles in the control of TB epidemics. In this review, we discuss the challenges with the current treatment of the disease and shed light on the remarkable potential of nanotechnology to provide more effective treatment and prevention for TB.

**Keywords:** Tuberculosis; Nanotechnology; liposome; Polymeric nanoparticle; non-polymeric nanoparticle.

#### Introduction

Tuberculosis (TB) is an infectious disease caused by bacteria whose scientific name is *Mycobacterium tuberculosis* [1]. It was first isolated in 1882 by a German physician named Robert Koch who received the Nobel Prize for this discovery. Worldwide, approximately 2 billion people are currently infected with

*Mycobacterium tuberculosis*, representing about 30% of the global population [2]. After HIV/AIDS, TB is the second most deadly infectious disease [3]. Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air, when people who have the disease cough, sneeze, or spit [4]. Most infections in humans result in an asymptomatic, latent infection, and about one in

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ten latent infections eventually progresses to active disease, which, if left untreated, kills more than 50% of its victims.

The classic symptoms are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss. Infection of other organs causes a wide range of symptoms. Diagnosis relies on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture of bodily fluids. Treatment is difficult and requires long courses of multiple antibiotics. Contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in (extensively) multi-drug-resistant tuberculosis. Prevention relies on screening programs and vaccination, usually with Bacillus Calmette-Guérin vaccine.

A third of the world's population are thought to be infected with *M. tuberculosis*, [5] and new infections occur at a rate of about one per second [6]. The proportion of people who become sick with tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute number of new cases is still increasing [6]. In 2007 there were an estimated 13.7 million chronic active cases, 9.3 million new cases, and 1.8 million deaths, mostly in developing countries [7]. In addition, more people in the developed world are contracting tuberculosis because their immune systems are compromised by immunosuppressive drugs, substance abuse, or AIDS. The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5-10% of the US population test positive [1].

### **Pathogenesis of tuberculosis**

The primary source of infection is viable tubercular bacilli, expelled in the environment by coughing, sneezing, shouting, and singing of a patient with active TB; the air is contaminated with these bacilli. Inhaled bacilli in a person are inoculated into his respiratory bronchioles and alveoli, usually towards the apex of the lung. When the inhaled microorganisms multiply to a

sufficient extent, an antigen-antibody interaction is evoked by the cell-mediated T-lymphocytes. Tubercles are then formed because of accumulation of macrophages at the site of infection [8-10]. This may lead to permanent suppression of infection or some microbes may survive in the focii and may become the source of post primary infection when these focii break down under the conditions of weak host defense mechanisms [11,12]. This may happen immediately or months or years later. The hilar lymph nodes may get easily infected because of the spreading of infected macrophages having active bacilli. The released microorganisms are circulated through lymph and blood vessels to different parts of the body and infect-

- (i) Reticuloendothelial system (e.g., liver, spleen, and lymph nodes),
- (ii) Serosal surfaces and sites with high oxygen pressure (apices of lungs, renal cortex, and epiphyses of growing bones).

Because of multiplication of organisms at these sites, numerous small focii develop throughout the body [13,14]. This type of wide-spread of infection is known as miliary TB. In some patients, the focii formation leads to temporary suppression of the infection, whereas microorganisms may still be present in the focii. During coughing, the caseous material containing microorganisms is expelled leaving cavities in the lungs. These active bacilli may then be swallowed by the same patient or inhaled by a healthy patient in a crowded area with poor personal and public hygiene resulting into infection of trachea, larynx, or bronchii. Infection of the oropharynx, larynx, and tracheobronchial tree respond fairly well to anti-TB drugs, whereas infections in the gastrointestinal tract, urinary tract, or lymph nodes respond partially to the treatment [15,16].

### **Historical background**

The history of *M. tuberculosis* is very old [17]. Fragments of the spinal column from Egyptian mummies 2400 BCE show definite pathological signs of tubercular decay. Exact pathological and

anatomical description of the disease appeared in the 17th century. In 1882, Robert Koch's scientific brilliance led to the discovery of *M. tuberculosis* as the causative agent of the disease. Different means of TB curtailment were developed from time to time. In the 19th century, a French bacteriologist Calmette together with Guerin created the Bacilli Calmette-Guerin (BCG) vaccine. Even though relatively ineffective, it is still in widespread use. In the 20th century, during World War II, came the final breakthrough, the greatest challenge to the bacterium that had threatened humanity for thousands of years chemotherapy.

The history of TB in India [18,19] also dates back to 600BC where in a Sushruta Samhita, the disease is known as Kshaya, "wasting disease," or RajaYakshmaa, "the king of diseases." The four causes of the disease proposed were overstrain, suppression of natural urges, wasting (e.g., because of grief, anxiety, or longing), and a promiscuous diet, any of which could cause the three morbid humors Vata, Pitta, and Kapha to flare up. A treatment based on the principles of Ayurveda, the classical Indian system of health and healing, was provided. In addition, medicines and dietary prescriptions were detailed. Alcohol in moderate quantities, the flesh of birds and animals, which inhabit dry areas, and goat's milk were among the items recommended. TB was rare until the second half of the 19<sup>th</sup> century. Concomitant with the growing population density caused by industrialization, its incidence has increased progressively since then[20,21].

### Current antituberculosis therapy

Recent years have produced no radical changes in tuberculosis treatment for adults or children. However, new drugs are on the horizon, and there is an increasing appreciation of the special requirements of adults and children when determining drug doses. There are, at present, five 'first-line' antituberculosis agents: INH (isoniazid), RIF (rifampicin), PYZ

(pyrazinamide), ETB (ethambutol), SM (streptomycin) and Rifabutin which is cited in table 1. The dearth of tuberculosis drug research is indicated by the fact that these agents, and the regimens that include them, have now been in use for 30 years. Recognised 'second-line' agents include ethionamide (ETH) or prothionamide, kanamycin (KM) or amikacin (AM), terizidone/cycloserine, capreomycin, viomycin and para-aminosalicylic acid. Without any formal evaluation, the fluoroquinolones have also attained a prominent position in regimens for the treatment of drug resistant tuberculosis; clinical studies are underway that may lead to their inclusion in 'first-line' regimens.

INH remains the cornerstone of antituberculosis regimens, a position earned by its high early bacterial activity (EBA), outstanding pharmacokinetics and relatively low toxicity. Because of its high EBA, INH is also often the first agent against which resistance develops. Only recently has its role been questioned [22]. In regimens containing RMP and PZA, the function of INH is the rapid elimination of metabolically active bacilli present in tuberculosis cavities and the protection of companion drugs from resistance. Only in regimens not containing RMP or PZA does INH have a sterilising role, and this requires 12–18 months of treatment. When the first antituberculosis drugs were introduced it was soon apparent that resistance developed following monotherapy with any agent, but that resistance could be prevented by multidrug therapy [23]. The major concern in early regimens was consequently the prevention of resistance. It was also known that prolonged therapy was required for permanent cure. As late as 1960, 2 years' treatment was considered necessary to establish a stable cure of pulmonary tuberculosis [24]. It was a considerable advance when 6-month regimens containing RMP and PZA produced a stable cure with low relapse rates.[25] It is recommended that EMB be included in the 2-month 'intensive phase' of treatment with INH, RMP and PZA in areas with

a high incidence of initial resistance to INH, to prevent the acquisition of MDR [26].

Nanotechnology-based platforms for systemic delivery of anti-TB drugs could have similar advantages. Controlled-release delivery systems can enhance their half-lives, keeping them in

	Drug	Brand names [Company]	Dose(mg)
First-line Drugs	Isoniazid	Rimifon® (Roche); Cotinazin® (Pfizer); Ditubin® (Schering) Rifampicin Rifadin® (Sanofi-Aventis); Abrifam® (Abbott); Rifaprodin®(Almirall)	300 300
		Pyrazinamide	Zinamide® (Merck & Co.); Pezetamid® (Hefa-Frenon); Pyrafat® (Fatol)
	Ethambutol hydrochloride	Myambutol® (Dura Pharmaceuticals); Etibi, Tibutol	400
	Streptomycin	Sesquisulfate-AgriStrep® (Merck & Co.); Streptobrettin® (Norbrook)	500
	Rifabutin	Mycobutin® (Pfizer).	150
Second-line Drugs	Clofazimine	Lamprene® (Novartis).	100
	Ethionamide	Trecator® (Wyeth); Nisotin®; Trescatyl® (M&B); Aetina®; (Bayer).	500
	Clarithromycin	Biaxin® (Abbott); Clathromycin® (Taisho); Naxy® (Sanofi Winthrop).	500
	Cycloserine	Closina®; Farmiserina® (Farmitalia); Seromycin® (Lilly).	500
	Moxifloxacin	Actimax® (Sankyo); Vigamox® (Alcon). Proflox® (Esteve); Octegra® (Bayer).	400
	Gatifloxacin	Tequin® (Bristol-Myers Squibb); Zymar® (Allergan).	400
	Linezolid	Zyvox®, Zyvoxid® (Pfizer).	400
	Capreomycin	Capastat® (Dista), Capastat sulphate® (Eli Lilly).	1000
	p-Aminosalicylic Acid	PASER® (Jacobus); Rezipas®. (Bristol-Myers Squibb)	500

Table 1- First and Second line antituberculosis drug

### Role of nanotechnology in Tuberculosis

Nanotechnology is a multidisciplinary field, which recently has emerged as one of the most propitious field in tuberculosis treatment. Nanotechnology is definitely a medical boon for diagnosis, treatment and prevention of tuberculosis. Using nanotechnology, it has become possible to achieve improved delivery of poorly water soluble drugs, targeted delivery of drugs to specific cells or tissues and intracellular delivery of macromolecules [27].

circulation at therapeutic concentrations for longer periods of time. This could have major implications in improving adherence to the drugs. Nanoscale delivery systems also enhance and modulate the distribution of hydrophobic and hydrophilic drugs into and within different tissues due to their small size. This particular feature of nanoscale delivery systems appears to hold the most promise for their use in clinical treatment and prevention of tuberculosis.

## **Nanotechnology in tuberculosis diagnosis**

Tuberculosis is diagnosed by chest radiology and occasionally followed by sputum collection and microbiological culture [28]. Radiological testing has low sensitivity and requires trained staff to operate the imaging system. Tuberculosis cultures are usually more sensitive than chest radiology but the microbiological test relies on bacterial growth and may take several weeks to yield a conclusive result [28,30]. Since tuberculosis is contagious, a level 3 biosafety lab is required for growing the cultures. These requirements render such diagnostic techniques inappropriate for the developing world.

For latent tuberculosis, diagnosis is even more challenging, since typical symptoms (coughing, fever, sputum production, etc.) are not present. The tuberculin skin test (TST) is the current standard test for latent infection. This test measures an individual's immune response to purified protein derivative (PPD) a mixture of more than 200 tuberculosis antigens [31]. Individuals with previous exposure to the pathogen (e.g. latent infection) exhibit an immune response and the formation of an induration (localized inflammation) on the forearm where the antigens were subcutaneously injected. The test is inexpensive and non-invasive but it requires two visits within a specific time period to a clinic. Furthermore, the test often yields false positive results in patients previously vaccinated with the Bacillus Calmette–Gurin (BCG) vaccine, and false negative results in patients co-infected with HIV who have low T-cell counts [29].

Recently, blood-based diagnostic tests with higher specificity have been developed to measure the production of INF- $\gamma$  by T-cells following exposure to TB antigens [28]. These tests are known as interferon gamma release assays (IGRAs) [31]. A test based on enzyme-linked immunospot assay methods (ELISPOT) is the T SPOT-TB test, and uses RD1 antigens to stimulate T-cells. ELISA-based tests include the Quantiferon test, which measures INF- $\gamma$

following exposure to the PPD antibody mixture and the Quantiferon Gold (which uses RD1 antigens) [30]. The RD1 antigens (CFP-10 and ESAT-6) are superior to PPD antibodies because they are specific to *M. tuberculosis*. As a result, the immune response to RD1 is also specific to *M. tuberculosis* and there is less interference from previous BCG vaccinations. Nonetheless, patients with compromised immune systems (e.g., patients co-infected with HIV) can give false negative results because these patients tend to have low T-cell counts. It is clear that there are major bottlenecks in diagnosing tuberculosis and that developments in the diagnosis of TB have been slower than other infectious pathogens. This presents an interesting challenge and opportunity for nanotechnologists. For example, the highly effective Quantiferon Gold assays do not react with PPD antibodies and do not require two clinical visits, but do require some laboratory equipment (such as an incubator). The ability of nanoparticles to potentially detect extremely small quantities of pathogens on a stand-alone platform may be particularly useful in the rapid detection of TB.

## **Nanotechnology based drug delivery for TB treatment**

### **(A) Polymeric nanocarrier**

#### **Dendrimer**

Dendrimers are precisely defined, synthetic nanomaterials that are approximately 100 nanometres in diameter. They are made up of layers of polymer surrounding a central core. Functionality dendrimers are archetypically comprised of three different topological components of chemical significance: a poly-functional core, interior layers, and a multivalent surface. The poly-functional focal core can encapsulate various chemical species and exhibits unparalleled properties due to the special nano-environment surrounded by extensive dendritic branching [32]. Due to this unique structure, these molecules represent attractive candidates for the encapsulation and delivery of anti-TB

agents for diverse administration routes, though only a few research works have been reported. To target the drug delivery to macrophages, Kumar *et al.* developed RIF-loaded mannosylated 5<sup>th</sup> generation (5G) PPI dendrimeric nanocarriers [33]. Surface modification with sugar molecules (e.g., mannose) recognizable by lectin receptors located on the surface of phagocytic cells improved the selective uptake of the drug-loaded nanocarriers by cells of the immune system. RIF encapsulation extents were approximately 37% with hydrophobic interactions and hydrogen bonding contributing to the physical binding of the drug to the core. The solubility of RIF within

unmodified dendrimers was ~52 mg/mL, while

the superficial mannose molecules sterically hindered the complexation and encapsulation of the drug and the solubilization of RIF was substantially less efficient at approximately 5 mg/mL (2-fold when compared to the aqueous solubility of RIF). High haemolysis levels shown by amine-terminated dendrimers precludes their clinical application. Mannosylation significantly reduced the haemolytic toxicity of the nanocarrier materials from 15.6 to 2.8%. When the RIF-containing dendrimers were assayed, findings indicated a beneficial effect of the carrier, reducing the intrinsic haemolytic effect of free RIF from 9.8 to 6.5%. A similar trend was observed when the viability of a kidney epithelial cell line was tested; encapsulation improved the

survival of the cells from ~50% for free RIF to

~85%. Drug release studies conducted *in vitro*

showed that the modified dendrimers sustained the release for about 120 h, as opposed to the fast

delivery (b10 h) found with regular dendrimers. The phagocytic uptake of RIF and RIF-loaded dendrimers was investigated with alveolar macrophages harvested from rat lungs. A clear increase in the intracellular concentration of the antibiotic was apparent.

Using a similar approach, a more recent work investigated the suitability of RIF-containing 4G and 5G PEGylated-PPI dendrimers to sustain the delivery of RIF [34]. PEGylation resulted in a significant increase of the percentage of drug entrapment from 28 and 39% to 47 and 61% for G4 and G5 derivatives, respectively. Also, the surface modification led to a better control of the release profile; i.e., 97.3 and 46.3% cumulative release values were respectively found for 4G PPI and PEG-PPI after 36 h. Finally, PEG-grafted dendrimers showed a minimal haemolytic activity (1–3%) as opposed to the NH<sub>2</sub>-terminated ones (14–20%).

### Cyclodextrins

Cyclodextrins are a group of structurally related natural products formed during bacterial digestion of cellulose. These cyclic oligosaccharides consist of ( $\alpha$ -1, 4)-linked  $\alpha$ -D-glucopyranose units and contain a somewhat hydrophobic central cavity and a hydrophilic outer surface and thus able to host other hydrophobic molecules [35].

Several works reported on the complexation of RIF by means of different CD molecules, though results regarding the efficiency of this approach are ambiguous. Ferreira and collaborators prepared inclusion complexes of RIF with HP $\beta$ CD [36]. The aqueous solubility of the drug increased linearly with the concentration of the CD, with the CD/RIF ratio in the complex being 1:1. Also, the solubilization was pH-dependent. The analysis of the chemical shift data of <sup>1</sup>H and <sup>15</sup>N NMR of free and complexed RIF revealed important changes in peaks of the side chain of the piperazine ring of the drug, suggesting the interaction of this region with the hydrophobic core of HP $\beta$ CD. Koester *et al.* explained the complexation of ofloxacin with  $\beta$ -cyclodextrin and the complexes showed a water

solubility enhancement of approximately 2.6 times.[37]. Rao et al. carried out a comparative investigation of RIF complexation with  $\beta$ -CD and HP $\beta$ CD in order to improve the chemical stability and aqueous solubility of the drug [38]. According to phase solubility studies, a 1:1 molar ratio was apparent; the stability constants found to be 58.13 and 76.37 for the pristine and the modified CD, respectively. These results indicated a stronger interaction between the drug and the HP $\beta$ CD. IR spectroscopy revealed that the interaction was through the piperazin group of RIF. However, only a 2-fold solubilization extent was apparent with  $\beta$ -CD when a common solvent technique was employed. As opposed to the findings by Ferreira et al. [36], herein no solubility improvement of RIF was found with HP $\beta$ CD. On the other hand, all the complexes improved the thermal stability of the drug. Additionally, when the antibacterial activity was assayed in *M. tuberculosis*, a significant decrease in the MIC from 64 to 32  $\mu$ g/mL was observed, this phenomenon likely due to a better permeation of the drug through the wall of the bacilli. Another drawback to the low aqueous solubility of a drug is the inability to conduct preliminary biological and clinical evaluations of new drug candidates [39]. In this context, the poorly-water soluble nitroimidazole P-824, a new anti-TB drug under study, has shown activity against drug-sensitive and multi-drug resistant bacilli. Aiming to conduct in vivo experiments in a short-course murine infection model, a complex with HP- $\gamma$ -CD was developed and a cyclodextrin/lecithin formulation prepared [40]. A reduction in the bacterial load in the lungs was observed with 50 and 100 mg/kg doses. CD have been also investigated as carriers for local delivery to the lung [41].

### Polymeric micelles

Polymeric micelles are nanocarriers generated by the self assembly of amphiphilic polymers in water above the CMC [42]. The hydrophilic blocks are exposed to the aqueous medium forming the micellar shell that facilitates the solubilization of the amphiphile in water and

stabilizes the aggregate. In contrast, the hydrophobic blocks form the inner micellar core, a hydrophobic domain that enables the incorporation of poorly-water soluble drugs by physical interaction or chemical conjugation leading to higher solubility extents [43]. Commercially available and FDA-approved poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) block copolymers (linear poloxamers and branched poloxamines) are among the most important micelle-forming materials [44]. Preliminary studies that investigated the solubilization of RIF within polymeric micelles of a variety of linear and branched PEO–PPO with a broad spectrum of compositions showed a minimal solubilization

effect ( $\sim$ 2-fold) [45]. These findings suggest that

the size of the micellar core strongly limits the incorporation of the extremely bulky RIF molecule. Other amphiphilic block copolymers synthesized by linking mono and bifunctional PEG precursors of different molecular weight with PCL enabled the fine tuning of the HLB and the enlargement of the micellar core, improving the solubilization extent 5- to 7-fold [45].

The micelle-forming prodrug showed a 5.6-fold increase in antituberculous activity against *M. tuberculosis* in vitro when compared to the free drug. The mechanism would primarily involve the micelle uptake and the latter intracellular release of the drug after the hydrolysis of the linkage. The same synthetic pathway was pursued in order to encapsulate PYZ [46,47] and RIF [47]. The size of the micelles would prevent renal filtration, increasing the residence times in the blood stream. Moreover, a stronger antimycobacterial activity was apparent. To overcome resistance, Jin and collaborators designed INH lipid derivatives [48]. The new amphiphilic molecules formed monolayers at the air/water interface. The aggregation behavior was intimately related to the character of the hydrophobic tail. Flexible medium-long tails

formed nano-sized vesicles. In contrast, short lipid tail-derivatives displayed weak hydrophobic interactions and they did not self-assemble. Molecules with very long tails led to the formation of crystal-like structures. Finally, they showed promising antibacterial activity against *Mycobacterium* due to a more lipophilic structure that enhanced the penetration of the drug into the pathogen.

### **Polymeric nanoparticles**

PNP have been extensively explored as means for drug solubilization, stabilization and targeting [49,50]. Depending on the technology employed for their production, two kinds of systems can be generated, namely nanocapsules and nanospheres. In the former, the drug solubilized in aqueous or oily solvents is surrounded by a polymeric membrane. In contrast, the latter is comprised of solid matrices of variable porosity where the active molecules are homogeneously distributed through the particle and, often, dispersed at the molecular level. A broad spectrum of biomaterials is available for the production of PNP [51]. Advantageous features such as high stability, high loading capacity of hydrophilic and hydrophobic drugs and feasibility of administration by different routes have made PNPs one of the most popular approaches for drug encapsulation [51]. PNP are removed from the body by opsonization and phagocytosis [52]. In order to prevent recognition by the host immune system and to prolong circulation times in the blood stream, the modification of the surface with highly hydrophilic chains (e.g., polyethylene glycol) has been pursued. This approach has been one of the most extensively investigated with respect to antituberculosis drug delivery systems.

Anisimova *et al.* investigated the encapsulation of RIF, INH and streptomycin within PBCA and PIBCA nanoparticles and tested the accumulation in human blood monocytes *in vitro* toward the development of a drug depot [53]. Encapsulated INH, streptomycin and RIF showed 4–8-, 7- and 22–25-fold increases in the intracellular concentration with respect to the extracellular

concentration. In contrast, free INH and RIF showed intracellular levels similar to and 5 times higher than the extracellular concentration, respectively. Streptomycin was not detectable. More recently, moxifloxacin-loaded PBCA NPs were produced by means of the anionic polymerization of poly(butyl-2-cyanoacrylate) in the presence of the drug [54,55]. Cytotoxicity studies indicated that the moxifloxacin loaded NPs were more toxic to the macrophages than the free drug [54]. When infected cells were exposed to the drug in the free and the encapsulated form, a pronounced increase in the intracellular concentration from 125–175 to 375  $\mu\text{g/mL}$  was found [54]. These results relied on the NP uptake by the phagocytic cells. In addition, encapsulated moxifloxacin was more effective than the free form to kill intracellular bacilli [54]. Evaluation of the anti-TB activity in mice infected with *M. tuberculosis* showed a significant decrease in the mycobacteria count in the lungs after IV administration [55]. More recently, the encapsulation of different first-line anti-TB drugs (RIF, INH and PYZ) within biodegradable PLGA nanoparticles was investigated by Khuller's group [56]. Findings showed that, as opposed to the biodistribution profile observed for the free drugs that were cleared from circulation after 12–24 h, nanoencapsulated drugs were detected in plasma for up to 9 days and therapeutic concentrations in tissues were maintained for 9–11 days. Khuller *et al.* also produced anti-TB drug-loaded alginate nanoparticles (235 nm diameter) by means of ionotropic gelation [57]. Encapsulation efficiency ranged between 80–90% for RIF, 70–90% for INH and PYZ and 88–95% for ETB.

### **(B) Nonpolymeric nanocarrier**

#### **Liposome**

Liposomes, ranging in size between 25 nm and several microns, are microscopic vesicles that comprise one or more phospholipid bilayers which surround an aqueous core [58]. They are prepared from natural or synthetic phospholipids and cholesterol, and may also additionally



include other lipids and proteins. The aqueous core facilitates the entrapment of hydrophilic drugs, while hydrophobic drugs are bound to or incorporated in the lipid bilayer. When administered, liposomes are recognised as being foreign, and are immediately taken up by cells of the mononuclear phagocytic system (MPS). In order to prevent elimination and extend circulation times, liposomes are usually PEGylated. A pioneering work explored the incorporation of gentamicin into liposomes and compared the antibacterial activity to that of the free drug in a mouse model of disseminated *M. avium* complex infection [59]. The encapsulated drug significantly reduced the bacterial count in spleen and liver. In addition, a dose-related reduction of the bacterial load, though no sterilization, was found. Similar results were obtained with different liposome-entrapped second-line antibiotics [60-62]. Deol and Khuller produced lung-specific Stealth® liposomes made of phosphatidylcholine, cholesterol, dicetylphosphate, O-steroyl amylopectin and monosialogangliosides/distearylphosphatidylethanolamine- poly(ethylene glycol) 2000 for the targeted delivery of anti-TB drugs to the lung [63]. Biodistribution experiments of different liposome types were conducted in healthy and tuberculosis infected mice after IV injection. Findings showed a pronounced increase from 5.1% with conventional liposomes to 31% with PEGylated systems containing O-steroyl amylopectin in the accumulation of the nanocarriers in the lungs after 30 min. The accumulation extent was associated to the composition of the vesicles. Moreover, pretreatment of both healthy and infected animals with conventional liposomes (1 h before the administration of modified liposomes) saturate the reticulo-endothelial system and the uptake levels in the lungs rose to approximately 40% for the modified nanocarriers, after 30 min. On the contrary, modified liposomes showed reduced (30– 50%) uptake and accumulation in the liver and spleen. Also, the biodistribution in the different organs was similar in both animal groups. The extent of drug incorporation was 8–

10 and 44–49% for INH and RIF, respectively. The cytotoxicity of the drug-loaded nanocarriers was evaluated in peritoneal macrophages and compared to that of the free drugs. A significant decrease in the toxic effects was observed. This phenomenon was associated with a more controlled release of the drug. Evaluation of the hepatic activity following the administration of the free and the encapsulated drugs indicated a statistically significant decrease in the hepatotoxic activity of the anti-TB agents upon encapsulation. Then, the therapeutic activity of free and encapsulated INH and RIF was evaluated in both therapeutic and sub-therapeutic dose [64]. A 12 mg/kg dose of free INH reduced the number of CFU in the lungs to about 4.5 log units, while the liposomal drug resulted in a decrease to 3.9 log units. A 10 mg/kg free and encapsulated RIF dose reduced the CFU to 4.3 and 3.8 log units, respectively. A similar trend was observed in the liver and spleen. Moreover, administration of sub-therapeutic doses (4 and 3 mg/kg for INH and RIF respectively) led to a higher decrease in CFU, as compared to the free drugs administered at a therapeutic concentration. Overall, a significant increase in the anti-TB activity was found.

With the aim of improving the anti-TB activity, reducing the toxicity and enabling the parenteral administration of highly lipophilic clofazimine, drug-loaded liposomes were produced [65] and preclinically evaluated in acute and chronic murine infections [66]. Encapsulation reduced the *in vitro* and *in vivo* toxicity of the drug and enhanced the anti-TB activity in both acute and chronic models. This inhibition was markedly higher in the liver and lung. In addition, chronically infected mice treated with the encapsulated drug showed total clearance from the liver and spleen with no signs of recovery 2 months post-treatment. In the lungs, a gradual decrease in CFU was observed, though a rebound was found 2 months post-treatment. Regardless of the apparently total clearance of the bacilli following a second treatment with the liposomal drug, a similar phenomenon was observed after 2 months. In more recent investigations, PYZ- [67]

and rifabutin containing liposomes [68] were also produced, stressing the great versatility and potential of these nanocarriers.

### **Nanoemulsion**

Nanoemulsions are transparent or translucent oil-in-water (o/w) or water-in-oil (w/o) droplets with a mean droplet diameter between 10-100 nm [69]. Nanoemulsions are also known as submicron emulsions, mini-emulsions, ultrafine emulsions, and unlike the thermodynamically stable microemulsions, nanoemulsions are kinetically stable with great stability in suspension due to their small droplet size. Due to their small droplet size, nano-emulsions may appear transparent, and Brownian motion prevents sedimentation, creaming and flocculation hence offering increased stability. In contrast to microemulsions, nanoemulsions are metastable and can be diluted with water without changing the droplet size distribution [70]. In the recent studies nanoemulsions are of considerable interest in anti-tubercular drug delivery.

Ahmed and co-workers developed different o/w nanoemulsions of RIF for IV administration using pharmaceutically acceptable excipients: Sefsol® 218 as the oil phase, Tween® 80, Tween® 85 and saline water as the surfactant, the cosurfactant and the aqueous phase [71]. The mean droplet size ranged between 47 and 115 nm, the lower sizes being for systems containing lower oil contents. The entrapment efficiency was over 99% and the visual homogeneity was excellent for all the nanoemulsions. In vitro drug release studies indicated an initial burst effect ranging from 40 to 70% after 2 h, followed by a more moderated release afterwards. Finally, stability assays over 3 months indicated slight increases in the droplet size and the viscosity of the systems at 4 and 25 °C.

### **Nanosuspensions**

Nanosuspensions are sub-micron colloidal dispersions of pure drugs stabilized with surfactants [72]. Nanonization (reduction of the average size of solid drug particles to the nano-

scale generally by top milling or grinding) is a useful methodology to improve the solubility of drugs displaying strong solute-solute interactions and high melting points and, in general, both poor water and lipid solubility. The solid and dense states of the pure drug particles confer a maximal mass per volume ratio, especially critical in systems demanding high drug loadings. Despite its potential, only a few studies aiming to optimize the pharmacotherapy of tuberculosis have been reported. Peters and collaborators developed a clofazimine nanocrystalline suspension in order to overcome the toxicity and the low solubility (0.3 µg/mL) of the drug [73]. To evaluate the suitability of the formulation for IV administration they compared the effectiveness of the nanosuspension in the treatment of murine *Mycobacterium avium* infection to that of drug-loaded liposomes. Application of 10-cycle discontinuous and continuous homogenization processes resulted in particles of 600 and 300 nm, respectively, both of which are small enough to prevent capillary embolism (less than 5 µm). At approximately 100 nm, the clofazimine-loaded liposomes included in the study were substantially smaller and these liposomes showed preclinical effectiveness despite limited physical stability over time, difficult reconstitution of freeze-dried samples and low drug loading capacity. An extensive size and size distribution characterization indicated that the nanosuspensions display more homogeneous size distributions, lower amounts of sub-2 and -5 µm particles and physical stability for more than 2 years. In vivo assays were conducted and shows continued treatment led to a significant reduction of bacterial counts in all the organs evaluated. Effectiveness levels were comparable to those of liposomal clofazimine, however, the ease of preparation and the higher physical stability of the nanosuspension were distinguishing. Reverchon et al. used supercritical carbon dioxide-assisted atomization to produce RIF sub-micronic particles of controlled size and size distribution that fit the range of injectable [74] and aerosolizable drug delivery systems (b5 µm) [75].

Of the different solvents used to solubilize the drug, DMSO was the most appropriate for nanonization.

### Niosomes

Niosomes are thermodynamically stable liposome-like vesicles produced with the hydration of cholesterol, charge-inducing components such as charged phospholipids (e.g., dicetylphosphate and stearyl amine) and non-ionic surfactants (e.g., monoalkyl or dialkyl polyoxyethylene ether) [76]. They were conceived as alternative DDS that overcome a number of drawbacks shown by the liposomes, which were mainly associated with sterilization, high production costs, scale-up difficulties and the instability of the phospholipidic components after light exposure even at room temperature. Niosomes can host hydrophilic drugs within the core and lipophilic ones by entrapment in hydrophobic domains. In an early study, Jain and Vyas prepared micro-sized (8–15  $\mu\text{m}$ ) RIF-loaded niosomes containing Span 85 as the surfactant [77]. In vivo studies showed that by adjusting the size of the carrier, up to 65% of the drug can be localized in the lungs. In a more recent investigation, the same group of scientists extended the investigations and evaluated the biodistribution of niosomes with smaller sizes (1–2  $\mu\text{m}$ ) produced with different sorbitan esters (Span® 20, 40, 60, 80 and 85) and cholesterol in a 50:50 percent mol fraction ratio [78]. The entrapment extents gradually increased with the increase of the hydrophobicity of the surfactant and ranged between 20 and 35%. In vitro release studies showed 80% maximal and 52% minimal levels for Span-20- and Span-85-based systems, respectively; the more lipophilic the surfactant was, the slower the drug release in the aqueous medium. Niosomal formulations attained substantially higher RIF concentrations in thoracic lymph nodes via the i.p. route (46.2% of the administered dose) as opposed to 13.1% for the free drug. These findings suggested that compartmentalization of the drug took place in the lymphous tissue. In contrast, when the drug-loaded carriers were injected intravenously, only

7.3% of the drug was found in the thorax, with the accumulation extent being lower than the 11.5% obtained by free RIF.

Mullaicharam and Murthy studied the organ biodistribution of RIF niosomes (5 mg/mL) following IV and i.t. administration and compared it to that of the free drug in albino rats [79]. In general, a significant increase in the total drug concentration in the lungs, liver, kidneys and blood serum was apparent for rifampicin-loaded niosomes. After IV, niosomes preferentially accumulated in the lung, liver and kidney with the organ to serum AUC ratios being 117,060 for lung/serum, 67 for liver/serum and 3068 for kidney/serum. In contrast, administration of free RIF resulted in a less selective delivery (558.3 for lung/serum, 16.1 for liver/serum and 332.6 for kidney/serum). After i.t. administration, the lung/plasma ratios were 128,585 and 885 for niosomes and free drug, respectively, representing a 145- fold increase in the accumulation capacity of RIF-loaded niosomes in the lungs as compared to the free drug.

### Solid lipid nanoparticles (SLN)

Solid lipid nanoparticle (SLN) dispersions have been proposed as a new type of colloidal drug carrier system suitable mainly for intravenous administration. The system consists of spherical solid lipid particle in the nanometer range, which is dispersed in water or in aqueous surfactant solution. Generally they are made of solid hydrophobic core having a monolayer of phospholipid coating. The solid core contains the drug dissolved or dispersed in the solid high melting fat matrix. The hydrophobic chains of phospholipid are embedded in the fat matrix. They have potential to carry lipophilic or hydrophilic drug or diagnostics. SLN not only combine the advantages of traditional colloidal drug carrier systems like liposomes, polymeric nanoparticles and emulsions but also avoid the problems associated with them [80].

The antitubercular drug loaded SLNs were prepared by the emulsion solvent diffusion technique to co-incorporate rifampicin, isoniazid

and pyrazinamide. The chemotherapeutic potential of the formulation was evaluated via the respiratory route in the guinea pigs. It was observed that a sustained drug release was maintained for 5 days in plasma and for 7 days in the organs. The pharmacokinetics was unaltered in healthy as well as TB infected guinea pigs. Seven weekly doses in the organs of TB infected guinea pigs, replacing 46 conventional doses. This was the first report of the chemotherapeutic efficacy of SLNs in experimental TB[81]. Next the studies were carried out via the oral route and better results were obtained as the drug levels could be maintained in the plasma for 8 days and in the organs for 9-10 days. Five oral doses of the formulation spaced 10 days apart were as efficacious as 46 doses of oral free drugs in terms of producing bacterial clearance in TB infected mice [82].

### **Local delivery of anti-TB drugs to the lung**

The potential advantages of direct delivery of the TB drug to the lungs include the possibility of reduced systemic toxicity, as well as achieving higher drug concentration at the main site of infection. Moreover, in contrast to the oral route of administration, inhaled drugs are not subjected to first-pass metabolism. A possible obstacle to using nanocarriers for pulmonary delivery is that their mass median aerodynamic diameter, an essential parameter for the particle deposition in the lungs, is often too small.

Nevertheless, the effectiveness of pulmonary drug delivery using nanoparticles was demonstrated in a number of studies [83]. The pharmacokinetics and antibacterial effect of the nanoparticle bound anti-TB drugs administered via respiratory route was investigated in guinea pigs [84]. The dose was delivered via a suitable facemask connected to the compressor–nebulizer system. A single nebulization of RIF, INH, and PYZ coencapsulated in PLG nanoparticles to guinea pigs resulted in sustained therapeutic drug levels in the plasma for 6 to 8 d and in the lungs for up to 11 d. This effect was similar to that

obtained after oral administration of the nanoparticulate formulation of the same drugs. In nebulization of nanoparticles to *M. tuberculosis*–infected guinea pigs at every 10th day, no tubercle bacilli could be detected in the lung after only five doses of treatment, whereas 46 daily doses of orally administered drug were required to obtain an equivalent therapeutic benefit.

Administration to infected guinea pigs of nebulized RIF, INH, and PYZ coencapsulated in wheat germ agglutinin– functionalized PLG nanoparticles was even more effective: three doses administered fortnightly for 45 d were sufficient to produce a sterilizing effect in lungs and spleen [85]. A sterilizing effect was also achieved when the drugs were loaded in solid lipid nanoparticles [86]. No tubercle bacilli could be detected in the lungs/spleen after seven doses of treatment of infected guinea pigs with drug-loaded solid lipid nanoparticles. It is noteworthy that the solid lipid nanoparticles display important advantages, such as the composition (physiologic compounds) and the possibility of large-scale production favored by the feasibility to avoid organic solvents in the manufacturing process [87].

### **Nanotechnology based vaccines for tuberculosis treatment**

The aerosol vaccine- under development through collaboration between Harvard University and the international not-for-profit Medicine in Need (MEND) could provide a low-cost, needle-free TB treatment that is highly stable at room temperature. While most new TB vaccines continue to call for needle injection, but this new vaccine could provide safer, more consistent protection by eliminating these injections and the need for refrigerated storage. A successful result of aerosol delivery using nanoparticle technology offers a potentially new platform for immunization. Among guinea pigs vaccinated with the aerosol treatment and subsequently exposed to TB, less than 1 percent of lung and spleen tissue showed effects of the disease. By contrast, in animals treated with the same dose of

the traditional injected vaccine, some 5 percent of lung tissue and 10 percent of spleen tissue showed symptoms following TB exposure.

In the aerosol vaccine, particles form at micrometer and nanometer scales and in spherical and elongated shapes, a combination that appears to improve dispersal in the mouth. While commonly used with food, cosmetics, and pharmaceuticals, this spray drying of small and large molecules is seldom used for drying cellular material. The new technique enables TB vaccines, and potentially other bacterial and viral-based vaccines, to sidestep the traditional problems associated with keeping vaccines chilled. Furthermore, a nanotechnology-based vaccine adjuvant for TB was developed by the U.S firm, Biosante, in 2002 [88].

## CONCLUSIONS

Although identifying novel anti-TB agents remains a priority, the development of the nanoparticle-based delivery systems for currently used agents may represent a cost-effective and promising alternative. The above data suggest that nanoparticles have a considerable potential for treatment of TB. Their major advantages, such as improvement of drug bioavailability and reduction of the dosing frequency, may create a sound basis for better management of the disease, making directly observed treatment more practical and affordable. Another important advantage of the nanoparticles is the feasibility of the versatile routes of drug administration, including oral and inhalation routes. In addition, high stability of the nanoparticles suggests long shelf life.

It can be expected that future research will concentrate on the development of the vectorized delivery systems combining advantages of the colloidal carriers, such as large payloads of a drug, with active targeting to the infection sites. Moreover, development of innovative formulation technologies suggests that nanoparticles can be incorporated into various solid dosage forms (microparticles, granules, or tablets), which can release the nanoparticles at the site of action, preserving their original

properties. These approaches would further improve efficacy and practicability of the nanoparticle-based formulations.

Finally, the success of this technology will probably depend on toxicologic issues associated with understanding of the fate of nanocarriers and their polymeric constituents in the body, as well as elimination of the risk of the residual organic solvents. However, further research is necessary in order to bring nanoparticulate formulations into the market.

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