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REVIEW

Nanomedicines towards targeting intracellular *Mtb* for the treatment of tuberculosis

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Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), causes the most human deaths than any other diseases from a single infectious agent. Treatments are long and costly and have many associated side effects. Intracellular bacilli are slow growing and difficult to target, which is augmenting the emergence of multi-drug resistance. A hallmark trait of TB is the formation of granulomas, chronic cellular aggregates, which limit bacterial growth but provides a survival reservoir where bacilli may disseminate from. Targeting intracellular *Mtb* is challenging, but nanomedicine may offer a solution. Nanomedicine is a significantly growing research area and offers the potential for specific disease targeting, dosage reduction, and intracellular drug delivery. This review discusses the application of the various forms of nanomedicine towards targeting of *Mtb*.

Tuberculosis

Tuberculosis (TB), caused by the bacillus *Mycobacterium tuberculosis* (*Mtb*), remains a global crisis. The World Health Organization states that TB kills 1.6 million people annually, 10 million more develop the disease each year, and approximately one-quarter of the world's population are latently infected.(WHO, 2018a) In September 2018, the first-ever high-level meeting on TB was held, where world leaders met at the United Nations (UN) Heads of State General Assembly to address the problems faced in eradicating the disease. It is costly and very difficult to treat, requiring multi-drug therapy over long periods (6–24 months).(Tiberi *et al.*, 2018; WHO, 2018a) For drug-sensitive TB, the World Health Organization guidelines recommend daily administration of rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol for 2 months, followed by INH and RIF for a further 4 months.(WHO, 2018a) The development of anti-TB drugs lacks investment with only two new drugs, bedaquiline and delamanid, being approved (for multi-drug-resistant [MDR] TB) in the past 50 years.(Ferlazzo *et al.*, 2018) Poor therapy management and patient noncompliance can lead to complications (such as MDR), and the adverse reactions to anti-TB drugs can cause significant problems.

The most common route of infection is the respiratory route whereby bacteria enter through aerosol droplet nuclei (1–5 μm), passed from an individual infected with pulmonary or laryngeal TB to a susceptible individual through coughing and sneezing. *Mtb* virulence is often defined by its transmission and intracellular survival ability, which is complex and is yet to be fully understood.(Simeone *et al.,* 2015) It is established that the pathogen can adapt, allowing it to avoid the hostile environment of the phagosome, but how *Mtb* achieves intracellular survival is not fully elucidated, as the lysosome is a complex organelle, which holds many different enzymes with the capability of degrading many microorganisms.

The human immunodeficiency virus (HIV) causes AIDS (acquired immunodeficiency syndrome) in humans. There are an estimated 36.9 million people infected with HIV,(UNAIDS, 2018) and owing to immunosuppression, these patients are highly susceptible to contracting active TB and/or reactivation of latent TB infection. Tuberculosis and HIV are among the leading causes of mortality worldwide, and in 2017, there were 300,000 TB deaths among HIV-positive people.(WHO, 2018a; WHO, 2018b) Coinfection makes treatment very problematic; for example, RIF induces the activity of the CYP3A enzyme system in the liver, and this cytochrome metabolizes most antiretroviral drugs and therefore decreases the concentration of these drugs in the blood.(Swaminathan and Narendran, 2008)

Following the UN TB meeting in 2018, which has given a new boost to TB funding and a renewed interest in anti-TB drug research, the aim of this review is to highlight current work in the field, the benefits of exploiting nanotechnology for targeted infectious disease research, and, finally, outline the potential towards clinical trial development, which has yet to be achieved for nano-therapeutic-focused TB treatment.

Nanomedicine

Nanotechnology holds great promise to improve human health and is predicted to significantly benefit all human society.(Etheridge *et al.,* 2013; Chang *et al.,* 2015; Saravanan *et al.,* 2018) There has been a rapid global growth of nanomedicine research in recent years, demonstrated by a 280% increase in scientific citations for the term nanomedicine in the last 5 years, and around a 7000% increase in the last 15 years (SciFinder keyword search; nanomedicine [January 2019]). In commercial terms, nanomedicine represents a significant growth area with the global market growth rate of 12.3% predicted to rise to $177.6bn between 2013 and 2019.(Transparency Market Research, 2018) The ability to create nanoscaled materials has allowed advancements in medicines, targeted drug delivery, and diagnostic tools as well as offered a novel set of antimicrobial agents.(Byrne *et al.,* 2011; McDonald *et al.,* 2013; Donnellan *et al.,* 2015) There are many advantages to using nano-formulations for therapeutic uses, such as the possibility of lowering the drug dose administered to patients, thus causing fewer side effects and possibly reducing treatment time. This is achieved through improved targeting of the drug-bearing nanoparticle (NP) to the required target, therefore enhancing the drug concentration at specific sites while decreasing delivery to nontarget sites.(Byrne *et al.,* 2011) Targeting (which can be active or passive) can be achieved by modifying NP surfaces with polymers and/or through bio-conjugation of antibodies and specific ligands. This can prevent NPs from binding with nonspecific blood components and targets them to specific receptors.(McCarron *et al.,* 2008; Kamaly *et al.,* 2012) This can also increase the blood circulation time of nanomedicines, which may be achieved by reducing the phagocytic clearance of a drug.(Kamaly *et al.,* 2012) Addition of the polymers, such as polyethylene glycol, to the surface of NPs renders the NP hydrophilic. This addition reduces reticuloendothelial system uptake of NPs (e.g., the liver and spleen), thus allowing it to stay in circulation longer.(Jokerst *et al.,* 2011) Polyethylene glycol is also reported to reduce the formation of aggregates. Additionally, if drugs are encapsulated by NPs (e.g., liposomes), they can be protected from enzymatic degradation (e.g., in the blood), and this could also improve drug stability. Nanocarriers can be designed to control drug release (e.g., some carriers will only release drugs at a certain pH) possibly further enhancing drug absorption at specific sites.

Nanoparticles have a large surface area relative to their volume, and their size is comparable with that of intracellular macromolecules and organelles such as proteins and DNA. Their small size allows nanomedicines to interact with targets both on cell surfaces and internally.(Navalakhe and Nandedkar, 2007) Macrophages uptake and phagocytose smaller entities more readily than do larger forms of the same material.(Clift *et al.,* 2008; Nasiruddin *et al.,* 2017) Therefore, if drugs are in the nano-form, this could be advantageous in treating some diseases where bacteria reside in immune cells (e.g., TB) or in cancer

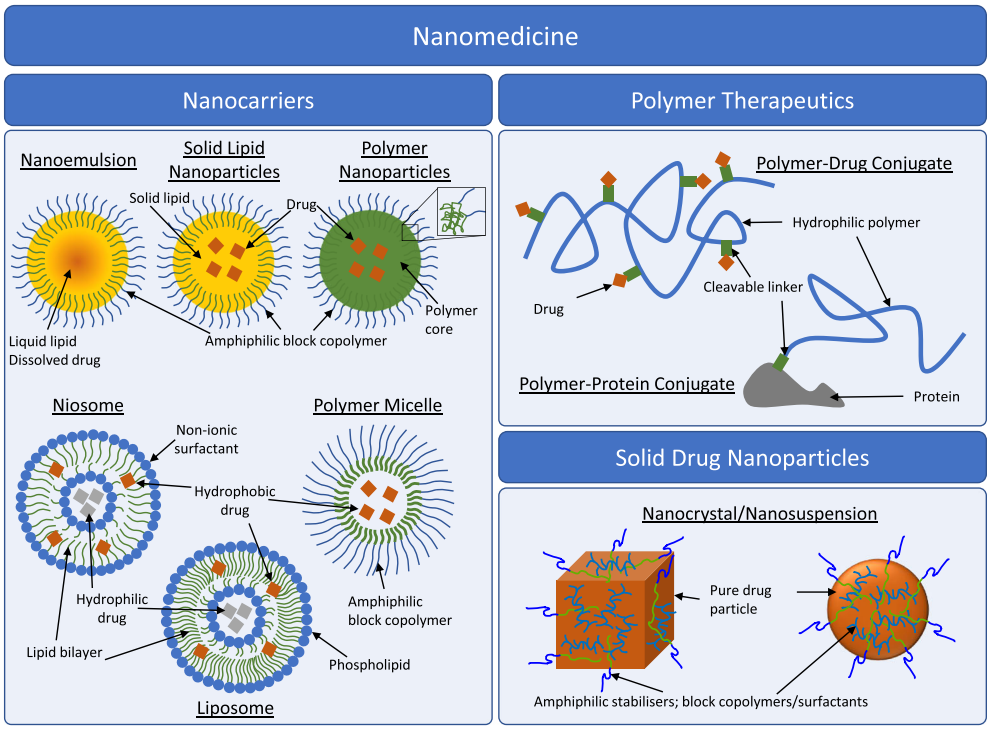

Figure 1. Schematic of the three overarching nanomedicine forms. [Colour figure can be viewed at wileyonlinelibrary.com]

treatments, where it is reported that liposomes < 130 nm have a higher level of selectivity and tumor accumulation over larger liposomes.(Meerovich *et al.*, 2008) This is a phenomenon of the "enhanced permeability and retention" effect, another means of targeting NPs to specific sites (size dependent), where NPs direct drug away from sites with tight epithelial junctions in the healthy vasculature and instead accumulate in areas where fenestrations (gaps) exist following new, abnormal tissue growth (i.e., tumors), thus enhancing permeation. Retention occurs as lack of lymphatic drainage from tumors leads to poor removal of NPs. Additionally, in the nano-form, drug bioavailability can be increased owing to the relatively high surface area available allowing for higher local concentration.

Nanotechnology has many applications within the medical field, but in the context of therapeutics, there are three overarching nanomedicine types that will be discussed herein: nanocarriers, polymer therapeutics, and solid drug nanoparticles (SDNs) (Fig. 1).

Nanocarriers

The largest category of nanomedicine, in which nanoscale materials act as vehicles to transport drugs that are either encapsulated within the nanocarrier core or adhered to the surface.(Torchilin, 2006) In both cases, adherence of drug to the carrier is generally through noncovalent attachment. There are several subcategories of nanocarriers, summarized in Figure 1. *Nanoemulsions* and *solid lipid nanoparticles* (SLNs) are conceptually identical where in both cases, biologically compatible lipids are used to solvate and encapsulate hydrophobic drugs forming a lipid nanocarrier.(Müller *et al.*, 2000; Mehnert and Mäder, 2001; Date *et al.*, 2010; Gordillo-Galeano and Mora-Huertas, 2018; Tayeb and Sainsbury, 2018) Surface stabilizers, such as amphiphilic block copolymers (i.e., linear or branched chain polymers, which incorporate both hydrophobic and hydrophilic sections to their structure) are incorporated into the nanocarrier to provide colloidal stability. The primary difference between nanoemulsions and SLNs is the use of liquid or a solid core, respectively, with the choice of lipid allowing for controlled release rates of the encapsulated drug. *Polymer NPs* are similar; however, they incorporate no lipid. Instead, hydrophobic drugs are encapsulated into a hydrophobic polymer core, which forms through the collapsing and aggregation of hydrophobic polymer chain in aqueous solvent.(El-Say and El-Sawy, 2017) The structure forms from amphiphilic block copolymers, which entrap the hydrophobic drug upon self-assembly in aqueous media with the hydrophilic segment of the polymer protruding

from the surface into the aqueous solvent. The amphiphilic polymers thus provide both the core and colloidal stability. Provided that biologically safe polymers and monomers are used, drugs are released through physiological biodegradation of the polymer structure. Self-assembled structures are common in nanocarrier design, with *liposomes*, *niosomes*, and *polymer micelles* formed through the assembly of amphiphilic smaller molecules to generate spherical structures, which encapsulate drugs. Both liposomes and niosomes are vesicle-type structures formed through generation of a liquid encapsulated within a lipid bilayer. Liposomes are generally formed through use of phospholipids, which consist of two hydrophobic fatty acid chains attached to a hydrophilic phosphate group head. (Torchilin, 2005; Allen and Cullis, 2013) In aqueous media, the molecules assemble to form a bilayer where the hydrophobic parts of the molecule are protected from the external aqueous environment. Thus, an aqueous region is present in the center of the nonpolar hydrophobic membrane. Niosomes are conceptually identical; however, the key difference is the use of biodegradable nonionic surfactants to form the bilayer, which are often relatively nontoxic, more stable, and inexpensive when than are liposomes.(Kuotsu *et al.*, 2010; Ag Seleci *et al.*, 2016) In both cases, single bilayer (unilamellar) or multiple bilayers (multilamellar) structures can form with either hydrophobic or hydrophilic drugs encapsulated in the membrane or the aqueous center, respectively. Polymer micelles form through the self-assembly of amphiphilic block copolymers, thus similar to polymer NPs; however, in this case, a wet, flexible core is formed as opposed to a solid/semisolid core.(Ahmad *et al.*, 2014; Karayianni and Pispas, 2016) In polymer micelles, the amphiphilic polymers arrange themselves, so the hydrophobic part of the polymer sits in the center of the spherical structure where hydrophobic drugs will be encapsulated. The hydrophilic, polar part of the polymer on the surface in contact with the surrounding aqueous solvent provides colloidal stability.

Polymer therapeutics

Drugs are delivered through the conjugation to a biologically compatible polymer, which are cleaved and released upon delivery.(Duncan, 2003; Khandare and Minko, 2006; Duncan, 2011; Seidi *et al.*, 2018) Polymer therapeutics include polymer–drug conjugates, where molecular drugs are delivered; polymer–protein conjugates, where a therapeutic protein is delivered; and polyplexes, which are formed through a DNA–polymer complex. Polymer therapeutics are often referred to as prodrugs, and although they lack particulate characteristics and thus do not fit with the conventional view of nanoparticulate design, they are included in many nanomedicine definitions owing to their size range of around 2–100 nm and their physiological behavior. Drugs are covalently attached to the polymer through a linker and as such are not released until they encounter a specific biological trigger, thus preventing wide physiological distribution. Cleavage is typically achieved using pH-responsive polymers or through enzymatic cleavage via disease-specific enzymes. The polymers serve several purposes: they increase the overall drug molecular weight, thus increasing its circulatory half-life; they lower the rate of clearance, thus varying the biological distribution of the drug; and they can incorporate functionality to address issues such as active site targeting or drug solubility. Multiple drug conjugates can be incorporated into the polymer for either direct or sustained combinational drug delivery.

Solid drug nanoparticles

Solid drug nanoparticles are the simplest form of nanomedicine, often referred to as nanosuspensions or nanodispersions.(Rabinow, 2004; McDonald *et al.*, 2015) The NP is composed of the drug molecule itself with surface-adsorbed stabilizers providing colloidal stability (Fig. 1). Reduction in particle size through SDN formulation increases the surface-to-volume ratio of the drug particle, thus exposing greater drug content with respect to the nonformulated, native drug, as illustrated in Figure 2.

The drug molecules employed are predominantly hydrophobic with very low water solubility. There are two main synthetic methods for SDN production: top-down and bottom-up approaches.(Junghanns and Müller, 2008; Möschwitzer, 2013; Wais *et al.*, 2016) Top-down approaches are where larger particles are repeatedly broken down into smaller participles until a size range distribution of less than 1000 nm is achieved. Examples include nano-milling or homogenization. Bottom-up approaches are where controlled crystallization or precipitation of dissolved drugs takes place. In both cases, amphiphilic stabilizers are present, which adsorb onto the surface of the SDN as they develop. The stabilizers are either polymer or surfactants or a combination of both. Colloidal stability is provided through either steric interaction (i.e., close-proximity repulsions between particles with long-chain polymer stabilizers) or

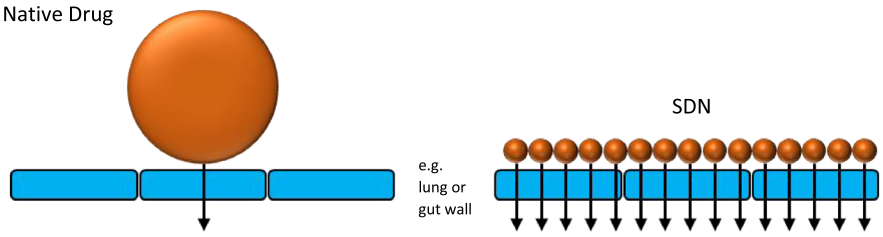

Figure 2. Representation of solid drug nanoparticles (SDNs) compared with conventional drug (image not to scale) illustrating the large surface area and surface-to-volume ratio. [Colour figure can be viewed at wileyonlinelibrary.com]

electrostatic interactions (i.e., electrostatic repulsion of similarly charged particles bearing cationic/anionic polymers or surfactants). Drug release is achieved as SDNs ultimately dissolve in vivo, with dissolution rates varied owing to SDN composition. The aggregated drug at the core of the SDN can either be crystalline or amorphous. Crystalline SDNs (often referred to as nanocrystals) are generally slower to dissolve than are amorphous SDNs; however, they may offer greater advantages in increasing drug circulatory half-life as well as longer-term drug product storage.

Nanomedicine and tuberculosis

Delivering an effective concentration of anti-TB drugs to sites where *Mtb* resides in the immune cells, deep within the lungs, is a huge feat, as often drugs dissolve rapidly and are absorbed by the blood.(Mizoe *et al.*, 2008) Granulomas are clusters of *Mtb*-infected macrophages surrounded by many immune cells, fatty acids, and cholesterol, securing *Mtb*. Their function is to localize and contain the infection, preventing both its growth and replication due to the acidic conditions with low oxygen availability, but they are unable to destroy all bacilli or prevent bacilli from generating energy. (Smith, 2003; Pieters, 2008; Ehlers and Schaible, 2012) Granuloma formation is a hallmark trait of *Mtb* infection (particularly in latent TB infection) and plays a pivotal role in immune-pathogenesis, where in 10% of cases bacilli from granulomas will escape and go on to cause active disease.(Silva Miranda *et al.*, 2012) These structures are poorly vascularized,(Grobler *et al.*, 2016) and therefore, it is very difficult to target drugs to these high-content bacilli constructs. Cells take up molecules/particles through phagocytosis, endocytosis, and pinocytosis. By engulfing NPs, particularly in the case of macrophages where *Mtb* resides, the particles may be colocalized with the pathogen. This would allow for very accurate targeting, for both antimicrobial NPs and drug delivery. Furthermore, it is accepted that smaller NPs are efficient at crossing epithelial barriers following oral administration, hence the branching of nanotechnology into TB research. (Hussain *et al.*, 2001) As such, nanomedicine has led to an original path of research undertaken within the therapeutic field of TB.

Literature on using nanomedicines to target TB is predominantly based on the use of polymers or liposomes with first-line drugs RIF or INH.(Pandey *et al.*, 2003; Pandey and Khuller, 2005; Pandey and Khuller, 2006; Ohashi *et al.*, 2009; Hirota *et al.*, 2010; Aboutaleb *et al.*, 2012; Raj *et al.*, 2012; Chuan *et al.*, 2013; Dube *et al.*, 2013; Vieira *et al.*, 2017; Hakkimane *et al.*, 2018) Studies have highlighted the possibilities of encapsulating anti-TB drugs within biodegradable polymer NPs, thus increasing the concentration of drugs at specific sites (e.g., infected macrophages) through targeting. NPs containing anti-TB drugs are usually made from biodegradable materials (e.g., alginate or solid lipids), and most TB research focuses on the use of poly-lactic-*co*-glycolic acid (PLGA) synthetic polymers.(Gelperina *et al.*, 2005; Ohashi *et al.*, 2009; Sung *et al.*, 2009; Hirota *et al.*, 2010; Onoshita *et al.*, 2010; Kalluru *et al.*, 2013)

Hirota *et al*. carried out a study to determine the uptake and cytotoxicity of PLGA microspheres (MSs) loaded with RIF on an NR8383 cell line derived from rat alveolar macrophages.(Hirota *et al.*, 2010) It was determined that RIF PLGA-MS had little/no cytotoxicity against the NR8383 cells and were readily engulfed, significantly more than when compared with RIF as an aqueous, native drug. Additionally, they reported that high concentrations of RIF were detected within phagosomes, suggesting that RIF entrapped in PLGA-MS can enter membranes of macrophages (probably through phagocytosis) more readily that can native RIF. NR8383 cells were then infected with *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) and exposed to the RIF PLGA-MS. The RIF PLGA-MS was found to be bactericidal against intracellular *Mycobacterium bovis* BCG, significantly more so than native RIF at both

concentrations tested (0.25 and 2.50 μg/mL) over a period of 7 days.(Hirota *et al.,* 2010) Sung *et al*. formulated RIF-encapsulated PLGA NPs with aerosol delivery directly into the lungs. They found the RIF concentration in the lungs over time was significantly improved when compared with native drug.(Sung *et al.,* 2009) Comparing RIF administered via a nanocarrier to a native solution in an in vitro setting offers encouraging results to go forward to animal and human trials. An important factor to consider is the delivery means, that is, whether directly into the airways (e.g., nebulizer) or through a systemic route. The advantages of a more targeted drug delivery for *Mtb* infections are numerous, especially if it can allow for the lowering of dosing.

Vieira *et al*. created nanostructured lipid carriers (NLCs) as nanocarriers of RIF for selective delivery. (Vieira *et al.,* 2017) To increase selectivity to macrophages, the NLCs were coated with mannose(Jain *et al.,* 2010) to target macrophage sugar receptors, and they were evaluated for cellular uptake and their impact on cell viability. The authors treated bone marrow-derived macrophages (BMDMs) with mannosylated RIF NLCs, RIF NLCs, and native RIF drug. (Vieira *et al.,* 2017) They followed cellular uptake using fluorescence microscopy and then quantified using flow cytometry. The mannose-coated RIF NLCs displayed a 14.5-fold increase in cellular uptake, which is encouraging but also highlights the power of targeting. Next, the authors infected bone BMDMs with *Mycobacterium avium* strain 2447 and treated them with mannosylated RIF NLCs, RIF NLCs, and native RIF. At three time points (0, 1, and 7 days), cells were lysed and bacteria suspensions collected. Colonies were counted after a further 7 days' incubation. Mannose-coated NLCs were reported to be more successful in decreasing the growth of intracellular *M. avium* strain 2447 than are controls. Finally, the authors report that NLCs containing RIF drug exhibited lower levels of cytotoxicity to BMDMs than did NLCs with no drug and account this to the surface charge of the NPs; however, the inclusion of the mannose coating onto RIF NLCs did reduce cell viability.(Vieira *et al.,* 2017)

Hakkimane *et al*. prepared nano-formulations of PLGA NPs loaded with RIF and IH2 (INH modified into INH benz-hydrazone).(Hakkimane *et al.,* 2018) The characterization of the NPs shape and morphology was analyzed using Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) was employed to visualize the encapsulation of drugs inside the NPs. Reversed-phase High Performance Liquid Chromatography (HPLC) was used to calculate the exact amount of drug encapsulated, and NP stability tests using dynamic light scattering were undertaken at different pH levels.(Hakkimane *et al.,* 2018) Dynamic light scattering measures hydrodynamic diameter, the size of primary particles if dispersed, or agglomerates along with the shell of ions/water molecules associated with the surface, by measuring the scattered light that passes through a solution. The zeta potential, a measure of the charge carried by NPs when suspended in an aqueous environment, gives an indication of the stability of NPs in a solution. Polydispersity index (a measure of size variation) suggested the NPs were stable and nonagglomerated, and drug release was described as slow and steady following an initial burst phase. Drugs encapsulated in NPs were found to be more stable at different pH levels than were free, native drug. After 24 h, 70% (pH 4.5) and 50% of RIF had degraded (pH 7.4), whereas when encapsulated, drug was released in a profoundly slower, sustained process over a period of days.(Hakkimane *et al.,* 2018) Mycobacterial susceptibility tests were carried out using the traditional MGIT™ system(Tortoli *et al.,* 1999) against the *Mtb* laboratory strain H37Rv. As growing *Mtb* utilize oxygen, the MGIT™ 960 system records the level of oxygen depletion via fluorescence readings, thus indicating both mycobacterial presence and growth. Comparing RIF-loaded PLGA NPs with native RIF drug, the authors report that at a concentration of 0.70 μg/mL, H37Rv developed resistance to native RIF but remained sensitive to RIF PLGA NPs, in which 100% inhibited bacilli growth.(Hakkimane *et al.,* 2018)

Aboutaleb *et al*. created RIF-loaded solid lipid NPs (SLNs [NPs with a solid hydrophobic core coated with a phospholipid monolayer]) for intravenous administration.(Aboutaleb *et al.,* 2012) The aim was to create spherical, stable RIF SLNs $<$ 100 nm in diameter. They found that RIF was released over a 120-h period in vitro in a biphasic pattern when in SLN form, thus indicating that the drug release was controlled and did not occur instantaneously. The antimycobacterial properties of the SLNs were tested against a surrogate strain of *Mycobacterium fortuitum* in an extracellular environment, and results show an eightfold higher efficacy at inhibiting growth when delivered in the SLNs compared with native RIF solution. Controlled release could offer the potential to lowering dosages.

Dube *et al*. have designed a 1,3-β-glucan (Glu) functionalized chitosan shell (CS), PLGA core NPs loaded with RIF drug (Glu–CS–PLGA + RIF) with the aim of both

stimulating some antimicrobial responses that *Mtb* suppress within the host macrophage (e.g., the suppression of reactive oxygen species [ROS]) and delivering RIF intracellularly.(Dube *et al.,* 2013) The spherical Glu-CS-PLGA + RIF NPs created were approximately 280 nm in size. With the use of human alveolar-like-macrophages, NP uptake was achieved through incubation (6 h) and confirmed by confocal microscopy. Cytotoxicity testing proved no significant change in macrophage viability when incubated with the NPs (although there was great variation in the data).(Dube *et al.,* 2013) The focus of this work was on *Mtb*'s ability to prevent the host cell (macrophage) from producing bactericidal ROS, reactive nitrogen species (RNS), and pro-inflammatory cytokines (e.g., IL-12 and IFN-γ). This suppression aids in *Mtb*'s intracellular survival. In the design of Glu-CS-PLGA + RIF NPs, 1,3-β-glucan was selected as it can interact with Dectin-1 macrophage surface receptors, which promote both pro-inflammatory cytokine production and ROS/RNS generation and also enhance the process of phagocytosis.(Dube *et al.,* 2013) Results suggest that CS-PLGA + RIF NPs successfully increased the antimicrobial activities of macrophages; TNF-α, IFN-γ, and IL-12 secretion was all enhanced by the NPs. Interestingly, it was reported that RIF could be delivered at a concentration four times higher than the mean concentration into macrophages when delivered as an NP construct rather than as a free RIF solution (in vitro); however, the efficacy of the drug in this NP formulation is not known as no tests with *Mtb*-infected macrophages were carried out.(Dube *et al.,* 2013)

In another study, Edagwa *et al*. demonstrated that PLGA NPs encapsulate RIF and a modified INH drug (INHP), localized in subcellular organelles within phagosomes in monocyte-derived macrophages (MDMs).(Edagwa *et al.,* 2014) Results displayed confocal microscopy images of PLGA RIF and INHP in endosomal compartments. To determine whether these NPs were trafficked to the same compartment as mycobacteria, the MDMs were infected with *Mycobacterium smegmatis* and then treated with the NPs. Imaging suggested that colocalization also occurred for the particles and mycobacteria, within the MDM cells. (Edagwa *et al.,* 2014) To assess their antimycobacterial activities, MDMs were exposed to RIF PLGAs (loaded with approximately 10% drug) and INHP PLGAs (loaded with approximately 5% drug) for 24 h; then MDMs were infected with *M. smegmatis* for 1–10 days. The same experiment was carried out for RIF and INHP in native forms. Comparing the results between the two types, the PLGA NPs exerted over 1.3-fold greater toxicity after 1-day incubation with *M. smegmatis* at 300 μm. (Edagwa *et al.,* 2014) It was found that when MDMs were infected with mycobacteria following 10 days of exposure to the PLGAs or native drugs, the PLGA NPs inhibited mycobacterial growth by 50%, whereas the native drugs had no effect on mycobacterial growth intracellularly. This suggested that in the nano-form, drugs are retained for extended periods inside macrophage cells. It must be noted, however, that this was a somewhat unorthodox means of measuring antimycobacterial activity, as the macrophage cells were first treated with the drug (in both nano-forms and native forms) before being infected with mycobacteria. It is unclear whether this is a means to prevent infection rather than clearing an infection. Overall, however, these results were encouraging from a nanomedicine perspective. With published reports demonstrating PLGA NPs carrying anti-TB drugs being engulfed by macrophages, the possibility of targeting granulomas has begun to be investigated. Granulomas harbor mycobacteria, which could cause reactivation to a diseased state or lead to the development of MDR TB. Grobler *et al*. have reported that delivering NPs to granulomas is possible.(Grobler *et al.,* 2016) With the use of a colloidal Pheroids (NPs with oil, gas, and water phases that are filled with RIF [dissimilar to PLGAs]) and an in vitro granuloma model, it was possible to target NPs to the macrophages. Pheroids are a patented drug delivery system, with the ability to control the rate of drug clearance and offer protection to drugs (e.g., from enzyme degradation) in vivo.(Steyn *et al.,* 2010) The analytical model presented by Grobler *et al*. demonstrates that when RIF was delivered using Pheroids, when compared with native RIF, there was a significantly higher concentration of RIF in the macrophages than in the blood (of 16 patients following their fourth daily dose of drug).(Grobler *et al.,* 2016) Additionally, using the delivery system, higher drug concentrations were measured at several positions in the granuloma model when compared with the native RIF. This work correlated nicely with the previous reports; when RIF was administered in a nano-formulation (loaded on PLGAs, SLNs, or packaged in Pheroids), compared with a solution of native RIF, a higher concentration of drug reached the desired site.

We have developed SDNs of first-line drugs RIF, INH, ethambutol, and PZA.(Donnellan *et al.,* 2017) The work was conducted following our same developmental procedure, which led human clinical trials of SDN-

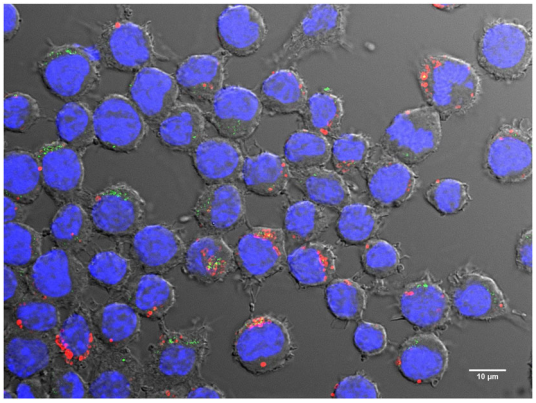

Figure 3. J774 cells infected with *Map* K10 (at a MOI 1:10) treated with RIF SDNs. J774 cells (blue) and *Map* K10 GFP (green) with 20 μg/mL RIF SDN (red) at 63×. RIF, rifampicin; SDN, solid drug nanoparticle [Colour figure can be viewed at wileyonlinelibrary.com]

formulated HIV antiretroviral drugs efavirenz(Mcdonald *et al.*, 2014) and lopinavir(Giardiello *et al.*, 2016) (EudraCT number 2013-004913-41). We screened the efficacy of the NPs against a reporter strain of *M. avium* subsp. *paratuberculosis* (*Map* K10/GFP(Harris *et al.*, 2002)). We found that RIF SDNs were 50-fold more efficacious, as evidenced by a reduced IC_{50} value (0.02 compared with 1.06 μg/mL) than was native drug. Additionally, RIF SDNs had a faster kill rate against *Map* than had native RIF.(Donnellan *et al.*, 2017) Next, we treated *Map*-infected macrophage-like cells (J774s) with SDNs and imaged uptake to determine if SDNs could be trafficked to mycobacteria residing in macrophages (Fig. 3). Solid drug nanoparticles and mycobacteria were found to be colocalizing within the cells. Additionally, we reported that the intracellular kill rate of the SDNs was more effective than that of the native drug.(Donnellan *et al.*, 2017)

Conclusions

The intracellular delivery of drugs remains a challenge to the TB research community, especially for treating the latent form of the disease, where bacilli reside within granulomas. A major problem in TB treatment is patient noncompliance, attributed to the lengthy treatment periods and daily, multi-drug dosing. If nanomedicines could aid in lowering drug dosages and treatment periods, this could help reduce this problem. Tuberculosis and HIV are closely associated, with TB being the most common cause of AIDS-related death; therefore, treatments need to be developed to consider coinfected patients. With a clinical trial for SDN-formulated HIV therapy underway, this field should also include TB treatment, as evidenced by all the in vitro studies outlined within. Nanomedicine is a rapidly expanding area and could provide multiple research avenues towards improving treatment outcomes, reducing drug dosing, and improving targeting. Currently, there are 50 clinically FDA (United States Food and Drug Administration)-approved nanomedicines. Of the 50 in clinical use, the most commonly used NP types are polymer (34%), nanocrystal (30%), and liposome (20%). When searching the term "nano" on ClinicalTrials.gov, a further 66 clinical trials included are listed as "recruiting" or "active" (as of January 2019). However, none of the approved nanomedicines or upcoming trials are aimed at TB treatment. There are numerous examples, however, of nanomedicine strategies utilizing polymer NPs, liposomal/noisomal delivery systems, and SLNs towards TB therapy, each showing good preclinical data and thus presenting themselves as good candidates for potential clinical studies.(Madeeha *et al.*, 2016; Nasiruddin *et al.*, 2017)

Overall, the pipeline for TB drug development is not as stagnant as it once was. There are more avenues and exciting areas being explored with nanotechnology being a driving force behind this. As evidenced here, there is plenty of preclinical work within the TB/nanofield being undertaken, but this needs to be fast-tracked and taken to the clinic. Additionally, a further understanding of how drugs penetrate the lung cavities would be beneficial and may allow a more tailored, nano-based regime to be designed, to exploit the unique properties of nanomedicines. Nanomedicine may also offer relief to the financial burden in TB drug development. For example, by using SDNs of first-line antibiotics, it is exploiting existing FDA-approved pharmaceutical ingredients, therefore saving time and the inordinate cost involved in novel drug development and testing.

This review aimed to highlight the use of nanotechnology towards the cellular targeting of drugs towards TB therapy, as well as the significant growth and range of nanomedicine strategies, not all of which have been adopted by TB research. These must be addressed and accelerated to meet the UN target of hastening progress towards the eradication of TB.

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Author contributions

Both authors contributed equally to the manuscript.

Conflict of interest

We declare no competing financial interests.

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