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Nanobead-based interventions for the treatment and prevention of tuberculosis

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Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the most devastating bacterial diseases to affect humans. *M. tuberculosis* is a robust pathogen that has evolved the capacity to survive and grow inside macrophage phagosomes. A cocktail of antibiotics has long been successfully used against *M. tuberculosis* but is becoming less effective owing to the emergence of multidrug resistance. The only available preventive vaccine, using *Mycobacterium bovis* bacille Calmette–Guérin, is considered to be ineffective against adult pulmonary TB, the most prevalent form of the disease. Here, we review the potential use of biodegradable nanoparticle-based anti-TB drug delivery systems that have been shown to be more effective against *M. tuberculosis* in animal models than conventional antibiotic treatment regimens. This technology also has substantial potential for vaccination and other therapeutic strategies against TB and other infectious diseases.

Until the introduction of antibiotics in the 1940s, tuberculosis (TB), the disease caused by *Mycobacterium tuberculosis*, was a feared scourge that caused hundreds of millions of human deaths. The availability of antibiotics such as isoniazid (INH) and rifampicin (RIF) led to the widespread hope that the disease could eventually be eradicated. Alas, such optimism proved premature and the disease, which is also referred to as the ‘white

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FURTHER INFORMATION
Global Alliance for TB Drug Development: http://www.tballiance.org/home/home.php
Stop TB Partnership tuberculosis vaccine candidates – 2009: http://www.stoptb.org/wg/new_vaccines/assets/documents/TB_vaccine_Pipeline_2009.pdf

plague' or 'consumption', has gradually increased in severity to the point that it is now the bacterial infection that kills the most people worldwide[1]. A staggering one-third of the world's population is latently infected and, according to the WHO, ~1.8 million people die every year, with an estimated 9.8 million new infections per year[2]. This pandemic is being driven by the additional complications of the emergence of multidrug-resistant (MDR) *M. tuberculosis* strains and the increase in patients with TB who are co-infected with HIV. For the past 80 years, one live attenuated bacterial vaccine based on *Mycobacterium bovis* bacilli Calmette–Guérin (BCG), which has lost several virulence genes, has been extensively used for the prevention of TB. Although this vaccine, given at birth, seems to offer some protection against childhood TB, especially tuberculous meningitis, a consensus is now emerging that it is not effective against adult TB. Therefore, the development of an improved vaccine is currently an international research priority[2,3].

Drug-susceptible TB can be effectively treated with a cocktail of four 'front-line' drugs — RIF, INH, pyrazinamide (PZA) and ethambutol (ETB) — given daily for 6–8 months or longer by the oral route (usually, all four drugs are administered for 2 months, followed by INH and RIF for the remaining period). However, the fundamental problem in the treatment of TB is the long duration of therapy required to cure the patient, which can hamper patient lifestyle and induces patient non-compliance, treatment failure and development of drug-resistant strains[4]. Furthermore, the recalcitrance of *M. tuberculosis* to eradication by the current anti-TB drugs is thought to result from its ability to achieve a non-replicating state in the host. Because RIF, INH and ETB (but not PZA) require bacterial replication for their action, the non-replicating state is thought to render *M. tuberculosis* phenotypically resistant to otherwise bactericidal antibiotics[3,4].

A few years after the introduction of streptomycin for TB therapy by Selman Waksam in 1944, the first signs of drug resistance were noted, and the same was seen later with RIF and INH. The situation deteriorated further when *M. tuberculosis* strains resistant to multiple drugs emerged; MDR *M. tuberculosis* is defined as being resistant to both RIF and INH[4,5]. Even more worrying now is the emergence of extensively drug-resistant (XDR) strains in many parts of the world. In addition to resistance to RIF and INH, XDR *M. tuberculosis* strains are resistant to at least one second-line, injectable drug (such as capreomycin, kanamycin or amikacin) and to any fluoroquinolone drug (for example, ciprofloxacin, levofloxacin, moxifloxacin or ofloxacin)[4]. These drugs are more expensive, inherently more toxic and less efficacious, need a higher dosage and must be used for up to 24 months. The use of second-line drugs is also a move towards broader-spectrum antibiotics, a strategy that can select for resistance among other, coexisting pathogens. The molecular mechanisms used by *M. tuberculosis* to induce the MDR and XDR state have been discussed recently[5].

The fear of the spread of XDR strains and the diminishing arsenal of effective treatment options reinforce the need to develop new, effective anti-TB drugs to overcome the problem of drug resistance and to shorten the treatment course. However, there are currently fewer than ten compounds in clinical development — perhaps too few to guarantee even a single new anti-TB drug in the near future[6] (see Further information for the Global Alliance for TB Drug Development). Conversely, current anti-TB drugs are still effective,

and strategies allowing more efficient delivery of these drugs are called for. In this context, nanotechnology is one of the most promising approaches for the development of more effective and more compliant drug delivery systems for the treatment of TB. This technology also offers a potentially powerful strategy for the development and delivery of new-generation TB vaccines. Here, we discuss the potential use of biodegradable nanoparticle-based delivery systems for drug therapy and vaccination against TB.

The cell biology and immunology of TB

In humans, TB is predominantly a disease of the lungs. *M. tuberculosis* infection starts with the inhalation of infectious bacteria and their deposition in the alveolar space of the lungs (FIG. 1). The bacteria are taken up by phagocytes in the lung, in particular by alveolar macrophages, and reside within intracellular phagosomes. Many receptors have been implicated in the uptake process, including mannose receptors, Toll-like receptor 2 (TlR2) and TlR4, surfactant protein A receptors, CD14, scavenger receptors, complement receptors and immunoglobulin receptors[7].These receptors are all potentially interesting for targeting nanoparticles to TB-infected macrophages (see below). Under ideal conditions, pathogen-enclosing phagosomes fuse sequentially with early and then late endocytic organelles to become bactericidal phagolysosomes. However, *M. tuberculosis* can prevent phagosome maturation and phagosome–lysosome fusion, thereby avoiding exposure to the lower pH and hydrolytic environment of the phagolysosome[8]. Several mechanisms have been implicated in the ability of *M. tuberculosis* to arrest phagosome maturation, but our understanding of this process is far from complete. Macrophages are key effector cells in mycobacterial killing but can also provide a niche for *M. tuberculosis* multiplication. Dendritic cells (DCs) also engulf bacteria but lack killing ability and may serve as a ‘Trojan Horse’ (REF. 9). DCs engulfing *M. tuberculosis* migrate to the draining lymph nodes and prime the naive T cells that subsequently return to the lungs to control the infection (FIG. 1).

These events lead to the formation of the granuloma at the site of infection, resulting in containment of the infection. This balanced status between host and mycobacterium is called ‘latency’, during which clinical signs of disease are absent and the bacteria persist in a non-transmissible form[3]. However, this balance is disturbed by factors such as malnutrition, immunosuppression, steroid use, anti-tumour necrosis factor (TNF) therapy or HIV infection, causing the bacteria to switch to high metabolic activity and initiate disease[3].

It is generally accepted that a cell-mediated immune response involving both $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells plays an important part in protection against TB. $CD4^+$ T cells enhance the antibacterial activity of macrophages by releasing cytokines such as interferon-γ (IFNγ) and TNF, whereas $CD8^+$ T cells kill infected macrophages — and, possibly, *M. tuberculosis* — by releasing cytotoxic mediators such as perforins, granzymes and granulysin[9]. Despite our improved knowledge of the complex cellular immune responses to *M. tuberculosis*, the type of immune response that is required to mediate protective immunity, and that should therefore be induced by vaccination, is not fully understood.

Nanobead-based therapies

Over the past 20 years, the potential has been explored for replacing the administration of antibiotics or other drugs in the ‘free’ form with an approach using drugs that are encapsulated in a nanoparticle (<1000 nm; some authors restrict this definition to <100 nm) or microparticle (>1000 nm) made up of a biodegradable polymer, allowing a slower, more sustained release of the drug than with conventional free-drug delivery.

Polymeric nanobeads (FIG. 2a) are composed of solid matrices that can have various porosities, depending on the degree of physical or chemical cross-linking of the polymer network, and throughout which the drug molecules are homogeneously distributed. The most extensively used polymer, poly(lactic-co-glycolic acid) (PLGA), is soluble in organic solvents and has been used in many drug release applications[10]. Alternative water-soluble systems such as alginates or chitosans[10] have also been described (see Supplementary information S1 (table)). Nanoparticles can be prepared by intramolecular cross-linking of the polymer chains or by adding a chemical agent. The density and size distribution of the particles can be controlled by manipulating the polymer concentration, the amount of cross-linking agent and the stirring rate of the solution during the preparation of the cross-linked particles.

The use of nanoparticles in this way is now considered to be increasingly important in biomedicine; an estimated two dozen nano-based therapeutics are approved for clinical use in cancer therapy (for example, poly-lactide-based particles enclosing taxol are used to treat breast cancer) and for treating infectious diseases (for example, polyethylene glycol (PEG)–interferon-α2a is used to treat hepatitis B and hepatitis C)[10,11]. Several other nanoparticle formulations are currently being evaluated in clinical or preclinical trials for the treatment of different diseases[11,12]. With the availability of several cheap, biodegradable natural and synthetic polymers approved for human use, both hydrophobic and hydrophilic molecules (including current anti-TB drugs) can be easily encapsulated in nanoparticles with almost no effect on drug shelf life and efficacy.

Nanoparticles and TB

Some of the most striking data have come from studies developing polymer-based antibiotic therapies against *M. tuberculosis* in animal models, including mouse, rat, guinea pig, rabbit and monkey models[13]. The caveat is that none of these models recapitulates all of the features of human TB. Most of these studies have used PLGA in combination with RIF, INH or both. Because of space limitations, we do not discuss alternative delivery systems that have shown promising results in the delivery of antibiotics targeting *M. tuberculosis* (for a brief summary, see BOX 1 and Supplementary information S2 (table)), such as liposomes[14] and solid–lipid nanoparticles[15]; in general, these particles are less stable than their polymer-based counterparts.

The basic idea is that the polymer provides a protective coat for the drug after its administration through injection or, more preferably, through oral or aerosol routes (FIG. 2a,b). After oral or aerosol administration, the particles bind to the apical surfaces of

epithelial cells (for example, microfold (M) cells) and are actively transported across the epithelial layer by transcytosis before being taken up by phagocytic cells, such as macrophages. This is an attractive scenario for treating TB, because macrophages are the main cell type to harbour the bacteria, especially in the lungs. These cells can phagocytose any kind of particle in a certain size range (usually ~200 nm and up to ~10 μm)[16], a property that is dependent on the broad range of 'nonspecific' receptors that are present on their surface[17]. Macrophages containing *M. tuberculosis* in a specialized phagosome can subsequently phagocytose an antibiotic-containing bead[18,19], which will most likely be targeted to a distinct phagolysosome, such that the beads and the *M. tuberculosis* do not colocalize[20]. Once inside the phagolysosome, the bead polymer is degraded[21], albeit slowly, and its contents are released in a sustained manner over several days, first locally, to kill the intramacrophage pathogens, and then systemically into the blood[22]. PLGA beads can be degraded non-enzymatically through ester hydrolysis in aqueous solutions, but they are degraded more rapidly in the low pH of the phagolysosome[23].

In vitro experiments using macrophages show that the delivery of antibiotics such as RIF inside nanobeads leads to a substantial increase in the drug concentration inside the cells relative to the concentration outside (up to 20-fold) and relative to that observed when the drug is added in the free form[13,20,24,25]. Obviously, for a pathogen that lives inside the macrophage, such as *M. tuberculosis*, this is a crucial issue. A sustained and increased concentration of first-line drugs inside the cells harbouring *M. tuberculosis* has the potential to prevent the development of drug-resistant strains. In studies using PLGA beads, both in aqueous solution in the test tube and in macrophages, a burst of drug release (up to 40%) occurs in the first few hours followed by a slower, sustained release over the subsequent days. This rapid burst is considered to be due to drug adsorbed on the bead surface[26].

The concept of slow and sustained release from a biodegradable particle is a crucial aspect of nanobead delivery. When freely administered antibiotics are given orally or through inhalation into the lungs of *M. tuberculosis*-infected guinea pigs, the drugs reach a high concentration in the plasma in hours but are then rapidly degraded or excreted (FIG. 3). By contrast, when the same overall dose of antibiotic is delivered through a single administration of PLGA beads by either delivery route, the plasma levels of the antibiotics remain above the minimum inhibitory concentration for up to 12 days[27] (FIG. 3). In a striking example using RIF and INH co-encapsulated into PLGA beads, only three oral applications of the beads gave the same therapeutic protection against *M. tuberculosis* in guinea pigs as 45 daily doses of the free antibiotics[27]. Similar data were shown using mice[28]. Thus, this technology has the potential to substantially reduce the dosing frequency and the 'pill burden' for patients with TB, leading to improved patient compliance, which would improve the treatment success rate and reduce the development of drug resistance.

Several second-line antibiotics have also been successfully administered against *M. tuberculosis* through the nanobead approach, including moxifloxacin[20] and capreomycin[29]. Also worth mentioning are studies showing that anti-*M. tuberculosis* drugs that cannot pass into the blood system when taken orally, such as streptomycin and econazole, can have effective therapeutic properties when delivered through PLGA or alginate beads, respectively[30,31]. These observations provide strong indirect evidence that the beads can

cross the gut epithelial barrier, in agreement with the direct evidence from electron microscopy studies[32]. Furthermore, encapsulating the more toxic, second-line anti-*M. tuberculosis* drugs can substantially reduce overall systemic toxicity[10], owing to their slow release. In general, the overall lethal dose of antibiotics in beads is many-fold lower than that for free drugs.

Work in this field is pushing towards the application of these promising therapeutic tools for use against human TB. Phase I clinical trials have been initiated at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, using PLGA nanoparticles encapsulating first-line anti-TB drugs. PLGA has long been approved by the US Food and Drug Administration for human use for implants and sutures. Nevertheless, there is some concern that the organic solvents used to dissolve this polymer may have undesirable side effects, especially for vaccination trials (see below). For this reason, alternative, water-soluble polymers may be preferred, and alginate nanoparticles enclosing antibiotics have also been used successfully against *M. tuberculosis* through oral and aerosol administration in mice and guinea pigs[33].

Polymers can be formulated by well-established recipes to make particles of different sizes; for example, PLGA beads can be made in sizes from 10 nm to many μm[34]. Most studies have reached the conclusion that 200–1000 nm particles are more effective for drug delivery than smaller or larger particles. However, the best results using different kinds of polymers and different animal models of *M. tuberculosis* have been achieved with particles of between 200 nm and 400 nm[22]. Earlier studies showed that some particles up to 10 μm in size can transverse epithelial cells and reach the underlying Peyer's patches to access phagocytic and immune cells[32]. However, there is no consensus on the upper size limit[35].

Nanoparticles in TB vaccine delivery

Given the lack of success of the *M. bovis* BCG vaccine, efforts are ongoing to develop more effective vaccines using a range of strategies[36] (see Further information for details on the vaccine pipeline).The development of nanoparticle- and microparticle-based delivery systems for new-generation TB vaccines is an exciting emerging field, using proteins, peptides or DNA that are protected by encapsulation in the particles (FIG. 2b); these particles can be administered by different mucosal and systemic routes. This process can also serve as a depot for the slow release of antigens, leading to a prolonged immune response[10]. In principle, multiple antigens, such as different stage-specific antigens of *M. tuberculosis*[37], can be co-encapsulated with or without immune stimulants or adjuvants. The bead size has been shown to influence the type of immune response induced (FIG. 2c), and nanoparticle vaccines can be formulated to induce cellular and/or humoral immunity, activating $CD8^+$ and/or $CD4^+$ T cells for improved efficacy[38,39]. One challenging problem is the delivery of exogenous protein subunit vaccine candidates to major histocompatibility complex (MHC) class I molecules to induce the cytotoxic T cell response, and nanoparticles using endosomal escape strategies have been engineered for this purpose[40,41].

For subunit vaccine delivery, the idea of using a water-insoluble polymer such as PLGA, in which the antigen must be exposed to a harsh, probably denaturing solvent, is theoretically

less attractive. Nevertheless, different *M. tuberculosis* protein or peptide subunit vaccine candidates have been evaluated in animal models using PLGA microparticle-based delivery systems through parenteral (injection) or respiratory routes, and these vaccines induced strong B cell and T cell responses[42–46]. In one study, mice that were immunized parenterally with the *M. tuberculosis* cell wall 71 kDa protein carried in PLGA microparticles exhibited a robust clearance of bacteria from the lungs and liver after challenge[42]. In another mouse study, immunization by the intranasal route using PLGA microparticles containing early secretory antigen target 6 (ESAT6) induced a strong immunogen-specific effector and memory T cell response in the lungs and thoracic lymph nodes44. Similarly, PLGA microparticles have been shown to enhance the *in vitro* T cell response to a TB10.4 (ESAT6 protein family member; also known as EsxH)–antigen 85B (Ag85B) fusion protein after aerosol adminstration[46]. Recently, an aerosol subunit vaccine using Ag85Bcontaining PLGA microparticles gave promising results in a mouse model[47]. Aerosol boost with this vaccine in *M. bovis* BCG-primed mice imparted protection against *M. tuberculosis* challenge and reduced the number and size of granulomas in the lungs[47]. In general, the aerosol route is especially attractive for nanoparticle delivery for TB vaccination and therapy, although microparticles have often been more effective than nanoparticles when delivered by this route[48].

A few groups have attempted to formulate nanobead or microbead DNA vaccines against *M. tuberculosis*. An earlier study in mice showed promising vaccination using *fbpA* DNA (which encodes Ag85A) adsorbed to cationic PLGA microparticles[49], and anti-mycobacterial immunity was also induced by a single injection of a *Mycobacterium leprae* heat shock protein 65-encoding plasmid in biodegradable PLGA microbeads[50]. A chitosan–DNA nanoparticle vaccine encoding human T cell epitopes of six *M. tuberculosis* proteins was evaluated in transgenic mice[51], and in a more recent study, a DNA vaccine encoding latency antigen Rv1733c associated with PLGA–polyethyleneimine nanoparticles was evaluated using a DNA prime–protein boost vaccination regimen in a mouse model[52]. Both of these nanoparticle-based DNA vaccines induced DC maturation and robust T cell responses after aerosol delivery. Evidently, degradation of the DNA by nucleases before it reached the target cells was not a substantial problem in these studies.

An alternative, innovative approach was developed using genes of interest loaded into nanoparticles that are then attached to the surface of attenuated bacteria[53]. This approach could theoretically be used to target TB granulomas, as super-infecting *M. tuberculosis* bacilli have been shown to enter pre-existing granulomas in the zebrafish and mouse models of TB[54]. These ‘microbots’ (miniature robots) using attenuated mycobacterial strains attached to nanoparticles encapsulating TB vaccines, therapeutics or immune stimulants could be developed for granuloma-specific delivery. Attempts have also been made to adsorb *M. bovis* BCG onto nanoparticles[55] or to encapsulate it in microparticles[56] for improved delivery and efficacy. In a guinea pig model, aerosol delivery of *M. bovis* BCG adsorbed on leucine nanoparticles imparted much better protection against *M. tuberculosis* challenge than parenteral immunization[55,57], whereas in mice oral delivery of *M. bovis* BCG encapsulated in alginate microparticles induced a stronger T cell response and greater protection than oral vaccination with free *M. bovis* BCG[56].

The addition of specific ligands to the surface of nanobeads and microbeads is an attractive option to facilitate vaccination (FIG. 2b,c); for example, *M. tuberculosis* surface adhesin could enable a targeted delivery of TB vaccines to lung mucosa. Nanoparticles can be alternatively formulated into hollow, low-density, dried particles called porous nanoparticle-aggregate particles for effective delivery of TB vaccines or therapeutics to the lungs[58], and recombinant viral vector-based TB vaccines can be improved into 'smart' nanoparticulate vaccines[59]. A nanoparticulate TB vaccine intended for intranasal administration would target nasal mucosa and nasal-associated lymphoid tissue (NALT), whereas a vaccine intended for oral vaccination would target gut-associated lymphoid tissue (GALT), including the Peyer's patches, where M cell-, epithelial cell- and DC-targeting strategies could be used[60].

Other potential uses in TB therapy

Several antimicrobial peptides have been shown to have bactericidal effects against *M. tuberculosis in vitro* and/or *in vivo*. These include ll37 (the 37-amino acid polypeptide cleaved from the precursor cathelicidin antimicrobial peptide)[61,62], defensins and human neutrophil peptide 1 (HNP1; also known as neutrophil defensin 1)[63]. MDR *M. tuberculosis* strains are generally less fit than non-MDR *M. tuberculosis* strains, and they are more susceptible to killing by peptides derived from granulysin[64]. However, MDR *M. tuberculosis* strains do not necessarily need to lose fitness[65]. In addition to incorporating anti-*M. tuberculosis* peptides inside nanobeads, it may be even more interesting to test the cDNAs encoding these peptides, as well as the signal peptide sequence that is needed to direct the newly synthesized protein into the endoplasmic reticulum.

It has been shown that phenothiazines, especially thioridiazine, have interesting therapeutic effects against *M. tuberculosis*[66]. Although the molecular effects of thioridiazine and other phenothiazines are not clearly-defined, they seem to block the efflux pumps of mycobacteria and other bacteria; they show bacteriostatic effects at low concentrations and bactericidal effects at high concentrations against both *M. tuberculosis* and MDR *M. tuberculosis*. Moreover, they are somehow concentrated (up to 100-fold) in macrophages, and TZ shows impressive killing of *M. tuberculosis* and MDR *M. tuberculosis* in macrophages[66]. For such drugs, slow-release nanoparticles offer an attractive alternative, especially in combination with more conventional classes of antibiotics.

Finally, another idea would be to use nanobeads to enclose drugs that enhance the innate immune mechanisms of macrophages against *M. tuberculosis*. For example, when protein kinase B (PKB; also known as AKT1) is inhibited, *M. tuberculosis* (and *Salmonella enterica* subsp. *enterica* serovar Typhimurium) loses the ability to arrest phagosome–lysosome fusion, and the bacteria are effectively killed[67]. Using nanobeads to selectively target inhibitors of such kinases to infected macrophages is an attractive option to consider. Such drugs could also be combined with more traditional antibiotic therapy.

Conclusions and future directions

Nanobead and microbead technology has enormous potential for the different strategies that must be developed to both prevent and treat TB and other diseases in humans. The

results summarized here for antibiotic delivery using nanoparticles make us optimistic that the nanoparticle approach will provide a substantial advantage over conventional therapy for human TB, owing to its enormous potential to reduce the 'pill burden' and improve patient compliance. This sustained delivery system can also be used to administer new TB drugs as they become available, to treat latent and active disease and to shorten the treatment course. Future efforts can also be focused on combining imaging agents and therapeutic agents in the same particle to revolutionize the treatment of TB. The technology is now being prepared for the transition from bench to clinic, with the initiation of Phase I therapeutic clinical trials in India. The use of nanoparticles and microparticles is one of many 'promising' approaches being followed at present for improving vaccination against TB; in this context, it is much more difficult to predict which approach (if any) will be successful in protecting the human population against the white plague.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1 |

Alternative delivery systems for the treatment of tuberculosis

During the past decade, liposomes have been extensively evaluated as a drug delivery system for the treatment of tuberculosis (TB) in animal models[13,14] and have been approved for human use to treat fungal infections and breast cancer using amphotericin B and doxorubicin, respectively[11,12]. Liposomes are spherical vesicles with a bi-layered membrane composed of natural or synthetic amphiphilic lipid molecules. They can be coated with polymers for stabilization of the structure and to prolong circulation half-life, or functionalized with specific ligands for targeted cell or organ delivery. Their unique ability to encapsulate both hydrophobic and hydrophilic drugs makes them excellent as therapeutic carriers. However, liposomes are suitable for administration by limited routes (for example, intravenous injection and inhalation). Another class of polymeric substances that has attracted a great deal of interest for drug delivery applications is dendrimers[68], although only one study has been reported for a TB application[69]. Dendrimers are highly branched, globular macromolecules with many arms emanating from a central core. Dendrimers have a very strong potential for anti-TB drug delivery and other applications, because their structure makes them suited for use in multivalent systems. In other words, one dendrimer molecule has hundreds of possible sites to couple to an active species. However, the production cost can be high. Alternative experimental delivery systems for drug delivery and other applications, such as fullerenes (for example, carbon spheres, or ‘buckyballs’, and carbon nanotubes) and metallic nanoshells (for example, gold nanoshells), are under development and have not yet been reported as having a TB application.

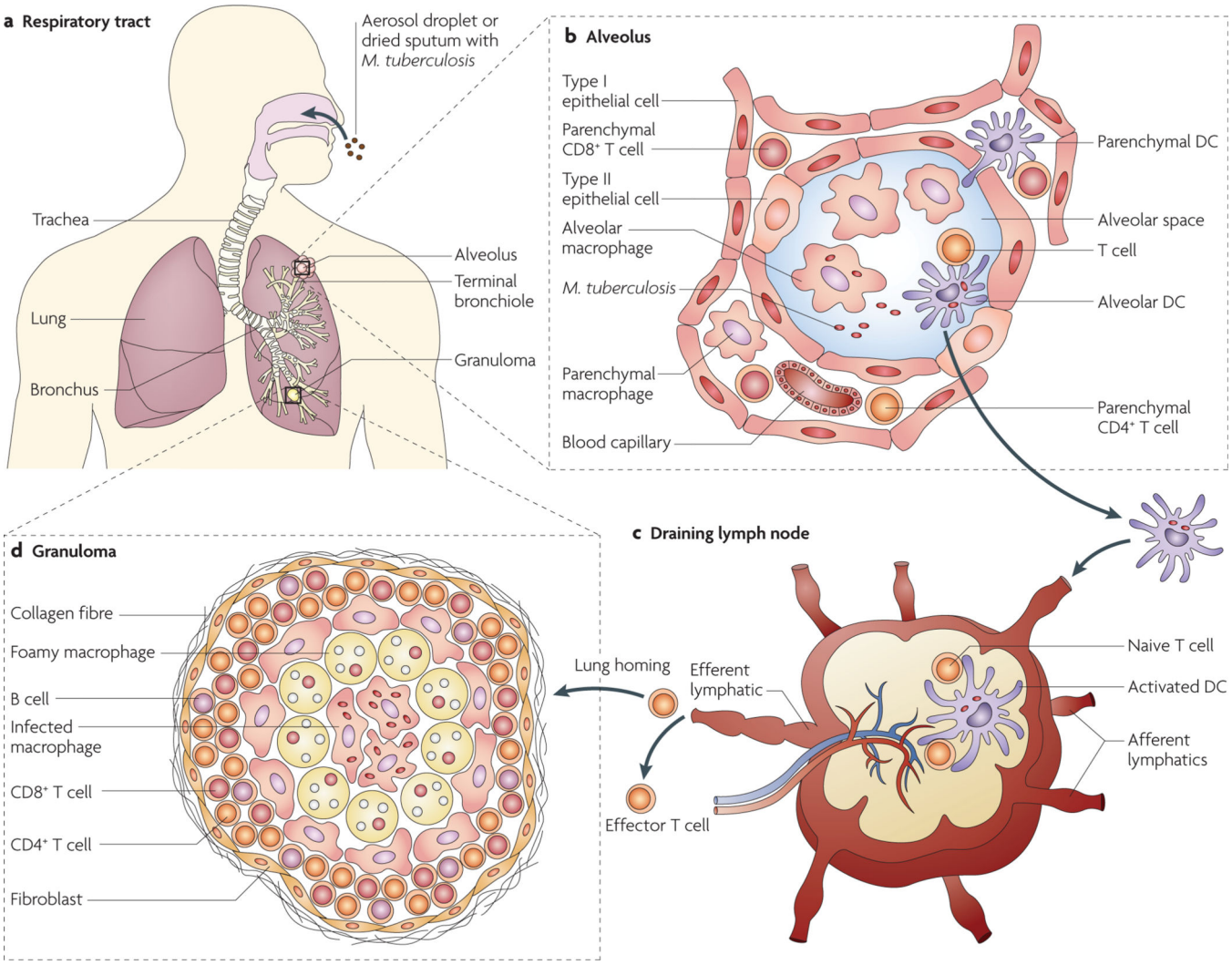

Figure 1 |. *Mycobacterium tuberculosis* infection and granuloma formation.

a | *Mycobacterium tuberculosis* infection starts with the inhalation of bacilli, either as an aerosol droplet generated by the cough of a patient with tuberculosis (TB) or as a dust microparticle of dried sputum, followed by deposition of the bacteria in the lung alveolar space. The lungs, where the major events of pulmonary TB are orchestrated, consist of the conducting airways, which are lined by mucosal tissue, and the lung parenchyma, which surrounds thin-walled alveoli that are specialized for gas exchange. **b** | The alveoli are lined by type I and type II epithelial cells and are separated by thin walls of interstitium containing pulmonary capillaries. in the alveolar cavity, the main hosts for the bacilli are alveolar macrophages. After initial bacterial multiplication in alveolar macrophages, the bacteria are taken up by dendritic cells (DCs), which carry *M. tuberculosis* to the draining thoracic lymph nodes[70]. Alternatively, DCs sampling the alveolar mucosa may carry bacilli to the lung parenchyma, leading to initiation of the local inflammatory foci. **c** | In the draining lymph nodes, DCs carrying bacilli undergo apoptosis, and the mycobacterial antigenic peptides that are released are presented by the activated lymph node-resident DCs to the specific naive cells through cross-presentation. On antigen presentation, activated T

cells proliferate, become Effector T cells (which type depending on the cytokine milieu; for example, single-versus multiple-cytokine-producing polyfunctional helper $CD4^+$ T cell subsets) and leave the lymph node to reach the blood circulation through the efferent lymphatics and the thoracic duct. **d** | effector T cells originating in the draining lymph nodes home back from the blood through pulmonary capillaries to the site of inflammation under the influence of chemokines and other mediators. extravasations of the mononuclear cells thus initiate the formation of signature ‘tubercle’ structures at the site of the infection, leading to containment of the infection. The classic TB granuloma is made up of a central core of infected macrophages surrounded by epithelioid and foamy macrophages and a peripheral rim of lymphocytes (B cells, $CD4^+$ T cells and $CD8^+$ T cells) in association with a fibrous cuff of extracellular matrix laid by fibroblasts.

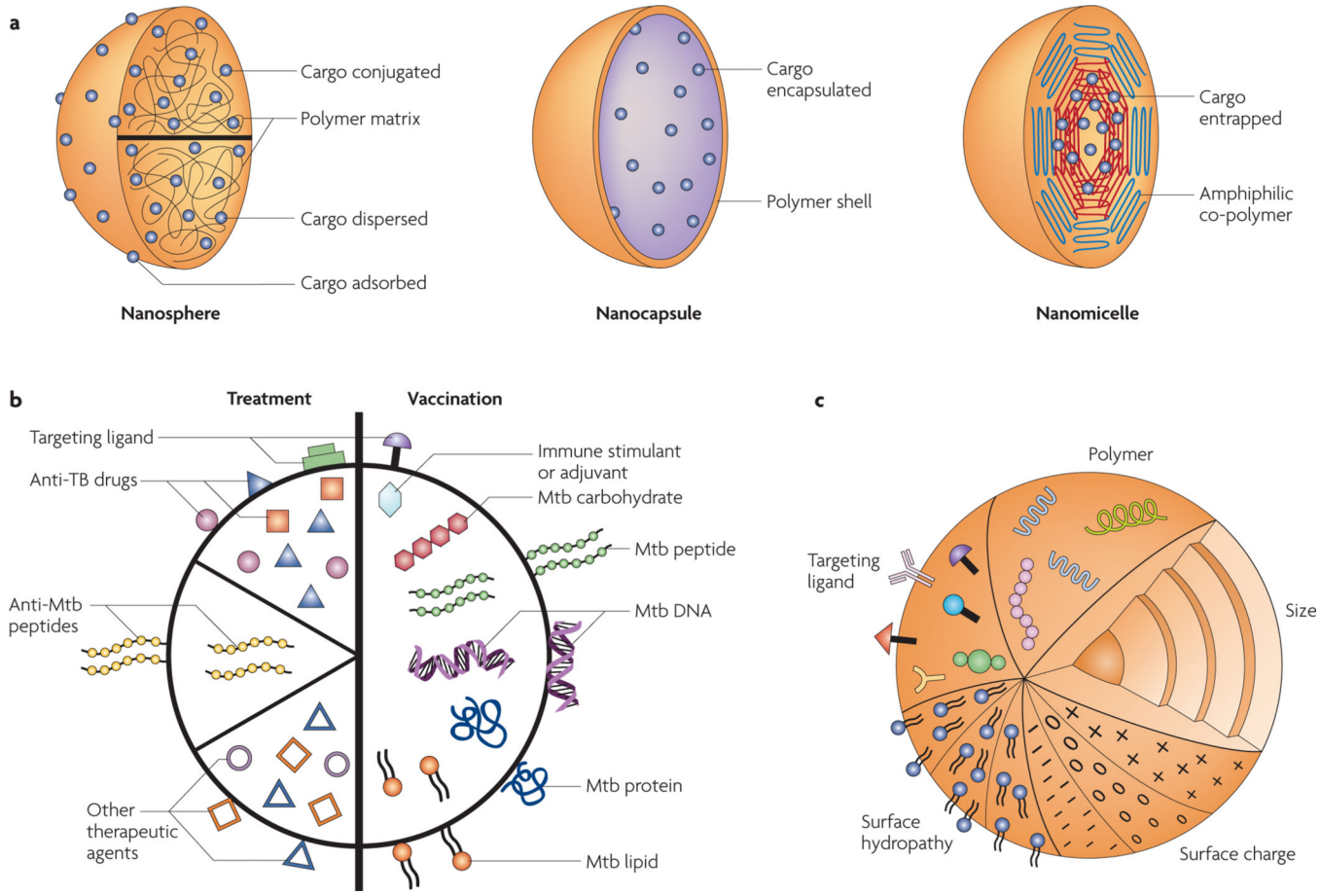

Figure 2 |. Nanobead properties.

a | Polymeric nanoparticles (schematic transections are shown) are sub-μm colloidal particles. They include: nanospheres, in which the cargo is dissolved, adsorbed or dispersed throughout the matrix, attached to the surface or attached to the polymer matrix; nanocapsules, in which the cargo is in solution and surrounded by a shell-like wall; and nanomicelles, in which amphiphilic co-polymers with hydrophobic and hydrophilic blocks self assemble to entrap the cargo. **b** | The different types of nanoparticles and microparticles that have been used for tuberculosis (TB) treatment (such as those encapsulating first-line and second-line anti-TB drugs alone or in combination) and for vaccination (such as those encapsulating or adsorbing *Mycobacterium tuberculosis* (Mtb) immunogenic proteins, peptides and DNA with or without adjuvants), as well as other, potentially useful particles that have not yet been used for TB applications (such as those encapsulating or adsorbing anti-*M. tuberculosis* peptides, other unconventional drugs and immunostimulants, or immunogenic *M. tuberculosis* lipids and carbohydrates). **c** | The main nanoparticle properties that can influence the uptake and efficacy of nanoparticle-based vaccines and therapies. The natural or synthetic polymer used for nanoparticle engineering profoundly affects the characteristics of the particle, such as its biocompatibility and biodegradability, its encapsulation or adsorption efficiency, its internalization or cellular uptake and its release of cargo, as well as affecting its adjuvant and immunological properties and its eventual clearance. The size of a nanoparticle affects its uptake route and its clearance[16] and also

influences the type of immune response that is induced[38,39]. Positively charged particles are preferentially taken up by living cells owing to the negative charge of the cellular membrane. surface hydrophobicity also increases nanoparticle uptake, whereas hydrophilicity (resulting from, for example, surface modifications with polyethylene glycol and poloxamer polymers) decreases uptake and phagocytosis, increasing the systemic circulation of the particle. Targeting ligands can also be used to direct nanoparticles to cells of interest. Toll-like receptor ligands, adhesins and antibodies for specific cell surface receptors and molecules have been used to this end[10,27,60].

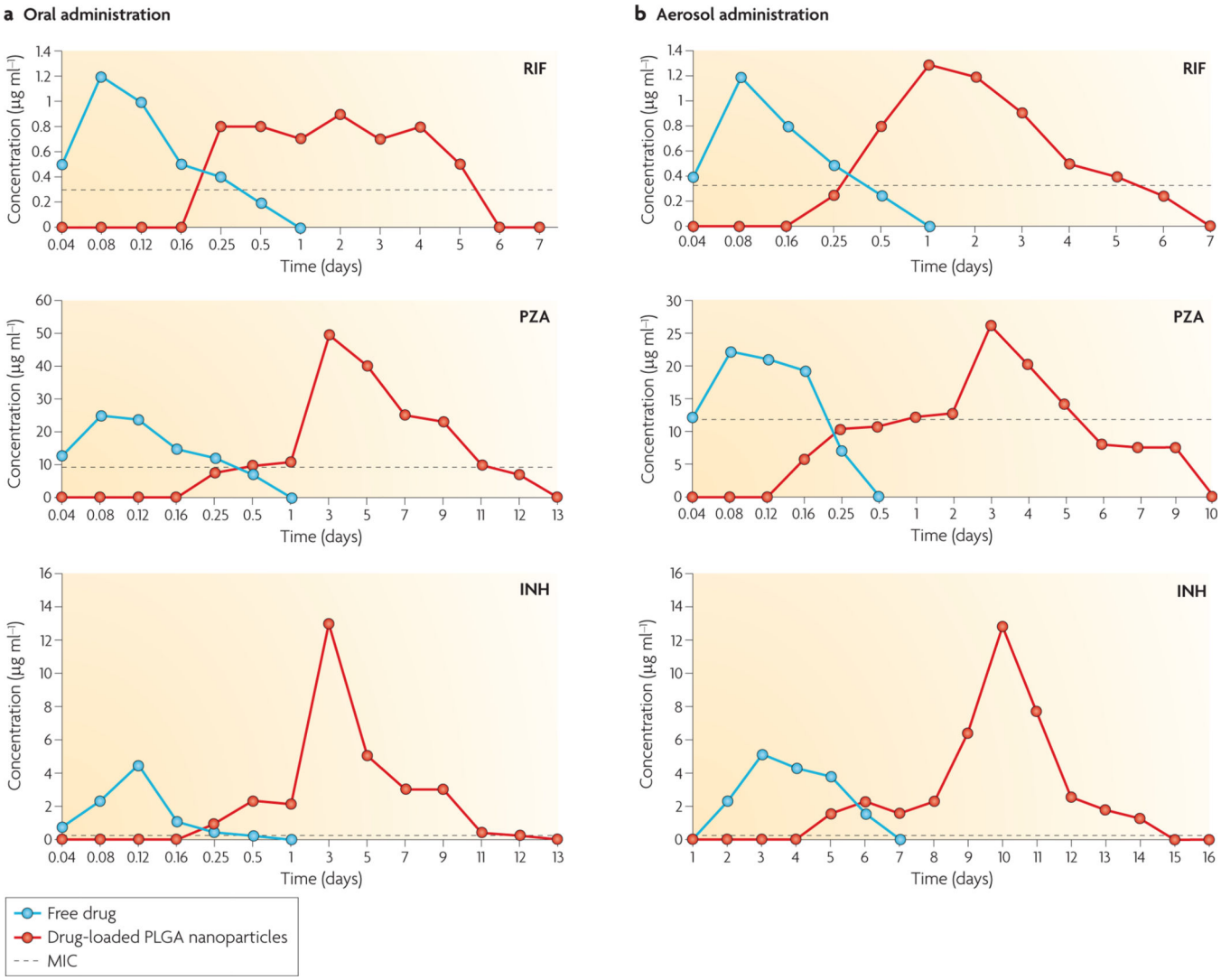

Figure 3 |. The principle of slow drug release with nanoparticles.

The kinetics of the accumulation of rifampicin (RIF), pyrazinamide (PZA) and isoniazid (INH) in the sera of guinea pigs after drug administration in the free form or using drug-enclosed poly-(lactic-co-glycolic acid) (PLGA) nanoparticles. **a** | Drugs administered through the oral route. **b** | Drug administration through the aerosol (lung) route. Note the substantial extension of the elevated drug concentration in the plasma after the administration of drugs using the nanoparticle system. These levels are significantly higher than the minimum inhibitory concentration (MIC) of the antibiotics when used to treat *Mycobacterium tuberculosis*. Data from REF. 27.