

Nanomaterials in tuberculosis DNA vaccine delivery: historical perspective and current landscape

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ABSTRACT

Vaccinations, especially DNA vaccines that promote host immunity, are the most effective interventions for tuberculosis (TB) control. However, the vaccine delivery system exhibits a significant impact on the protective effects of the vaccine. Recently, effective nanomaterial-based delivery systems (including nanoparticles, nanogold, nanoliposomes, virus-like particles, and virus carriers) have been developed for DNA vaccines to control TB. This review highlights the historical development of various nanomaterial-based delivery systems for TB DNA vaccines, along with the emerging technologies. Nanomaterial-based vaccine delivery systems could enhance the efficacy of TB vaccination; therefore, this summary could guide nanomaterial selection for optimal and safe vaccine delivery, facilitating the design and development of highly effective TB vaccines.

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1. Introduction

Tuberculosis (TB), causing 1.5 million deaths in 2020 (Ghebreyesus, 2022, Zaman, 2010), is a significant global threat to human health. In this year, an estimated 10 million people worldwide have been infected with TB (Ghebreyesus, 2022), with 1 million new patients (Ghebreyesus) reported in 2021 (according to the World Health Organization), and 842,000 (World Health Organization, 2020) new cases in China in 2020. On an average, 49% TB patients spend more than 20% (in the range of 19–83%) of their annual household income on TB treatment (Viney et al., 2021), which is a challenging process due to numerous reasons (Figure 1). It involves the circumvention of phagocytic fusion and its destruction (I), a neutralization of the acidic environment (II), an inhibition of envelope formation in apoptosis (III), the suppression of plasma-membrane repair and immune-cell activation (IV–V), and the restriction of pro-inflammatory responses (VI) (Sampath et al., 2021). Although a combination of first-line (isoniazid, rifampicin, ethambutol, and streptomycin) and second-line (amikacin, kanamycin, and capreomycin) drugs is useful for TB treatment, their utility is limited by the prevalence of multidrug-resistant TB (MDR-TB) (Marks et al., 2014), extensively drug-resistant TB (XDR-TB) (Mullerpattan et al., 2019), and HIV superinfection.

Vaccination that promotes host immunity is the most effective intervention for TB treatment (Sia & Rengarajan,

2019); the Bacillus Calmette–Guerin (BCG) vaccine is approved worldwide for the prevention of TB meningitis (Puvacic et al., 2004) and military TB (Trunz et al., 2006). Currently, more than 90% TB vaccines are manufactured using five variants, namely, the 1173P2 (Pasteur), 1331 (Danish), 1077 (Glaxo), 172 (Japanese), and D2PB302 (China) strains (Li et al., 2020). Although TB vaccination is effective for infants (Schrager et al., 2020), its protective efficacy for adolescents and adults is low (Lowrie et al., 1994). Additionally, the effectiveness of vaccination varies greatly in different countries owing to significant differences in immune capacities (Abubakar et al., 2013). However, administering the BCG vaccine at an age of 3–5 years provides protection against active TB for the next 20 years (Mangtani et al., 2018). Furthermore, the BCG vaccine exhibits significant protective efficacy against persistent new *M. tuberculosis* infections, as indicated by recent studies (Nemes et al. 2018). According to data from the United States National Library of Medicine (<http://clinicaltrials.gov>), 149 studies on TB have been conducted in different phases of clinical trials (105 have been completed and 15 are in recruitment). However, these vaccines exhibit a poor ability to generate humoral and cell-mediated immunity (Ojha et al., 2020), necessitating the development of novel vaccines and delivery systems for TB.

DNA vaccines, with facile preservation, transport, manufacture, good stability, and efficient immune responses,

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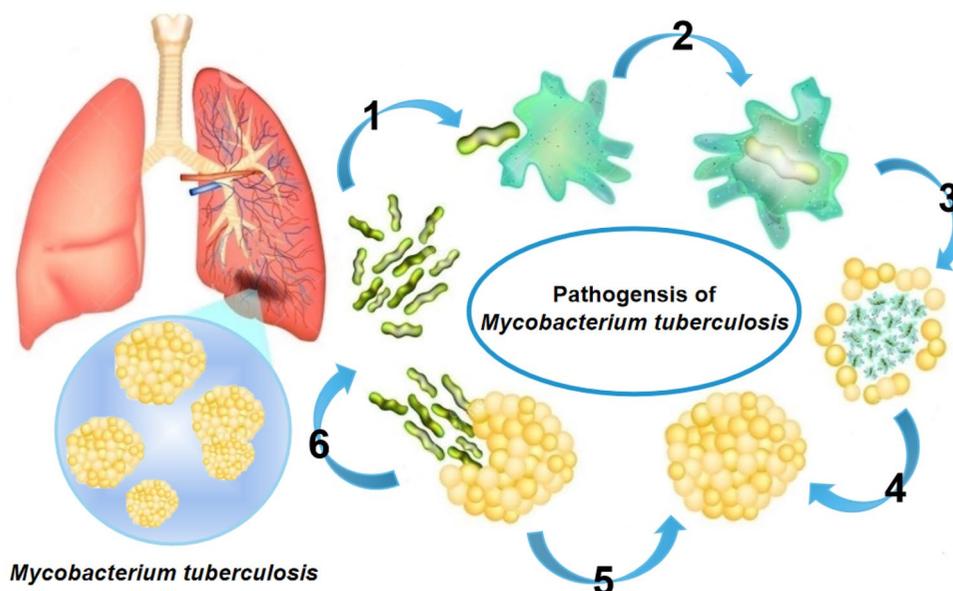


Figure 1. TB pathogenic mechanism. **Note:** *M. tuberculosis* escapes the influence of host immune defenses by the (I) circumvention of phagocytic fusion and phagocytic destruction, (II) neutralization of the acidic environment, (III) inhibition of apoptotic-envelope formation, and (IV) suppression of plasma-membrane repair, causing the spread of infection after macrophage necrosis, (V) suppressing the activation of the immune cells and (VI) restricting the pro-inflammatory responses.

are a useful strategy for the treatment and prevention of MDR-TB (Kutzler & Weiner, 2008). Currently, prophylactic and therapeutic DNA-vaccine vectors for delivery include hemagglutinating virus of Japan (HVJ)-liposomes and their envelopes, adenoviral or lentiviral vectors, and adeno-associated viral vectors (Okada et al. 2007, Yoshida et al., 2006); the DNA-vaccine antigens are HSP65 (Goncalves et al., 2007), MPT64 (Kamath et al., 1999), Ag85A (Tanghe et al., 2001), ESAT6 (Wang et al., 2004), Mtb10, and Mtb41 (Skeiky et al., 2000). DNA vaccines recombine the foreign gene encoding a specific antigen protein into a eukaryotic expression element to form recombinant plasmid DNA (Liang et al., 2008). Subsequently, this plasmid is directly transformed into living cells *in vivo*, its dominant gene is released and enters mRNA in the nucleus *via* the nuclear pore complex, which is translated into a protein *via* ribosomes and transfer RNA (tRNA). After being delivered by the APC to drainage lymph nodes, it induces cellular and humoral immune responses (Tejeda-Mansir et al., 2019). The greatest drawback of DNA vaccines is their lack of immunogenicity in large mammals because of poor delivery efficiency (Simmons et al., 2018). Therefore, to enhance the immune responses elicited by TB DNA vaccines, it is vital to improve vaccine delivery to the nucleus and optimize the antigen combinations (Figure 2). This stimulates the host immune system to express internalized plasmid DNA *via* somatic cells, which is subsequently presented to the CD8⁺ T cells *via* class I MHC complexes (Sefidi-Heris et al., 2020). Hence, the design and development of safe and efficient delivery systems has attracted immense attention in the field of DNA-vaccine development.

Nanomaterials, with good biocompatibility and biodegradability, easy modification and processing, and surface properties with a controllable mucosal absorption rate promote the entry of functional molecules into cells and protect

against degradation, facilitating efficient drug delivery (Jiao et al., 2018). Various nanomaterials, including nanoparticles, liposomes, virus-like particles, and self-assembled proteins, have been developed for TB DNA-vaccine delivery (Figure 3) (Ho et al., 2021); recent reports indicate that nanomaterial-based delivery systems cause high immune responses (Wang et al., 2015). Thus, a systematic review of the progress in nanomaterial use for TB DNA-vaccine delivery could facilitate the development of safe and effective TB vaccines.

2. Nanomaterials in TB DNA-vaccine delivery

2.1. PLGA polymer nanoparticles in vaccine delivery

Polymer nanoparticles can be divided into two categories, namely, synthetic and natural (such as, poly (D, L-lactic-co-glycolic acid) (PLGA)) (Chan et al., 2010); these materials are widely used as they are easy to obtain and exhibit low production costs, good stability, and high physicochemical versatility. Additionally, PLGA exhibits immunological characteristics, including a receptor-recognition ability *via* cytokine stimulation and APCs, that protect vaccines from enzyme attack and provide more immune-safety than live bacterial carriers (Saxena et al., 2013, Thomas et al., 2019). Furthermore, PLGA can be used to independently design nanostructures with adjustable sizes and different surface properties (Franck et al., 2021). Thus, PLGA polymer nanoparticles, exhibiting good biocompatibility, favorable cellular interactions, and biodegradability, are widely used in the design and development of TB DNA vaccines (Mikusova & Mikus, 2021). Plasmid DNA and other molecules (such as, trehalose dimycolate and dimethyl dioctyldecyl ammonium bromide) have been incorporated into PLGA microspheres with up to 60% encapsulation efficiency (Khademi et al., 2018). As an efficient DNA-vaccine delivery system, PLGA

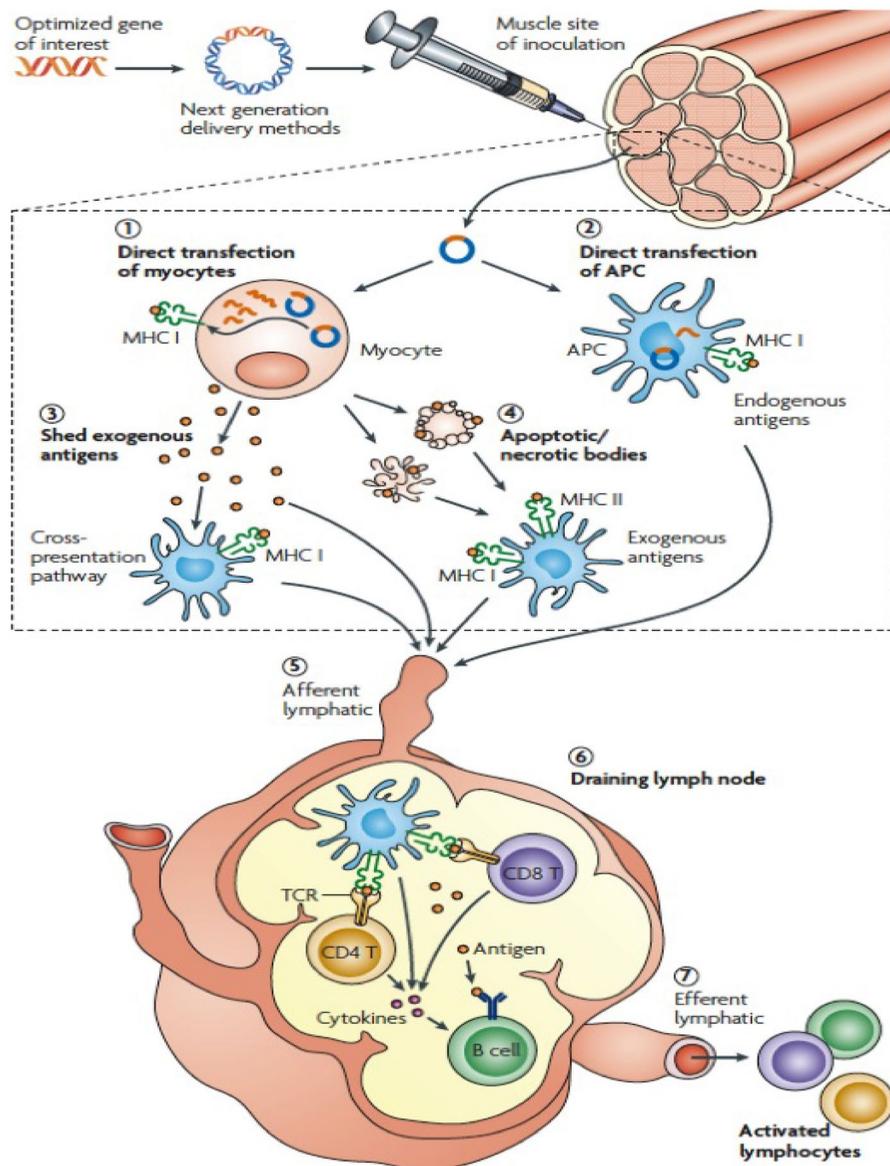
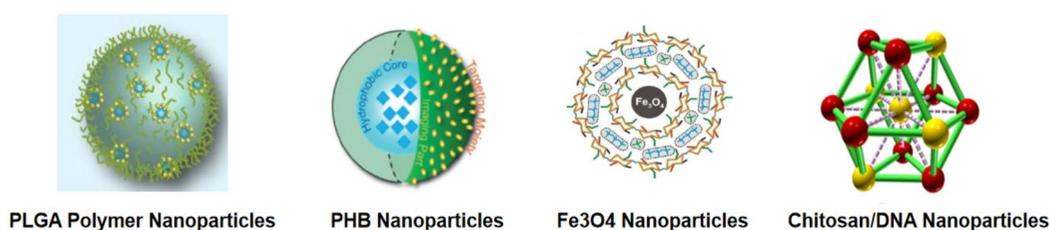


Figure 2. TB DNA-vaccine action mechanisms. **Note:** TB DNA vaccines induce immunogenicity and immune response in the host by improving the delivery process of the vaccine to the nucleus and optimizing the precise combinations of antigens.

nanoparticles prevent DNA degradation, facilitate pathogen stimulation, and enhance the internalization of APCs (Newman et al., 2002, Jiang et al., 2005). By encapsulating, capturing, and delivering plasmid DNA, PLGA nanoparticles deliver the vaccine materials to cells and maintain antigen release at a slow rate due to gradual biodegradation, stimulating the host to produce dual and long-acting cellular and humoral immune responses (Dykman & Khlebtsov, 2017) (Figure 4). Furthermore, PLGA exhibits potential to be used as a plasmid-DNA transfection mediator targeting phagocytes, such as macrophages, and prevents nuclease biodegradation (Wang et al., 1999). Numerous vaccines, such as, the 2020-Mtb72F-TB10.4/CpG-PLGA vaccine (Dalirfardouei et al., 2020) and 2006-hsp65-DNA-TDM-PLGA vaccine (Coelho et al., 2006), utilize PLGA as the delivery system. In the development of TB DNA vaccines, the aforementioned immunostimulants are combined with plasmids loaded with TB-dominant antigen genes and subsequently encapsulated in the

biopolymer material PLGA. Novel TB vaccines encapsulating plasmid DNA and immunostimulants using PLGA nanoparticles induce higher levels of interferon gamma (IFN)- γ and immunoglobulin (Ig) G2a subtype antibodies in mice *via* injection compared to naked plasmid-DNA vaccines (Lima et al., 2003), indicating a strong Th1 polarization-directed response (Wedlock et al., 2002). Additionally, after a challenge with *M. tuberculosis* H37Rv, a single dose of TB DNA nano-vaccine significantly decreased the bacterial colony-forming units in the lungs of mice and improved the granulomatous reactions of the lungs. Thus, *M. tuberculosis* is efficiently eradicated, without inhibiting its growth, on reducing the plasmid DNA by 10-fold. Additionally (Khademi et al., 2018), polymer nanoparticles with PLGA enhance the mucosal immune response to TB DNA vaccines and are suitable for the administration of non-parenteral and parenteral TB DNA vaccines. Therefore, non-biodegradable synthetic polymer materials and natural polymers cause significantly



Nanomaterials for tuberculosis DNA vaccines delivery

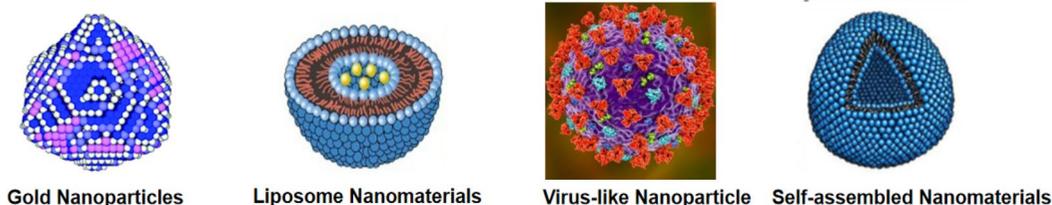


Figure 3. Nanomaterials in TB DNA vaccines. **Note:** Nanomaterials used for TB DNA-vaccine delivery include nanoparticles, liposomes, virus-like particles, and self-assembled proteins.

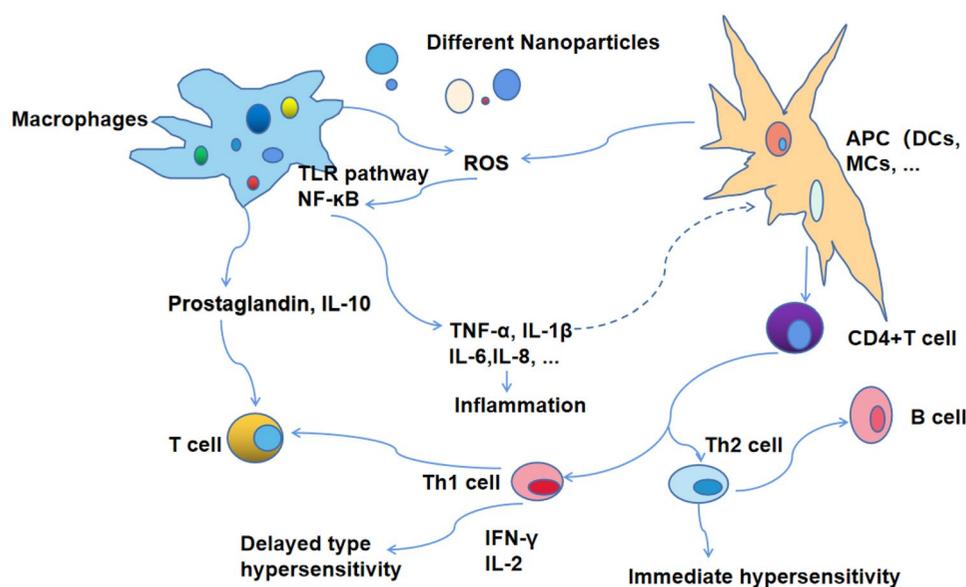


Figure 4. Nanoparticle materials in vaccine delivery. **Note:** Nanoparticle TB vaccines are taken up by macrophages and delivered to antigen-presenting cells, stimulating T and B cells to produce long-acting cellular and humoral immune responses.

higher humoral and cellular immune responses than naked TB plasmid DNA (Rancan et al., 2014).

2.2. Chitosan (CS) and its derivative nanoparticles in vaccine delivery

The core scaffold of the biopolymer of chitins and its derivative nanoparticles can be modified with functional molecular adjuvants or copolymers, such as, dimycolates (TDM) and N, N, N-trimethyl chitosan (TMC), to enhance the plasmid transfection and antigen expression of TB DNA vaccines. TDM, located in the outer layer of the *M. tuberculosis* cell wall, acts as an immunomodulator with adjuvant effects; in

combination with recombinant human hepatitis B virus vaccines, it produces cellular immune responses against the antigen and increased antibody levels (Koike et al., 1998). TMC is a cationic CS derivative that can load plasmid DNA via electrostatic interactions (Thanou et al., 1999; Amidi et al., 2006). CS nanoparticles have been used to formulate the existing BCG vaccine and other multi-epitope (T-cell epitopes) and pPES (multi-gene family protein) DNA vaccines. Several unique characteristics, such as, good bio-adhesion, effective antigen transport via instantaneous opening of tight junctions in cells, good biocompatibility, and low toxicity, make this nanoparticle delivery system particularly important for nasal DNA-vaccine delivery (Csaba et al., 2009).

2.3. Polyhydroxy biopolyester nanoparticles in vaccine delivery

Polyhydroxy biopolyester nanoparticles include poly(3-hydroxybutyrate) (PHB) and polyhydroxyalkanoates (PHA); PHB is commonly extracted and purified from *E. coli* and *Lactococcus lactis*. Antigens carried on specific nanoparticles are preferentially recognized and presented, enhancing the ability of cells to respond to immunogens (Khader et al., 2007). TB DNA vaccines containing PHB nanoparticles with ESAT-6 or Ag85A (the dominant antigens of *M. tuberculosis*) on the surface of biological beads induce high levels of cytokines interleukin (IL)-2, IFN- γ , tumor necrosis factor (TNF)- α , IL-17A, and IL-6 on intramuscular injection. According to Parlane et al., these bovine-TB vaccines cause a high T-cell immune response, with both CD4⁺ and CD8⁺ involved in the induction of IFN- γ release (Parlane et al., 2014). In TB DNA vaccines, PHA nanoparticles have been produced using bacteria (*via* bioengineering) as intracellular contents when carbon sources are abundant (Grage et al., 2009).

2.4. Fe₃O₄-Glu-polyethylenimine nanoparticles in vaccine delivery

Fe₃O₄-Glu-polyethylenimine nanoparticles encapsulated in a DNA vaccine (Ag85A-ESAT-6-IL-21) have been used as prophylactic vaccines in mouse models of *M. tuberculosis* infection (Yu et al., 2012). These nanoparticle vaccines induce a stronger immune response than naked DNA, overcome the inability of plasmid DNA to easily cross the cell membrane, stimulate IFN- γ production, and significantly reduce the bacterial burden after challenge in vaccinated mice (Pereira et al., 2014, Zhao et al., 2017). This vaccine exhibits a strong protective efficacy against *M. tuberculosis*, possibly due to the encapsulation of plasmid DNA in the Fe₃O₄-Glu-polyethylenimine nanoparticles. After intramuscular injection, a reservoir is formed at the injection site, initiating an immune response and gradually releasing the antigen, thereby establishing a long-term response. Both materials show strong protection against *M. tuberculosis* and should be studied in depth (Khan et al., 2007, Zaman et al., 2011).

2.5. CS/DNA-complex nanoparticles in vaccine delivery

A CS/DNA-complex nanoparticle system containing three ESAT-6 T cell epitopes (ESAT-6/3e) and FMS-like tyrosine kinase 3 ligand (FL) genes (termed ESAT-6/3e-FL) enveloped with CS nanoparticles has been reported to improve mucosal delivery and immunogenicity (Feng et al., 2013). This CS/DNA vaccine significantly improves the secretory IgA mucosal response level (Wu et al., 2017). Additionally, the CS/DNA nanoparticles exhibit a high cellular uptake and can be used to deliver TB DNA vaccines. Compared with traditional systems, the new design enables extracellular-matrix nucleic acids to be transferred more efficiently and remain longer on the nasal mucosal surface. Thus, the nanomaterial induces significantly higher levels of secretory IgA and a longer duration of mucosal immunity than naked DNA vaccines in animal

studies; additionally, the vaccine exhibits minimal cytotoxicity (Wu et al., 2017). According to recent research, CS/DNA complex nanoparticles are safe and effective DNA-vaccine delivery systems (Fries et al., 2021). These nanoparticles have been developed as a potential platform for DNA packaging, to facilitate the crossing of DNA through different physiological barriers to elicit effective mucosal immunity.

2.6. Gold nanoparticles in vaccine delivery

The low transfection rate and necessity for repeated vaccinations are the main disadvantages of plasmid-DNA vaccines. According to observations made in 1992, the delivery of plasmid DNA coated with gold nanoparticles, administered in the epidermis using a gene gun loaded with pressurized helium, enhances the expression of foreign proteins and produces antibodies. Subsequently, it has been confirmed that the bombardment of the epidermis with gene-gun-assisted DNA delivered *via* gold nanoparticles preferentially triggers Th2-type cellular immune responses (IgG1 and IgE isotope antibodies) and produces Th2-characteristic cytokines (such as IL-4) (Feltquate et al., 1997, Tighe et al., 1998). However, the failure of vaccination programs indicates that the expression and transformation of exogenous genes in the host cells requires improvement. The 2007-Ag85A-EP + GM-CSF (Zhang et al., 2007), 2015-Ag85B-IL-33-EP (Villarreal et al., 2015), and 2018-EP vaccines (Tang et al., 2018) confirm the efficacy of AuNPs for DNA vaccination by injection *via* T cell activation and protection. Future studies should focus on the delivery of different-sized AuNPs loaded with plasmid DNA using various types of materials to develop new TB-protection strategies.

2.7. Liposome nanomaterials in vaccine delivery

Liposomes, small vesicles formed by hydrophobic and hydrophilic interactions, have been utilized for the delivery of TB vaccines (Pinheiro et al., 2011). Liposome-nanomaterial-based delivery effectively prevents DNA degradation by endonucleases outside the cell *via* interactions with the cell membrane, improving the efficiency of plasmid-DNA transfection presentation compared to naked plasmid-DNA vaccines (Schwendener et al., 2010). The surface charge and particle size of liposomes significantly influence their vaccine delivery (Song et al., 2014), possibly due to their physicochemical (size and charge) and immunogenic (combined with adjuvants and targeted ligands) abilities to attract, interact with, and activate APCs (such as, dendritic cells (DCs), macrophages, and B cells). The positively charged surfaces of cationic liposomes efficiently interact with the negatively charged surfaces of DCs, promoting the transmission and uptake of antigens. DCs are of great importance because they are one of the main inducers of T cell-mediated immune response and mediate antigen presentation to T cell receptors *via* the MHC. Modified liposomes with appropriate targeting ligands stimulate and activate cells through a pattern-recognition receptor, causing the maturation of APCs, and the processing and presentation of antigens

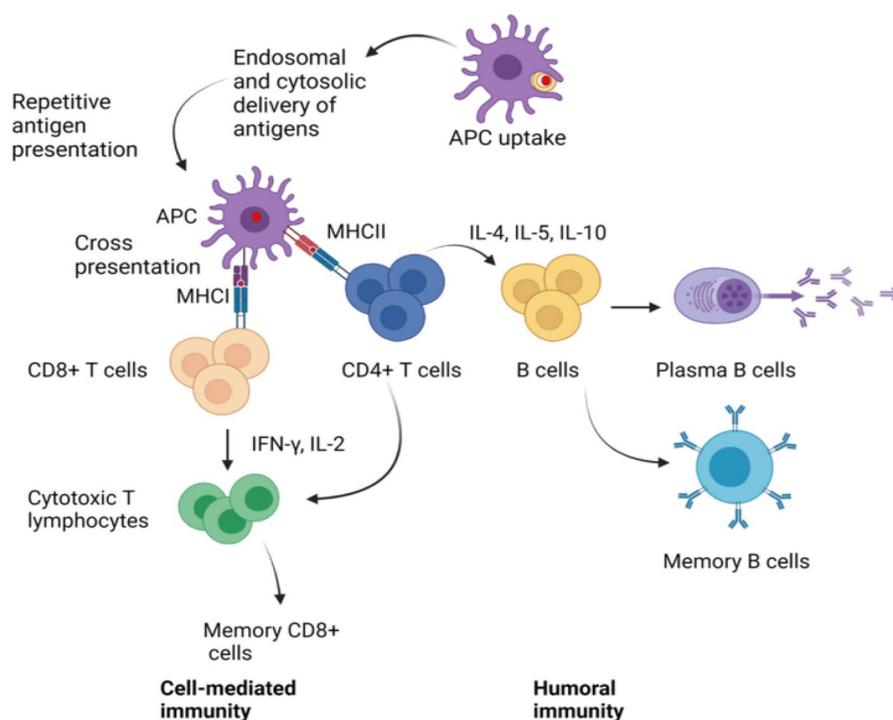


Figure 5. Liposome nanomaterials in vaccine delivery. **Note:** Liposome vaccines with targeting ligands are captured by DC and activate cells through pattern recognition receptors, producing T cell-mediated immune responses, and mediating antigen presentation to T cell receptors, causing APC maturation with antigen processing and presentation.

(Chatzikleantous et al., 2021) (Figure 5). DNA contains phosphate groups; thus, it firmly binds to plasmid DNA through electrostatic interactions, and is more widely used than the other two forms of liposome encapsulation and transfection. A liposome vaccine composed of a newly developed liposome of dimethyl dioctadecyl ammonium (DDA) and pattern recognition receptor agonists (mono-phosphoryl lipid A [MPLA] and trehalose 6,6'-dibenzoate [TDB]) has been reported. This vaccine significantly reduces the ζ potential compared to naked plasmid-DNA delivery and exhibits a higher storage stability with slower and more sustained antigen release. As a Toll 4-like receptor agonist, MPLA elicits a strong humoral immune response and reduces the surface charge of DMT liposomes through electrostatic interactions, increasing the stability of the DNA vaccine. Furthermore, DDA/MPLA liposomes induce a stronger IFN- γ and IL-17 response than DDA alone (Agger et al., 2008). DDA-MPLA-TDB liposomes have been used for the design of TB DNA vaccines and the construction of a plasmid pCMFO that secretes the fusion of four multistage antigens (Rv0577, Rv3044, Rv2875, and Rv2073c). This vaccine elicits a more significant response level of IL-2, induces a Th1-biased response, and provides enhanced and more durable protection against *M. tuberculosis* infection than pCMFO or pCMFO/DDA. Additionally, liposomes enable the simultaneous slow release and deposition of plasmid DNA and the two receptor agonists TDB and MPLA at the injection site after immunization, to form a reservoir (Tian et al., 2018). DDA-based liposomes are moderately or strongly responsive chemical molecule adjuvants that elicit a humoral response and powerful cell-mediated immunity against different types of antigens in the laboratory and large animals.

These liposomes, in combination with immunomodulatory compounds, enhance vaccine immunogenicity (Hilgers & Snippe, 1992), induce DC maturation, and enhance plasmid-DNA uptake (Thanou et al., 2002; Slutter et al., 2009). Moreover, liposomes can be combined with viral vectors to form novel DNA delivery vectors (such as HVJ). HVJ-liposomes can be used as gene transfer vectors, mainly *via* the HVJ viral-cell fusion mechanism, to directly and effectively introduce DNA into host cells. Their complete DNA gene transfection efficiency is 30–100 times greater than that of naked DNA (Yoshida et al., 2006) and more than three times greater than that of cationic liposome delivery (Nakamura et al., 2001). Some studies have compared intramuscular injection of the HVJ-liposome HSP65 DNA vaccine with that of the IgHSP65 DNA vaccine using a gene gun. The IgHSP65+mIL-12/HVJ vaccine significantly induces T-cell activation, produces cytotoxic CD8⁺ T cells and T cell helper responses, increases IFN- γ and cytokine concentrations in the supernatant, and reduces granulomas and pathological conditions in the lungs of challenged mice (Hobson, 2003; Huckriede et al., 2005). HVJ-liposomes could be developed as new anti-TB DNA-vaccine delivery systems in the future owing to their high antigen expression ability, low cytotoxicity and inflammatory response, and repeated injections that do not affect transfection efficiency (Pineiro et al., 2011).

2.8. Virus-like nanoparticles and virosomes in vaccine delivery

Virus-like nanoparticles (VLP) and virosomes, containing viral envelope proteins, encapsulate plasmid DNA; the viral

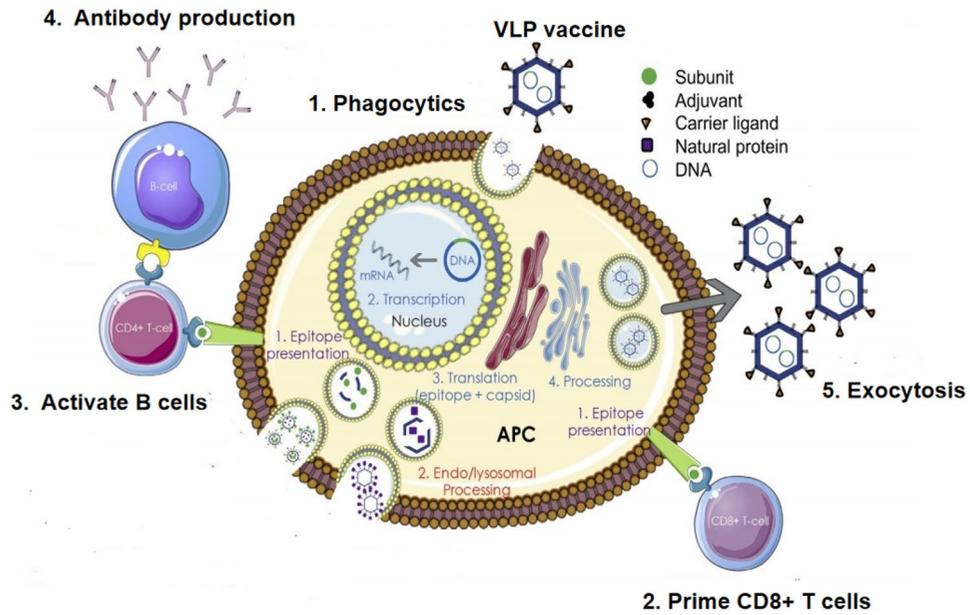


Figure 6. Virus-like nanoparticles in vaccine delivery. **Note:** VLP or virosome vaccines bind to targeted/non-targeted cells, and the targeted virus causes anti-TB protection with infectious doses that are significantly lower than those involving non-targeted cellular pathogens. The replication mechanism remains intact, and is used to replicate the viral vector vaccine, produce more viruses, and infect other APCs. The antigen is displayed on the cell surface and stimulates CD4⁺ and CD8⁺ T cells.

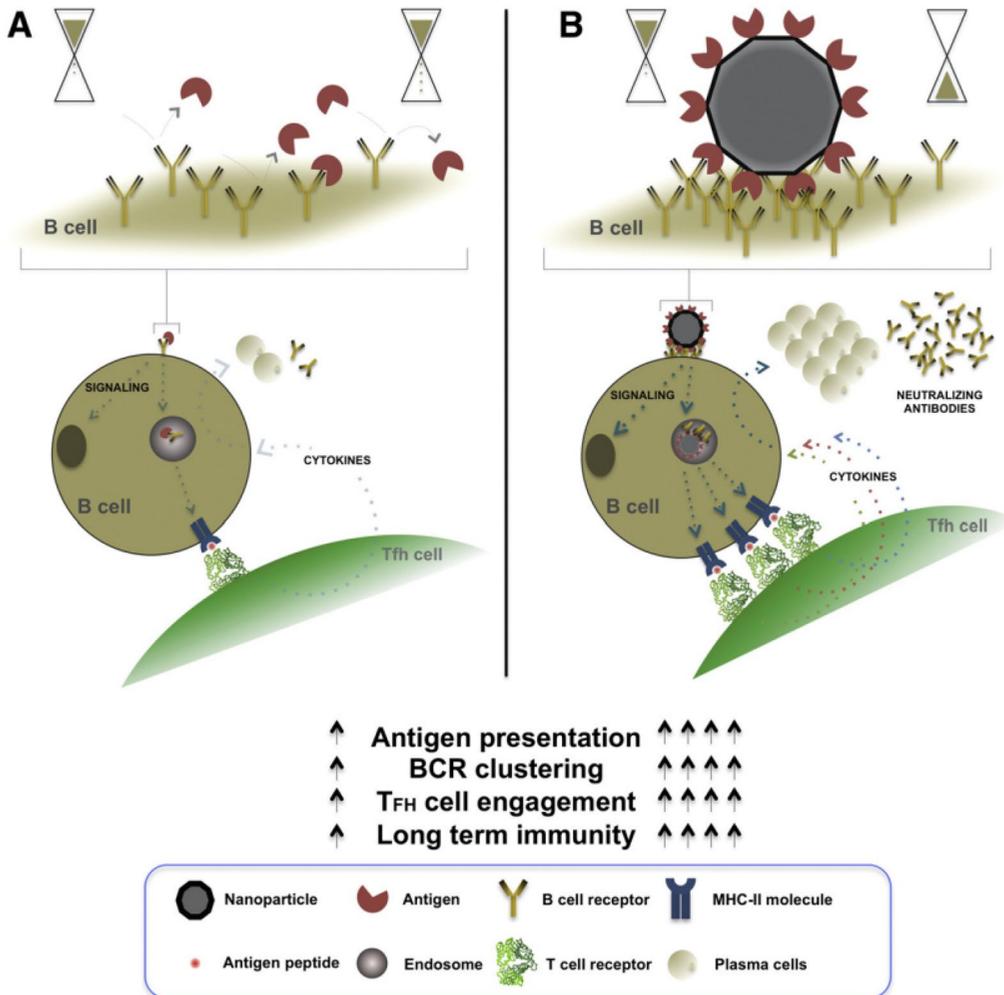


Figure 7. Self-assembled protein nanoparticles in vaccine delivery. **Note:** Nanoparticle vaccines with self-assembled proteins facilitate potent generation and long-lived immuno-protection in germinal centers. These nanoparticles, loaded with the desired antigen, are designed to present multiple copies of the pathogen epitope in a highly ordered manner on the surface of the self-assembled nanoparticle.

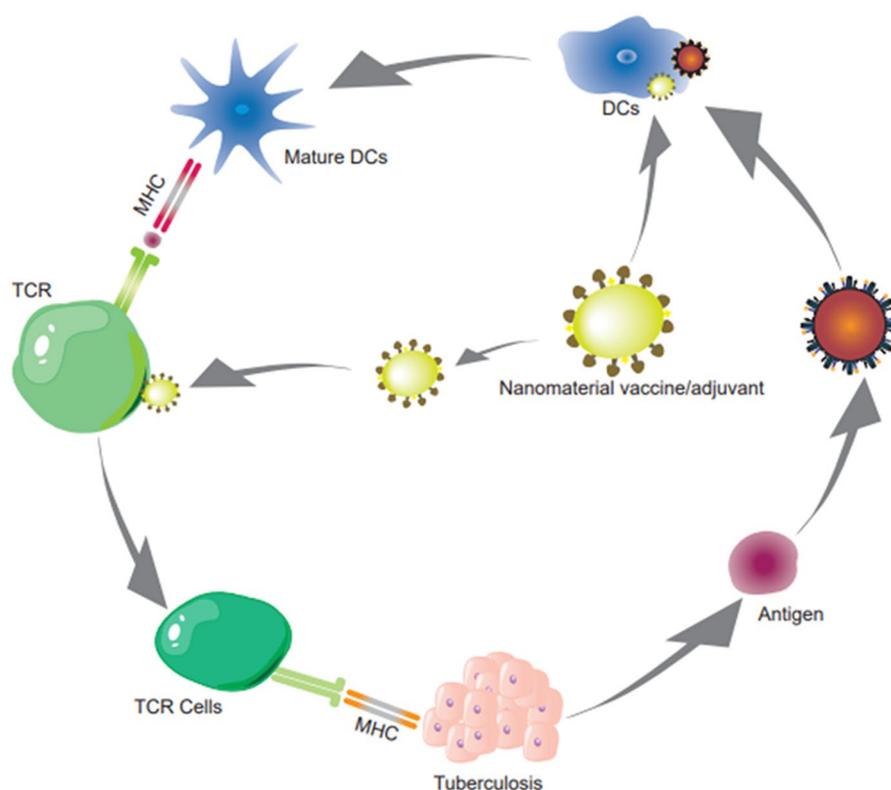


Figure 8. Nanomaterial vaccine delivery route. **Note:** Nanomaterial delivery in the antigen or adjuvant of DNA-TB vaccines delivers vaccines into DCs, causing their maturation, and activates CTL to clear TB infection. These vaccines are also activated by TCR and effectively cross-present via APC cells, initiating CD8⁺ T-cell immunity *in vivo*.

envelope protein makes VLP more immunogenic than polymer nanoparticles and liposomes during DNA-vaccine delivery against *M. tuberculosis* (Shakouri et al., 2016). The mechanism of action of VLP vaccines has been described in detail in previous publications (Figure 6); VLP mimics a virus but lacks a genome, and therefore cannot replicate. VLP and virosome vaccines bind to targeted/non-targeted cells; subsequently, the targeted virus exhibits anti-TB protection by reduced infectious doses compared with non-targeted cellular pathogens. VLP and virosomes can replicate or non-replicate and contain the genomic information of the necessary antigens in their genome. Their replication mechanism remains intact on use, and replicates the viral vector vaccine to produce more vaccine viruses and infect other APCs. The antigen is displayed on the cell surface and stimulates CD4⁺ and CD8⁺ T cells (Chung et al., 2020). Recently, a novel Ag85A-ESAT6 fusion antigen vaccine containing (LV-AEG/SVGmu), a new DC-targeted recombinant lentivirus targeting the *M. tuberculosis* lentiviral vector, has been constructed; it is highly inclined to transfect DC-SIGN-expressing cells and expresses its fusion antigen *in vitro* and *in vivo*. This vaccine induces an antigen-specific lymphoid proliferation response and increases the secretion of Th1 cytokines (including IL-2 and IFN- γ) (Shakouri et al., 2016, Safar et al., 2020). Furthermore, a new DNA vaccine virus vector, pUMVC6, containing an IE promoter expression vector for cytomegalovirus and a kanamycin marker at the 5' end (Safar et al., 2020, Hanif et al., 2010) has recently been constructed. Its delivery

virus vector contains human IL-2 peptides as immunostimulants, allowing the antigen gene to secrete cytoplasmic proteins in the cell, triggering a strong immune response in healthy volunteers (Wu et al., 2016). As viral vectors enhance the ability of the BCG vaccine to combat TB compared with non-targeted lentiviral vectors, targeted lentiviral vectors should be designed for enhanced delivery using heterologous proteins in the future.

2.8. Self-assembled peptides and proteins in vaccine delivery

Self-assembled peptides and protein nanomaterials are injectable, biodegradable, and biocompatible. Through self-assembly, peptide nanomaterials can be applied in numerous fields, such as, drug delivery (small molecules and large molecules), regenerative medicine, and nano-biotechnology (Yu et al., 2016). The multi-epitope DNA vaccine HSP65 scaffold is a novel transplantation strategy that uses a protein-engineering backbone design. Five T-cell epitope antigens derived from the PE19, PPE25, MTB10.4, ESAT-6, and Ag85B proteins of the H37Rv *M. tuberculosis* strain of the pPES epitope-HSP65 scaffold, have been inserted into the scaffold protein to enhance epitope processing expression and immune response. Enzyme-linked Immunospot results indicate that the five T-cell epitope proteins expressed by this scaffold-construct induce IFN- γ ⁺ and polyfunctional CD4⁺ T-cell responses, significantly enhancing the immune response of CTL in B cells and IL-2 levels (CD8⁺ T-cell

Table 1. Nanomaterials used in TB DNA vaccines.

Classification	DNA vaccine	Adjuvant	Administration	Mice	Action	References
Polymer Nanoparticles	H37Rv -PLGA	TDM	Intramuscular	BALB/c	Produces high levels of IL-6, TNF- α , IL-12, IL-10, IFN- γ , NO, and IL-4	(Lima et al., 2001)
	hsp65-PLGA	TDM	Intramuscular	BALB/c	Produces high levels of IgG2a subtype antibody and IFN- γ	(Lima et al., 2003, Cai et al., 2004)
	Ag85B/MPT-83/ESAT-6	DDA	Intramuscular	C57BL/6	Produces IgG and IFN- γ	(Cai et al., 2005, Afrough et al., 2020)
	Ag85B/MPT-83/MPT-64-PLGA	DDA	Intramuscular	C57BL/6	Produces IgG and IFN- γ	(Poecheim et al., 2016)
	Ag85A-TMC	MDP	Intramuscular	C57BL/6	Increases the Th1-associated antibody levels and the number of IFN- γ -producing T-cells	(Dalirfardouei et al., 2020)
	Mtb72F-PLGA	TB10.4/CpG	Intramuscular	BALB/c	Induces Th1 cytokine production	(Dalirfardouei et al., 2020)
	Esat-6/3e-CS	FL	Intramuscular	C57BL/6	Elicits enhanced T-cell responses and protection against H37Rv in an <i>M. tuberculosis</i> challenge	(Feng et al., 2013)
	Ag85A-ESAT-6-Fe3O4-Glu-PEI	IL-21	Intramuscular	C57BL/6	Produces IFN- γ	(Bettencourt et al., 2020, Shakouri et al., 2016)
	Ag85A-ESAT-6-PHB bio-beads	/	Intramuscular	C57BL/6	Induces IFN- γ , IL-6, IL-17A, IL-2, and TNF- α	(Satti et al., 2014, Yoshida et al., 2006)
Liposomes	Hsp65-HVJ	IL-12	Intramuscular	BALB/c	Enhances T-cell activation and IFN- γ production	(Tian et al., 2018)
	Rv2875-Rv3044-Rv2073c-Rv0577-pCMFO	DDA-MPLA-TDB)	Intramuscular	C57BL/6	Elicits responses of Th1-biased and, more significantly, IL-2 cell responses	(Bettencourt et al., 2020, Shakouri et al., 2016)
Virus-like particles	Ag85A-ESAT6-LV-AEG/SVGmu-DC	/	Intramuscular	BALB/c	Elicits a strong immunity of T-helper 1 (Th1) cells and produces high levels of IFN- γ and IL-2	(Safar et al., 2020, Stylianou et al., 2015)
	pe35-esxa-rv3619c-rv3620c-pUMVC6	IL-2	Intramuscular	BALB/c	Induces an Th1 immune response	(Safar et al., 2020)
Virus carriers	Ag85A	GMCSF	Intramuscular electroporation	BALB/c	Activates the T-cell response of CD4 and CD8. and enhances the CTL activities of T cells.	(Tang et al., 2018)
	Ag85B-ESAT-6-Rv2660c	/	Intramuscular electroporation	BALB/c	Induces surprisingly high levels of CD8+ T cells in blood and increases the IFN- γ levels	(Zhang et al., 2007)

responses) compared to other DNA constructs (naked DNA, epitope, or tandem HSP65 protein).

The delivery of protein expressed as an adjuvant molecule with plasmid DNA causes enhanced protective efficacy against a challenge infection with *M. tuberculosis* H37Rv, emphasizing the significant role of CD4⁺ helper T cells (Th1-type) in protection (Tanghe et al., 2001, Yang et al., 2011). Figure 7 shows self-assembled protein nanoparticles in vaccine design. B- and T-cell stimulation and activation, and the subsequent secretion of antigen-specific antibodies by plasma cells depend on the effective cross-linking between B-cell surface immunoglobulins (B-cell receptors, BCRs) and recognition patterns presented by the pathogen. The high-density and structurally ordered antigenic array presented by nanoparticle vaccines facilitate multiple binding events to occur simultaneously between the self-assembling protein nanoparticles and host-cell BCRs (Lopez-Sagaseta et al., 2016). Several paradigms have been reported for the design of TB DNA-vaccine delivery vectors using

protein-backbone engineering. The synthetic antimicrobial peptide KLKL5KLK exhibits effective immunostimulant properties that enhance and prolong immune responses against *M. tuberculosis* in combination with DNA vaccines (Li et al., 2008). Therefore, these self-assembled peptide and protein nanomaterials represent novel TB DNA-vaccine delivery systems with immense application prospects.

3. General review of nanomaterial delivery

DNA vaccines represent a third generation of new and improved vaccines for the prevention and treatment of TB. The acquisition of plasmid DNA is simple and does not require protein purification; the principle of TB DNA vaccination is also relatively simple (Huygen, 1998). For vaccine preparation, the dominant antigen gene fragment of TB is synthesized and amplified using primers, followed by an insertion of the DNA into the expression plasmid, its purification and amplification using transformed bacteria,

combination with novel nanomaterial vectors, and finally immunization of the host, followed by screening and evaluation (Hobernik & Bros, 2018). Nanomaterials are used as antigens or adjuvants in DNA vaccines, which affect T cells or DCs and activate CTL to clear TB infection (Figure 8) (Yan et al., 2019). DCs are the most effective cross-presenting type of APC to initiate CD8⁺ T cell immunity *in vivo*, which is conducive to antigen presentation and uptake (Jorritsma et al., 2016). Novel nanomaterial-based mucosal administration enables barrier crossing *via* perioral, intranasal, and intratracheal routes, inducing local or systemic immunity at the mucosal site (Jin et al., 2019). Additionally, the use of novel nanomaterials, electroporation (Covello et al., 2014), micro-needling (Noh et al., 2022), and needle-free delivery systems (Mooij et al., 2019), such as gene guns or biological syringes which enhance skin-based delivery (Table 1), significantly enhance the transfection efficiency.

4. Future research

Despite immense scientific progress in recent years, TB remains a significant threat to public health; nanomaterials could facilitate the development of better TB DNA vaccines and alleviate this global threat (Xu et al., 2018). Various nano-carriers have been investigated for use in TB vaccines, such as, lipid, polymeric, carbohydrate, and amino acid nanoparticles and viral nanoparticulate vectors. Other than immense applications in vaccine delivery, polymeric nanoparticles are promising adjuvants that enhance the protection of TB vaccines *in vivo*. Despite promising *in-vitro* and *in-vivo* results, further investigation is required before the clinical use of nanomaterial-based therapeutics and vaccines. From molecular mechanisms and detection tools to therapeutic strategies and vaccine development, nanomaterial research has focused on the generation, transmission, and role of 'microscopic' molecules. The emergence of fusion proteins has facilitated the development of TB vaccines. Non-viral vector nanomaterials for gene therapy load nontoxic short- or large-sized genetic materials that are effective in dividing and non-dividing cells. This material overcomes the difficulty of delivering traditional plasmid DNA to the nucleoplasm *via* the nuclear membrane and exhibits a low transfection rate and a high intracellular and nuclear uptake, gene delivery, and transfection efficiency (Mullick Chowdhury et al., 2016). Physical methods, such as electromechanical destruction, and the utilization of nanomaterials of cell membranes to deliver DNA molecules to the nucleus and cytoplasm with high throughput, enable the rapid expression of DNA molecules (Ding et al., 2017). Further preclinical (small and large animal studies) and clinical validation of toxicity and efficacy is required before the 'bench to bedside' transition of nanomaterial-based therapeutic vaccines. Nanomaterials could significantly reduce TB treatment duration, reduce the adverse effects of medications, and mitigate the development of multidrug resistance, facilitating a 'TB-free world' (Xu et al., 2018). Summarizing, novel nanomaterial-delivery vaccines against *M. tuberculosis* will play a crucial role in TB research and the development of future applications to control TB infection.

Disclosure statement

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