Polymeric Nanoparticles in Tuberculosis

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Abstract Tuberculosis (TB) is an infectious disease caused by the bacteria *Mycobacterium tuberculosis (Mtb).* TB causes the most human deaths than any other diseases from a single infectious agent. Treatments of TB are long and costly and have many limitations. Intracellular bacilli are slow growing and difficult to target, which is augmenting the emergence of multidrug resistance. Targeting intracellular *Mycobacterium tuberculosis* is very 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. Different polymers of natural or synthetic origin which are commonly used for the fabrication of nanoparticles with antitubercular drugs are outlined in this chapter. Polymeric nanoparticles have recently attracted increasing attention in tuberculosis treatment due to their unique properties. Polymeric nanoparticles provide an innovative therapeutic alternative to improve the limitations and disadvantages of the conventional available treatments for tuberculosis.

Keywords Polymeric nanoparticles · Tuberculosis · Natural polymers · Synthetic polymers

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1 Introduction

Tuberculosis (TB) is an infectious disease caused by the bacteria *Mycobacterium tuberculosis (Mtb).* TB is the world's second greatest cause of death from an infectious disease. The requirement of daily medicine delivery for long periods of time is a significant obstacle in treating the disease (up to 9 months). This often leads to poor adherence by patients, which is not only a risk to the patient's well-being, but due to its highly infectious nature, it represents a serious risk to public health. The current approach to tackle poor adherence is directly observed treatment, where each day during their treatment patients is observed taking their medication by a healthcare worker, which presents a considerable burden to the healthcare system, both in cost and time [1]. Since this therapy is carried out for a long duration, chances of patients discontinuing the course before cure are high, and this compromises the patient's compliance and adherence to treatment. The failure of treatment could lead to the appearance of multidrug-resistant and extensively drug-resistant TB. The drug-resistant strains pose challenges for treatment and eradication of TB [2]. In addition, the drugs currently used for the treatment of TB suffer from serious adverse side effects, such as hepatotoxicity, as well as short plasma half-life and rapid clearance [1]. Traditional TB treatment also entails precise dosages and frequencies, and lengthy treatment periods that lead to patient noncompliance. So, despite the availability of current antibiotics, there is still a large demand for other treatment options [3]. In tuberculosis, the lung is the main target organ. The pathogen is an intracellular agent, with alveolar macrophages serving as both a reservoir and a therapy target. Various polymeric carriers derived from natural or synthetic sources have been employed to target drugs to the deep lung in recent decades. These carriers have particle sizes that range from micro to nanometers. Despite the advantages of micron-sized carriers, nanoparticles have lately become essential in pharmaceutical science due to their unique properties [4].

The delivery of antituberculosis drugs (ATDs) with nanoparticle (NP)-based controlled-delivery devices is one of the promising approaches. Several reports have been published on the advantages of NP drug delivery systems for infectious diseases. Some of the drug delivery systems have been accepted for clinical treatment of different infectious diseases, while a few others are currently under different phases of clinical and preclinical trials. The polymeric NPs offer unique benefits to achieve a slow and sustained release that has the potential to treat chronic diseases like TB [2].

This chapter highlights the pathogenesis of TB, conventional treatments of TB, and its limitations along with the different natural and synthetic polymers used for the preparation of nanoparticles in TB.

2   Pathogenesis of Tuberculosis

Mtb is one of the most successful human pathogens, due to its ability to carry a primary infection to a state of dormancy, persisting in the body even in immune-competent people. In this regard, it is important to mention that there are two billion people infected worldwide and only nine million develop the disease annually [5]. The presence of hereditary or acquired deficiencies of the immune system markedly increases the risk of progression to active TB.

The first stage of tuberculosis is initiated with inhalation of droplets generated by a person with active tuberculosis. These droplets can remain for a longer time in the air. When inhaled, a single droplet may be enough to cause the disease. Most droplets end up in the upper respiratory tract, where the microbes are killed, but a few penetrate further down. The bacteria reach the alveoli in the lungs, where the alveolar macrophages phagocytose them. Several receptors are involved in the uptake process including mannose receptors, Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4), surfactant protein A receptors, CD14, scavenger receptors, complement receptors, and immunoglobulin receptors [6]. Sometimes macrophages fail to destroy the bacteria either because compounds produced by the microbe inactivate them or because phagosome-lysosome fusion mechanisms are inhibited by *Mtb*, thereby avoiding low pH exposure and hydrolytic surroundings of phagolysosomes.

In the second stage, mycobacterium multiplies in the macrophage, eventually causing its lysis. This results in the cellular damage which attracts the inflammatory cells and blood monocytes to the area. Monocytes differentiate into macrophages and attempt to attack the microbe which is ingested by the macrophages and grow inside the phagocyte. These macrophages again lyse and die due to bacterial load [7]. Two to three weeks after infection, the third stage begins. T cell immunity develops, and lymphocytes drift to the region of infection. Presentation of mycobacterial antigens to the T cells causes their stimulation, resulting in the release of γ-interferon and other cytokines. The γ-interferon activates macrophages to secrete IL 12, TNF-α, IL-8, and other proinflammatory cytokines. Fast growth of the *Mtb* stops, and, at this stage, the host cell develops cell-mediated immunity. Those that are outside of cells are resistant to antibody-activated complement attack due to the high lipid content of mycobacterial cell wall. Cell-mediated immunity is also responsible for much of the pathology of tuberculosis. Tissue damage can also take place when activated macrophages release lytic enzymes, reactive intermediates, and various cytokines. It is at this stage that the immune system, specifically the macrophages, will enclose the microorganisms inside tubercles. In between these structures, the atmosphere is anoxic and acidic and prevents the growth of mycobacteria. In-between these structures is anoxic and acidic, preventing the growth of mycobacteria. This balance between host and mycobacterium is called latency which is one of the hallmarks of TB. In the fifth and final stage, the tubercles may dissolve by many factors such as malnutrition, immunosuppression, steroid use, or HIV infection. For unknown reasons, the centers of tubercles may liquefy,

providing an outstanding growth medium for the microbe which now begins to grow rapidly in the extracellular fluid. The large number of bacteria and the immune response against them eventually cause the lung tissue near the tubercles to become necrotic and form a cavity [8]. Most tuberculosis infections stop at stage three.

3   Conventional Treatment and Its Limitation

In 2014, TB strategy was adopted by the World Health Assembly with a goal to design a blueprint for sustainable strategies to reduce the number of TB deaths by 90% by 2030 [9]. Currently the short-term treatment (6 months duration) for tuberculosis is composed of a combination of different anti-tuberculosis drugs (known as first-line drugs), including rifampicin (RIF), isoniazid (INH), pyrazinamide (PYR), ethambutol (ETB), and streptomycin (STM) [9]. The World Health Organization (WHO) and the International Union against TB have suggested a combination of at least two first-line drugs in one dosage form to cope with the complex nature of disease progression. These are commonly known as Fixed Dose Combinations (FDC) [10]. Thus, prescription errors can be reduced and adherence to the treatment can be improved. Conventional treatment regimen for TB is treated with first-line drugs, second-line drugs, and third-line drugs. First-line drugs are used as an oral combination therapy with isoniazid, rifampin, pyrazinamide, and ethambutol for several months. Second-line anti-TB therapy is used to circumvent with multidrug resistance TB. Second-line drugs are more lethal and are more expensive than first-line drugs and treatment may last longer [11–13]. The clinical efficacy of third-line anti-TB drugs is not established. Third-line drugs are also not listed by WHO [13].

In 2016, an estimated 490,000 people all over the globe developed multidrug-resistant TB (MDR-TB) [9]. The main reasons behind the emergence of MDR-TB include mismanagement of TB treatment and person-to-person transmission. Inappropriate use and premature treatment interruption of anti-TB drugs may lead to drug resistance. In this context, in spite of the availability of anti-TB drugs since a longer time, TB still remains to be one of the main preventable causes of death by an infectious disease. Hence, it is important to develop new drug delivery systems that ensure high treatment adherence and low adverse effects and that are adequate for both adults and children [14].

4   Polymeric Nanoparticles

Several nanosized carriers are available for drug delivery purposes that include liposomes, solid lipid nanoparticles (SLNs), polymeric nanoparticles (PNPs), nanosuspensions, nanoemulsions, and many other excellent multifunctional nanosystems, leading to better pharmacokinetics, biodistribution, and bioavailability. Polymeric nanoparticles possess very good biocompatible and biodegradable features that

make them sustainable candidates for use as drug delivery carriers [15]. The choice of polymer to develop the polymeric nanoparticles is dependent on different factors like size of the nanoparticles (NPs) required, inherent properties of the drug, surface characteristics, biodegradability, biocompatibility, toxicity, and desired drug release profile [16].

5 Polymer Used

Natural polymers and synthetic polymers are used for the preparation of nanoparticles in TB drug delivery. Natural polymers are of interest for the preparation of nanoparticles in TB treatment such as: (i) polysaccharide-based polymers and (ii) polypeptide- and protein-based polymers. These natural polymers are mainly used for the fabrication of nanoparticles due to their properties, e.g., biodegradability, biocompatibility, and low toxicity. Apart from the use of natural polymers, synthetic polymers are also used for the preparations of nanoparticles [4].

5.1 Polysaccharide-Based Polymers

Polysaccharides are a significant class of hydrophilic polymers with natural origin and biocompatibility that find frequent use in water-based polymer systems and in nanotechnology in particular, which is mainly due to their favorable properties in biological systems, e.g., biodegradability, biocompatibility, and low toxicity. These properties constitute considerable requirements for the utilization of NPs and thus polysaccharides represent an ideal class of building blocks for NP fabrication [17]. Chitosan is the most widely studied polysaccharide to develop polymeric nanoparticles [16].

5.1.1   Chitosan Nanoparticles

Chitosan has multiple properties that can aid in the treatment of TB. For instance, it is known for its biocompatibility and biodegradability which promotes good adhesion to mucosal surfaces. It also increases the absorption of vaccines and drugs, lengthens the duration of therapeutic effects for drug effectivity, and facilitates drug delivery to specific body sites [3]. Specific qualities such as solubility, stability, and improved mucoadhesion through physical and chemical alterations allow chitosan to better serve its purpose and expand its uses. Furthermore, the US Food and Drug Administration has declared chitosan to be safe for human consumption. Traditional TB treatment also includes exact dosages and frequencies, as well as long treatment periods, which might contribute to patient noncompliance. Therefore, there is always a demand for other treatment options so that the problems associated with

traditional treatment can be solved [3]. Therefore, several research and efforts are being made by researchers on chitosan-based nanoparticles in TB treatment.

Pourshahab et al. [18] have prepared the spray-dried inhalable powders containing INH-loaded chitosan/tripolyphosphate (TPP) nanoparticles for sustained delivery of the drug to the lung. For the preparation of nanoparticles, they have used ionic gelation method. From the *in vitro* drug release study, they found that the rate of drug release from nanoparticles was decreased with increasing the amount of chitosan. Nanoparticles were spray-dried using excipients such as lactose, mannitol, and maltodextrin alone or with leucine. The *in vitro* deposition data indicated that spray drying of isoniazid-loaded nanoparticles with lactose in the presence of leucine resulted in the production of inhalable powders with the highest fine particle fraction (FPF) (45%) [18].

Garg et al. [19], in the year 2016, have prepared the chitosan nanoparticles (CNPs) by ionic gelation technique followed by spray drying for sustained delivery of anti-tubercular drugs, INH, and RIF, to the lungs. For the preparation of nanoparticles, they have selected the chitosan with low molecular weight. They further investigated the chemotherapeutic efficacy and toxicity against experimental murine TB. They found that the CNPs had a smooth spherical shape with an average size of 230 ± 4.5 nm, with a polydispersity index of 0.180 ± 0.021. Drug encapsulation efficiency was observed to be 70.8 ± 6.62 for RIF and 68.8 ± 7.02 for INH. The higher drug encapsulation was observed for CNPs due to the higher drug/polymer ratio, as well as an excellent ionic gelation between tripolyphosphate (TPP) and chitosan. Kinetic analysis of drug release from optimized formulations indicates that the drug is released from the nanoparticles by a diffusion mechanism. An initial burst release of RIF and INH from nanoparticles could be due to rapid dissolution of drug crystals located on the surface or present beneath the nanoparticle surface. They found that the smooth surface nanoparticles are easily captured by the alveolar macrophage of the lungs, and the low PDI of nanoparticles suggested a homogenous dispersion. Both drugs were detected in different organs (lungs, liver, spleen, and kidney) until 24 h post nebulization. They found that the optimized formulations showed lower cytotoxicity and a significant reduction in the number of bacilli in the lungs, as compared to free drug. Finally, they have concluded that the CNPs may be exploited as a prospective tool for drug delivery to direct drugs to the lung tissues for the treatment of TB [19].

Rawal et al. [20] have developed RIF-loaded nanoparticles by ionic gelation probe sonication method. They further analyzed the prepared nanoparticle with respect to its direct targeting potential of lungs. The size range and the drug entrapment efficiency of the nanoparticles were assessed from 124.1 ± 0.2 to 402.3 ± 2.8 nm and $72.00 \pm 0.1\%$, respectively. The results of the cumulative in vitro drug release studies exhibited that the drug release from the developed nanoparticle sustained up to 24 h. Additionally, pharmacokinetic and toxicity studies carried out with prepared NPs dry powder inhalation (DPI) formulations and compared with conventional DPI and marketed formulation showed rifampicin release for extended periods. From their findings, they suggested the freeze-dried rifampicin nanoparticles as a better targeted delivery system for developing treatment strategy for tuberculosis [20].

RIF is one of the most efficient anti-TB medications and a key component of DOTS (directly observed treatment, short-course) therapy. However, inadequate bioavailability, increased drug resistance, decreased cell permeability, failure to achieve enough drug concentrations at the infected site, and degradation before reaching the target site have all hampered this medicine's usefulness [21]. Therefore, they have used a novel hydrophobic derivative of chitosan (octanoyl chitosan) for the preparation of nanoparticles containing RIF. They have prepared octanoyl chitosan (OC) nanoparticles by using double emulsion solvent evaporation technique without cross-linking. They did not use cross-linking method because this method involves the use of potential toxic agents such as glutaraldehyde and after formulation the removal of cross-linking agents makes the method less effective and tedious. They further optimized the OC NPs by using 3^2 full factorial design. They found that the optimized batch of OC NPs exhibited a smooth and spherical morphology and had a mean hydrodynamic diameter of 253 ± 19.06 nm (PDI 0.323 ± 0.059) and entrapment efficiency of $64.86 \pm 7.73\%$ for rifampicin. They further studied MTT assay for the determination of biodegradability and non-cytotoxicity of the polymer, and their results suggested its likely safety in clinical use. They also concluded that further evaluations in animals are required to evaluate its utility and potential clinical use [21].

The drug loading of aminoglycoside (AG) loaded chitosan nanoparticles is very low. This drug loading is low because of the electrostatic repulsion between the positively charged AG and positively charged chitosan [22]. Therefore, dextran sulfate (Mw 500 000), a polyanion, has been used to shield the positively charged AG in order to improve the drug loading. Lu et al. have prepared the AG (streptomycin, gentamicin, and tobramycin)-loaded chitosan nanoparticles with the aim of high drug loading. They further conducted the test of in vivo oral efficacy of streptomycin (SM)-loaded chitosan nanoparticles in a Mycobacterium tuberculosis chronic infection mouse model. They concluded that the chitosan nanoparticles may provide a promising oral drug delivery formulation for AG which usually, in tuberculosis treatment, is administrated as an injectable preparation [22].

In 2018, Wardani et al. [23] worked on the in vitro antibacterial activity of chitosan nanoparticles against Mycobacterium tuberculosis. They have concluded from their work that chitosan nanoparticles have a relatively rougher surface with an uneven structure which exhibited highly amorphous feature, and it has promising anti-tubercular activity by preliminary in vitro techniques. Therefore, they also concluded it has the definite potential as a source of compounds that may be developed further into antimycobacterial drugs [23]. A new nanomedicine antibacterial agent, based on dihydroartemisinin (DHA) and chitosan (CS), has been developed by Gu et al. [24] to overcome MTB's drug-resistant. To enhance DHA's solubility, they have prepared nanoparticles of DHA-loaded CS by an ionic crosslinking method with sodium tripolyphosphate (STPP) as the crosslinking agent. They found that the DHA-CS NPs exhibited an excellent antibacterial effect on the rifampicin-resistant strain (ATCC 35838) and, at a concentration of 8.0μg/ml, the antibacterial impact reaches up to $61.0 \pm 2.13\%$ ($n = 3$). From their findings, they have concluded the DHA-CS NPs combined with rifampicin may have potential use for TB treatment [24]. A few examples of chitosan-based nanoparticles in TB are given in Table 1.

Table 1 Chitosan-based nanoparticles in TB

Carrier material	Specification of chitosan used	Encapsulated drug	Particle size	Drug encapsulation efficiency	Remarks	Refs.
Chitosan nanoparticles	Chitosan (CS) (degree of deacetylation: 93%)	Rifampicin (RIF)	221.9 nm	44.17 ± 1.98%	In vitro study suggests that oral sustained release CNs of rifampicin might be an effective drug delivery system for tuberculosis.	[16]
Chitosan nanoparticles	Chitosan (CIF, Cochin)	Isoniazid	661.8–823.8 nm		Chitosan nanoparticles are physically and chemically stable and retain their pharmaceutical properties at various environmental conditions over a period of 3 months.	[25]
Hyaluronic Acid Coated Chitosan Nanoparticles	Chitosan (Analab Fine Chemicals, Mumbai)	Rifampicin	211.23–301.67 nm	82.56 ± 1.26% to 87.21 ± 0.98%	Nanoparticles could effectively target macrophage, a residual site of *Mycobacterium tuberculosis.*	[26]
Chitosan nanoparticles-based dry powder inhalation formulations	Low molecular weight of chitosan (>75% deacetylation)	Prothionamide	1.76μm (aerodynamic particle size)		Animal study also revealed the reduction of dose in pulmonary administration, which will improve the management of tuberculosis.	[27]
Chitosan nanoparticles		Isoniazid	620 ± 10.97 nm		Nanosystem is thus an efficient approach for antitubercular therapy.	[28]

5.1.2 Alginate Nanoparticles

Sodium alginate is a natural polymer with properties such as an aqueous matrix environment, high gel porosity, and biocompatibility, and is approved by the US Food and Drug Administration (USFDA) for oral use [29]. It is a natural polysaccharide, rich in carboxyl group and is easy to bind with positive charge cations such as Ca^{2+}. It is of low toxicity, good biocompatibility, and relatively low cost; therefore, it can be used for the preparation of nanoparticles [30].

Kumar and Bhatt [30] fabricated and evaluated the isoniazid-loaded sodium alginate nanoparticles. They prepared the nanoparticles using ionotropic gelation technique. The particle size, drug loading, and encapsulation efficiency of the fabricated nanoparticles were studied. The in vitro drug release study of the optimized formulation showed 66.56% drug release in 24 h. They concluded that the isoniazid-loaded sodium alginate nanoformulation has the potential to provide enhanced efficacy of isoniazid [30]. Shaji and Shaikh [31] have prepared D-cycloserine (D-CS)-loaded alginate-chitosan nanoparticles using ionotropic gelation method. They further designed and optimized the biodegradable polymeric nanoparticles of D-CS using 2^3 factorial design to study the influence of formulation variables on particle size and entrapment efficiency of polymeric nanoparticles. They found that the optimized batch exhibited the entrapment efficiency of $98.10 \pm 0.24\%$ with particle size 344 ± 5 nm. Further, in vitro release study of the optimized formulation in phosphate buffer saline (pH 7.4) showed a biphasic release pattern with initial burst release of about 34.49% of drug, followed by controlled release up to 24 h. They further concluded that the delivery system for D-CS could be a potential alternative to the existing conventional therapy in multidrug-resistant tuberculosis (MDR-TB) [31]. Alginate-based nanoparticles in tuberculosis treatment are summarized in Table 2.

5.1.3 Guar Gum Nanoparticles

Guar and its derivatives are widely used in many applications including food, drug delivery, and healthcare products because of their natural abundance and their low cost and other desirable functionalities [34]. Guar gum is a water-soluble polysaccharide, and it is composed of sugars, such as galactose and mannose. Additionally, swelling behavior of guar gum at acidic pH imparts an efficacy to protect the antigen in harsh gastric environment. Guar gum is a natural nontoxic, biodegradable, mucoadhesive, cost-effective polymer which can encapsulate a higher amount of antigen [35]. Kaur et al. [35], in the year 2015, developed an effective carrier system containing Ag85A-loaded guar gum nanoparticles for oral vaccination against tuberculosis. They have used nanoprecipitation for the preparation of nanoparticles. They found that the developed particles with an average diameter of 895.5 ± 14.73 nm and high antigen entrapment seem to be optimum for oral vaccine delivery. In vivo studies data revealed that the developed nanocarriers can induce a strong mucosal as well as systemic immune response. Finally, from the experimental evidence, they

Table 2 Alginate-based nanoparticles in TB

Carrier material	Specification of alginate used	Encapsulated drug	Particle size	Drug encapsulation efficiency	Route of administration	Remarks	Refs.
Alginate nanoparticles	Sodium alginate (medium viscosity, 3500 cps for a 2% w/v solution)	Isoniazid (INH), rifampicin (RIF) and pyrazinamide (PZA)	235.5 ± 0 nm	70–90% for INH and PZA and 80–90% for RIF	Pulmonary	Inhalable alginate nanoparticles can serve as an ideal carrier for the controlled release of antitubercular drugs.	[29]
Alginate nanoparticles	Sodium alginate (medium viscosity, 3500 cps for a 2% w/v solution)	ATDs (rifampicin, isoniazid, pyrazinamide and ethambutol)		70–90%	Orally	Potential for intermittent therapy of TB.	[32]
Sodium alginate coated with chitosan and Tween 80	Low viscosity sodium alginate (ALG, MW ~ 4500 Da, viscosity ~8 cP, 39% content of α-L-guluronate, and % 61 content of β-D-mannuronate)	Rifampicin (RIF) in combination with ascorbic acid (ASC)	324.0 ± 40.7 nm	0–36%	Pulmonary	Reducing the risk of systemic toxicity and hence improving the patient compliance.	[33]

concluded that the guar-gum nanoparticle can be utilized for safe and effective vaccine delivery via oral route [35]. Goyal et al. [36] in the year 2016, worked on chemotherapeutic evaluation of guar gum-coated chitosan nanoparticle against experimental tuberculosis. The major objective of their work was to develop and evaluate the therapeutic potential of ATDs-loaded natural polysaccharide comprising of galactomannan subunit in experimental TB. From their work, they have concluded that guar gum-coated chitosan nanoparticles could be a promising carrier for selective delivery of ATDs to alveolar macrophages for efficient management of TB with the interception of minimal side effects [36].

5.2 Polypeptide and Protein-Based Polymers

5.2.1   Gelatin-Based Polymers

Nanoparticles made of biodegradable polymers like proteins and polysaccharides can act as efficient drug delivery vehicles for controlled and targeted release, aiming to improve the therapeutic effects and also to reduce the side effects of the formulated drugs. Over the past few decades, there has been considerable interest in developing protein-based nanoparticles as GRAS (generally regarded as safe) drug delivery devices [37]. Gelatin is a denatured protein which is obtained either by partial acid or alkaline hydrolysis of animal collagen and has been extensively used for the preparation of nanoparticles [4, 37]. Gelatin is a natural versatile biopolymer, and it can be used in different applications because of its low cost, easy availability, biodegradable and biocompatible nature as well as the presence of abundant active groups. The gelatin nanoparticles (GNPs) can be prepared by several different techniques, including desolvation, coacervation-phase separation, emulsification-solvent evaporation, reverse phase microemulsion, and nanoprecipitation [38]. Saraogi et al. [39] developed and characterized the rifampicin-loaded gelatin nanoparticulate delivery system for the effective management of tuberculosis. Gelatin nanoparticles containing rifampicin were prepared by using two-step desolvation method. The gelatin nanoparticles were characterized for size measurements, drug entrapment, and in vitro drug release study. The size of nanoparticles was found to be 264 ± 11.2 nm with low PDI suggesting the narrow particle size distribution. They have conducted the TEM photomicrograph, and this study revealed the gelatin nanoparticles (GPs) were spherical in shape (Fig. 1) [39].

The drug release showed the biphasic pattern of release, i.e., initial burst followed by a sustained release pattern. The gelatin nanoparticles were evaluated for cytotoxicity study on J-774 macrophage cell lines and *in vivo* biodistribution and antitubercular studies on mice model. The biocompatibility of GPs was tested using MTT assay on J774 cells. They found that the cells incubated with GPs and RIF-GP remained nearly 100% viable when compared to the control group at concentrations as high as 1mg/mL (Fig. 2). They found that the cell viability was 94%, 91%, and 65%, respectively, for GPs, RIF-GPs, and RIF-treated cells, at 1 mg/mL
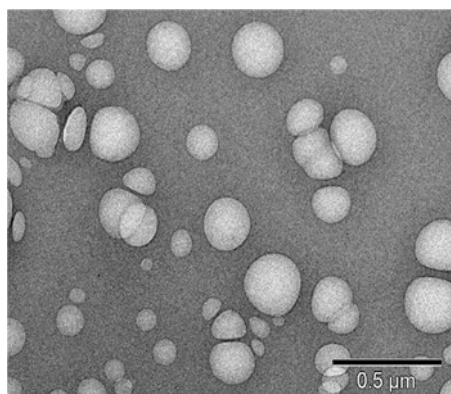
Fig. 1 TEM photomicrograph of gelatin nanoparticles [39]. Reused with permission from Elsevier
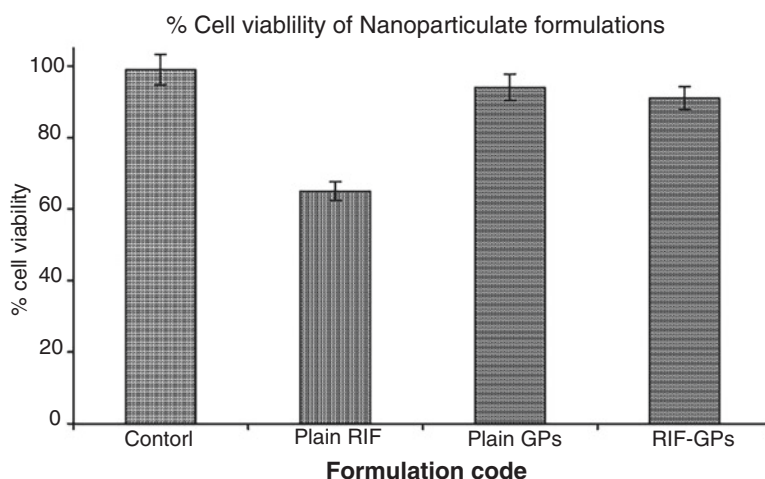

Fig. 2 Cell viability studies on J774 cells after 72 h exposure (values represent mean ± S.D., $n = 3$) [39]. Reused with permission from Elsevier

concentration. A significant evidence ($P \leq 0.05$) of lesser cytotoxicity was detected for GPs and RIF-GPs as compared to free RIF after 72 h of treatment. These results clearly indicated that GPs, even with a varied degree of modification, were biocompatible and nontoxic to normal J774 cells. They found that the gelatin nanoparticles showed improved chemotherapeutic efficacy of the drug as compared to conventional therapy. Therefore, they concluded that the prepared gelatin nanoparticle may be utilized as potential tool for the delivery of bioactives to the lung tissues leading to minimized side effects and improving the therapeutic efficacy of the drug [39].

Saraogi et al. [40] in the year 2011, prepared the mannosylated gelatin nanoparticles (Mn-GNPs) for the selective delivery of an antitubercular drug, INH, to the

alveolar macrophages. They have used gelatin type A (Bloom 300). They have pre-pared the gelatin nanoparticles using a two-step desolvation method and efficiently conjugated with mannose. The size of nanoparticles (both plain and Mn-GNPs) was found to be in the range of 260–380 nm, and the maximum drug payload was found to be 40–55%. The average particle size of Mn-GNPs was more, whereas drug entrapment was lesser compared to plain GNPs. The organ distribution studies proved the efficiency of Mn-GNPs for spatial delivery of INH to alveolar tissues. Intravenous administration of INH-loaded Mn-GNPs (I-Mn-GNPs) resulted in a significant reduction in bacterial counts in the lungs and spleen of tuberculosis-infected (TB-infected) mice and also a reduction in the hepatotoxicity of the drug. They found that the mannose-conjugated GNPs may be explored as a potential car-rier for safer and efficient management of TB through targeted delivery of INH when compared to plain GNPs and free drug [40].

Sharmah et al. [41] used gelatin (type B 75 bloom) as a potential drug carrier for controlled delivery applications. They have used cellulose whiskers (CWs) in con-trolling the release of the drug because CWs have the capacity to form strong hydro-gen bonds. These CWs also provide good strength to the drug carrier material. They have prepared CWs from filter paper cellulose by acid hydrolysis. They have attempted to prepare gelatin-CWs nanoparticles by desolvation method using iso-niazid as drug and glutaraldehyde as a crosslinking agent. They found the zeta potential values of the cross-linked gelatin nanoparticles in the range of −10.7 to −21.1 mV. The zeta potential values were decreased with the increase of CWs con-tent. The decrease in surface charge might be owing to an increase in electrostatic interaction between the protonated amino groups of gelatin matrix and –OH groups of CWs. Both the swelling degree (%) and cumulative release (%) decrease with the increased content of CWs. Cytotoxicity study revealed that CWs were nontoxic to human lymphocytes and also gelatin nanoparticles containing CWs were less toxic than CWs-free nanoparticles. Their results suggested that gelatin-CWs nanoparti-cles have the potential uses in controlled drug delivery [41]. Sarfraz et al. [42], in the year 2016, worked on the immune response to antituberculosis drug-loaded gelatin and polyisobutyl-cyanoacrylate nanoparticles in macrophages. They have loaded anti-TB drugs (moxifloxacin and rifampicin) into gelatin (type B, 225 bloom) and polyisobutyl-cyanoacrylate nanoparticles. They further characterized the prepared nanoparticles. They also determined the cellular immune responses and cellular viability. Finally, they have concluded that the NPs together with the chemotherapeutic drugs might be able to trigger an immune response in macro-phages. They found that the combined effect might be able to overcome mycobac-teria infections [42].

5.2.2   Albumin-Based Polymers

Bovine serum albumin (BSA) is one of the most useful drugs carriers in the NP form. It is a biodegradable, nontoxic carrier that can be metabolized in vivo with the formation of harmless degradation products that are bioavailable, easily purified, and soluble in water, which enables delivery by injection [43]. Nanoparticle

parameters (diameter, polydispersity, bioactive substance loading, and the yield of nanoparticle) are very important for drug transport through the bloodstream. Tazhbayev et al. [43] have used INH as the model drug and they have prepared BSA-INH NPs by an ethanol desolvation of an aqueous protein solution in the drug presence. They found that the properties of nanoparticles are significantly affected by the concentration of BSA, urea, L-cysteine, and the drug. The application of the Taguchi method is used for finding the optimal conditions for BSA-INH NPs [43]. Ma et al. [44] in the year 2022 have worked on the treatment of spinal tuberculosis in rabbits using bovine serum albumin nanoparticles loaded with isoniazid and rifampicin. They found that the INH-RFP-BSA-NPs showed the characteristics of sustained release in vivo and target biodistribution in focus vertebral body. They finally concluded on the basis of their findings that the therapeutic effect of the prepared formulations in rabbit spinal tuberculosis is much better than common INH and RFP [44]. Ge et al. [45] have prepared bovine serum albumin nanoparticles loaded with isoniazid and rifampicin (INH-RFP-BSA-NPs) by a modified self-emulsion solvent diffusion method, with albumin and polylactic acid used as carriers and to form the nanoparticles' structure. After that, they studied the drug release characteristics *in vitro*. They found that the drug loading and drug entrapment efficiencies were high, at 19.8% and 87.8% for isoniazid, respectively, and 20.1% and 98.0% for rifampicin, respectively. Drug release from the prepared nanoparticles was slow and sustained with 97.02% INH cumulative release at 6 days, and full release of RFP requiring 5 days [45].

Joshi and Prabhakar [46] have prepared rifampicin-loaded bovine serum albumin nanoparticles (RIF-BSA NPs) by desolvation method using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as the cross-linking agent. They found that the use of EDC reduces the time for cross-linking and makes the preparation method simple. RIF-BSA NPs confirmed the enhanced *in vitro* therapeutic efficacy assessed by MTb killing assay compared to the free drug suggesting the possibility of dose reduction. Further, they have found that the FITC-labelled RIF BSA NPs could be efficiently taken up by RAW264.7 cells infected with *Mtb* (H37rv), as confirmed using fluorescence microscopy. Finally, they concluded that the use of RIF-BSA NPs DPI formulation can be a promising strategy for the treatment of pulmonary tuberculosis [46].

5.3   Other Synthetic Polymers

Synthetic polymers used in pharmaceuticals such as polyesters (lactones) and acrylates, PLGA has been the most popular type [4]. Poly (lactic-co-glycolic acid) (PLGA) has been used most successfully for the fabrication of nanoparticles containing antitubercular drugs [4, 47].

5.3.1 PLGA Nanoparticles

Poly (lactic-co-glycolic acid) (PLGA) is one of the most successfully developed biodegradable polymers. Danhier et al. [47] reviewed on PLGA-based nanoparticles, and in their review, they present why PLGA has been chosen to design nanoparticles as drug delivery systems [47]. As patient non-compliance has been a major issue in the successful management of TB. Therefore, a lot more research works were going on the PLGA-based nanoparticles [47–49]. Horváti et al., in the year 2015, worked on antimycobacterial activity of peptide conjugate of pyridopyrimidine derivative against *Mycobacterium tuberculosis* in a series of in vitro and in vivo models. They found that PLGA nanoparticle for encapsulation of a pyridopyrimidine derivative exhibited improved antimycobacterial activity and low toxicity compared to the unencapsulated drug in an infected guinea pig model [49]. Sung et al. [50] have prepared PLGA nanoparticles containing rifampicin using a solvent evaporation process, spray-dried into porous nanoparticle-aggregate particle (PNAPs) containing varying amounts of nanoparticles. They further characterized the physical and aerosol properties of the prepared nanoparticles. *In vitro* release study of the prepared nanoparticles showed an initial burst of rifampicin, with the remainder available for release beyond eight hours [50]. Tripathi et al. [51] have prepared PLGA-based rifampicin nanoparticles using single and double evaporation method, solvent diffusion, and ionic interaction method. They further optimized the processing parameters involved in the method (drug/ polymer ratio, concentration of surfactant, phase ratio (organic phase/aqueous phase) and sonication time) to obtain small nanoparticles with maximum drug entrapment. They found that the release behavior of rifampicin showed a biphasic pattern described by an initial burst (11.26% in 1 days) release followed by a slower and continuous release (more than 30 days). They found that the technology would improve patient compliance, the lack of which is the major reason for the development of multidrug-resistant strains of mycobacterium. Further studies, such as confocal microscopy and study of accumulation of drugs in infected macrophages, can be done and are suggested as future scope of their work [51]. Malathi and Balasubramanian [52] worked on the synthesis of biodegradable polymeric nanoparticles and their controlled drug delivery for tuberculosis. They have attempted to develop a synthetic polymeric anti-TB nanodrug delivery system. A series of PLGA polymers with different molar feed ratios, i.e., 90/10, 75/25, 50/50, were synthesized by using direct melt polycondensation method. They prepared rifampicin-loaded PLGA nanoparticles by the double Emulsion-solvent evaporation method using PVA as a stabilizer. The average diameter of the PVA-coated PLGA-RIF nanoparticles is less than 250 nm. The in vitro release profile of the rifampicin-loaded PLGA nanoparticles showed an initial burst followed by sustained release. They found that the nanoparticles were remarkably advantageous in terms of high drug encapsulation efficiency, low polymer consumption, and better-sustained release profile. These systems could be cost-effective, feasible, and save valuable life and resources in the management of tuberculosis [52].

Hakkimane et al. [2] have prepared a nanoformulation of the two most effective first-line drugs, RIF and INH, which are used in both the phases of the 6-month TB therapy. Since INH is small and a highly hydrophilic molecule, it has low cellular penetration and also low drug loading efficiency in nanoformulation using hydrophobic USFDA-approved polymers like PLGA. This has led to a considerable hindrance in effective treatment with INH. To overcome this issue, they have modified INH into INH benz-hydrazone (IH2) by adding a hydrophobic moiety called benzaldehyde, a commonly used food additive, using Schiff base reaction. The newly formed IH2 is encapsulated in PLGA polymer, and its encapsulation in polymer is increased around 15-fold compared to INH encapsulation in PLGA. They found that RIF and IH2 loaded in NPs release in a slow and sustained manner over a period of 1 month, and they are more stable in NPs formulation compared to the free form. Finally, they have concluded that NP formulations will improve the efficacy of drug delivery for TB treatment [2].

Xie et al. [53] have developed PLGA nanoparticles encapsulating a conventional anti-TB drug (levofloxacin) to design more effective strategies against *Mtb*. They have prepared levofloxacin-nanoparticles using a double emulsification method. They further investigated the average diameter, zeta potential, polydispersity index, morphology, and drug release efficiency in vitro of the prepared LEV-NPs. In this study, the bactericidal effect and mechanism of LFLIU combined with levofloxacin-loaded PLGA nanoparticles on *M. smegmatis* in macrophages are investigated. The results support the potential of LFLIU combined with drug-loaded nanoparticles as a new, noninvasive, safe, and effective method for the treatment of TB [53]. In the same year, Liang et al. [48] prepared rifapentine (RPT)-loaded PLGA and PLGA–PEG NPs using premix membrane homogenization combined with solvent evaporation method. The aim of this work was to develop and characterize RPT-loaded PLGA-based nanoparticles for reducing dosing frequency. Their study revealed that in contrast to free drug, RPT-loaded NPs were more effective against *Mtb* in vitro [48].

6   Conclusion

Different polymers are used for the preparation of nanoparticles in tuberculosis treatment. As conventional treatment of tuberculosis is of long duration, so adherence to the treatment is difficult for the patients. Therefore conventional treatment has some limitations. Limitations of the conventional treatment can be overcome by preparing the polymeric nanoparticles. The polymeric nanoparticles possess good biocompatible and biodegradable characteristics. Therefore, nanoparticles effectively target macrophage. Most of the polymeric nanoparticles are prepared with anti-TB drugs with different manufacturing method, and these works are going on a laboratory scale. Still lot more effort and sophisticated techniques are needed for the development of polymeric nanoparticle for mass production from a laboratory scale. New technologies are necessary so that in near future these polymeric nanoparticles will be available in the market.

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