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Targeted Nanoparticle Based Interventions for the Management of Tuberculosis – A Holistic Approach

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Abstract –

Through the design of drug delivery systems and the opening of the possibility of controlling infections at the molecular level, nanotechnology has significantly improved therapeutics. Nanocarriers can cross biological barriers and are able to target cellular reservoirs of Mycobacterium tuberculosis (M. tuberculosis). Systems based on nanoparticles have a lot of potential for treating and preventing tuberculosis (TB). Numerous nanocarriers have been thoroughly examined as potential drug delivery systems for a range of administration methods. A promising approach to treating TB with enhanced drug absorption and decreased dose frequency involves targeting the pharmaceuticals to specific physiological areas, such as the lymph nodes. By reducing side effects and necessitating less frequent dosing regimens, nanotechnology-based rational targeting may increase treatment success. Ultimately leading to improved levels of adherence and more patient compliance. The current paper gathers the fundamental physiological characteristics of the illness as well as recent advancements in the study of nanoparticles used to treat tuberculosis.

Keywords- Mycobacterium tuberculosis, Nanocarriers, Nanoparticles, Drug delivery, Antituberculosis drugs

1. Introduction-

Tuberculosis (TB) is one of the most ancient diseases of mankind and has co-evolved with humans for many thousands of years or perhaps for several million years.[1] Mycobacterium tuberculosis complex, a group of closely related bacterial species, is the cause of tuberculosis (TB), a highly contagious chronic infection. Mycobacterium tuberculosis is currently the main cause of human tuberculosis. M. bovis, M. microti, and M. africanum are additional M. tuberculosis complex members that have been linked to tuberculosis. Infection with M. africanum is extremely rare, while M. bovis has a wider host range and is the primary cause of tuberculosis in other animal species. Typically, M. microti is not known to cause TB in humans. Humans become infected by ***M. bovis,*** usually

via milk, milk products or meat from an infected animal.[2,3] WHO claims that TB is an international pandemic. Following COVID-19 (behind HIV/AIDS), TB is the second infectious killer in the world and the 13th largest cause of death overall. TB exists in all nations and among all age groups. In 2021, 1.6 million individuals worldwide (including 187 000 persons living with HIV) passed away from TB. 10.6 million individuals worldwide, including six million men, 3.4 million women, and 1.2 million children, are predicted to have contracted tuberculosis (TB) in 2021.[4] It is anticipated that between 2020 and 2021, the incidence rate of TB (new cases per 100 000 people per year) increased by 3.6%.[5] In India, the incidence of TB is projected to be 210 per 100,000 people in 2021, down from the baseline year of 2015 (when it was 256 per lakh of population); this is a drop of 18%, which is 7 percentage points higher than the 11% global average.[6]The likelihood of contracting tuberculosis may rise in the presence of immunosuppressive conditions such diabetes, alcoholism, malnutrition, chronic lung disease, and HIV/AIDS.[7] The disease often manifests in the lungs, but it can also spread to other parts of the body, including the central nerve or circulatory systems, called extrapulmonary tuberculosis. The death rate from untreated active TB is roughly 50%.[8-10]

Gram-positive tubercle bacilli are long, nonmotile, and acid-fast. The TB bacteria can survive for a long time in the air before being breathed by humans and being consumed by alveolar macrophages, which are white blood cells, where they begin to reproduce within two to three weeks.[11] In 95% of cases, the germs can be carried by macrophages throughout the body without any outward signs of a disease. But if the germs are not entirely eliminated, they can lay dormant for a few days before reactivating years later. Latent tuberculosis infection (LTBI), which has no symptoms, or tuberculosis illness are the two possible outcomes of TB infection. The death rate for this illness is around 50% if untreated. (Figure 1)

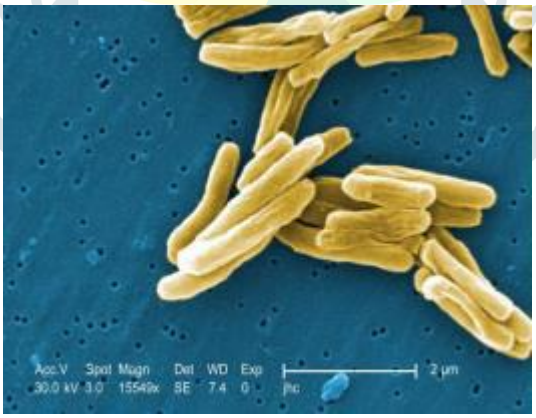

Figure 1: SEM of *M. tuberculosis*

Basic drugs used for treatment of tuberculosis

The current frontline treatment for drug susceptible TB includes a 2-month initiation phase of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (ETB), followed by a 4-month continuation phase of INH plus RIF.[12,13] The 194 Member States of WHO routinely report treatment success rates for drug-susceptible TB cases of at least 85%.[14] Reduce the establishment of drug-resistant strains by providing a mix of anti-TB medications with various mechanisms of action. But the creation of resistant strains has been attributed to erratic drug supply, disobedience with the therapy, improper drug regimens, and a lack of oversight. Low patient

adherence is caused by the lengthy course of treatment as well as the high percentage of side effects, which include gastrointestinal problems, hepatotoxicity, peripheral neuropathy, optic neuritis, ototoxicity, nephrotoxicity, and skin responses. [16]

Drug Resistance to anti-TB drugs-

Although TB management has historically been fraught with difficulties, the HIV epidemic and the rising prevalence of medication resistance pose two enormous risks to worldwide TB control today. In the nation most severely impacted by AIDS, the incidence of TB has increased significantly as a result of HIV-1 infection.[17] In the very early years of the chemotherapeutic era, the issue of resistance to anti-TB medications was also identified as a severe issue. The fact that Mtb may survive both intra cellular within macrophages and extra cellular within granulomas environments where traditional drug delivery is compromised—is one of the main challenges in treating tuberculosis. As a result, bacteria are exposed to low levels of antibiotics, allowing for the first development of phenotypic drug tolerance and later the acquisition of drug resistance.[18] TB drug resistance is characterized by both the types of drugs to which the bacteria lack susceptibility and the manner in which resistance was acquired. [19] Resistance to one first-line anti-TB drug only known as Mono-resistance, resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin known as Poly- resistance, resistance to at least both isoniazid and rifampicin known as **Multidrug resistance (MDR),** resistance to any fluoroquinolone, and at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance known as **Extensive drug resistance (XDR),** resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampicin, in the form of mono-resistance, poly-resistance, MDR or XDR known as **Rifampicin resistance (RR).** Resistance to single agents is the most common type; resistance to multiple agents is less frequent but of greater concern. Drug-resistant M. tuberculosis may develop over the course of treating a patient who was initially susceptible to the disease, or it may develop at the time of infection. By endangering the ability to control tuberculosis (TB), the issue of antibiotic resistance in Mycobacterium tuberculosis has been deemed a global health emergency by the WHO. About 484 000 cases of TB were brought on by RIF-resistant strains in 2018, 78% of which were MDR-TB. India, China, and the Russian Federation were the three nations most impacted by MDR-TB, accounting for half of all cases worldwide.[5] Second-line medicines (SLDs) must be used in the treatment of TB due to an increase in cases of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB worldwide. Treatment for MDR-TB is pricy, lengthy (18–24 months), and complex, requiring at least five different medications, some of which are injectable, and is also linked to a greater incidence of side effects.[20] (Table 1)

Table 1- Basic Drugs used for the treatment of Tuberculosis

First line		Second line		Extensively drug-resistant (XDR) TB drug		Novel generation of anti -TB
Oral Administration	Intravenous Administration	Oral Administration	Intravenous Administration	Oral Administration	Intravenous Administration	Oral Administration
Rifampicin (RIF) Isoniazid (INH Pyrazinamide (PZA) thambutol (ETB)	Streptomycin	Ethionamide Ethambutol Cycloserine Sodium Aminosalicylate Granules Pyriodoxine Levofloxacin Rifabutin	Kanamycin Amikacin Capriomycin	Amoxycillin & Potassium Clavulanate Clarithromycin Linezoid Moxifloxacin Clofazimine Thiacetazone Ethambutol	Capreomycin Injection	Delamanid pretomanid bedaquiline

The creation of a tuberculosis vaccine faces a variety of difficulties. Bacilli Calmette-Guerin (BCG), the only TB vaccine with a license to prevent infection, sadly has little effect on preventing pulmonary TB in adults. The immuno-protective effect of the BCG vaccination only lasts for 10 to 15 years, and the T cell mixed population is not efficiently stimulated by the BCG vaccine (particularly for CD8C T cells). The development of nanoscale aerosol vaccines against tuberculosis has also increased the amount of medication that reaches the target alveoli and can perfuse the entire respiratory system. A particle system with both micrometre and nanometer dimensions has recently been created by Garcia Contreas et al. for the aerosolized delivery of the BCG attenuated tuberculosis vaccine.[21] In comparison to a typical parenteral BCG formulation, aerosol application of BCG encapsulated nanomicroparticles in guinea pigs increased their resistance to tuberculosis infection. Alternately, when combined with antibiotics, the pulmonary route of nanoparticulate delivery of aerosolized IFN- has been demonstrated to be a safe and effective novel adjunct treatment for tuberculosis.[22,23] The particulate techniques for immunization for mucosal or parenteral delivery can be significantly influenced by particle size-dependent cell trafficking.[24]

To find a speedier, more effective, and less toxic treatment, it is evident that new methodologies are urgently needed in addition to traditional drug development techniques. With multiple examples in the literature involving different antibiotics and antibacterial agents, nanoparticles (NPs) have been demonstrated to be practical and adaptable instruments that can be employed as antibacterial agents as well as drug delivery systems. The development and implementation of novel treatments for MDR pathogens, including MDR- and XDR-TB, is being pursued by a number of organizations in Europe today, including universities, small and medium-sized businesses, research institutions, and pharmaceutical firms, through the use of research consortiums. [25]

Nanotechnology is the study, manipulation, control, design, synthesis, and development of various systems through the control of matter at the nm (nanometer scale) of 1-100 nm, where 1 nm=10-9, i.e. at the molecular and atomic level.[26] Medication formulations using nanoparticles are crucial for pulmonary medication delivery. Different nanoparticle complexes exhibit advantageous results involving the high potential for drug delivery

(intracellular), and enhancing dissolution properties makes it very uncouth to deliver the drug through the lungs.[27] Modern advances of science in drug delivery are very focused upon polymers (chain of monomers) and their types as they exert salient features and distinct biological functions.

Many nanoparticles are utilized to deliver medications with qualities including enhancing solubility, extending the action of formulations, varying degrees of lipophilicity or hydrophilicity, and lower toxicity in the fields of nanotechnology and nanomedicine. Different physicochemical characteristics of nanoparticle drug delivery systems exist, including surface characteristics, shape, size, therapeutic efficacy, drug system release, and loading. The pharmacokinetics, biodistribution, and biocompatibility of therapeutic molecules were all impacted by the surface characteristics of nanoparticles.[28] The determination of biodistribution and cellular internalization of nanoparticles were impacted by nanoparticle form.[29] The biodistribution, cellular internalization process, and protein adsorption of nanoparticles can all be significantly impacted by their size. Controlled drug release refers to a significant release of drug cargos that takes into account drug dispersion, matrix erosion, and desorption.[30] Drug loading can lessen the carrier's toxicity by utilizing less drug carrier.[31]

Nano drug delivery systems are often made from natural or synthetic substances (such as polymers and lipids) utilizing a variety of processes to produce particles with a diameter ranging from 10 to 1000 nm.[32] Because poly DL, lactic-co-glycolic acid (PLGA) is non-immunogenic, biodegradable, and has the ability to encapsulate both hydrophobic and hydrophilic compounds, many groups have employed it as the polymeric encasing material.[33] The primary targets for Mtb infection, macrophages and dendritic cells, were also found to take up PLGA selectively.[34] Additionally, PLGA's cytotoxicity was assessed in vitro and in vivo, and no harmful side effects were found.[36] Treatment delivery via nanoencapsulation offers the chance to add targeting tactics that will increase treatment effectiveness and reduce systemic toxic side effects for the patient. The dose and adverse effects of the medicine are reduced by functionalizing the surface of the particles with a targeting ligand, which increases bioavailability at the site of infection.[37] A successful targeting strategy will have a beneficial effect on drug delivery to the infection site, may guide drug trafficking into organelles that contain infections, and may enhance the release and potency of the active medicine.[38] In this review, we will discuss the recent nanotechnology approaches for the development of first and second-line TB drugs delivery. The hurdles and limitations for nanotechnology as future TB therapy will also be discussed.

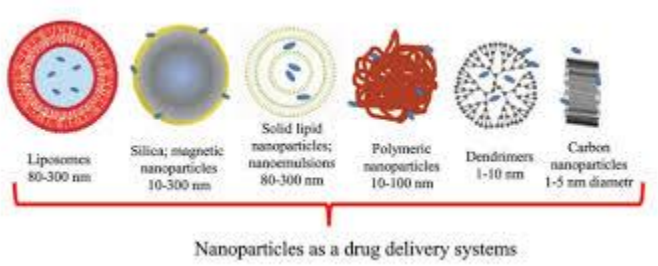

Figure 2- Nanoparticles used as drug delivery systems

Nanoparticles Based System for Treatment of Tuberculosis-

A microscopic item that functions as a single entity and is less than 1000 nm in size is referred to as a nanoparticle. They can be created using a variety of techniques, resulting in NPs with a variety of shapes and sizes, such as

biodegradable lipids, liposomes, solid-lipid NPs, polymers like poly lactic-co-glycolic acid (PLGA), or polysaccharides like chitosan.[39] Nanoparticles can be a helpful strategy for treating tuberculosis (TB) in two different ways: (i) for their inherent anti-mycobacterial activity; and (ii) as vehicles for well-known anti-tubercular drugs to reduce dosage and drug-associated side effects and allow administration via convenient administration routes like pulmonary or oral ones. A wide range of nanocarriers, including polymeric nanoparticles, nanocapsules, micelles, dendrimers, nanogels, and liposo, have been designed to access the reservoirs of Mtb. By physical encapsulation, adsorption, or chemical conjugation, therapeutic agents can be added to nanocarriers. Another significant benefit of employing nanocarriers is the ability to actively or passively target host cells.[40,41] Various researchers have explored several nanocarriers for tuberculosis management and are exemplified below.(Table 2)

Liposomes-

The term "liposome" refers to multilayer carriers that contain an aqueous volume inside of a membranous lipid bilayer. The most researched lipid nanocarriers for the delivery of different medicinal payloads, such as therapeutic and diagnostic substances, are liposomes. Liposomal particles are spherical vesicles made of cholesterol, which increases in vitro and in vivo stability, and natural or synthetic phospholipids, which are amphiphilic molecules.[42] These particles can entrap both hydrophobic and hydrophilic drugs because they can be made from phospholipids that are naturally produced by the lungs as surfactants.[43] Stealth® liposomes were created by Deol and Khuller for the specific delivery of anti-TB medications to the lung. Phosphatidylcholine, cholesterol, dicetylphosphate, O-steroyl amylopectin, and monosialogangliosides/distearylphosphatidylethanolaminepoly (ethylene glycol) 2000 were all included in the composition of liposomes.[44] The described liposomal carriers have demonstrated efficacy against Mycobacterium strains that are highly and multiple-drug resistant. The administration of vaccines by liposomes has been proven to be effective in the fight against tuberculosis.[45]

In order to target macrophages, Hardwaj et al.[46] created isoniazid and ciprofloxacin-co-loaded liposomes. These liposomes also contained 4-aminophenyl-D mannopyranoside. As pH-sensitive ingredients, cholesteryl hemi-succinate (CHEMS) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were employed to produce liposomes. The alveolar macrophage absorption of the pH-sensitive ligand-decorated liposomes was significantly higher than that of the unadorned liposomes. These liposomes reached the highest medication concentration in the lungs after pulmonary administration. The findings of this study provide important proof that pH-sensitive macrophage-targeting liposomes may be used to increase the bioavailability of anti-TB medicines. While preventing its release to other tissues, the drug's release from the liposome may aid in maintaining the drug's location inside the lungs. Liposomes-based drug delivery is superior; low toxicity, a wide range of sizes (20 nm-1 mm), ability to solubilize drugs with poor water-solubility, facilitating their nebulization, represent as a biobased pulmonary reservoir with prolonged residence period, minimize mucociliary clearance of drugs owing to their surface viscosity, used as a targeting agent within the lung, particularly to infected or impaired AMs and the lung epithelial tissue. There are numerous ways to send this special delivery technique and the nanocarriers to the lungs, including insufflations, nebulization, instillation, etc.[47]

Niosomes

Niosomes are similar to liposomes in that they are mostly made of non-ionic surfactants, either with or without lipids incorporated. Niosomes have qualities that make them suitable for use in pharmaceutical products, including non-toxicity, stability, long-lasting effects, biodegradability, the ability to alter drug distribution, and the potential to increase drug bioavailability. Ethionamide and D-Cycloserine dual drug-loaded self-assembled niosomes were created by Kulkarni et al. for the effective treatment of multidrug-resistant tuberculosis. The niosomes were characterized for atomic force microscopy, in vitro hemodialysis, osmotic shock, and antibacterial investigations using Box Behnken design. The experiments on hemodialysis demonstrated that intravenous delivery of dual drug-loaded niosomes is safe. In comparison to pure drug and single drug-loaded niosomes, the MIC of dual drug-loaded niosomes was the lowest. This efficiency was noted as a result of the first burst release of D-Cycloserine and the delayed release of lipophilic ethambutol. So, a dual medicine combination contained in niosomes worked synergistically to cure tuberculosis.[48]

The effect of surface features on the functionality of the niosomal particles was also studied in the research on niosome technology for TB. The loading of pyrazinamide in niosomes consisting of either Span® 60 or 85 and cholesterol in various molar ratios was examined by El-Ridy et al.[49] The particles with negative and positive surface charges were struck with dicetyl phosphate (DCP) and stearyl amine, respectively. The formulation with a molar ratio of 4:2 between Span® 60 and cholesterol had the highest level of entrapment. The best encapsulation efficiency was demonstrated by negatively charged niosomes, followed by neutral niosomes. It was discovered through in vivo biological tests on guinea pigs infected with M. tuberculosis that pyrazinamide's increased potency was caused by its encapsulation in niosomes.

Mehta et al.[50] examined the potential for the effective co-encapsulation of anti-TB medicines in niosomes in a subsequent investigation. Triton X 100, PEG 2000, water, and Span® 80 were used by the authors to create very stable niosomes, which were then used to encapsulate various first-line antitubercular medications (rifampicin, isoniazid, and pyrazinamide). For each drug individually, high encapsulation efficiency as well as favorable system compatibility and stability were noted. However, the generated multidrug-loaded niosomes' biological performance remained a mystery. Tyloxapol was used as a biocompatible surfactant in Mehta et al.[51] research on the encapsulation of rifampicin, isoniazid, and pyrazinamide into niosomes. It was found that the final formulations had good stability and had high encapsulation efficiency for each medication. Isoniazid and rifampicin were presumably present in the film bilayer, according to the localization investigation, whereas pyrazinamide was largely adsorbed on the surface of head groups. The difference in these medications' solubilities can be used to explain why isoniazid and pyrazinamide showed sustained drug release whereas rifampicin showed significantly rapid release. At this point, understanding the in vivo behavior of these multidrug-loaded niosomes would present potential for the creation of niosomes that would be compatible with the existing multidrug regimens for anti-TB therapy.

Polymeric nanoparticles (PNPs)

Since they provide a variety of appealing qualities in medication administration, polymers have attracted a lot of research in the last few decades. Numerous important characteristics of polymeric NPs make them perfect for

delivering antimicrobial medicines. PNPs are solid, colloidal nanoparticles (10–1,000 nm), composed of biodegradable polymers.[52-53] PNPs can be categorized as either nanospheres (matrix type) or nanocapsules (reservoir type) depending on how their structural organization is set up. In contrast to nanocapsules, which dissolve/disperse the drug in a liquid core of oil or water enclosed by a solid polymeric membrane, nanospheres type PNPs entrap the drug in the polymer matrix. Both PNP kinds allow for the chemical conjugation or adsorption of the medication on the surface (of the matrix or capsule).[54] Depending on the composition and required features of PNPs, various preparation techniques have been developed. These techniques can easily be divided into two groups: direct monomer polymerization and dispersion of premade polymers. Solvent evaporation, salting out, nanoprecipitation, dialysis, and supercritical fluid technology are some of the techniques used to disperse premade polymers. Emulsification polymerization, miniemulsion polymerization, microemulsion polymerization, interfacial polymerization, and controlled/living radical polymerization are some of the techniques that directly polymerize monomers. Rao and Geckeler have thoroughly examined each of these techniques.[52] PNPs have been created using a variety of biocompatible and biodegradable natural and synthetic polymers. Because they are biodegradable, these polymers are broken down into individual monomers inside the body and eliminated by regular metabolic processes.[55]

The synthetic polymers polylactic acid (PLA), polyglycolic acid (PGA), polylactic acid (PLGA), polyglycolic acid (PLGA), polycaprolactone (PCL), copolymer N-(2-hydroxypropyl) methacrylamide (HPMA), polyaspartic acid (PAA), and polyglutamic acid are the most often utilized ones. Albumin, alginate, chitosan, collagen, dextran, gelatin, and heparin are among the most frequently utilized natural polymers.[56] Compared to their colloidal counterparts, such as polymeric micelles (PMs) and liposomes, PNPs offer better stability on storage and in vivo (in the blood), higher drug payload, more homogeneous particle size distribution, better and controllable physicochemical properties, higher drug circulation times, and more controlled drug release.[57]

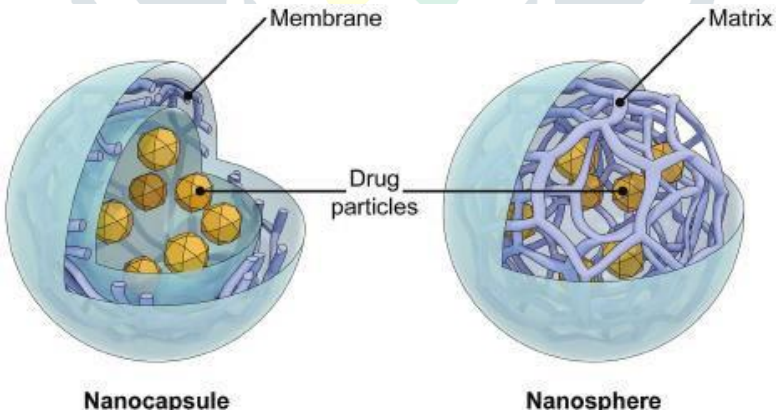

Figure 3: Structure of the polymeric nanospheres and nanocapsules

Polymeric nanoparticles are a desirable replacement for liposomes. Polymeric nanoparticles can be created with a narrower size dispersion and are, to some extent, sustainable. When synthesizing nanoparticles, various polymer lengths, surfactants, and organic solvents can be used to precisely control factors like size, zeta potential, and drug-release pattern. Additionally, with the help of drug moieties and targeting ligands, the functional groups on the surfaces of polymeric NPs can be altered chemically. There are now two types of polymeric NPs available for antimicrobial medication delivery. First is made when diblock copolymers having hydrophilic and hydrophobic

sections spontaneously self-assemble. The hydrophilic portion shields the hydrophobic portion from opsonization and degradation, while the hydrophobic portion generates a polymer core that contains the medicines. It is possible to alter the hydrophobic chain length to regulate the rate of medication release. The hydrophobic polymeric core was created using a variety of biopolymers, such as polycaprolactone (PCL) and poly(cyanoacrylate) (PCA), whereas the hydrophilic component was created using polyethylene glycol (PEG). In light of this, polymeric NPs are widely used to deliver medications that are less water-soluble due to the general hydrophobic characteristics of the NP core. [58] Sustained release poly(lactideco-glycolide) (PLG) nanoparticles with RIF, INH, and PYZ loaded were created by Pandey et al.[59] for oral delivery. Therapeutic concentrations in tissues were detected 9 to 11 days after a single oral dose of nanoparticles was administered, whereas free drugs were cleared from the plasma within 12 to 24 hours after administration in mice. For RIF and INH and PYZ, the drugs could be detected in the plasma for up to 4 days and 9 days, respectively. Coya et al. created mannose functionalized PNPs to enhance the response of innate immune human cells to overcome the limits seen in currently existing treatments against M. tuberculosis lung infections.[60] PNPs' capacity to stimulate inflammatory processes may be helpful in combating specific pathogens, but persistent inflammatory responses may be harmful to the host. Because of this, writers decided to modify the PNPs' surface with mannose in order to lessen their proinflammatory characteristics. Researchers discovered that human macrophages effectively internalize produced PNPs, which significantly alter the response of M. tuberculosis-infected ones. In order to boost the effectiveness of loaded molecules, grafting ligands onto the surface of PNPs may be a promising method for modulating cell metabolism via the immune response pathway. Similar to this, Malik et al.'s recent research examined the encapsulation of the bivalent H1 antigen, a fusion of the M. tuberculosis Ag85B and ESAT6 proteins, in PNPs to examine its function in immunomodulation and protection in a mouse model of M. tuberculosis lung infection.[61] Here, produced PNPs are effectively internalized by THP-1 human monocytes, and immunized animals produce much more total serum IgGs than control mice. In addition, immunized mice exhibit notable decreases in lung and spleen bacterial burden and extended survival in protection trials.

In order to create a drug depot system, Anisimova et al. created RIF, INH, and streptomycin-loaded PBCA (poly(n butylcyanoacrylate)) and PIBCA (poly(isobutylcyanoacrylate)) nanoparticles and investigated in-vitro cellular absorption in human blood monocytes. Encapsulated INH (4–8 fold), streptomycin (7 fold), and RIF (22–25 fold) all showed promising increases in intracellular concentration to extracellular concentration as compared to free drug INH (identical), streptomycin (undetectable), and RIF (5 times).[62] In a mouse model, injectable PLGA (poly(lactic-co-glycolic acid)) nanoparticle-based implants were also given subcutaneously. A single subcutaneous dose can keep medication levels in the plasma, lungs, and spleen for about a month while keeping bacterial counts in these organs nearly undetectable.[63] Alginate nanoparticles of an anti-TB medication with a diameter of 235 nm were created by Zahroor et al. using the ionotropic gelation process. the oral delivery of the drug to mice, Free medicines were eliminated from blood within 12 to 24 hours, however tissues (such as the spleen, liver, and lung) remained detectable the following day. In contrast, plasma levels of polymeric nanoparticles were up to 7 for ethambutol (ETB), 9 for RIF, and 11 for INH and PYZ. Organ drug levels were visible in tissues up until day 15 of the experiment.[64] Using the nanoprecipitation approach, du Toit et al. created emulsion-based polymeric nanoformulations of INH-loaded (77–414 nm). Precursors with a water or emulsion

base were both employed. By varying the polymer concentration, the size of the nanoparticles can be altered; lesser polymer concentrations produced smaller particles. Depending on the method utilized to prepare the nanoparticles, in vitro release experiments showed an initial burst release that might last up to 2 hours.[65]

With benefits like improved drug solubilization, decreased immunogenicity, regulated distribution, greater efficacy, and improved pharmacokinetics, the use of polymer-drug conjugation in drug delivery has significantly expanded over the past 20 years. In contrast, the majority of polymer-small molecule drug conjugates employed up to now contain non-biodegradable polymer carriers, including polyethylene glycol (PEG), that limit polymer size below the molecular cut-off of 40 kDa required for renal elimination.[66] In contrast, hydrolysable hydrophobic polyesters with limited utility for drug conjugation, such as polycaprolactone (PCL) and poly(lactide-co-glycolide) (PLGA), are frequently used in FDA-approved devices and are used to physically entrap pharmaceuticals within nanoparticulate carriers.[68] Many anti-tuberculosis medications, however, are highly water soluble, which makes them more likely to burst release in the systemic circulation and makes them easily leached out from the nanocarriers during manufacture.[69-72] Recently, polyketals have also been investigated as a new family of acid responsive and biodegradable polymers appropriate for drug conjugation.[73] In contrast to polyesters, polyketals give pH neutral hydrolysis products. Several polymers, including synthetic polymers like PLGA and natural polymers like gelatin and chitosan, have been investigated for isoniazid(INH) conjugation.[76] To introduce functional groups suitable for drug conjugation, these systems require additional chemical changes to the polymers. Chitosan-INH conjugates were made by Berezin and Skorik utilizing two alternative synthetic methods, either by treating the chitosan with acrylic acid or epichlorohydrin prior to INH conjugation. Due to the incomplete cleavage of INH from the polymer, modified chitosan polymers showed lower biodegradability and either similar (for N-(2-carboxyethyl)chitosan INH conjugates) or higher (for N-(3-chloro-2-hydroxypropyl)chitosan INH conjugates) minimum inhibitory concentrations compared to free drug.[75]

Solid Lipid Nanoparticles (SLN)

To create lipid nanoparticles with a size range of 50–1000 nm in SLN, the drug is primarily trapped in a solid lipid matrix using a hot or cold high pressure homogenization process. It is noteworthy that the solid lipid nanoparticles exhibit significant benefits, including composition (physiologic chemicals) and the ability to produce them on a large scale while avoiding the use of organic solvents in the production process.[76,77] In certain severe cases, TB infection can spread from the lungs to the lymphatic system. SLNs can distribute ATDs such INH, RIF, and PZA to the lymphatic and respiratory systems in addition to the lungs. These SLNs are transported to lymphoid organs after being phagocytosed by AMs and reaching the lungs. To assess the SLN delivery procedure and biodistribution via pulmonary delivery in mice, radio-labeled aerosolized SLNs were used. A prolonged release of the antibiotic payloads carried by the SLNs, which can effectively remove the viruses and bacteria housed at these lymphatic zones, was found after the lungs successfully absorbed radio-labeled SLNs.[78] Nemati et al. described formulation development[79], in which dry powder inhalation was used to study the pulmonary delivery of ethambutol encapsulated in SLN. Compritol® and Tween® 80 were used to make the ethambutol-loaded SLN, which had a very high drug encapsulation efficiency of 98%. The proposed formulations' excellent biocompatibility and lack of toxicity were demonstrated by the MTT tetrazolium salt test. The

synthesized SLN's potential to release sufficient ethambutol molecules to guarantee effective mycobacterial clearance, however, was not subjected to an examination of its antimycobacterial activity.

A good attempt at solid lipid particle research for tuberculosis in the context of multidrug therapy was shown in a study by Pandey et al.[80] The three separate antitubercular medications (rifampicin, isoniazid, and pyrazinamide) contained in the nebulized solid lipid particles under research were examined for their chemotherapeutic potential. These solid lipid particles were created utilizing the emulsion solvent diffusion method from stearic acid. The residence length and medication bioavailability in particular organs (lung, liver, and spleen) were significantly increased as a result of effective drug encapsulation. Compared to free medicines given orally, the drug-loaded solid lipid particles used in nebulization showed much more action in guinea pigs with M. tuberculosis. After 7 days of therapy in guinea pigs with the suggested formulation, no tubercle bacilli were found in the lung or spleen. Additionally, no hepatotoxicity was detected after the administration of the synthesized solid lipid particles, according to the biochemical study. However, as the mycobacterium is frequently concealed in the granuloma, basic investigations clarifying the uptake of the solid lipid particles by macrophages are required to enable the efficient treatment of latent tuberculosis.[81]

Nanoemulsions-

They are classified as isotropic blends of oils, surfactants, and cosurfactants or cosolvents that, when in contact with gastrointestinal fluids, can spontaneously produce (nano)emulsions with droplet sizes of 200 nm or less.[82] They have a low oil content (30% w/w) and high surfactant and cosurfactant content (>60%). Self-(nano)emulsifying drug delivery systems (S(N)EDDS) offer enhanced palatability, physical and chemical stability, and patient compliance because they may be put into capsules as a single dose and do not contain water. High drug loading, enhanced drug absorption, regulated distribution, targeting potential, and reduced unpredictability brought on by dietary effects are only a few of the specific benefits connected with S(N)EDDS.[83] In addition to being able to increase drug bioavailability, S(N)EDDS are also simple to produce and scale up because they are created using excipients that are regarded as safe. One of the technologies used to enhance the delivery of anti-TB medications is S(N)EDDS. In vitro drug release experiments revealed that nanoemulsions had significantly longer drug release times than commercial capsule formulations and drug suspensions, up to 24 hours. The relative bioavailability of ramipril nanoemulsion to that of a traditional capsule was 229.62%, and it was 539.49 for drug suspension, indicating the usage of the developed ramipril nanoemulsion for geriatric and pediatric patients.[84] Using chitosan and polymyxin B, Henostroza et al. created a cationic nanoemulsion with rifampicin that had a particular surface modification. Both the rifampicin nanoemulsion including polymyxin B and the rifampicin nanoemulsion containing chitosan had zeta potential values of +51.3 mV and +5.5 mV, respectively, with particle sizes of about 150 nm. The cationic nanoemulsion and negatively charged mucin interacted electrostatically, according to the results of an in vitro mucoadhesion research. This has the effect of increasing the created preparation's residence time at the target site, which increases its effectiveness in treating ocular tuberculosis.[85]

Nanosuspensions

Colloidal dispersions of pure pharmaceuticals at sub-micron sizes, supported by surfactants, are referred to as nanosuspensions. With a high melting point, excellent solute-solute interactions, poor lipid and water solubility, and reduction of particles to the nanoscale (also known as "nanonization"), this platform helps to improve and boost the drug's solubility. In procedures requiring massive loadings of the medication, the maximum mass per volume ratio provided by the solid and dense states of the pure drug particles is crucial.[86]

Mice were given an intravenous dose of the nanosuspension (385 nm) version of clofazimine. When compared to pharmacokinetic data, drug concentrations in these organs reached high concentrations, well in excess of the minimal inhibitory concentration for the majority of M. avium strains, which led to a significant reduction in bacterial loads in the liver (72.5 mg/kg tissue), spleen (81.4 mg/kg tissue), and lungs (35.0 mg/kg tissue) of mice infected with M. avium.[87] Clofazimine nanocrystals had effects that were comparable to those of the liposomal formulation utilized in this investigation as a control. In order to address the poor solubility and toxicity, this study was specifically designed. RIF sub micronic particles (400 nm to 3 m) were created by Reverchon et al. employing supercritical carbon dioxide-assisted atomization and are suited for parenteral and aerosolized drug delivery systems. They investigated how different solvents affected particle size and drug degradation, and they recommended using a nanoparticle production strategy for more practical TB pharmacotherapy that administers medication locally to the lungs.[88, 89]

Dendrimers

A family of nano sized macromolecules known as dendrimers—from the Greek words "dendros" and "meros"—are distinguished by their compact, spherical geometry in solution and highly homostructural, branched three-dimensional (3D) architecture. Dendrimers are globular macromolecules that have a highly branching 3D architecture and can have exquisite control over their size and structure. They exhibit a central core that is surrounded by an exponential number of dendritic branches with hydrophobic and hydrophilic moieties. With each generation, the number of surface groups grows exponentially while the dendrimer diameter increases linearly. While higher generation molecules are typically denser and more stiff, low-generation dendrimers are typically flexible.[90]

Recently, RIF loaded PEGylated 5G EDA-PAMAM dendrimers were emphasized by P. Dineshkumar and colleagues.[91] The PEGylation of 5G PAMAM dendrimers was verified by 1H-NMR spectra and the Fourier Transform Infrared Spectrophotometry (FTIR) method. It was discovered that the PEGylated Dendrimer's RIF entrapment efficiency was 99%. For PEGylated (in 120 h) and non-PEGylated (in 72 h) PAMAM dendrimers, respectively, the drug release rate of RIF is 81% and 98%. Due to the inhibition of red blood cell interaction with quaternary ammonium groups on the surface of dendrimers, hemolytic studies revealed that non-PEGylated PAMAM dendrimer revealed 11.6 to 25.3% of toxicity, whereas the PEGylated PAMAM dendrimer strongly showed lower toxicity effects (less than 2.5%). Similar effects were highlighted in early studies with dendrimers bearing cationic groups on their surface.[92]

Isoniazid (IND), an extremely potent anti-TB medication, also contains dendrimers. The loading of the IND with 1.5G PAMAM dendrimer utilizing a dialysis technique was described by N. Singh et al. (1993). Both FTIR and UV spectroscopic methods validated the loading of IND. This formulation showed that up to 24 hours, approximately 93.25 percent of IND was continuously released. Additionally, research on the kinetics of drug release showed that the kinetic is zero order and corresponds to a non-Fickian diffusion, which is most likely caused by the distinct boundary between the surface of the dendrimer and the empty spaces where IND is contained.[94]

Gold Nanoparticles

Recently, Gold NPs have drawn a lot of interest for use in medication delivery. Recently, gold nanoparticles (AuNPs) have been successfully and significantly used as a medication delivery mechanism for TB therapy.[95] The activity of GNPs and the plant extract that is trapped aid in the early recovery from TB. The increased gene expression in the redox process, which results in the death of bacteria and fungi, is the postulated mechanism for the antibacterial activity of GNPs. The antibacterial activity of GNPs was directly influenced by their nano size, surface area, and photothermal nature.[96] Another accepted theory is that GNPs connect intracellularly to the Sulphur base found in cells in the form of thiol groups in enzymes, which causes an abrupt disruption of the respiratory chain and the production of a significant amount of free radicals, which results in death.[97] According to a recent study by Gupta et al, ethanolic and hydroalcoholic extracts showed anti-tubercular activity at concentrations as high as 50 g/mL and 75 g/mL, respectively, but ethanolic and hydroalcoholic GNPs only showed anti-tubercular activity at MIC levels of 2.5 g/mL and 20 g/mL, respectively.[98]

Silver nanoparticle (AgNPs)

Among the several metallic nanoparticles used in biomedical applications, silver nanoparticles (AgNPs) are one of the most important and fascinating nanomaterials. Song et al.'s in vitro testing of tiny, non-biogenic AgNP measuring less than 10 nm against a variety of bacteria species, including M. tuberculosis, E. coli, S. aureus, and Salmonella typhi, was one of the earliest findings on the antimycobacterial impact of AgNP. At 10 ppm, the antimycobacterial action was noticed, and the suggested mechanism is based on the presence of AgNPs in the cytoplasm of mycobacteria and the subsequent bacterial-metabolic abnormalities.[99] In a different investigation, an antibacterial impact against field isolates and standard strains of Mycobacterium TB and Mycobacterium bovis was observed. This study used the antimycobacterial action of physicochemically synthesized, tetrahedral and spherical AgNP measuring 50 nm. The minimum inhibitory concentration (MIC) for standard cultures of M. tuberculosis and M. bovis was determined to be 1 and 4 g/mL. Higher dosages, ranging from 4-32 g/mL for M. bovis and 1-16 g/mL for M. tuberculosis, were required to inhibit the clinical isolates.[100] In a recently reported study, Zakharov et al. tested the effects of isoniazid and AgNPs in three experimental groups of MDR-TB-infected mice: group 1 received only isoniazid (50 mg/kg); group 2 received intramuscularly administered AgNPs at doses ranging from 12.5 to 125 g/kg; and group 3 received a combination of the treatments described for groups 1 and 2. The use of AgNPs in the treatment of TB brought on by MDR strains improves the effectiveness of isoniazid according to the histopathologic grading of lesions.[101]

Selenium Nanoparticles (SeNPs)

SeNPs (selenium nanoparticles) are less dangerous, biocompatible, and very good at targeting only certain cells. Many researchers have documented the potent antimicrobial effects displayed by some form of selenium via reaction with membrane peroxidases and, subsequently, induce the generation of oxygen-free radicals consisting of superoxide anion. The major antimicrobial mechanistic pathways of these NPs are still undefined.[102] Chitosan-stabilized Se NPs were created by Estevez et al. (2020), who also looked at how they affected Mtb and Mycobacterium smegmatis (Msm).[103] By weakening the integrity of the bacteria's cell envelope, they noticed that exposure to selenium nanoparticles prevented Mtb from going through its regular transformation stages. With MIC values of 0.195 g/mL and 0.400 g/mL, respectively, the effectiveness of Se NPs on mycobactericidal was tested against two varieties of the slow-growing Mtb and the rapid-growing Msm. To rule out the possibility of chitosan having a second influence on the bactericidal action of Ch-SeNPs, the antibacterial effectiveness of bovine serum albumin stabilized SeNPs (BSA-SeNPs) on the Mtb was also assessed. When compared to Ch-SeNPs at equivalent concentrations, the results showed that BSA-SeNPs had a greater ability to inhibit bacterial growth. The authors came to the conclusion that the SeNPs, rather than the stabilizing agent chitosan, were responsible for the nanoparticles' ability to kill mycobacteria. This study shown that Se NPs have significant antibacterial ability and can kill mycobacteria. They conducted additional analysis using cryo-EM and TEM (3A) to further support this discovery. The results of their analyses demonstrated that the contact interaction of the SeNPs with the cell walls of the Msm and Mtb cells resulted in the ejection of their cytoplasmic material and the destabilization of their integrity. The findings of this study are extremely important because they hold the promise of creating exceptional nanosystems with strong antimycobacterial potential using either Se NPs alone or in conjunction with antibiotics, which could represent a significant advance in the treatment of multi-drug resistant tuberculosis strains. The immunological escape of Mtb from phagolysosomal obliteration and the restricted drug delivery into infected cells have been two of the main problems for TB and drug-resistant TB therapy over the years. By combining TB immunology expertise and nanoscience, Pi et al. (2020) found a solution to this issue and created macrophage-targeted Se NPs for the synergistic bactericidal and antimicrobial eradication of Mtb in host cells.[104] The mannosylated Se NPs may also serve as an exceptional vehicle for the exact delivery of isoniazid into macrophages for increased intracellular Mtb eradication, in addition to the immediate eradication of Mtb.[105]

Table 2 – Nanoformulations for Antitubercular drugs and their results obtained

Nanoparticle Used	Drug	Formulation type	Outcome observed	References
Polymeric	Rifampicin (RIF) and Isoniazid (INH)	RIF-loaded poly lactic-co-glycolic acid(PLGA) NP	The NP prepared inhibited the colonization and growth of *Mtb* H37Rv strain at 70% of the MIC	106
Polymeric	Rifampicin (RIF) and Isoniazid (INH)	RIF-loaded poly lactic-co-glycolic acid (PLGA) NPs and INH modified as INH benz-hydrazone	RIF loaded in PLGA NPs consistently inhibited the growth at 70% of the minimum inhibitory concentration (MIC) of pure RIF (MIC level 1 µg/mL), and pure IH2 and IH2-loaded NPs showed inhibition at MIC equivalent to the MIC of INH (0.1 µg/mL). These results show that NP formulations will improve the efficacy of drug delivery for TB treatment.	107

SLN	Rifampicin (RIF)	Rifampicin (RFP)-loaded solid lipid nanoparticles (RFP-SLNs)	RFP-SLNs delivered markedly higher RFP into alveolar macrophages(AMs) (691.7 ng/mg in cultured AMs, 662.6 ng/mg in primary AMs). Under pulmonary administration of RFP-SLNs, the amount of RFP in AMs was significantly higher	108
Niosomes	Ethionamide and D-cycloserine	Dual niosomes(ETH + D-CS)	The dual drug loaded niosomes showed greater bacterial inhibition than the free drug combination. The optimized formulation displayed acceptable % entrapment efficiencies (>70%) and optimum particle size (137.4 nm) along with sustained release up to 3 days. *Mycobacterium Smegmatis* was used as a model organism to assess bacterial inhibition.	48
Liposomes	Rifampicin (RIF) and Isoniazid(INH) , Pyrazinamide (PZA), Streptomycin(SM).	NP-siRNA liposomes	The novel NP-siRNA liposomes functionalized with the anti-TB drugs and TGF-β1 siRNA were endocytosed efficiently by human macrophages The novel NP-siRNA liposomes functionalized with the anti-TB drugs and TGF-β1 siRNA were endocytosed efficiently by human macrophages .	109
AgNP	Rifampicin (RIF) and Isoniazid (INH)	AgNP aqueous suspension (Argovit-C, 10 mg/mL silver, in a concentration of 3.3%)	The patients enrolled in the AgNP group – showed faster healing of the laryngeal TB-lesion, including ulcerations and voice function compared to standard tuberculosis drugs.	110
Selenium Nanoparticles	Isoniazid (INH)	Ison@Man-Se/Man-Se NPs	Ison@Man-Se/Man-Se NPs promote the fusion of Mtb into lysosomes for synergistic lysosomal and Isoniazid destruction of Mtb and also induced autophagy sequestration of Mtb, evolving into lysosome-associated autophagosomal Mtb degradation linked to ROS-mitochondrial and PI3K/Akt/mTOR signaling pathways. This novel nanomaterial-assisted anti-TB strategy manipulating antimicrobial immunity and Mtb clearance may potentially serve in more effective therapeutics against TB and drug-resistant TB.	104
Nanoemulsions	Rifampicin (RIF)	Rifampicin containing Nanoemulsions droplet	The nanoemulsions had average droplet sizes of 40–60 nm, with narrow polydispersity indices. They exhibited desirable pH, surface tension, viscosity, refractive index, density and viscosity attributes for pulmonary rifampicin administration. All nanoemulsions demonstrated more than 95% aerosol output and inhalation efficiency greater than 75%.	111

Nanoparticle and Toxicology-

Human exposure to nanoparticles is inevitable because they are present in large quantities in the environment, both from natural and man-made sources. Given this ongoing exposure, it is crucial to comprehend the possible acute and long-term negative consequences that nanoparticles may have on people. According to a number of studies, nanoparticles smaller than 100 nm can enter cells, those less than 40 nm can penetrate cell nuclei, and those smaller than 35 nm can cross the blood-brain barrier to enter the brain. [112] The knowledge about nanotoxicity help to identify the key safety issues in implementing nanoscience for health application, by investigating interaction of nanoparticles and biological systems (i.e., proteins, cells) and elucidating the relationship between the physical and chemical properties like size, shape, surface chemistry, composition, and aggregation with respect to toxic biological responses. Oxidative stress induced by nanoparticles can enhance inflammation through up regulation of redox sensitive transcription. Nanoparticles can also change cellular redox signaling and mitochondrial function. However, it is crucial to look into the toxicity effects of developed carrier systems at the cellular level in addition to studies on pharmacological activity, efficacy, and pharmacokinetics. In order to minimize hazardous consequences, official authorities should use permitted materials while developing carrier systems. The International Alliance for Nano EHS Harmonization (IANH) is working to establish in vitro

and in vivo nanomaterial testing protocols that are validated to yield the same results in numerous laboratories around the world and to correlate the obtained results with obtained or predictive in-vivo data. [113]

Conclusion-

Even though identifying new anti-TB agents remains a top priority, developing nanoparticle-based delivery systems for drugs already on the market could be a viable and affordable alternative. According to the aforementioned research, nanoparticles hold great promise for the treatment of tuberculosis. Their key benefits, including increased drug bioavailability and decreased dosing frequency, may lay a solid foundation for better disease management and make directly observed treatment more practical and affordable. The viability of numerous drug delivery methods, such as oral and inhalation routes, is another significant benefit of nanoparticles. Furthermore, the nanoparticles' high stability suggests a long shelf life. Future studies are likely to focus on creating vectorized delivery systems that combine the benefits of colloidal carriers—like their ability to carry large medication payloads—with active targeting of infection sites. Nanoparticles can also be included into several solid dosage forms (microparticles, granules, or tablets), which can release the nanoparticles at the site of action while maintaining their original features, according to the development of revolutionary formulation technologies. The efficacy and viability of the formulations based on nanoparticles will be further enhanced by these strategies. Last but not least, the effectiveness of this technology will likely depend on toxicological issues related to knowledge of the fate of nanocarriers and their polymeric constituents in the body, as well as the elimination of the risk of the remaining organic solvents. This makes the idea of employing medication carriers manufactured from natural polymers (like chitosan or gold NPs) an appealing potential.

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