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Tuftsin-Plga Conjugate To Target Tuberculosis Cell

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ABSTRACT
Tuberculosis (TB) remains a major global health concern, with prolonged treatment regimens and increasing drug resistance posing significant challenges. Conventional anti-tubercular therapies often suffer from poor bioavailability, systemic toxicity, and low patient adherence due to extended treatment durations. Targeted drug delivery systems offer a promising solution to these limitations by enhancing drug accumulation at infection sites while minimizing side effects. In this study, we developed and characterized a Tuftsin-PLGA (Poly(lactic-co-glycolic acid)) conjugate designed to deliver anti-TB drugs directly to macrophages, the primary host cells for Mycobacterium tuberculosis. Tuftsin, a natural tetrapeptide (Thr-Lys-Pro-Arg), is known for its ability to enhance macrophage activation and facilitate receptor-mediated uptake. By conjugating Tuftsin with PLGA nanoparticles, we aimed to create a targeted, biocompatible, and sustained-release system for anti-tubercular drug delivery. The study demonstrates that Tuftsin-functionalized PLGA nanoparticles represent a promising approach for targeted TB therapy. By leveraging the macrophage-specific uptake of Tuftsin and the controlled release properties of PLGA, this system offers an efficient, patient-friendly drug delivery strategy. The sustained release mechanism reduces systemic side effects, enhances drug bioavailability, and ensures higher therapeutic efficacy, ultimately improving patient adherence to TB treatment regimens. Future in vivo studies and clinical trials are essential to validate the translational potential of this novel drug delivery platform. In conclusion, the development and characterization of Tuftsin-PLGA conjugates offer a novel, targeted, and sustained drug delivery approach for tuberculosis therapy.
KEYWORDS: Tuftsin, controlled release properties, TB therapy, PLGA

INTRODUCTION

Tuberculosis (TB) is grouped as a second largest infectious disease and a major cause of increased mortality rate mostly in developing countries. There is an increased inclination of the researchers in the field of tuberculosis management. Several organizations including WHO aim to work for the disease management. Priority of researchers is to come out with a validated result for effective management of this deadly disease. Historically tuberculosis was acknowledged by ancient Egyptians but it was the discovery of bacillus by Koch which highlighted the biological and physiological aspect of the disease [1]. Drug development was initially directed by streptomycin antibiotics which proved out to be active against positive agent mycobacterium tuberculosis. Further as a chain reaction following the lead antibiotic drug regimen for tuberculosis was developed which includes Isoniazid (INH), Rifampicin (RIF), Ethambutol(EMB),Pyrazinamide (PZA) .Which is the first line drug for tuberculosis nowadays[2].WHO estimated 8.6 million tuberculosis cases in 2012 and 1.3 million TB deaths. Asia being the largest continent which co exists with HIV and TB infection[3].

Developed country which includes Japan, America and New Zealand has a lower rate of these infectious diseases due to the high standard of living and updated medical facilities. At the global level it has been observed a continuous increment in TB cases in spite of available treatment, the reason pin pointed was the multi drug resistant tuberculosis which is the biggest hurdle in the way to attain a successful therapy of tuberculosis,[4].Nowadays researchers aim to increase and accelerate tuberculosis research and authenticate there outcomes. TB infection has also boomed due to the environmental condition which is occupied largely by the industrial dust and cigarette smoke which ultimately aggravate the situations. Treatment of TB include course of 6 to 9 month and it may be increased depending upon the drug given. Drugs are administered orally but the associated systemic effect which burdens liver and kidney has again attracted researchers to work for the other alternatives routes of delivery system as well as targeting, [5].Whereas the emergence of micro particulate and Nanocarrier delivery system has proved out best to reduce the dose frequency and greatly assisted in the management of disease[6]. It must be reviewed that nanotechnology has fulfilled the task of targeted delivery with use of various polymer and receptors associated with infected organs[7].

Table1: MAJOR PROBLEMS ASSOCIATED WITH THE CURRENTLY AVAILABLE TB TREATMENT

Problem	Details	References
Nonadherence to Treatment	Prolonged treatment duration and complexity lead to nonadherence, causing suboptimal responses, resistance, relapse, and continuous disease spread	[8]
Adverse Events	Adverse effects from anti-TB drugs contribute to nonadherence.	[9]
Multidrug-Resistant and Extensively Drug-Resistant TB	MDR (resistance to rifampin and isoniazid) and XDR (MDR + resistance to fluoroquinolones and aminoglycosides) are on the rise. Resistant TB forms in partially suppressive drug concentrations	[10]
Second-Line Drugs for Drug-Resistant TB	Second-line drugs are not universally available, are less effective, more toxic, and require longer use than first-line drugs.	[11]
Co-infection of TB and HIV	Treatment for both TB and HIV involves a high pill count, drug interactions, overlapping toxicity, and immune reconstitution syndrome.	[12]
Nonadherence in Latent TB Prophylaxis	Isoniazid prophylaxis for latent TB is associated with nonadherence. Attempts to shorten treatment with alternative drugs led to severe adverse events	[13]
Challenges in Current TB Treatment	Pulmonary TB remains prevalent, and current treatments (Rifampicin, Isoniazid, etc.) are prolonged (6 months), leading to side effects, poor adherence, and slow recovery, despite being highly effective.	[14]
Novel Drug Delivery Strategies	Efforts focus on enhancing treatment efficacy by exploring new drug delivery methods and routes to shorten treatment duration and improve compliance. Nanoparticles offer a solution for better bioavailability and decreased dosing frequency.	[15]
Nanoparticles in TB Treatment	Nanoparticles improve drug bioavailability, reduce dosing frequency, and improve adherence, addressing key challenges in TB control.	[16]

Importance of Nanoparticle-Based Anti-TB Drug Delivery	Nanoparticle formulations can potentially reduce treatment duration, decrease dosing frequency, enhance targeted delivery, and improve outcomes while lowering patient default rates.	[17]
Tuftsin-Conjugated Nanoparticles for TB Treatment	The study aims to develop tuftsin-conjugated nanoparticles loaded with Rifampicin for targeted lung macrophage activation, improving drug stability, dispersibility, and controlled release.	[18]
Innovative TB Management Approach	The study focuses on tuftsin-conjugated nanoparticles as a novel solution for lung-targeted delivery, improving the management and effectiveness of tuberculosis treatment.	[19]

PREPARATION & CHARACTERIZATION

For the effective and controlled treatment of tuberculosis high dosages & frequent administration are required for maintaining the drug therapeutic concentration over time [20]. Nanotechnology related drug delivery may improve therapeutic efficacy by minimizing adverse drug effects and also the dosing frequency. Mostly for the formation of nanoparticle as a carrier system various natural polymer such as cellulose, chitin, and chitosan. Poly lactide co-glycolide (PLGA) semi synthetic polymer is frequently used as a polymer in the formulation of drug carrier system because of their excellent biocompatibility and biodegradability. For the preparation of nanoparticles it is important to select an appropriate encapsulation technique by which biological as well as chemical stability of the incorporated drug should be maintained during the processs [21]. The aim of present study was to develop and characterize Tuftsin conjugated PLGA NPs for the delivery of Rifampicin at the site of action i.e. Macrophages. Emulsification solvent evaporation technique is chosen for the preparation of plain &Tuftsin conjugated NPs. Various process parameters were identified & optimized which affect the preparation and performance of carrier system. The prepared NPs were characterized for particle size, particle morphology, surface morphology.

MECHANISM OF TUFTSIN-PLGA CONJUGATE IN TUBERCULOSIS THERAPY

Tuftsin, a naturally occurring peptide (Thr-Lys-Pro-Arg), exhibits a strong affinity for macrophages. When conjugated with PLGA nanoparticles, it enables targeted drug delivery by binding to tuftsin receptors on the macrophage surface. This interaction enhances the intracellular uptake of drug-loaded nanoparticles and facilitates the phagocytosis of Mycobacterium tuberculosis-infected cells. Additionally, tuftsin possesses immunostimulatory properties, activating macrophages and enhancing their antimicrobial response, thereby improving the efficacy of tuberculosis treatment [22].

SYNTHESIS OF TUFTSIN-PLGA CONJUGATE

Initially protection of primary amino groups of tuftsin is needed to prevent self-reaction, which was carried out by using Fmoc (fluorenylmethoxy carbonyl succinimide) to form Fmoc protected tuftsin (Fmoc-Tu), the standard procedure for protection of primary amino groups was followed (Lapatsanis*et al.*, 1983). Briefly tuftsin (1mM) was dissolved in an excess of 10 ml of 9% Na_2CO_3 and was mixed with twice molar ice cold solution of Fmoc in Dimethylformamide (DMF). The mixture was stirred at room temperature for 15 min, diluted ten times and acidified with concentrated HCl to pH 2. The precipitate formed was extracted twice with 6–10 ml portions of ethyl acetate. The pooled ethyl acetate extract was washed three times with 10 ml portions of normal saline. The ethyl acetate portion was dried by using anhydrous sodium sulfate; volume was reduced to minimal quantities using a vacuum evaporator and finally dried in vacuum desiccators to get dry Fmoc-Tu. For the synthesis of amine terminated PLGA, calculated quantity of PLGA was dissolved in 10

mL Dichloromethane (DCM), and Dicyclohexylcarbodiimide (DCC) and N-Hydroxy succinimide (NHS) were added in five molar excess in order to activate free carboxylic group of PLGA. As a result precipitated dicylcohexyl urea was obtained which was removed by filtration, while the excess of NHS and DCC were removed by dialysis against distilled water for 6 h. Further, Ethylenediamine (EDA) was added to the above solution and pH of the mixture was adjusted to about 5.0 by addition of 1 N HCl. This resulted in amine terminated PLGA[23]. Fmoc-Tu was dissolved in a mixture of DCM and DMF (1:1) and solution of PLGA was added to it. Equimolar quantities of DCC and Hydroxybenzotriazole (HoBt) were added to it and the mixture was stirred overnight. The precipitated dicyclohexylurea (DCU) was filtered off and the filtrate containing PLGA conjugated to Fmoc-Tu was concentrated and precipitated by diethyl ether. Finally it was deprotected by treatment with 30% piperidine solution in DMF for 30 seconds to get TuPLGA[24]. The final product was purified by similar solvent precipitation using diethyl ether, followed by washing the precipitate with *n*-hexane to remove the unreacted impurities[25]. The synthesis is illustrated schematically in Fig.1.

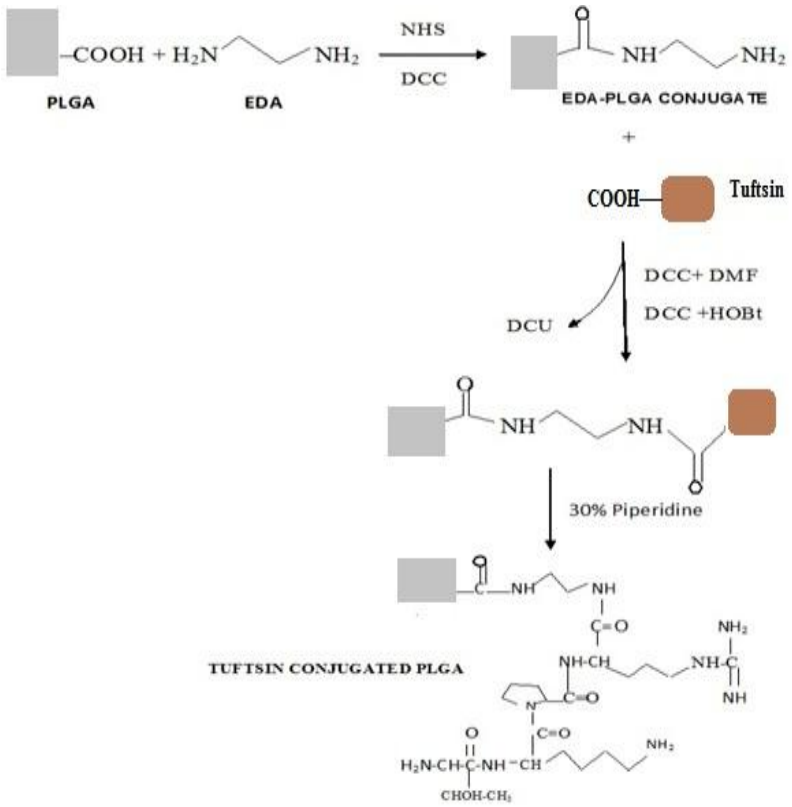

Figure 1: Schematically representation of synthesis of Tuftsin-PLGA Conjugate

CHALLENGES AND FUTURE PERSPECTIVES

The Tuftsin-PLGA conjugate therapy holds significant potential, it faces several challenges, including stability concerns, large-scale manufacturing complexities, and clinical translation hurdles. Further research is essential to refine the formulation, ensure long-term safety, and conduct clinical trials to validate its therapeutic efficacy. Moreover, investigating combination therapies using Tuftsin-PLGA nanoparticles loaded with multiple anti-TB drugs could offer a more comprehensive and effective strategy for tuberculosis treatment[26].

RESULTS AND DISCUSSION

The present study focused on the development and investigation of Tuftsin-conjugated nanoparticles for the targeted delivery of the antitubercular drug Rifampicin.Tuberculosis (TB), a widespread and highly contagious bacterial infection, remains one of the leading causes of mortality globally. Nanotechnology plays a crucial role in the design of drug carrier systems, enhancing therapeutic effectiveness while minimizing adverse effects. Additionally, nanoparticle-based delivery can reduce dosing frequency, thereby improving patient adherence to treatment. Conventional anti-tubercular drugs often fail to effectively eliminate Mycobacterium tuberculosis residing within macrophages.

CONCLUSION:

Thus, there is a pressing need to develop drug delivery systems specifically designed to target these immune cells. This study aimed to formulate a carrier system capable of delivering Rifampicin directly to macrophages while ensuring controlled drug release.

CONFLICT OF INTEREST

None

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REFERENCES

1. Brock, I., Ruhwald, M., Lundgren, B., Westh, H., Mathiesen, L. R., & Ravn, P. (2006) 'Latent tuberculosis in HIV positive, diagnosed by the M. tuberculosis specific interferon-γ test', *Respiratory Research*, 7(56). Available at: https://doi.org/10.1186/1465-9921-7-56
2. Dheda, K., Booth, H., Huggett, J. F., Johnson, M. A., Zumla, A., & Rook, G. A. W. (2005) 'Lung remodeling in pulmonary tuberculosis', *Infectious Diseases*, 192(7), pp. 1201-1209. Available at: https://doi.org/10.1086/444545
3. World Health Organization (2012) *Global tuberculosis report 2012*. Geneva: WHO.
4. Sharma, S. K., Mohan, A., & Kadhiravan, T. (2005) 'HIV-TB co-infection: Epidemiology, diagnosis & management', *Indian Journal of Medical Research*, 121(4), p. 550.
5. World Health Organization (2013) *Global tuberculosis report 2013*. Geneva: WHO.
6. Anisimova, Y. V., Gelperina, S. I., Peloquin, C. A., & Heifets, L. B. (2000) 'Nanoparticles as antituberculosis drug carriers: Effect on activity against Mycobacterium tuberculosis in human monocyte-derived macrophages', *Journal of Nanoparticle Research*, 2(2), pp. 165-171.
7. Mohan, H., Bal, A., Punia, R. P. S., & Bawa, A. S. (2005) 'Granulomatous prostatitis – An infrequent diagnosis', *International Journal of Urology*, 12(5), pp. 429-524. Available at: https://doi.org/10.1111/j.1442-2042.2005.01068.x
8. Munro, S. A., Lewin, S. A., Smith, H. J., Engel, M. E., Fretheim, A., & Volmink, J. (2007) 'Patient adherence to tuberculosis treatment: A systematic review of qualitative research', *PLoS Medicine*, 4(7), e238. Available at: https://doi.org/10.1371/journal.pmed.0040238
9. Tirore, L. L., Nadamo, S. A., Derilo, H. T., Erkalo, D., Sedore, T., Tadesse, T., & Ermias, D. (2021) 'Time to recovery from COVID-19 and its predictors among patients admitted to treatment centers of Southern Nations, Nationalities, and Peoples' Region (SNNPR), Ethiopia: A multi-center retrospective cohort study', *Infection and Drug Resistance*, 14, pp. 3047-3062.
10. World Health Organization (2006) *The world health report 2006: Working together for health*. Geneva: WHO.
11. Shegokar, R., Al Shaal, L., & Mitri, K. (2011) 'Present status of nanoparticle research for the treatment of tuberculosis', *Journal of Pharmacy & Pharmaceutical Sciences*, 14(1), pp. 100-116.
12. Cerrone, M., Bracchi, M., Wasserman, S., Pozniak, A., Meintjes, G., Cohen, K., & Wilkinson, R. J. (2020) 'Safety implications of combined antiretroviral and anti-tuberculosis drugs', *Expert Opinion on Drug Safety*, 19(1), pp. 23-41. Available at: https://doi.org/10.1080/14740338.2020.1694901
13. C Tortajada [1], J Martínez-Lacasa, F Sánchez, A Jiménez-Fuentes, M L De Souza, J F García, J A Martínez, J A Caylà; Tuberculosis Prevention Working Group Int J Tuberc Lung Dis. 2025
14. Tiwari, D., & Martineau, A. R. (2023) 'Inflammation-mediated tissue damage in pulmonary tuberculosis and host-directed therapeutic strategies', *Seminars in Immunology*, 65, 101672. Available at: https://doi.org/10.1016/j.smim.2023.101672
15. Elumalai, K., Srinivasan, S., Shanmugam, A., Dahanayake, M. H., & Jayasundera, A. C. (2024) 'Review of the efficacy of nanoparticle-based drug delivery systems for cancer treatment', *Biomedical Technology*, 5, pp. 109-122.

16. Dahanayake, M. H., & Jayasundera, A. C. (2021) 'Nano-based drug delivery optimization for tuberculosis treatment: A review', *Journal of Microbiological Methods*, 181, 106127.
17. Vieira, C. C., Peltonen, L., Karttunen, A. P., & Ribeiro, A. J. (2024) 'Is it advantageous to use Quality by Design (QbD) to develop nanoparticle-based dosage forms for parenteral drug administration?', *International Journal of Pharmaceutics*, 657, 124163. Available at: https://doi.org/10.1016/j.ijpharm.2024.124163
18. Vieira, A. C., Magalhães, J., Rocha, S., Cardoso, M. S., Santos, S. G., Borges, M., Pinheiro, M., & Reis, S. (2017) 'Targeted macrophage delivery of rifampicin-loaded lipid nanoparticles to improve tuberculosis treatment', *Nanomedicine*, 12(24), pp. 2721-2736.
19. Nabi, B., Rehman, S., Aggarwal, S., Baboota, S., & Ali, J. (2020) 'Nano-based anti-tubercular drug delivery: An emerging paradigm for improved therapeutic intervention', *Drug Delivery and Translational Research*, 10(4), pp. 1111–1121.
20. Astarie-Dequeker, C., Nigou, J., Passemar, C., & Guilhot, C. (2010) 'The role of mycobacterial lipids in host pathogenesis', *Drug Discovery Today: Disease Mechanisms*, 7(1), e33-e41.
21. Johnson, K. A. (1997) 'Preparation of peptide and protein powders for inhalation', *Advanced Drug Delivery Reviews*, 26(1), pp. 3-15.
22. Barnes, P. J. (2008) 'Emerging pharmacotherapies for COPD', *CHEST Journal*, 134(6), pp. 1278-1286.
23. Cheow, W. S., Ng, M. L. L., Kho, K., & Hadinoto, K. (2011) 'Spray-freeze-drying production of thermally sensitive polymeric nanoparticle aggregates for inhaled drug delivery: Effect of freeze-drying adjuvants', *International Journal of Pharmaceutics*, 404(1), pp. 289-300.
24. Fessi, H. P. F. D., Puisieux, F., Devissaguet, J. P., Ammoury, N., & Benita, S. (1989) 'Nanocapsule formation by interfacial polymer deposition following solvent displacement', *International Journal of Pharmaceutics*, 55(1), R1-R4.
25. Chan, H. K., & Li, J. (2015) 'Inhaled antimicrobial chemotherapy for respiratory tract infections: Successes, challenges and the road ahead', *Advanced Drug Delivery Reviews*, 85, v.
26. Dube, D., Agrawal, G. P., & Vyas, S. P. (2012) 'Tuberculosis: From molecular pathogenesis to effective drug carrier design', *Drug Discovery Today*, 17(13), pp. 760-773.