DEVELOPMENT OF DRUG-LOADED NANOPARTICLES SUITABLE FOR INHALATION AS TARGETED TREATMENTS FOR *MYCOBACTERIUM TUBERCULOSIS*

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Introduction

Tuberculosis (TB) is an infectious disease caused mainly by *Mycobacterium tuberculosis* (Mtb) bacteria. It generally affects the lungs and in some cases, other parts of the body. According to the World Health Organisation (WHO), millions of cases of TB are recorded yearly making it the one of the top causes of death worldwide especially for immunocompromised patients. Nowadays, there is a rise of multi-drug resistant TB (MDR-TB), whereby patients become resistant to first-line therapy. One of the major causes of MDR-TB cases is patient non-compliance to medications, due to the lengthy dosage regimens and side effects. The ultimate goal of this project is to develop a targeted therapy for tuberculosis, using inhalable Poly-Lactide-co-Glycolide (PLGA) nanoparticles (NPs) to overcome the issue of MDR-TB. PLGA NPs are one of the most clinically developed and FDA approved nanosystems. By loading anti-tubercular drugs (ATD) into PLGA NPs, the goal is to target drug delivery to the lungs via inhalation and enhance uptake by alveolar macrophages (AM) which are the host cells for *Mtb*. This targeted approach would reduce dosage regimens, toxicity and enhance efficacy, thus providing better prognosis for patients, and reducing the incidence rate of MDR-TB.

Materials and Methods

- PLGA NPs loaded with a fluourescent dye (Rhodamine B) were formulated in three different sizes 200, 300 and 500 nm approximately, by varying drug to polymer ratio (1:100, 1:10 and 1:50 (w/w%)), the organic phase volume and the stirring speed.
- The cellular uptake of the Rhodamine B PLGA NP was investigated using THP-1 derived macrophages, as an *in vitro* model of AMs, and was confirmed by confocal microscopy using Hoechst 33342 and CellMask green dyes to stain nucleus and cytoplasm, respectively.
- Then, Rifampicin (RIF), as a model TB drug, was loaded into PLGA NPs using nanoprecipitation method.

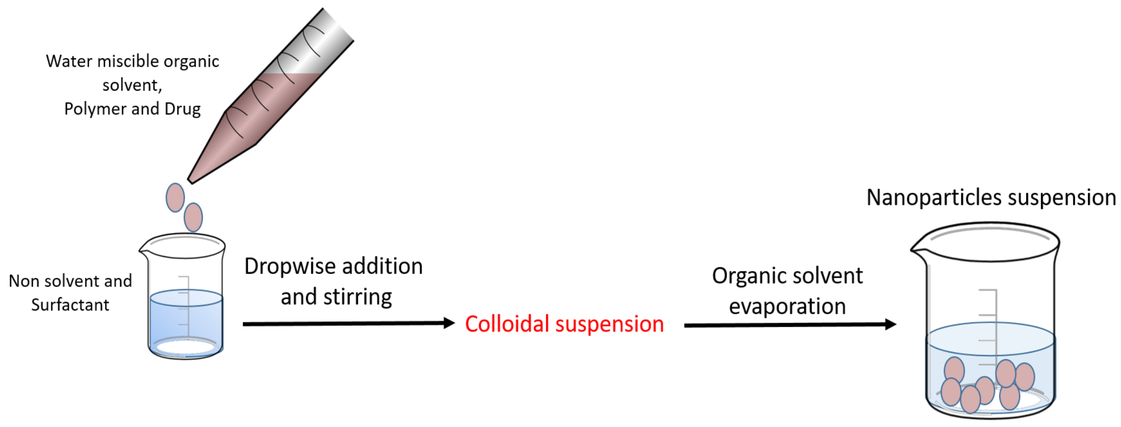

Figure 1: Nanoparticles manufacturing using nanoprecipitation method

Formulation and characterization of Rhodamine B labelled PLGA NPs

For Rhodamine B loaded PLGA NPs, a significant effect was seen on the particle size and PDI of the NPs when process parameters were altered including the organic phase volume, stirring speed and the dye content amount. Our results confirm the successful formulation of Rhodamine B-loaded PLGA NPs of sizes circa 200, 300 and 500nm for 1:100, 1:10 and 1:50 dye to polymer ratio (w/w%), respectively.

Table 1: Effect of drug to polymer ratio (w/w) on the physicochemical properties of Rhodamine B loaded PLGA nanoparticles using nanoprecipitation method. The average hydrodynamic size, polydispersity index (PDI) and zeta potential of PLGA NPs were measured by the Nanosizer ZS90 (Malvern, UK) using dynamic light scattering and electrophoretic mobility. Data shown as mean ± SD (n = 3).

Rhodamine B: PLGA Ratio (w/w)	Size (nm)	PDI	Zeta potential (mV)
Blank NPs	175.6 ±3.41	0.057 ±0.005	-3.02 ±0.98
1:10	283.5 ±5.18	0.053 ±0.021	- 1.28 ±0.28
1:50	485.7 ±122	0.451 ±0.11	- 2.85 ±0.11
1:100	201.1 ±2.30	0.035 ±0.02	- 3.81 ±0.17

Effect of nanoparticle size on macrophages cellular uptake

Extensive cellular uptake of all Rhodamine B-loaded PLGA NPs was evident in THP-1 derived macrophages with significant distribution within the cytoplasm for NPs 200-500nm.

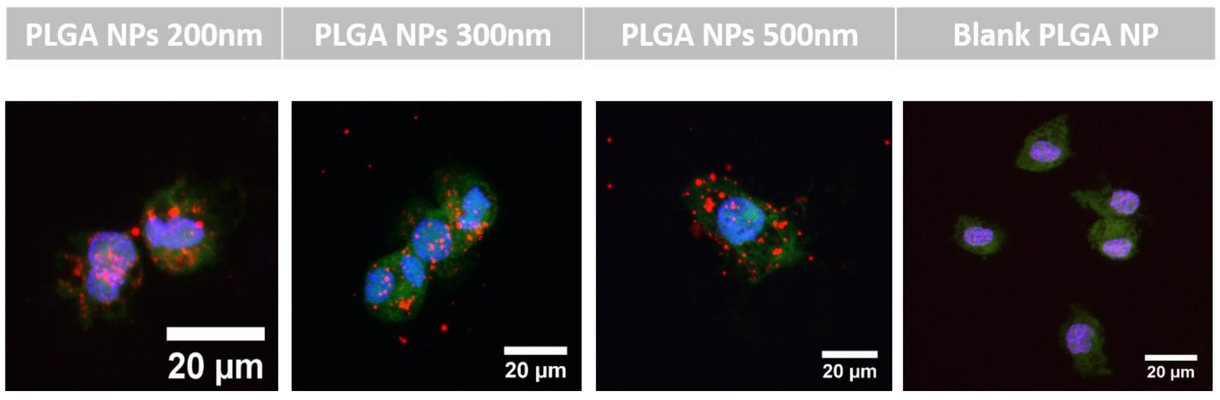

Figure 2: Representative images of the interaction of PLGA nanoparticles (NP) with differentiated THP-1 cells following PLGA-RhodamineNP treatment for 24 hrs. THP-1 derived macrophages were treated with 200nm, 300nm and 500 nm PLGA-Rhodamine B NPs (red) and viewed using confocal microscopy. Hoechst (blue), CellMask™ (green) were added to allow visualisation of the nucleus and cytoplasm respectively

Formulation and characterization of rifampicin (Rif) loaded PLGA NPs

RIF loaded PLGA NPs with a particle size <200nm were prepared. Polydispersity index (PDI) was consistent between batches and <0.2 using different purification methods. Zeta potential was almost neutral for all formulations (-0.8 - -2.1 mV). The encapsulation efficiency (EE) of RIF, however, within the all prepared NPs was very low and current work seeks to enhance this using alternative manufacturing methods.

Table 2: Effect of PLGA nanoparticles purification method on the physicochemical properties of Rfiampicin loaded PLGA nanoparticles using nanoprecipitation method. PLGA NPs were loaded with 1:10 drug to polymer ratio (w/w). The average hydrodynamic size, polydispersity index (PDI) and zeta potential of PLGA NPs were measured by the Nanosizer ZS90 (Malvern, UK) using dynamic light scattering and electrophoretic mobility. Data shown as mean ± SD (n = 2).

Formulation – purification method	Size (nm)	PDI	Zeta potential (mV)	EE%
PLGA-RIF – dialysis	159.6 ± 4.038	0.056 ± 0.016	- 2.47 ± 0.51	3.2
PLGA-RIF- centrifugation	196.0 ± 6.121	0.038 ± 0.021	- 1.63 ± 0.283	2.3
PLGA-RIF- ultrafiltration	158.4 ± 1.664	0.091 ± 0.013	- 3.94 ± 1.15	0.7

Conclusion and Future Perspective

- PLGA NPs within the size range 200-500nm demonstrated effective cellular uptake in alveolar macrophages.
- Data obtained from confocal microcopy indicates no difference in the uptake among different sized particles.
- Further quantitative analysis, using techniques such as high content cell analysis currently underway will better determine the correlation between the size of NPs and cellular uptake.
- Alternative manufacturing methods including double-emulsion solvent evaporation (DESE), microfluidics and supercritical fluid-based approaches are being explored to increase drug encapsulation efficiency and scale-up the process.

References

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