Tuberculosis (TB) is an infectious disease caused mainly by Mycobacterium tuberculosis (Mt) 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 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 Mt. 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 fluorescent 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.

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.

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.

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.

Conclusion and Future Perspective

- PLGA NPs within the size range 200-500nm demonstrated effective cellular uptake in alveolar macrophages.
- Data obtained from confocal microscopy 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.

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References


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