Inhalable ATRA-loaded Nanoparticles as Targeted Host-directed Immunotherapy for Tuberculosis

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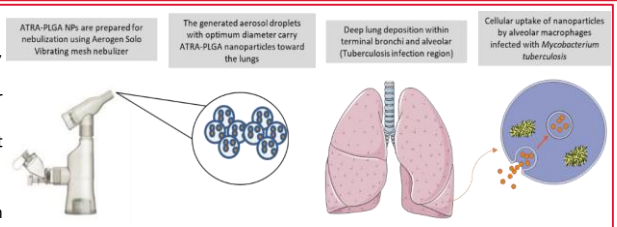
Background

- Tuberculosis (TB) is the top bacterial infectious disease killer and disruption of essential TB services, due to the Covid-19 pandemic, has led to a sharp increase in cases and deaths in 2021
- The emergence of strains of multiple drug-resistant tuberculosis (MDR-TB) strains has pushed our available stock of anti-TB agents to the limit of effectiveness.
- An adjunctive, host-directed therapy (HDT) such as all trans retinoic acid (ATRA) designed to boost the host immune response to kill the bacteria could help address this issue.

Hypothesis

Delivering ATRA-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles (ATRA-PLGA NPs) via inhalation could reduce mycobacterial growth and provide a means of cellular level targeting to the alveolar macrophages, the TB host cells.



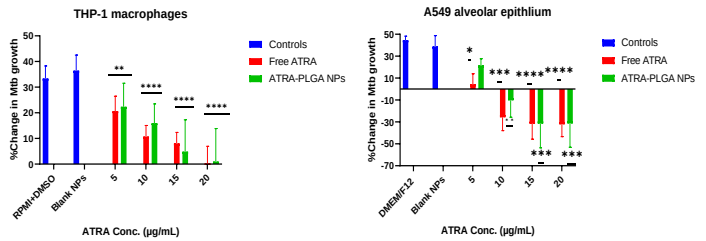
Graphical abstract: ATRA-PLGA nanoparticles are nebulized using Aerogen Solo vibrating mesh nebulizer to target alveolar macrophages which are the niche of *Mycobacterium tuberculosis* in the lung

1. Successful development of a scalable manufacturing protocol of ATRA-PLGA NPs with desired physicochemical characteristics

Manufacturing method	Size (nm)	Polydispersity Index (PDI)	Surface charge (mV)	Encapsulation Efficiency%
Bench Scale (nanoprecipitation)	251.6±9.7	0.187±0.030	-1.80 ±0.410	69.8±12.4
Microfluidics (Nanoassemblr Ignite)	260.8±9.49	0.187±0.011	- 1.90±0.620	76.4±5.4

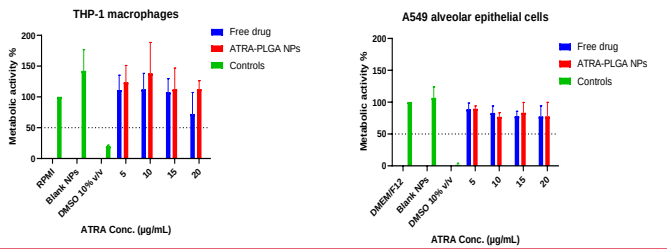
2. ATRA-PLGA NPs showed a dose response effect in reducing bacterial growth in macrophages and alveolar epithelial TB *in vitro* infection models

ATRA treatment arrests growth of Mtb (H37Ra) after 5 days of treatment in infected THP-1 derived macrophages and A549 alveolar epithelial cells. Efficacy was assessed by monitoring the change in bacterial growth (%), using the BacT/ Alert® 3D system (n=3). Statistical analysis was done using two-way ANOVA with Tukey's post-hoc test comparing treatment groups to RPMI+DMSO and Blank NPs groups as reference.

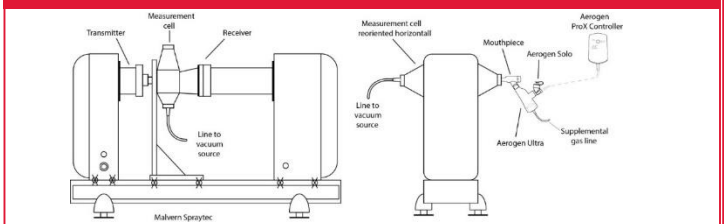


3. ATRA treatments are not associated with toxicity to airway cells *in vitro*

Cell viability of THP-1 macrophages and A549 alveolar epithelial cells was determined by MTS assay 72 hrs post-treatment. Toxicity studies were carried out in the absence of Mtb infection (H37Ra). Dotted lines represents 50% viability as cut-off toxicity value. Results were plotted as (%) metabolic activity relative to cell media group (n = 3) and using DMSO 10% as + ve control.



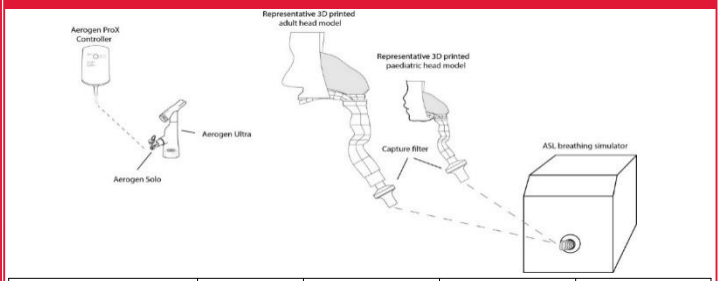
4. Nebulized ATRA-PLGA NPs demonstrated an optimum droplet size required for aerosol deposition in the lungs (1-5 µm)



Aerodynamic characteristics of nebulized droplets of ATRA-PLGA nanoparticles aerosolized using Aerogen Solo vibrating mesh nebulizer were measured by laser diffraction via Malvern Spraytec with inhalation cell.

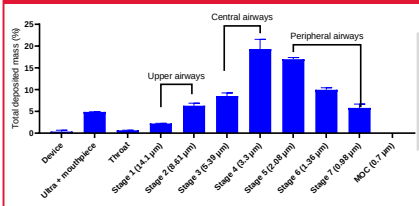
Dv10 (µm)	DV50 (VMD) (µm)	Dv90 (µm)	FPF (%) <5µm	Flow rate (mL/min)
0.78 ± 0.06	3.00 ±0.18	8.33 ± 0.26	71.78 ± 1.85	0.27 ± 0.04

5. High ATRA-PLGA NPs dose delivered at the level of trachea through 3D printed head models under simulated adult and paediatric breathing profile



Model	Tidal volume (mL)	Inhalation to exhalation ratio	Breathing rate (Breaths per minute)	Tracheal dose (%) of nominal dose
Adult	500	1:1	15	47.05 ± 3
Paediatric	300	1:2	20	20.15 ± 3.46

6. MMAD of nebulised ATRA-PLGA NPs of 2.13 µm as measured by cascade impaction



~ Half of the delivered dose deposited in the stages of the impactor equivalent to the terminal bronchi and alveolar region (TB-infection area)

MMAD: mass median aerodynamic diameter; GSD: geometric standard deviation; FPF_{ND}: fine particle fraction of nominal dose; FPF_{ED}: fine particle fraction of emitted dose.

MMAD (µm)	GSD	FPF_{ND} (%)	FPF_{ED} (%)	Mass Balance (%)
2.13 ± 0.06	1.93 ± 0.04	58.59 ± 6.29	81.81 ± 1.162	88.5

7. Conclusions and future perspective

- **Scalable and reproducible manufacturing** protocol for ATRA-PLGA NPs using Nanoassemblr Ignite microfluidics system developed
- Demonstrated the ***in vitro* efficacy and cell biocompatibility** of ATRA-PLGA-NPs in macrophages and alveolar epithelial cells
- Successful integration of the ATRA nanoformulation with Aerogen Solo® nebulizer to enable **efficient delivery to the site of TB infection** in the lungs
- Efficient delivery of the formulation under simulated normal adult and paediatric breathing patterns and through 3D printed head models
- Future work will include the assessment of efficacy and toxicity of ATRA-PLGA NPs in a clinically relevant ***in vivo* model**

References:
1) Bahlool, AZ *et al*, (2022), *Current Research in Immunology* 3: 54-72
2) Bahlool, AZ *et al*, (2022), *Pharmaceutics*, *14*(8), 1745

Acknowledgment:
RCSI Strategic Academic Recruitment (StAR) PhD program
DDL Career development grant 2022

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