

In vitro activity of inhalable microparticles containing anti-TB drugs and an efflux pump inhibitor against *Mycobacteria* infections

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Mycobacteria Infections



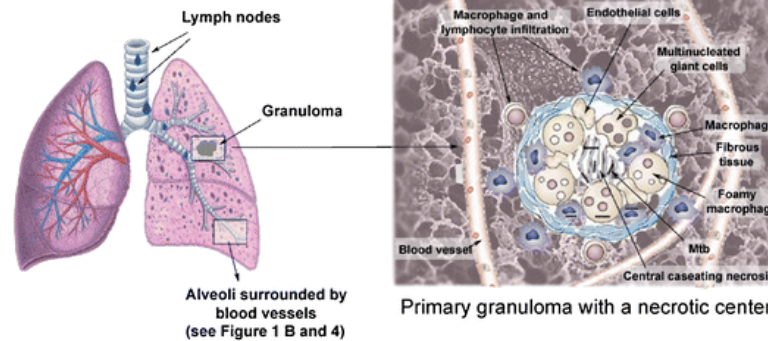
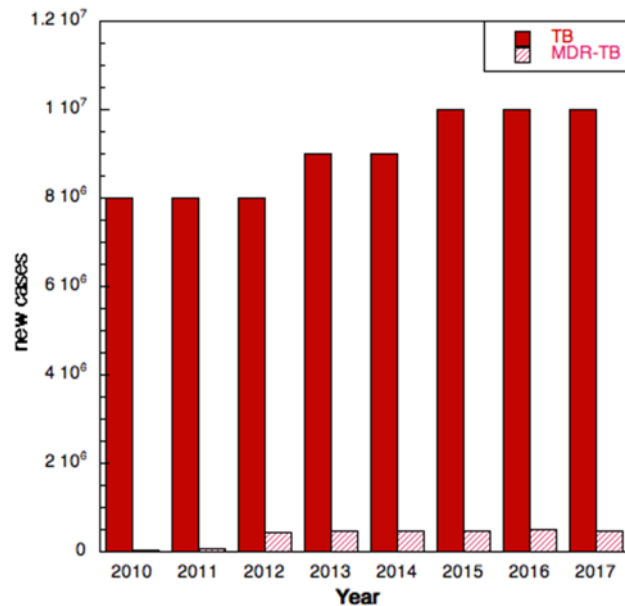
TB Epidemiology

TB Pathogenesis

NTM

Mycobacterium tuberculosis

Nontuberculous *mycobacteria*

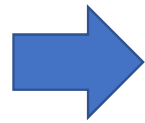


Muttil et al. Pharm. Res. 2009; 26(11): pp2401-2416

- Over 150 different species
- Great deal of attention in many geographical regions
- Cause of superinfections in immunocompromised subjects
- Mode transmission not defined
- Pulmonary infection

Conventional Therapy & **RESISTANT STRAINS**

First-line Drugs



MDR
XDR



New Molecules



Alternative/Combined
Drug Delivery

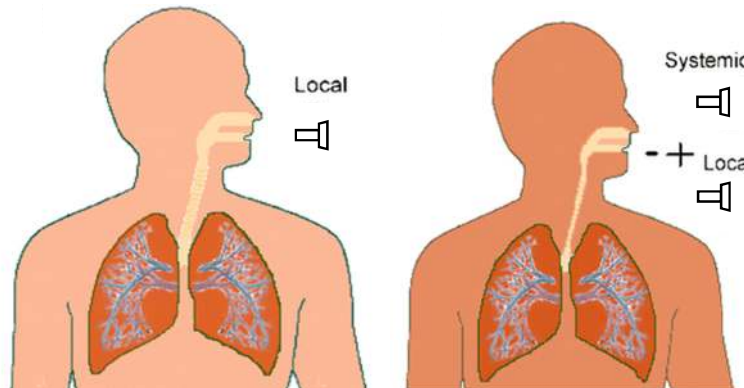
High Dose Delivery

Targeted
delivery to
AMs

Extended release



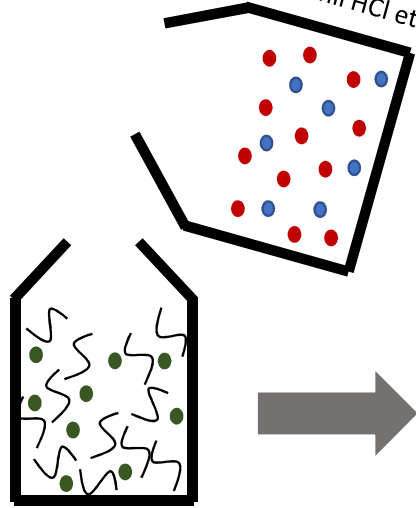
Second-line Drugs



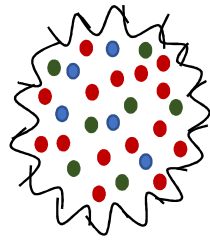
Muttill et al. Pharm. Res. 2009; 26(11): pp2401-2416

Sodium Hyaluronate Nanocomposite Microparticles

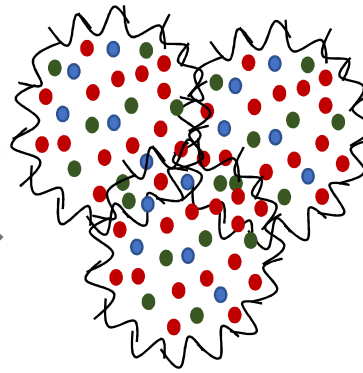
Rifampicin + Verapamil HCl ethanol solution



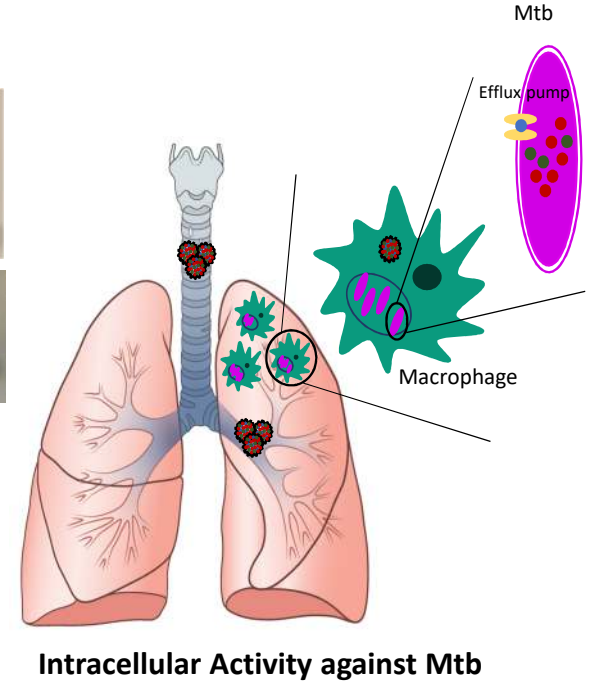
HYA LMW + Isoniazid water solution



480 nm
Nanoparticle



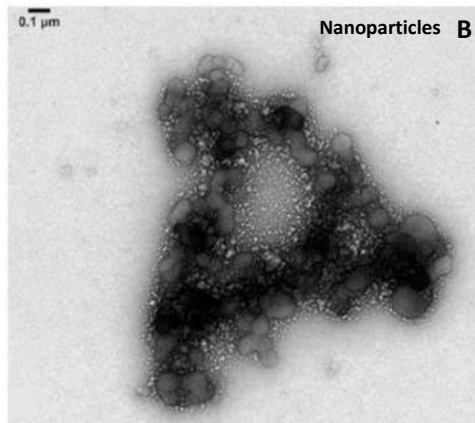
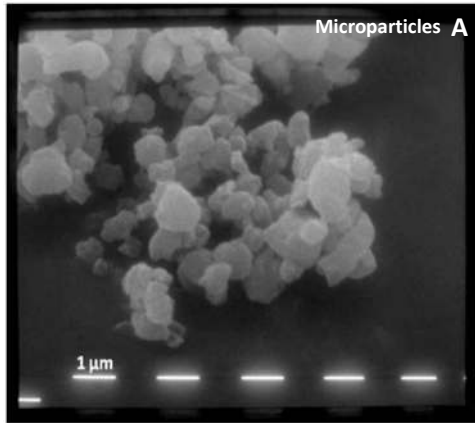
1 μm
Nanocomposite microparticles



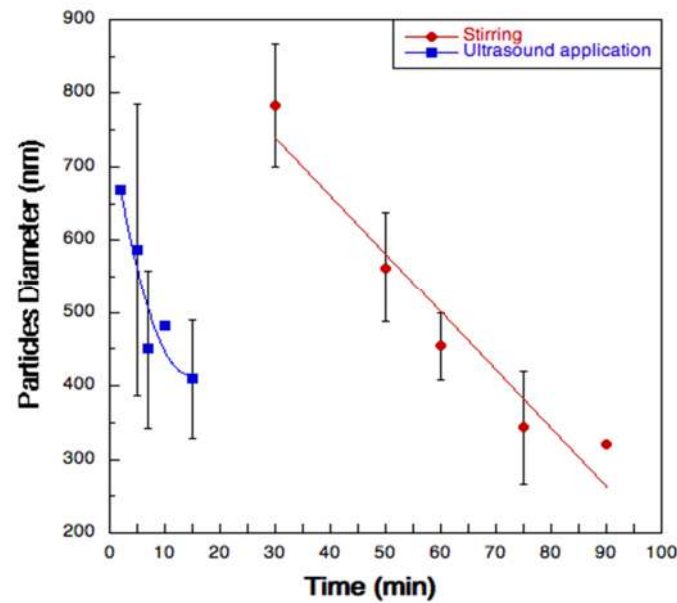
Intracellular Activity against Mtb

HYA LMW	35%
Isoniazid	18%
Rifampicin	35%
Verapamil HCl	12%

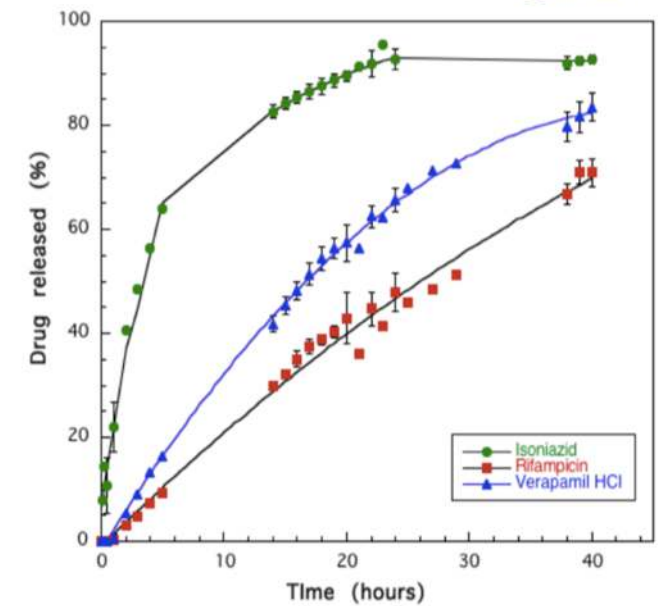
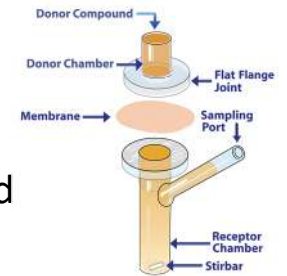
Morphology, Redispersion & *in vitro* Powder Dissolution

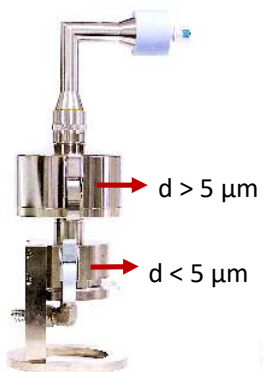


Microparticles de-aggregation to re-generate starting nanoparticles

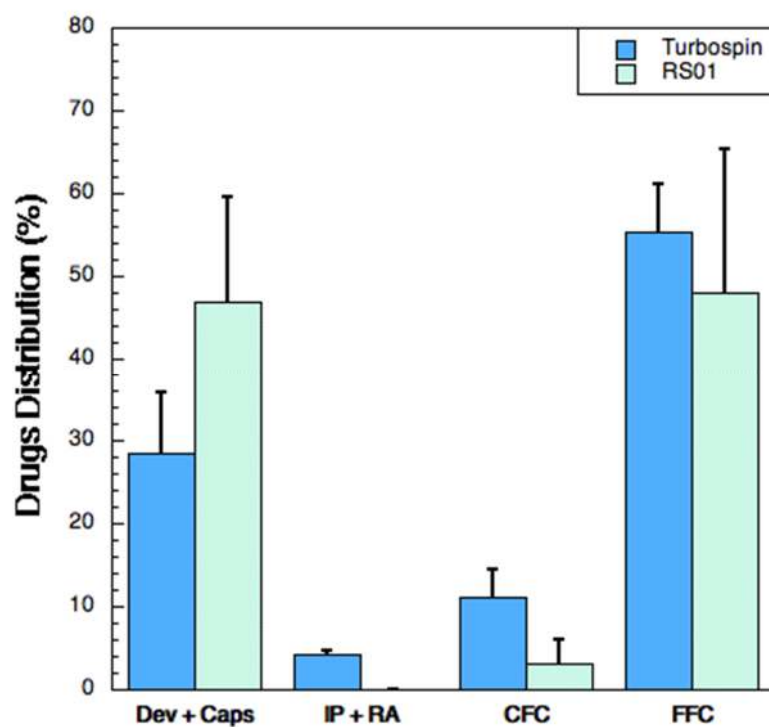


Drugs released





In vitro Aerodynamic Performance & Stability

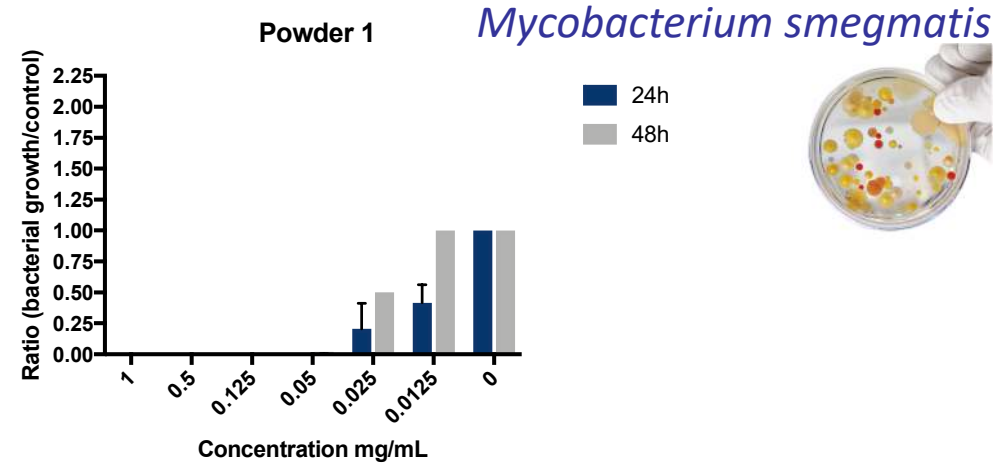
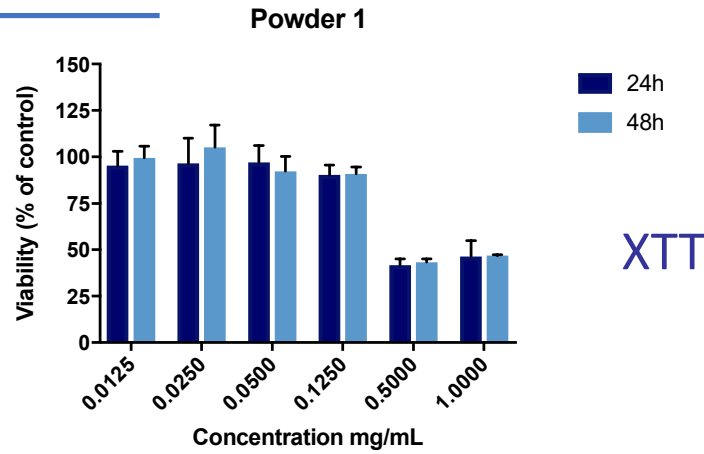


	Emitted Fraction* (%)			Fine Particle Fraction* (%)		
	T ₀	1month /T amb	24h /50°C	T ₀	1month /T amb	24h /50°C
Isoniazid	50.73 (17.20)	61.99 (5.92)	58.75 (1.96)	90.62 (8.15)	92.34 (1.28)	87.95 (0.57)
Rifampicin	48.96 (15.78)	58.56 (6.07)	55.81 (3.11)	93.55 (5.69)	90.81 (1.21)	83.62 (0.83)
Verapamil	51.90 (17.70)	62.83 (5.35)	59.54 (4.24)	93.89 (5.72)	92.26 (0.85)	88.81 (1.33)

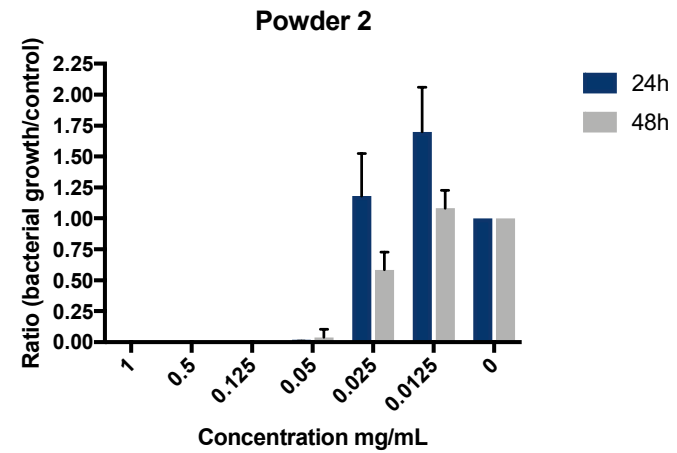
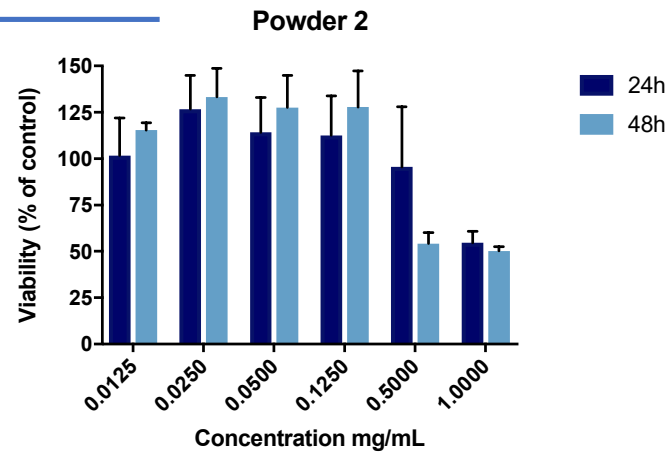
* RS01

In vitro cytotoxicity & Bactericidal Activity

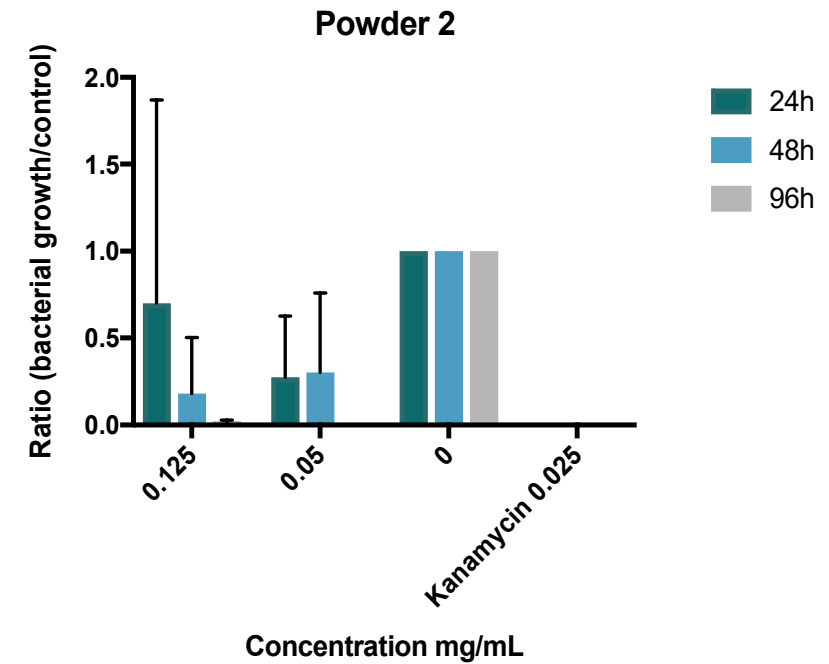
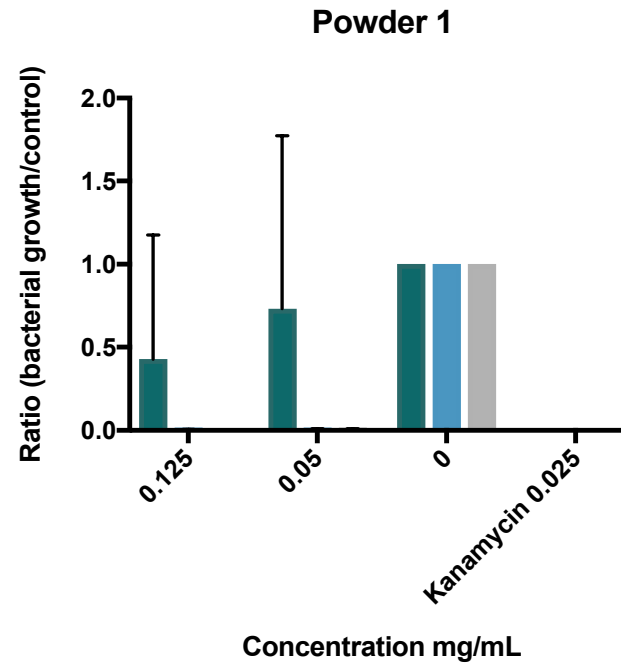
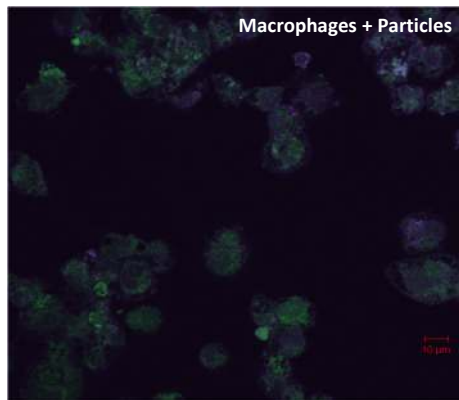
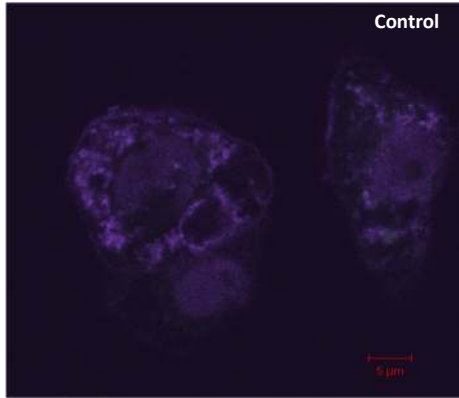
HYA LMW
Rifampicin
Isoniazid
Verapamil



HYA LMW
Rifampicin
Isoniazid



In vitro Macrophages Infection and Treatment



Conclusions

- Although it is a preliminary stage of development, this powder is very promising as candidate for effective host-targeted therapy
- HYA LMW 1 micron-sized aggregates of nanoparticles loaded with two different first-line TB antibiotics and one MB and AMs efflux pump inhibitor have been successfully developed
- The highly respirable dried powder is able to de-aggregate into nanoparticles having the same size of the starting NP, that release drugs in a period of time compatible with complete AMs uptake
- This powder (Powder 1) reports a greater ability to inhibit *Mycobacterium smegmatis* growth at lower concentrations compared to a similar powder formulated without Verapamil
- Although it is slightly more toxic at higher concentration, Powder 1 shows a superior bactericidal activity even at sub-MIC rifampicin concentration (powder concentration = 0.05 mg/mL) after 48 h of incubation



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