

Three-dimensional culture systems for mechanistic insights in Tuberculosis

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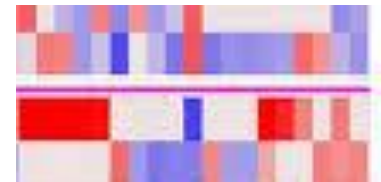
Tuberculosis – an overview

- Caused by *Mycobacterium tuberculosis*
- Responsible for the death of around 1.5 million people in 2020 worldwide
- India – the country with highest burden of TB
- Growing problem of multidrug resistant TB worldwide
- Severe need to understand host-pathogen interactions and develop new treatments

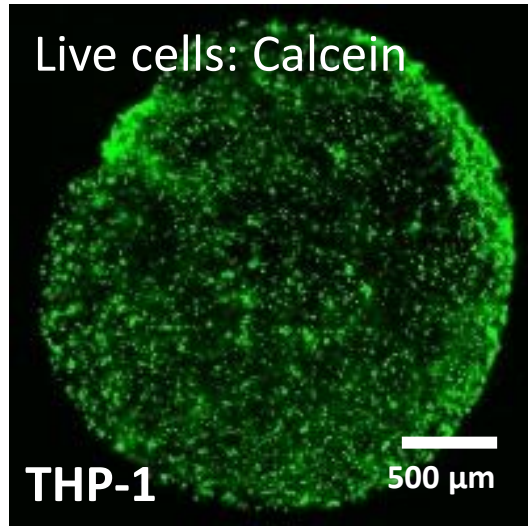
*Global Tuberculosis Report, 2021

Current culture systems are not representative

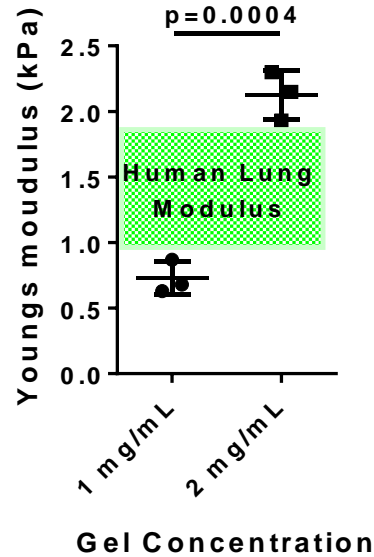
- **2D vs 3D:** current 2D cultures are not representative. Lack of dimensionality and extra-cellular matrix
- **High stiffness:** Lungs have low modulus ($\sim 1-10\text{kPa}$) compared to 2D culture plates ($\sim \text{GPa}$)
- **Short time duration:** Only allow investigation for a few days
- **High variances in gene expression:** Gene expression seen is not representative of human infection
- **Unreliable drug efficacy:** Pyrazinamide, a frontline TB drug does not show efficacy in any *in vitro* culture system



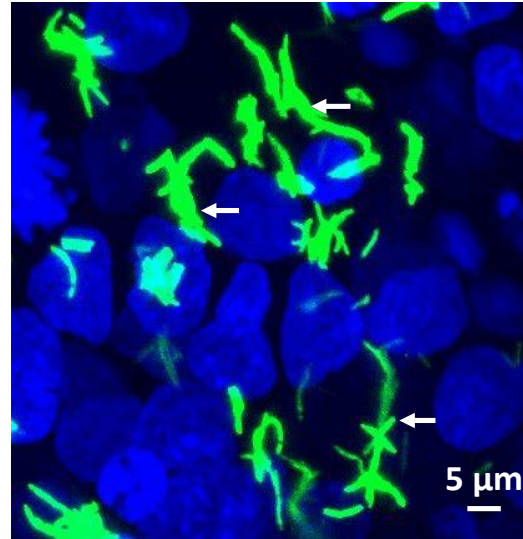
3D system mimics *in vivo* phenotypes



3D Hydrogel culture of mammalian cells

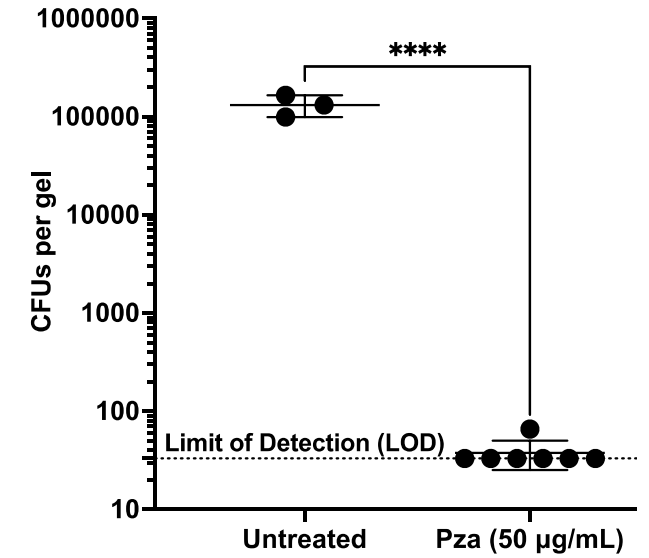


Tunable mechanical properties



Cord formation

Mimics *in vivo* phenotype



Faithful drug efficacy

- We have previously developed a 3D hydrogel based culture system (unpublished work) that overcomes several shortcomings of current culture systems
- In this project, we plan to build upon this platform, screen drug libraries and provide mechanistic insights on host-pathogen interactions such as drug tolerance, bacterial persistence, gene and metabolomic associations etc.

Learnings and major techniques

Students working on this project will develop following expertise:

- Problem solving ability and time management!
- Engineering materials for biological applications
- Designing and engineering organs and microfabrication
- Mammalian and bacterial cell culture
- Understanding of bacterial persistence, antibiotic tolerance, genetic and metabolic association
- Microscopic techniques, histology and cryo-sectioning
- Working with clinical samples and in biosafety level 3 facilities

Further reading

- Mishra R, Targeting redox heterogeneity to counteract drug tolerance in replicating *Mycobacterium tuberculosis*. *Science Translational Medicine* 11 eaaw6635 (2019)
- Mishra R, Heterogeneous Host–Pathogen Encounters Coordinate Antibiotic Resilience in *Mycobacterium tuberculosis*. *Trends in Microbiology*. S0946-842 (2020)
- Tezera LB, Dissection of the host-pathogen interaction in human tuberculosis using a bioengineered 3-dimensional model. *Elife* 6, (2017)

For more details, please visit:

<https://be.iisc.ac.in/~rachit/index.html>

<http://cidr.iisc.ac.in/amit/>