In vitro granuloma models of tuberculosis: potential and challenges

Paul Elkington[1*], Maria Lerm[2], Nidhi Kapoor[3], Robert Mahon[4], Elsje Pienaar[5], Dongeun Huh[6], Deepak Kaushal[7], Larry S Schlesinger[8*]

[1]NIHR Biomedical Research Centre, Faculty of Medicine, University of Southampton, UK.

[2]Department of Clinical and Experimental Medicine, Faculty of Medicine and Health Sciences, Linköping University, Linköping, SE-581 85, Sweden

[3]Translational Research Institute for Metabolism and Diabetes, Florida Hospital-Adventist Health System, Orlando, FL, United States

[4]Division of AIDS, Columbus Technologies, Inc. Contractor to National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

[5]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, United States

[6]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, United States

[7] Tulane Center for Tuberculosis Research, Covington, LA, United States

[8] Texas Biomedical Research Institute, San Antonio, TX, United States

Summary: This review summarizes the arguments for developing more advanced human tuberculosis granuloma cell culture models and critically reviews those available to date. We discuss the utility of complementary approaches such as organoids and mathematical modelling and future challenges and opportunities.

*** Address for correspondence:**

Paul Elkington
Clinical and Experimental Sciences
University of Southampton
Southampton SO16 1YD
UK
p.elkington@soton.ac.uk

Larry Schlesinger
Texas Biomedical Research Institute
P.O. Box 760549
San Antonio,
TX 78245-0549
LSchlesinger@txbiomed.org

Running title: Modelling the tuberculosis granuloma

Word count: 3816 (see cover letter). Abstract: 199

Abstract

Despite intensive research efforts, several fundamental disease processes for tuberculosis (TB) remain poorly understood. A central enigma is that the host immune response is necessary to control disease, and yet is also essential for promoting persistence and transmission by causing lung immunopathology. Our inability to distinguish these processes makes it challenging to design rational novel interventions, such as vaccines or host-directed therapies that accelerate bacterial clearance, without augmenting tissue damage. Elucidating basic immune mechanisms is likely to require both *in vivo* and *in vitro* analyses, since *Mycobacterium tuberculosis* is a highly specialized human pathogen. The classic immune response is the TB granuloma organized in three dimensions within extracellular matrix. Several groups are developing advanced cell culture models reported to recapitulate granulomas. In January 2018, NIAID convened a workshop, entitled "3-D Human *in vitro* TB Granuloma Model" to advance the field by bringing together investigators involved in granuloma models as well as bioengineers and mathematical modelers. Here, we summarize the arguments for developing more advanced TB cell culture models and critically review those available to date. We discuss how integrating complementary approaches, such as organoids and mathematical modelling, can maximize their utility, and conclude by discussing future challenges and opportunities.

Key words: Granuloma, tuberculosis, tissue culture models

Why advanced cell culture models may be required?

Mycobacterium tuberculosis (Mtb) kills more people than any other single infectious disease and new interventions to control the ongoing pandemic are urgently required [1]. From the FDA and other regulatory agencies' perspective, this necessitates preclinical data that reliably predicts efficacy in humans. A fundamental challenge for the field is the complexity of the host-pathogen interaction, particularly within multicellular tissue granulomas [2]. Granulomas are organized cellular aggregates containing multiple cells, primarily with a central core of mature macrophages, surrounded by T cells, B cells and fibroblasts, and are proposed to form in response to a persistent stimulus. Although granulomas have been well described since the invention of the microscope, mechanisms that regulate cellular dynamics, behavior and maintenance are only recently being fully understood. For example, while the granuloma has traditionally thought to be necessary to limit infection, more recent data from the zebrafish model suggest that it may facilitate mycobacterial proliferation and dissemination [2] and the molecular determinants of macrophage reprogramming are only recently emerging [3]. Specific cellular events typical of TB granulomas, such as the formation of multinucleate giant cells or development of central necrotic caseation, are incompletely understood.

In this regard, development of more advanced *in vitro* systems to study human TB granulomas may be worthwhile, but conversely it could be argued that human Mtb infection is so prolonged and complex that only *in vivo* models can provide meaningful results [4]. Herein we first outline potential benefits of *in vitro* granuloma models (Table 1). We discuss what an optimal model may include, and how to resolve the inherent tension between complexity, tractability and throughput. We summarize recent progress and review how parallel developments in organ-on-a-chip systems and mathematical modelling may feed into the field. We address the need for validation of *in vitro*

models with *in vivo*-derived information. Finally, we conclude by suggesting a roadmap for potential future developments and outcomes.

Potential benefits of a human model

A fundamental issue within the TB field is our failure to fully understand the determinants of control versus disease progression. Cell culture systems permit mechanistic and dynamic investigations that are not possible in patients and may be challenging in animal models. Mtb is predominantly a human pathogen, so infection of another host makes assumptions that fundamental biological pathways are common to both. Mtb and humans have undergone a prolonged co-evolution for approximately 70,000 years [5], thus human systems are essential to confirm or refute findings in other model systems. Therefore, an advanced human TB cell culture model that recapitulates core features of human infection would permit dissection of relevant basic biological processes and also the testing of new diagnostic, therapeutic and vaccine interventions. An early model was developed by Altare [6], which was used to investigate specific aspects of host immunity to Mtb [7, 8], and helped inform the more recently developed models.

For an optimal model we propose that the pathogen should be virulent Mtb, since although *Mycobacterium bovis* has 99.95% genetic homology to Mtb, it does not cause the same disease [9]. Primary human cells should be used since cell lines or cells from other species do not reliably predict responses of primary human cells. The composition should include mononuclear phagocytes and lymphocytes as they constitute the prominent cell types of TB granulomas, as well as fibroblasts and epithelial cells [2]. The model should permit 2- or, preferably, 3-Dimensional (2-D, 3-D) organization, since the human immune response is spatially organized in 3-D [10], and incorporate physiological extracellular matrix, since this regulates host cell survival and Mtb growth [11, 12]. Longer-term experiments are preferred, since the host-pathogen interaction is prolonged [4]. Current models

already permit longer experiments than standard cultures (up to 4 weeks), but are arguably still relatively short compared to human infection and should be extended as feasible. Furthermore, an ideal system would permit the continued addition of immune cells, since granulomas are dynamic environments with the recruitment of new cells [13]. Readouts should be multi-parameter, incorporating both host and pathogen physiology, since any single intervention will likely have multiple reciprocal effects on host and pathogen. The approach is ideally modular, so that individual components or factors can be varied within the system to test specific hypotheses. Finally, it should be possible to modulate the environment over time, since conditions change dynamically, e.g., reduced oxygen tension and altered drug pharmacokinetics [14, 15], and the model should allow for interventions of different conditions and resultant analyses.

However, there is no point in adding complexity to a model system without a specific goal, therefore an equally pertinent question is what is the minimal composition of a model system to permit investigation of fundamental disease mechanisms that are predictive of the human response. Organoid models are teaching us that it may not be necessary to incorporate all elements to obtain results that recapitulate the *in vivo* situation [16]. Increasing complexity may detract from the tractability, with a balance ranging from genuine high throughput to very intricate but low replicate numbers. Equally relevant to defining the minimum requirement of a model system is the determination of what approach is appropriate for *in vivo* validation. Finally, each model system should allow for integration of co-morbidities, e.g., HIV, diabetes and cigarette smoke exposure, as well as assessment of responses in the very young and old [1]. However, an open question is when to introduce these variables into the model.

Cell culture systems of TB granulomas developed to date

Collagen matrix model of Mtb dormancy

The 3-D model developed by Kapoor et al. uses human peripheral blood mononuclear cells (PBMCs) in an extracellular matrix to form spatio-temporal 3-D structures and microscopic granulomatous aggregates in response to virulent Mtb [17]. This model demonstrates features of human TB, e.g., multinucleated giant cell formation, increase in $CD4^+CD25^+$ T cells, decrease in activated macrophages and increase in cytokine and chemokine secretion by immune cells in response to Mtb (Figure 1). Critically, the model demonstrates development of Mtb latency and reactivation upon immune suppression caused by anti-TNF-α treatment.

Therefore, this model can be used to understand the host-pathogen interaction during latency and resuscitation since it displays fundamental characteristics of latency, such as a non-replicating state [18], development of resistance to rifampicin, loss of acid-fastness and accumulation of lipid bodies [19, 20]. Consequently, it has potential to identify agents active against dormant bacilli, a key consideration in the development of short course regimes [1]. Limitations of the model include relatively low throughput, technical challenges of adding further cells to permit modelling dynamics over time and the requirement of collagenase to release cells from the collagen matrix for downstream analyses.

Multicellular lung tissue model

Lerm and colleagues introduced Mtb into an existing human *in vitro* lung tissue model in order to study early granuloma formation [21]. A collagen matrix supported by a filter membrane forms a scaffold for a human fibroblast cell line, which grows and differentiates before addition of primary macrophages/monocytes and a human epithelial cell line (Figure 2). After the cells have formed a tissue, the apical side is exposed to air, which causes the epithelial cells to secrete mucus. This organotypic mucosa is representative of lung tissue, both in anatomical and functional aspects [22].

To achieve an Mtb infection representative of human TB, macrophages carrying the bacilli are introduced as “trojan horses”. Monocytes, which easily migrate in the model [21], cluster at the infected macrophages to form granuloma-like structures. To avoid artefacts due to physical sectioning of the structures for interrogation, cryosectioning can be replaced by optical sectioning using confocal microscopy, which also has the advantage that a microtome is not needed in the BSL3 facility [23]. The relevance of using such a biomimetic human tissue model has been emphasized [24]. This model has allowed the group to validate observations made in animal models.

Granulomas are highly dynamic rather than static structures [25] and one important question raised is whether granulomas restrict or promote Mtb growth [2]. A recent study in zebrafish demonstrated that *M. marinum* hi-jacks newly recruited macrophages to disseminate in the tissue [2]. This observation could be confirmed with Mtb in the human lung tissue model, showing that Mtb uses its virulence factors to trigger granuloma formation, pointing towards a bacterial advantage [21]. Further, matrix metalloproteinases (MMPs) secreted by host cells in infected tissue as a consequence of mycobacterial virulence factors can promote granuloma formation in zebrafish [2] and are implicated in human TB pathology [26, 27]. These observations have been investigated and confirmed using the human *in vitro* lung tissue model, dissecting the set of MMPs induced by Mtb infection and demonstrating that MMP inhibition reduces both granuloma pathology and bacterial load [28]. Furthermore, these findings are consistent with subsequent reports of the benefit of MMP inhibition in a mouse model of TB [29].

Limitations of this model include the difficulty with adding other immune cells such as lymphocytes due to MHC incompatibility, or neutrophils, which the developers have attempted, and so primarily models the macrophage-Mtb interaction. In addition, translation to high throughput is challenging. The model has potential to study diverse aspects of host protection, such as epigenetic modulation

to improve protection, and as a secondary assay for testing novel TB drugs, which underpins its usefulness for applied TB research.

Granuloma model to assess the impact of the human immune response

The Schlesinger lab group has created a human PBMC-based granuloma model with two goals in mind: 1) to address how the human immune status may dictate early granuloma formation and bacterial response; and 2) to enable tractability for potential translational applications (Figure 3) [30]. The model uses human PBMCs isolated from naïve or latent TB infected (LTBI) individuals, autologous serum and virulent Mtb, and is being interrogated to discriminate host and bacterial determinants in individuals with and without LTBI. The model demonstrates the significant influence of immune memory on granuloma formation, bacterial survival, lymphocyte proliferation, pro- and anti-inflammatory cytokines, and lipid body accumulation. Moreover, there is a specific transcriptional signature of Mtb associated with survival depending on the host immune status. Specifically, with LTBI, Mtb converts to a latency signature early (within 7 days) indicating early adaptation to the granuloma environment.

Limitations of the published model include the absence of fibroblasts and matrix, which have since been added and found to influence the kinetics of granuloma formation and stability. The model also lacks the continual influx of mononuclear phagocytes to maintain the dynamic structures over longer periods of time. HIV has been added to the model providing insight into its effects on the nature and timing of granuloma formation and dissolution. The model allows for comparative analysis to other granulomatous diseases such as sarcoidosis [31] and is scalable for throughput analysis of potential therapeutic compounds. Therefore, further development and evolution of this system can investigate basic biology, therapeutics and also the effect of co-morbidities.

Bioelectrospray 3-dimensional model

The Southampton TB group have developed a system based on cell encapsulation within microspheres, using cross-linking of alginate when immersed in a gelling bath of calcium chloride [32]. The resulting microspheres have a matrix and cellular composition that is highly tractable (Figure 4), while cells can readily be released from the spheres for downstream analysis by dissolving the microspheres in EDTA or Sodium citrate. The group has used the system to investigate diverse aspects of the host-pathogen interaction and translational application. First, comparison of spheres with or without a collagen provided evidence that the extracellular matrix regulates the host-pathogen interaction, as first suggested from transgenic mouse studies [11]. Subsequently, the group used the system to investigate different aspects of the host immune response, such as studying spheres exposed to different cytokines or with augmented Mtb-responsive T cells [12]. The ability to incorporate different cell types into multiple spheres, and then study the effect for over 21 days, is a potentially powerful application to determine protective versus pathological immune responses. Importantly, Mtb is pyrazinamide sensitive when in 3-D microspheres, but not in standard 2-D culture or broth, demonstrating that Mtb is under similar stress as *in vivo* conditions [33]. Since the cells and bacteria are held within the microspheres, the model can be readily adapted to microfluidic pharmacokinetic modelling, and accelerated Mtb killing can be shown with increasing concentrations of rifampicin [33]. Finally, the group has used the model to study the effect of MMP inhibition with doxycycline to limit TB-driven immunopathology [27].

Limitations of the model are broadly similar to those outlined above, including the further addition of immune cells (simulating cell recruitment [13]), which is challenging once cells are encapsulated within the microspheres. Further development is required to model the multiple

231 microenvironments that Mtb is thought to reside in during human infection [4]. For example, dual
232 encapsulation would permit modelling of the caseous central core, while generation of larger
233 spheres would provide an oxygen gradient from the periphery to a hypoxic core [14]. Modulation of
234 the microsphere composition will be required to permit cellular ingress to mimic recruitment to
235 granulomas.
236
237 **Lessons from animal models of TB granuloma formation**
238
239 The models outlined above demonstrate the diverse potential of *in vitro* modelling using human
240 cells, but at this point all lack the complexity and chronic host-pathogen interaction that occur in
241 human disease. Therefore, animal modelling will continue to be an essential component of fully
242 dissecting the host-pathogen interaction and findings from animal model systems must be used to
243 further inform the development of human systems.
244
245 The classically described granuloma has a caseous center surrounded by an inner myeloid and an
246 outer lymphocytic layer [2]. Eventually, the granuloma becomes necrotic and/or fibrotic, and may
247 lead to mineralization or cavitation. Signaling cascades involving myeloid cells and IFN-γ from T-cells
248 contrive to eliminate Mtb within the lesion, although sterilization is rarely achieved. Thus, the
249 granuloma environment permits mechanisms that both promote and inhibit bacterial killing.
250 Specialized granuloma architecture of human lung TB is not modeled in the prototypical mouse
251 granuloma, although mouse models that allow the unstable or stable development of necrotic, or
252 fibrotic lesions have recently been developed [34]. Murine granulomas also do not permit immune
253 control in a latent state. Guinea pigs, rabbits and macaques develop human-like necrotic and
254 organized granulomas, and develop hypoxia, especially in the necrotic regions [34]. Rabbits have
255 been used, under the right conditions, to study of cavitary lesions.
256

Animal models have raised important questions about how specific mechanisms prevalent within lung granulomas can drive the balance of Mtb killing and survival. As one example, upon coming into contact with Mtb, myeloid cells intensely express indoleamine dioxygenase (IDO), a tryptophan catabolic enzyme [35]. Dr Kaushal's work shows that IDO may inhibit optimal anti-TB T cell responses and *in vivo* blockade of this host pathway improves granuloma-specific killing of Mtb by invoking stronger adaptive responses and permitting lymphocytes access to the core of the lesion, where Mtb-infected macrophages are present. Thus, immunosuppressive pathways may be prevalent in animal, and potentially human, lung granulomas. IDO has been detected in human patients [36]. Since Mtb promotes a robust Th1-response resulting in chronic granulomatous inflammation, it is counterintuitive for Mtb to be deliberately immunogenic as the resulting immune response could eliminate infection and cause tissue damage [37]. Thus, Mtb induction of novel regulatory mechanisms like IDO could potentiate its survival in the face of this immune stress. Using zebrafish, Tobin and colleagues demonstrate that granuloma formation is accompanied by reprogramming events driven by E-cadherin creating cellular tight junctions [3]. Inhibition of this signaling pathway caused lesions to become disordered, with greater immune cell access to the granuloma-resident bacilli and eventually better clearance [3]. Thus IDO and E-cadherin represent two of likely several pathways which prevent efficient killing of Mtb in the granulomas. Understanding fundamental mechanisms of such signaling events will require reciprocal interchange between *in vivo* and *in vitro* models.

How can parallel advances in other fields inform TB granuloma models?

Organ on a chip

The last two decades have seen considerable research efforts to leverage microfabrication technologies initially developed for the semiconductor industry to create new types of *in vitro* cell

283 culture models. Using microfabricated patterns and fluidic channels that can be engineered at the
284 size scale of single cells, these advanced *in vitro* systems have demonstrated unprecedented
285 capabilities to control the cellular microenvironment with high spatiotemporal precision and to
286 present cultured cells with various types of biochemical and mechanical signals in a physiologically
287 relevant context [38-40] . Recent advances in this microsystems approach have led to a new wave of
288 microengineered cell culture models known as 'organs-on-chips' (aka microphysiological systems)
289 designed to mimic microarchitecture, dynamic environment, and integrated biological function of
290 complex human physiological systems [38, 41, 42].
291
292 As the proof-of-principle demonstration of this technology, a human breathing lung-on-a-chip model
293 provides a great example of how living human cells can be combined with a synthetically designed
294 culture environment to reverse engineer the salient features of a complex organ *in vitro* (Figure 5)
295 [43-45]. By enabling co-culture of human alveolar epithelial cells and lung microvascular endothelial
296 cells on the opposite sides of a thin porous membrane undergoing cyclic stretch, this microdevice
297 replicates the lung alveolar-capillary interface and its dynamic mechanical activity during breathing
298 (Figure 5B). Importantly, this system also offers unique capabilities to model complex organ-level
299 physiological functions such as protective immune responses to bacterial infection and
300 environmental exposures (Figure 5C). Similar approaches have been applied successfully to
301 emulating the functional units of other organs such as the liver, heart, bone, kidney, brain, intestine,
302 bone marrow, and placenta [41].
303
304 One critical aspect of organ-on-a-chip technology that has garnered attention recently is the
305 possibility of developing specialized *in vitro* models of complex disease processes. From the
306 perspective of TB research, this is an area of significant potential that needs further exploration for
307 modeling TB granulomas. In particular, to precisely control and manipulate cells and their
308 microenvironmental cues may be instrumental in recapitulating granuloma complexity. For example,

309 microdevices can be designed to contain two or more layers of interconnected yet individually
310 addressable cell culture chambers in order to co-culture multiple relevant cell types (e.g., infected
311 macrophages, epithelial cells, T and B cells, fibroblasts) in physiologically relevant arrangements and
312 to modulate their environment both spatially and temporally. Such devices would provide a robust
313 platform to mimic cellular heterogeneity and granuloma 3-D structural organization, thus facilitating
314 mechanistic investigation of complex intercellular interactions in the development and progression
315 of TB. Further, such systems may be useful to study healing mechanisms within the lung and tissue
316 regeneration, addressing the often-overlooked issue of pulmonary scarring that follows TB and leads
317 to long-term morbidity.
318
319 Recent advances in organ-on-a-chip technology make it possible to engineer complex 3-D networks
320 of self-assembled perfusable blood vessels by replicating the process of vasculogenesis and
321 angiogenesis [46, 47], which could be integrated into an *in vitro* granuloma model to allow for
322 continued recruitment of blood-borne immune cells, offering a means to reconstitute the
323 dynamically replenished environment of *in vivo* granulomas. When constructed with human cells
324 and tissues, organ-on-chip models of granulomas may provide a basis for novel preclinical research
325 platforms for identification and validation of new therapeutic targets and high-content screening of
326 lead compounds for TB [48]. These types of systems may serve as a significant contributor to timely
327 and cost-effective translation of research discoveries for TB.
328
329 ***Reciprocal interchange between* in vitro *and mathematical modelling***
330
331 Several mathematical and computational models have been developed to describe various aspects
332 of TB disease, spanning from bacterial metabolic scale to human population scale [49-53]. These
333 models have helped inform experimental studies and expand our understanding of the mechanisms

underlying TB disease. This raises the question: what role can mathematical and computational models play in the development and application of *in vitro* TB granuloma models?

First, *in vitro* models are relatively easily manipulatable in model inputs, can measure more outputs at high resolution, and can easily collect longitudinal data. Therefore, the wealth of data produced by *in vitro* systems could contribute to development of computational models that represent the biology with a high degree of detail and confidence. Second, computational models that are well calibrated to the experimental systems could help address the tractability question for the ideal *in vitro* granuloma model. Computational models can be used to quickly and cost-effectively screen large numbers of interventions, thereby narrowing the treatment design space to be explored and the number of interventions to be experimentally tested in model systems. Third, it might not be feasible to develop the ideal *in vitro* system outlined above. Some models might be better suited for some studies than others (for example, varying drug concentrations versus including multiple cell types), or types of measurements (long term dynamics versus high resolution spatial data). Computational models could help integrate data from multiple *in vitro* systems, thereby extending their individual predictive capabilities. For example, computational methods have successfully integrated data from various *in vitro* cell proliferation, differentiation and death assays to predict interventions for pulmonary fibrosis [54]. In short, using computational approaches to complement and integrate granuloma model systems could accelerate their development and application.

Future developments and potential benefits

The authors believe that advanced human granuloma cell culture models will make significant contributions to our understanding of TB pathogenesis going forward. The close co-evolution of host and pathogen means that studying the natural pairing is necessary to understand this persistent human infection, and such cellular systems permit the mechanistic and dynamic investigations

required to fully dissect granuloma biogenesis and allow for multiple types of interrogations. As one example, the combination of advanced models with single cell sequencing and other "omics" approaches would permit unique dissection of the host-pathogen interaction in a human system. Similarly, 3-D live cell imaging of granulomas over time may provide new insights into granuloma biology, just as it has been so informative in the zebrafish model of *M marinum* [2].

Granuloma models may uncover novel regulatory pathways and therapeutic approaches such as host-directed therapies, while predicting both the beneficial and potentially harmful outcomes of each intervention in clinical trials. Similarly, if protective innate or adaptive immune responses can be fully characterised in these models, these will inform new vaccination strategies and their efficacies when used in clinical studies. The primary challenge is defining the key attributes of any *in vitro* model that are required to reflect events *in vivo*. Simply developing ever more complex models is wasteful of resources if they are not aimed at answering specific pathophysiological questions. Development of granuloma systems must be cross-correlated with events in patients and in animal models to ensure relevance to human disease. Thus, a multidisciplinary approach bringing together advanced cell culture modelers with bioengineers and mathematical modelers, in tandem with *in vivo* models is needed to bridge the requirements of complexity, tractability, and throughput. Ultimately, we will only control TB if we fully understand its pathogenesis. Further advancing cell culture models and related technologies can serve as a central pillar of that effort in bridging *in vivo* experimentation and clinical studies.

Footnote page

All authors state no conflicts of interest. This publication and workshop (convened in January, 2018 and entitled "3-D Human *in vitro* TB Granuloma Model") have been funded in whole or in part with Federal Funds from the Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health (NIH), Department of Health and Human Services under contract number HHSN272201600001G – Research Support Services for the Division of AIDS. Funding support was also provided in part by grants from the Medical Research Council MR/P023754/1 to PE, the National Institutes of Health (NIH) (1DP2HL127720-01 to DH, R33AI102239 to PE, UL1TR001070 to LSS), the National Science Foundation (CMMI:15-48571) to DH and the Swedish Research Council #2014-02289 to ML. The authors thank our fellow workshop presenters, Eusondia Arnett, Daniel Barber, Louis Joslyn, Smita Kulkarni, Lucie Low, Matyas Sandor and Ankur Singh, as well as Liku Tezera and Magdalena Bielecka for their insights and lively discussion.

References

1. Wallis RS, Maeurer M, Mwaba P, et al. Tuberculosis-advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis **2016**; 16:e34-46.

2. Pagan AJ, Ramakrishnan L. The Formation and Function of Granulomas. Annu Rev Immunol **2018**; 36:639-65.

3. Cronan MR, Beerman RW, Rosenberg AF, et al. Macrophage Epithelial Reprogramming Underlies Mycobacterial Granuloma Formation and Promotes Infection. Immunity **2016**; 45:861-76.

4. Fonseca KL, Rodrigues PNS, Olsson IAS, Saraiva M. Experimental study of tuberculosis: From animal models to complex cell systems and organoids. PLoS Pathog **2017**; 13:e1006421.

5. Brites D, Gagneux S. Co-evolution of Mycobacterium tuberculosis and Homo sapiens. Immunol Rev **2015**; 264:6-24.

6. Puissegur MP, Botanch C, Duteyrat JL, Delsol G, Caratero C, Altare F. An in vitro dual model of mycobacterial granulomas to investigate the molecular interactions between mycobacteria and human host cells. Cell Microbiol **2004**; 6:423-33.

7. Puissegur MP, Lay G, Gilleron M, et al. Mycobacterial lipomannan induces granuloma macrophage fusion via a TLR2-dependent, ADAM9- and beta1 integrin-mediated pathway. J Immunol **2007**; 178:3161-9.

8. Peyron P, Vaubourgeix J, Poquet Y, et al. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. PLoS Pathog **2008**; 4:e1000204.

9. Garnier T, Eiglmeier K, Camus JC, et al. The complete genome sequence of Mycobacterium bovis. Proc Natl Acad Sci U S A **2003**; 100:7877-82.

10. Marakalala MJ, Raju RM, Sharma K, et al. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nat Med **2016**; 22:531-8.

11. Al Shammari B, Shiomi T, Tezera L, et al. The Extracellular Matrix Regulates Granuloma Necrosis in Tuberculosis. J Infect Dis **2015**; 212:463-73.

12. Tezera LB, Bielecka MK, Chancellor A, et al. Dissection of the host-pathogen interaction in human tuberculosis using a bioengineered 3-dimensional model. eLife **2017**; 6:e21283.

13. Schreiber HA, Harding JS, Hunt O, et al. Inflammatory dendritic cells migrate in and out of transplanted chronic mycobacterial granulomas in mice. J Clin Invest **2011**; 121:3902-13.

14. Belton M, Brilha S, Manavaki R, et al. Hypoxia and tissue destruction in pulmonary TB. Thorax **2016**; 71:1145-53.

15. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis **2013**; 208:1464-73.

16. Clevers H. Modeling Development and Disease with Organoids. Cell **2016**; 165:1586-97.

17. Kapoor N, Pawar S, Sirakova TD, Deb C, Warren WL, Kolattukudy PE. Human Granuloma In Vitro Model, for TB Dormancy and Resuscitation. PLoS ONE **2013**; 8:e53657.

18. Gomez JE, McKinney JD. M. tuberculosis persistence, latency, and drug tolerance. Tuberculosis (Edinb) **2004**; 84:29-44.

19. McCune RM, Jr., Tompsett R. Fate of Mycobacterium tuberculosis in mouse tissues as determined by the microbial enumeration technique. I. The persistence of drug-susceptible tubercle bacilli in the tissues despite prolonged antimicrobial therapy. J Exp Med **1956**; 104:737-62.

20. Daniel J, Deb C, Dubey VS, et al. Induction of a novel class of diacylglycerol acyltransferases and triacylglycerol accumulation in Mycobacterium tuberculosis as it goes into a dormancy-like state in culture. J Bacteriol **2004**; 186:5017-30.

21. Parasa VR, Rahman MJ, Ngyuen Hoang AT, Svensson M, Brighenti S, Lerm M. Modeling Mycobacterium tuberculosis early granuloma formation in experimental human lung tissue. Dis Model Mech **2014**; 7:281-8.

445 22. Nguyen Hoang AT, Chen P, Juarez J, et al. Dendritic cell functional properties in a three-
446 dimensional tissue model of human lung mucosa. Am J Physiol Lung Cell Mol Physiol **2012**; 302:L226-
447 37.
448 23. Braian C, Svensson M, Brighenti S, Lerm M, Parasa VR. A 3D Human Lung Tissue Model for
449 Functional Studies on Mycobacterium tuberculosis Infection. J Vis Exp **2015**.
450 24. BeruBe K, Gibson C, Job C, Prytherch Z. Human lung tissue engineering: a critical tool for safer
451 medicines. Cell and tissue banking **2011**; 12:11-3.
452 25. Via LE, Schimel D, Weiner DM, et al. Infection dynamics and response to chemotherapy in a
453 rabbit model of tuberculosis using [(1)(8)F]2-fluoro-deoxy-D-glucose positron emission tomography
454 and computed tomography. Antimicrob Agents Chemother **2012**; 56:4391-402.
455 26. Elkington P, Shiomi T, Breen R, et al. MMP-1 drives immunopathology in human tuberculosis and
456 transgenic mice. J Clin Invest **2011**; 121:1827-33.
457 27. Walker NF, Wilkinson KA, Meintjes G, et al. Matrix Degradation in Human Immunodeficiency
458 Virus Type 1-Associated Tuberculosis and Tuberculosis Immune Reconstitution Inflammatory
459 Syndrome: A Prospective Observational Study. Clin Infect Dis **2017**; 65:121-32.
460 28. Parasa VR, Muvva JR, Rose JF, Braian C, Brighenti S, Lerm M. Inhibition of Tissue Matrix
461 Metalloproteinases Interferes with Mycobacterium tuberculosis-Induced Granuloma Formation and
462 Reduces Bacterial Load in a Human Lung Tissue Model. Frontiers in microbiology **2017**; 8:2370.
463 29. Xu Y, Wang L, Zimmerman MD, et al. Matrix metalloproteinase inhibitors enhance the efficacy of
464 frontline drugs against Mycobacterium tuberculosis. PLoS Pathog **2018**; 14:e1006974.
465 30. Guirado E, Mbawuike U, Keiser TL, et al. Characterization of host and microbial determinants in
466 individuals with latent tuberculosis infection using a human granuloma model. mBio **2015**; 6:e02537-
467 14.
468 31. Crouser ED, White P, Caceres EG, et al. A Novel In Vitro Human Granuloma Model of Sarcoidosis
469 and Latent Tuberculosis Infection. Am J Respir Cell Mol Biol **2017**; 57:487-98.

32. Workman VL, Tezera LB, Elkington PT, Jayasinghe SN. Controlled Generation of Microspheres Incorporating Extracellular Matrix Fibrils for Three-Dimensional Cell Culture. Adv Funct Mater **2014**; 24:2648-57.

33. Bielecka MK, Tezera LB, Zmijan R, et al. A Bioengineered Three-Dimensional Cell Culture Platform Integrated with Microfluidics To Address Antimicrobial Resistance in Tuberculosis. mBio **2017**; 8:e02073-16.

34. Foreman TW, Mehra S, Lackner AA, Kaushal D. Translational Research in the Nonhuman Primate Model of Tuberculosis. ILAR J **2017**; 58:151-9.

35. Gautam US, Foreman TW, Bucsan AN, et al. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of Mycobacterium tuberculosis. Proc Natl Acad Sci U S A **2017**.

36. Isa F, Collins S, Lee MH, et al. Mass Spectrometric Identification of Urinary Biomarkers of Pulmonary Tuberculosis. EBioMedicine **2018**; 31:157-65.

37. Russell DG. The evolutionary pressures that have molded Mycobacterium tuberculosis into an infectious adjuvant. Curr Opin Microbiol **2013**; 16:78-84.

38. Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. Trends Cell Biol **2011**; 21:745-54.

39. Folch A, Toner M. Microengineering of cellular interactions. Annu Rev Biomed Eng **2000**; 2:227-56.

40. El-Ali J, Sorger PK, Jensen KF. Cells on chips. Nature **2006**; 442:403-11.

41. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol **2014**; 32:760-72.

42. Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE. Microengineered physiological biomimicry: organs-on-chips. Lab Chip **2012**; 12:2156-64.

43. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Science **2010**; 328:1662-8.

44. Huh D, Leslie DC, Matthews BD, et al. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med **2012**; 4:159ra47.

45. Huh D, Kim HJ, Fraser JP, et al. Microfabrication of human organs-on-chips. Nat Protoc **2013**; 8:2135-57.

46. Kim S, Lee H, Chung M, Jeon NL. Engineering of functional, perfusable 3D microvascular networks on a chip. Lab Chip **2013**; 13:1489-500.

47. Osaki T, Sivathanu V, Kamm RD. Vascularized microfluidic organ-chips for drug screening, disease models and tissue engineering. Curr Opin Biotechnol **2018**; 52:116-23.

48. Esch EW, Bahinski A, Huh D. Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov **2015**; 14:248-60.

49. Linderman JJ, Cilfone NA, Pienaar E, Gong C, Kirschner DE. A multi-scale approach to designing therapeutics for tuberculosis. Integr Biol (Camb) **2015**; 7:591-609.

50. Beste DJ, Hooper T, Stewart G, et al. GSMN-TB: a web-based genome-scale network model of Mycobacterium tuberculosis metabolism. Genome Biol **2007**; 8:R89.

51. Sershen CL, Plimpton SJ, May EE. Oxygen Modulates the Effectiveness of Granuloma Mediated Host Response to Mycobacterium tuberculosis: A Multiscale Computational Biology Approach. Front Cell Infect Microbiol **2016**; 6:6.

52. Pienaar E, Lerm M. A mathematical model of the initial interaction between Mycobacterium tuberculosis and macrophages. J Theor Biol **2014**; 342:23-32.

53. Magombedze G, Mulder N. A mathematical representation of the development of Mycobacterium tuberculosis active, latent and dormant stages. J Theor Biol **2012**; 292:44-59.

54. Warsinske HC, Wheaton AK, Kim KK, Linderman JJ, Moore BB, Kirschner DE. Computational Modeling Predicts Simultaneous Targeting of Fibroblasts and Epithelial Cells Is Necessary for Treatment of Pulmonary Fibrosis. Front Pharmacol **2016**; 7:183.

Figure legends

Figure 1: The collagen matrix model. (A) Infected PBMCs, (B) uninfected PBMCs, (C) H & E staining showing multinucleated giant cells, (D) Fluorescent staining of granulomas sections with DAPI (nuclear stain), CD68 (macrophage marker-shown in red) and CD3 (T cells-shown in green) monoclonal antibodies. Reproduced from (Ref #17). Permission to reprint this figure provided by

Figure 2: The human *in vitro* lung tissue model. Cells are sequentially layered onto a collagen matrix and then infected macrophages are added to model the early events of human lung infection.

Figure 3: The host immune response impact on granulomas. Human PBMCs from either naïve or LTBI individuals are incubated with autologous serum and Mtb. The model has shown that host immune status has significant impacts on granuloma formation and function, and bacterial responses.

Figure 4: The bioelectrospray microsphere model; Immunoaugmentation with Mtb-specific T cells. **A.** Cellular aggregates within microspheres after 14 days. Scale bar 20µm. **B.** Microspheres imaged after 4 days show early granuloma formation (yellow). **C.** Addition of either ESAT-6 responsive T cells (red) or CFP-10 responsive T cells (blue) increases Mtb growth compared to infected PBMCs without supplemented T cells (black).

Figure 5. A human breathing lung-on-a-chip. (A) The alveolar system is modeled in a microfluidic device consisting of two overlapping parallel microchannels separated by a thin porous membrane. (B) The alveolar-capillary interface is created in this system by culturing human alveolar epithelial cells and lung microvascular endothelial cells on either side of the membrane. To mimic breathing,

cyclic vacuum is applied to the hollow chambers adjacent to the cell culture channels to stretch the membrane in the lateral direction. (C) Introduction of E. coli into the alveolar compartment of this model induces adhesion (top row) and transmigration (middle row) of neutrophils flowing in the lower vascular chamber. The recruited neutrophils then phagocytose the bacteria (bottom row). Portions of figure from Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Science **2010**; 328:1662-8. Reprinted with permission from AAAS.

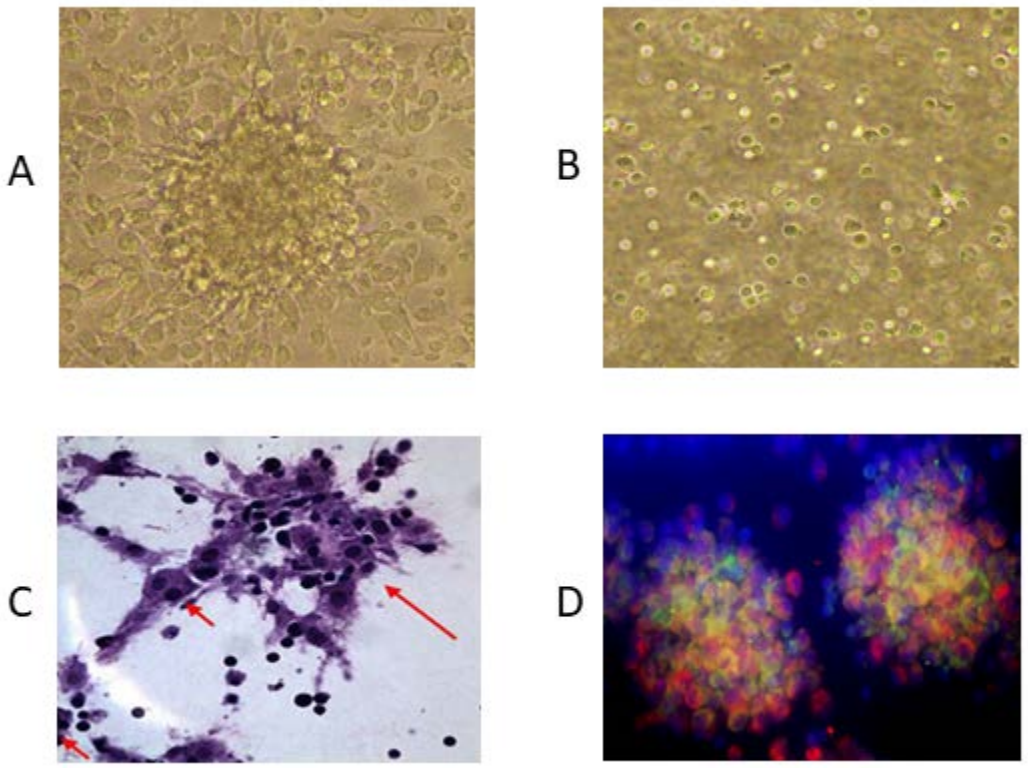

Figure 1: The collagen matrix model. (A) Infected PBMCs, (B) uninfected PBMCs, (C) H & E staining showing multinucleated giant cells. (D) Fluorescent staining of granulomas sections with DAPI (nuclear stain), CD68 (macrophage marker-shown in red) and CD3 (T cells-shown in green) monoclonal antibodies.

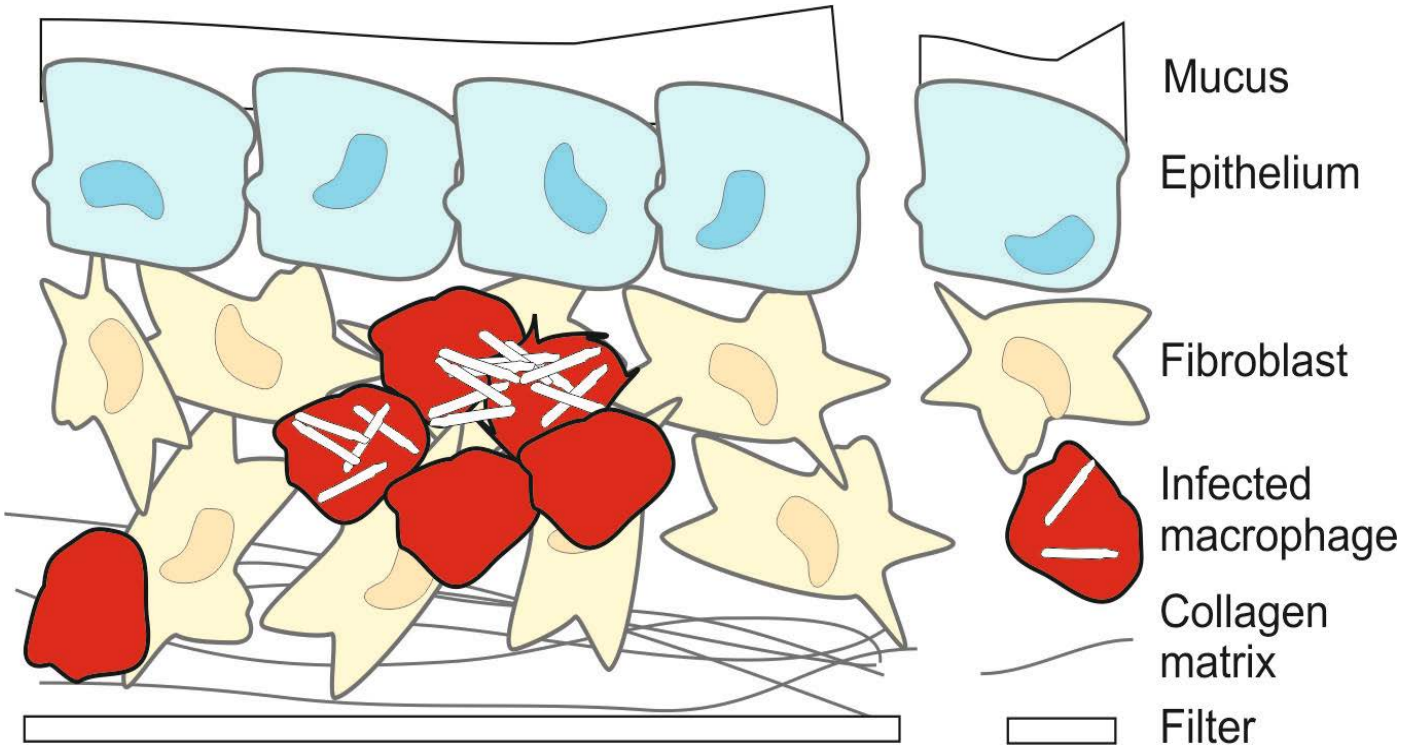

Figure 2: The human *in vitro* lung tissue model. Cells are sequentially layered onto a collagen matrix and then infected macrophages are added to model the early events of human lung infection.

Naive

Host response:

- Lesser pro-, anti-, and regulatory cytokine responses:

IFNg, TNF, IL-12p40, IL-2, IL-10 and IL-13

Bacteria response:

- Increased expression of genes involved in glycolysis

Latent

Host response:

- Increased proliferative activity
- Increased lipid body accumulation

Bacteria response:

- Increased expression of genes involved in:
  - Mannose donor and LAM biosynthesis, fatty acid degradation and utilization, Krebs cycle, and glyoxylate shunt

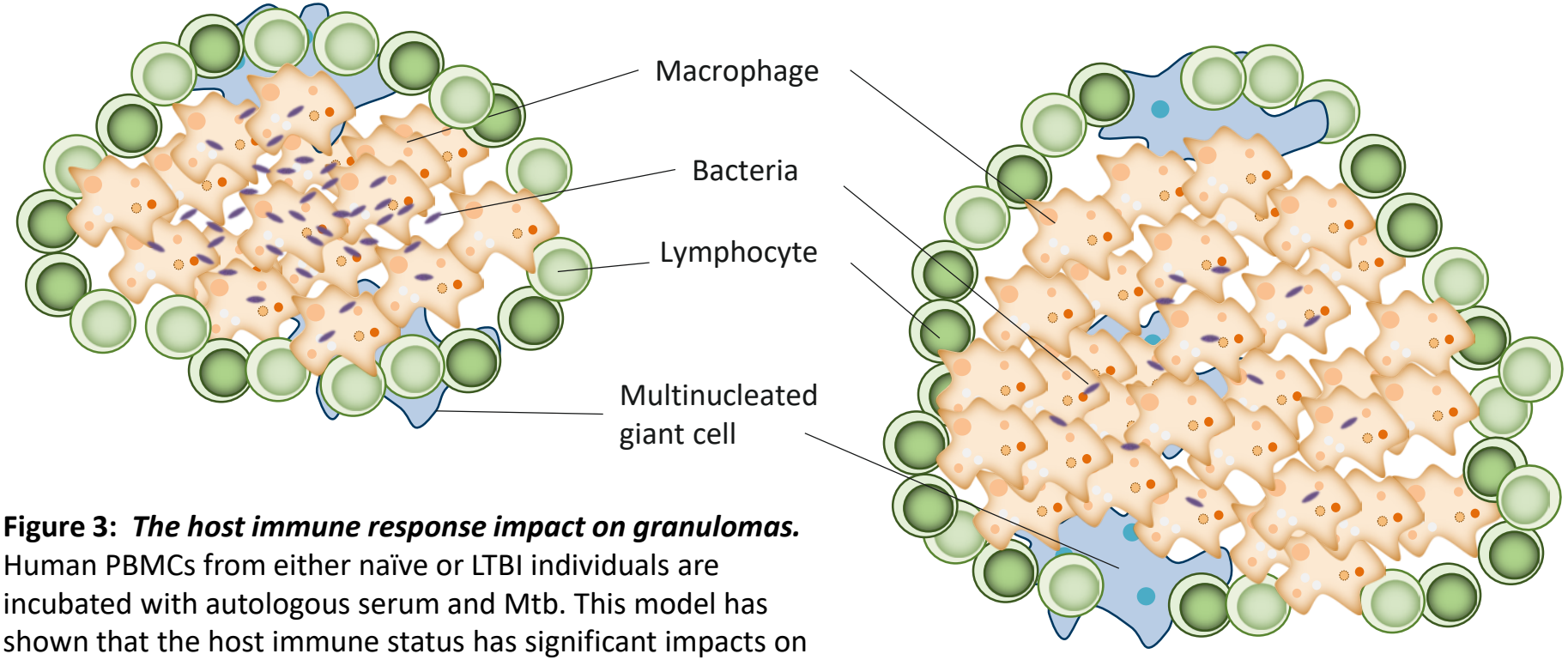

Figure 3: ***The host immune response impact on granulomas.*** Human PBMCs from either naïve or LTBI individuals are incubated with autologous serum and Mtb. This model has shown that the host immune status has significant impacts on granuloma formation and function, and bacterial responses.

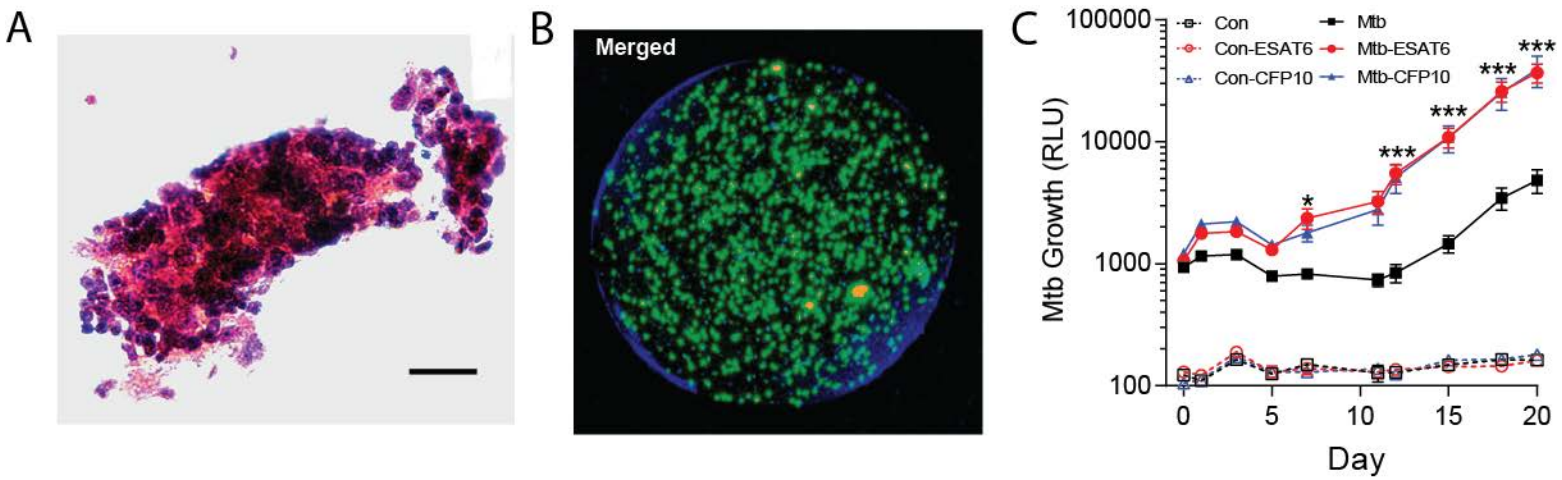

Figure 4: The bioelectrospray microsphere model; Immunoaugmentation with Mtb-specific T cells. A. Cellular aggregates within microspheres after 14 days. Scale bar 20µm. **B.** Microspheres imaged after 4 days show early granuloma formation (yellow). **C.** Addition of either ESAT-6 responsive T cells (red) or CFP-10 responsive T cells (blue) increases Mtb growth compared to infected PBMCs without supplemented T cells (black).

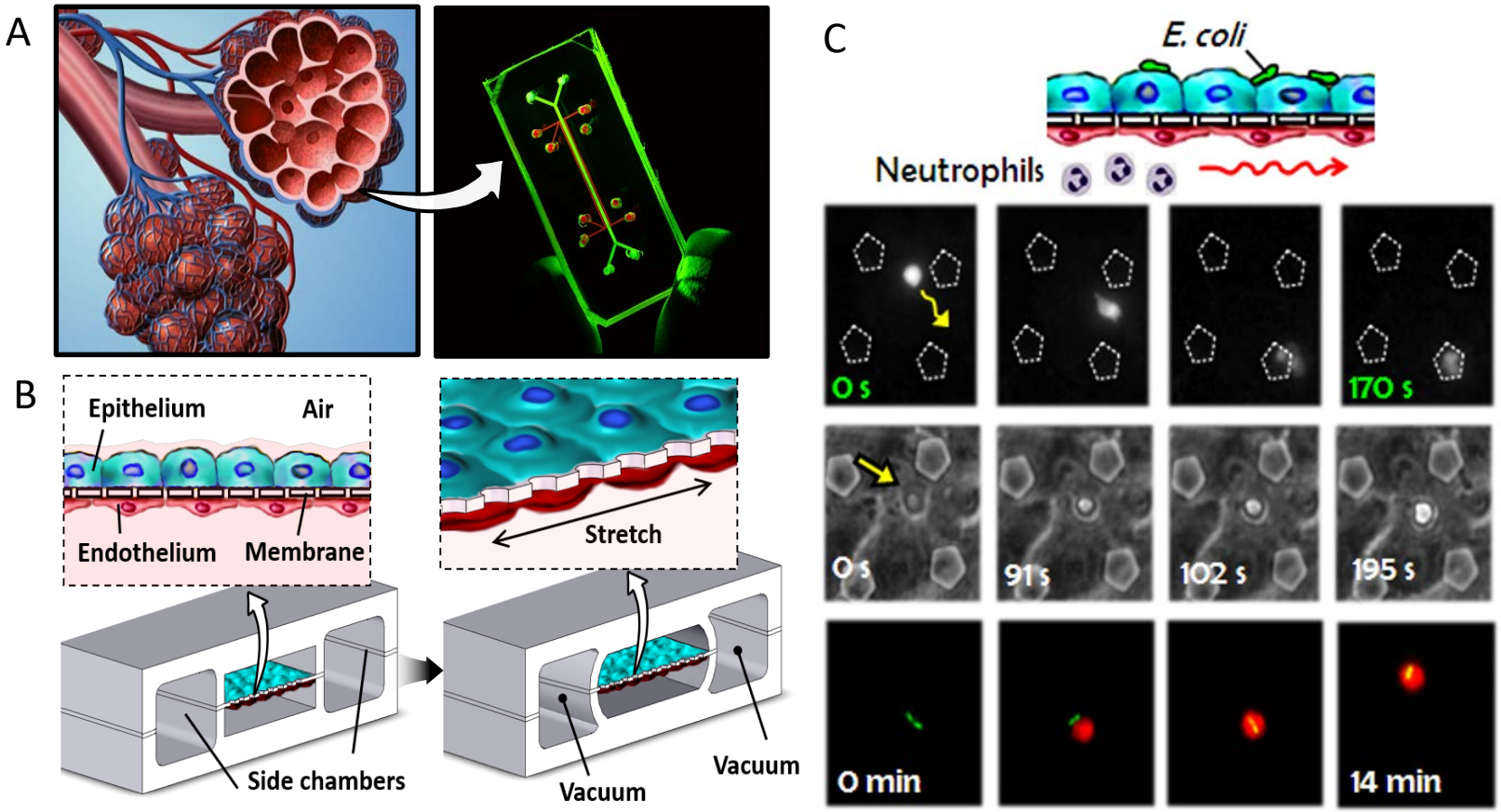

Figure 5. A human breathing lung-on-a-chip. (A) The alveolar system is modeled in a microfluidic device consisting of two overlapping parallel microchannels separated by a thin porous membrane. (B) The alveolar-capillary interface is created in this system by culturing human alveolar epithelial cells and lung microvascular endothelial cells on either side of the membrane. To mimic breathing, cyclic vacuum is applied to the hollow chambers adjacent to the cell culture channels to stretch the membrane in the lateral direction. (C) Introduction of E. coli into the alveolar compartment of this model induces adhesion (top row) and transmigration (middle row) of neutrophils flowing in the lower vascular chamber. The recruited neutrophils then phagocytose the bacteria (bottom row).