Review Article

Marina Cañadas-Ortega, Clara Gómez-Cruz, Juan José Vaquero, and Arrate Muñoz-Barrutia*

The contribution of microfluidics to the fight against tuberculosis

https://doi.org/10.1515/ntrev-2022-0004
received June 7, 2021; accepted October 27, 2021

Abstract: The high mortality associated with tuberculosis brings forward the urgency of developing new therapies and strategies against the disease. With the advance of drug-resistant strains, traditional techniques have proven insufficient to manage the disease appropriately. Microfluidic devices have characteristics that can enhance treatment prescription and significantly advance our knowledge about the disease and its interaction within the human body. In addition, microfluidic systems provide advantages in terms of time and costs, which are particularly important in countries with low income and resources. This review will highlight how microdevices can help bridge the gaps in disease management, including their use for drug testing and development, drug susceptibility, basic research, and novel approaches to anti-TB vaccines and organ-on-chip studies.

Keywords: microfluidics, tuberculosis, microdevices, infectious diseases, respiratory diseases

*** Corresponding author: Arrate Muñoz-Barrutia,** Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, 28911 Leganés, Spain; Grupo de Aplicaciones Tecnológicas Avanzadas y Salud Humana, Instituto de Investigación Sanitaria Gregorio Marañón, 28009 Madrid, Spain, e-mail: mamunozb@ing.uc3m.es
Marina Cañadas-Ortega: Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, 28911 Leganés, Spain, e-mail: macanada@pa.uc3m.es
Clara Gómez-Cruz: Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, 28911 Leganés, Spain, e-mail: clgomezc@pa.uc3m.es
Juan José Vaquero: Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, 28911 Leganés, Spain; Grupo de Aplicaciones Tecnológicas Avanzadas y Salud Humana, Instituto de Investigación Sanitaria Gregorio Marañón, 28009 Madrid, Spain, e-mail: juanjose.vaquero@uc3m.es

1 Motivation

Infectious diseases of the respiratory airways are the third leading cause of death worldwide. Among them, tuberculosis (TB) constituted the sixth most deadly disease in 2000–2016. Around one-fourth of the global population is infected by *Mycobacterium tuberculosis* (*Mtb*), making TB a global disease. Approximately 10 million new cases are diagnosed every year, and the number of deaths associated with TB per year exceeds a million. These cases mainly occur in developing countries and low-income areas, such as South-East Asia, Africa, and the Western Pacific [1]. Although most infections remain latent, in which the bacterium is inactive and asymptomatic, around 10% of cases develop active TB, where the bacterium is multiplying and invading tissues, mainly the lungs [2].

Numerous factors hinder the advances of the scientific community in the fight against TB, including the appearance of new drug-resistant strains of *Mtb* (DR-TB) and the unmet requirements for both diagnosis and treatment.

The most used method for diagnosing pulmonary TB is sputum smear microscopy because of its low price and quickness, at the expense of low sensitivity and specificity. Tuberculin skin tests or chest X-rays are also used, with the disadvantage of presenting a low potential for point-of-care (POC) testing. The detection of antibodies specific for mycobacterial antigens and lipoarabinomannan (LAM), a lipopolysaccharide on the *Mtb* bacterial cell wall, can also be used for diagnosis. However, the former presents low cost-effectiveness and accuracy, while the second lacks sensitivity. Finally, the use of molecular genotypic analysis (primarily based on nucleic acid amplification tests) is widely spread. However, it presents some disadvantages since most of the tests require tight temperature control and complex steps. For this reason, their use is limited to laboratories and requires trained staff. These methodologies are more extensively covered in the numerous reviews regarding the issue [3–5].

The development of DR-TB adds complexity to the diagnosis. This resistance emerges from patients' poor

adherence to the long therapies against TB, insufficient monitoring of disease development, and other factors such as improper use of antibiotics in the food industry [6]. These drug-resistant strains can be of different types: multidrug-resistant (MDR) – not responding to treatment with isoniazid and rifampicin, the two first-line more powerful anti-TB drugs – or extensively drug-resistant (XDR) – resistant to the two previously mentioned ones, as well as to any fluoroquinolone and at least one of the three main second-line injectable drugs (such as capreomycin, kanamycin, or amikacin) [7].

This reduced drug susceptibility limits the treatment options, forcing the clinicians to use complex combinations of different anti-TB drugs. In addition, they often include multiple second-line drugs, which usually entails more extended hospital stays resulting in increased medical costs and higher human costs due to the severe side-effects caused by these second-line drugs [8–10].

Furthermore, drug resistance is usually harder to diagnose. For example, it requires phenotypic assays (like culture and microscopy of the strains, requiring several weeks to enable proper guidance) or the genotyping of DNA regions connected with drug resistance, which needs laboratories with appropriate biosafety levels.

These demands are critical in areas with technical constraints, such as underdeveloped or developing countries, where most cases are localized. However, the need for expensive laboratory infrastructure and thoroughly trained healthcare staff is one of the most significant barriers preventing these communities from accessing proper diagnosis or treatment at the POC [8]. Therefore, there is an urgent need for new diagnostic tools for active and latent TB, as well as for testing the drug susceptibility of these strains. Moreover, it is necessary to advance the development of new anti-TB drugs to increase the therapeutic efficacy in terms of costs, time, and side effects.

Microfluidic devices allow the manipulation of liquid volumes in the microscale [11], covering these needs in terms of costs, portability, number, and time of assays. These miniaturized devices work based on the application of electrical or mechanical properties to cover biological demands. The reduced volume and precise manipulation also result in lower reagent use and a minimization of the costs [12]. Regarding the development of new drugs and their testing, this technology enables multiplexing, designing devices with different channels to perform several reactions simultaneously. Additionally, several microchips can be coupled and imaged using a single microscope. These advantages lead to shorter testing times of potential drug candidates during preclinical development by reducing the assay time compared with traditional drug susceptibility tests. Thus, this technology allows performing more trials in less time, leading to quicker prescriptions [13].

These devices can be classified into three different groups: (i) high-throughput, automatized systems with precise fluid control, aimed to facilitate laboratory research; (ii) those for low-scale experiments, dedicated to 3D *in vitro* cell culture (for organ-on-a-chip or drug screening applications), and (iii) simple and disposable devices to be used without advanced training or facilities [14]. Most devices used for drug susceptibility and fundamental research purposes fall into the second class. Diagnostic devices tend to fall in the third category, and a detailed summary can be found elsewhere [15]. In this manuscript, we review the last decade's advances regarding the use of microfluidic devices applied to the field of TB, focusing on their role in drug testing and development, basic research, and emerging approaches.

2 Drug testing and development

The increasing number of drug-resistant strains of *Mtb* urgently requires the development of new fast and reliable tools to identify this resistance. To guide treatment prescription, it is thus essential to identify the particular drug resistance of specific strains by carrying out drug susceptibility tests (DSTs). Typically, they evaluate the growth or metabolic inhibition of the bacterial cells in drug-free or drug-containing media. More recently, new techniques for detecting mutations in drug-resistance associated genes have been established [16,17].

Mutations that result in drug-resistant strains are usually detected alongside *Mtb* diagnosis itself. However, more knowledge about the drug susceptibility of these strains is required due to the differences between genotypic and phenotypic resistance of *Mtb* and to the inability of current methods to detect all mutations involved in resistance (as they only cover a limited number of genomic regions) [18].

Furthermore, besides determining whether a specific strain is resistant to the first- or second-line TB drugs, it is essential to find new medicines that can control and eliminate these resistant strains. Situated farther from the POC but still necessary for the pharmaceutical industry are the microdevices oriented to developing new drugs. The goal of these devices consists of analyzing death processes and studying the effect of the compounds (mostly at the phenotypic level or through morphological changes) rather than identifying the susceptibility of a particular strain. The same chips developed for drug

discovery can also be used for DST, basic research, or other purposes.

In the drug testing and development process, microfluidic devices provide a system that allows the monitoring of colonies in the long term. Microdevices coupled with a microscope for time-lapse imaging of the growing bacterial colonies enable researchers to understand how the antibiotics affect the bacterial cells, either at metabolic or physiological levels, and to elucidate their death mechanism [19]. It is worth reminding that these technologies offer benefits such as the low amounts of compounds required or the possibility of multiplexing the chips, testing several drugs simultaneously [20].

Microdevices must trap bacteria in the microscope's focal plane to image the colonies and prevent movement during image acquisition, which can take up to several weeks. Different strategies have been developed to accomplish this, either using matrices or culture chambers in microfluidic devices to confine the cells in a single plane.

2.1 Culture of bacterial cells embedded in matrices

Different matrices have been used to trap the cells. Golchin *et al.* [21] used a thin layer of alginate hydrogel matrix to immobilize bacteria and promote planar growth, preventing the cells from going out of focus during long incubations. Dividing cells were recorded using time-lapse confocal imaging to observe phenotypic variations and antibiotic killing in individual mycobacteria cells. The nutrients were provided through media perfusion across a cellulose dialysis membrane from polydimethylsiloxane (PDMS) and a glass flow cell, sealed with a mechanical clamp (Figure 1 left). The group of Choi *et al.* utilized 3D agarose matrices to immobilize *Mtb* for rapid DST, developing the disc agarose channel (DAC) chip [22]. *Mtb* cultures are loaded in 24-well plexiglass chips, mixed with the liquid agarose, which is allowed to solidify (Figure 1 right). The antibiotics are flooded with the medium and allowed to diffuse in the agarose matrix; then, the chip is sealed and incubated to analyze the drug susceptibility. The 3D agarose structure allowed the immobilization and tracking of specific cells for 13 days with an automated imaging system. The susceptibilities of different strains (non-resistant/control, MDR, and XDR-TB strains) were tested with the DAC and compared with the results of liquid culture DST to validate the system. The agreement rates of specificity and sensitivity of different drugs obtained were around or above 90% when compared with liquid culture, thus proving its capacity for DST. The time required for the DST was 9 days using the DAC system. However, *Mtb* cells used to assess the validity were obtained from pre-cultured colonies on a liquid medium, which requires one or 2 weeks of culture to get a significant number of cells. Although the system reduces the turnover of conventional methods, which require 4–12 weeks (1–6 of sputum culture and 2–6 of DST), the sample culture still needs a long time.

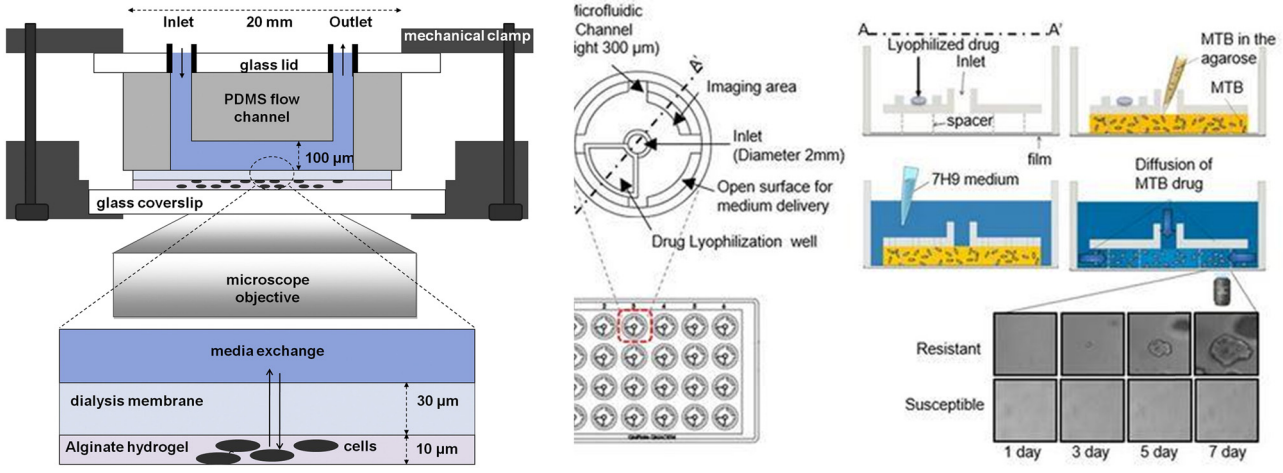

Figure 1: Left: Schematic of the microfluidic device presented in ref. [21], based on the hydrogel matrix. On top of a coverslip, cells are embedded in the thin alginate hydrogel, covered by a cellulose dialysis membrane that allows media diffusion. Mechanical clamps and a glass lid seal the PDMS flow channel structure. Right: Sketch of the automatic disc agarose channel chip and trapping methodology in agarose matrix used in refs [18,22]. (leftmost) Top view of the chips within 24-well plate, with channels, inlet, and drug reservoirs depicted. (rightmost) The experimental process of loading *Mtb* cells within an agarose matrix, covering with media and diffusion of anti-TB drugs within the media can be observed. Images reproduced with permission from TB (left, [21]) and Applied Microbiology and Biotechnology (right, [22]).

Two years later, in 2018, the same DAC chip was used to overcome complications regarding the inoculum effect [18] (increment in the minimal inhibitory concentration (MIC) of antibiotic required when the size of the inoculum, *i.e.*, the number of organisms inoculated, increases [23]). This effect constitutes one of the main problems of culture-based DST methods, as a standardized inoculum size is required to obtain reproducible DST results; additionally, it can cause a reduction in the drug effectiveness due to the increased number of bacteria [23,24]. Therefore, the DAC platform was used to evaluate the consistency of MIC values obtained from different inoculum sizes compared to the liquid culture reference method. Moreover, the critical concentration of 12 anti-TB drugs was determined from 254 clinical isolates (including pan-susceptible and resistant strains) without measuring the inoculum size. The tested anti-TB drugs gave consistent results with 96.3% overall agreement within 7 days. However, the isolates required a lengthy subculture on a liquid medium before the test, as in the previous study. In a recent 2021 study, the DAC system improved with a modified channel to increment the antibiotic diffusion into media to reduce turnaround times for DSTs. This fully automated DST system was named QuantaMatrix microfluidic agarose channel (QMAC) and integrated mycobacteria growth indicator tubes [25].

2.2 Monitoring in culture chambers

Culture chambers within microdevices have been widely used to analyze drug effects on *Mtb*. However, to achieve this, bacterial cells need to be encapsulated in the chambers. To this respect, Delincé *et al.* [26] developed InfectChip, consisting of a cellulose semipermeable membrane between a micropatterned coverslip and a PDMS chip. The coverslip is patterned with circular microchambers where the cells are trapped, grow, and divide, whereas the medium, is continuously flowing through a microchannel in the PDMS chip (Figure 2 left).

This device was used to study the dynamics and interactions of single-cell behavior between *Mtb* and motile eukaryotic phagocytes in a host-cell model, providing a platform to visualize the different stages of infection. However, despite its advantages, it presents several limitations, including a limited lifespan of the macrophages, which constrains the visualization of the infection process.

The design shares similarities with the device developed by Wakamoto *et al.* [29], used for basic research (Figure 4). It can be used to test new antibiotics. For example, they tested faropenem [30], and it was also used to analyze the effects of inhibitors against drug resistance mutation for isoniazid [31].

Other systems have been developed to encapsulate bacterial cells in microfluidic devices. For example, Sala *et al.* [27] proposed a model to analyze the effect of different drugs on dormant cells using a previously developed microdevice [28]. The microdevice design includes a PDMS layer patterned with long grooves, covered by a transparent membrane, and sealed with a thick PDMS layer with flow channels. Cells get trapped in the grooves between the membrane and the PDMS layer (Figure 2, right). This system differs from the previous ones [26,29] as the cells are not inside culture chambers in the coverslip but directly growing on PDMS grooves.

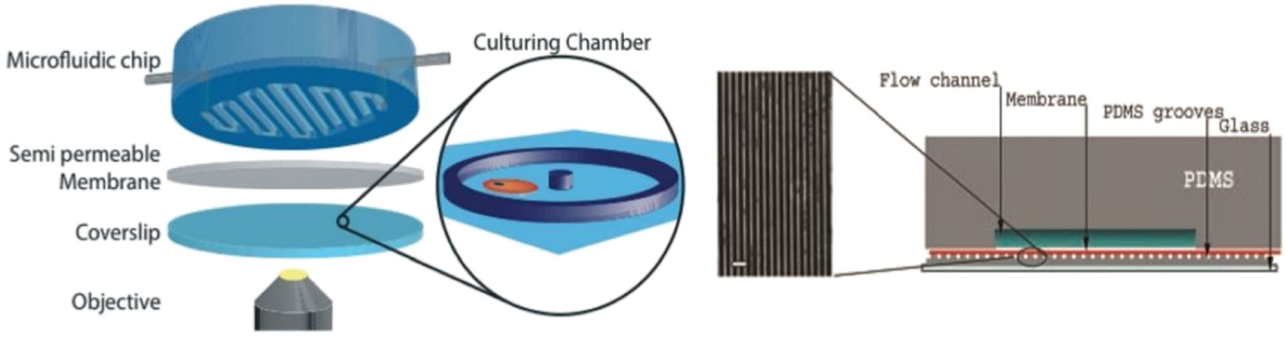

Figure 2: Microfluidic devices that use PDMS channels to trap cells. Left: 3D model of the microfluidic device from ref. [26], showing the semipermeable membrane that covers the patterned microchambers in the coverslip. The PDMS chip diffuses media through the membrane. Right: Representation of the microdevice used in ref. [27] to culture cells in PDMS grooves covered by a membrane, fed by diffusion through a flow channel encapsulated in a PDMS chip. Images reproduced with permission from Lab Chip (left, [26]) and Science (right, [28]).

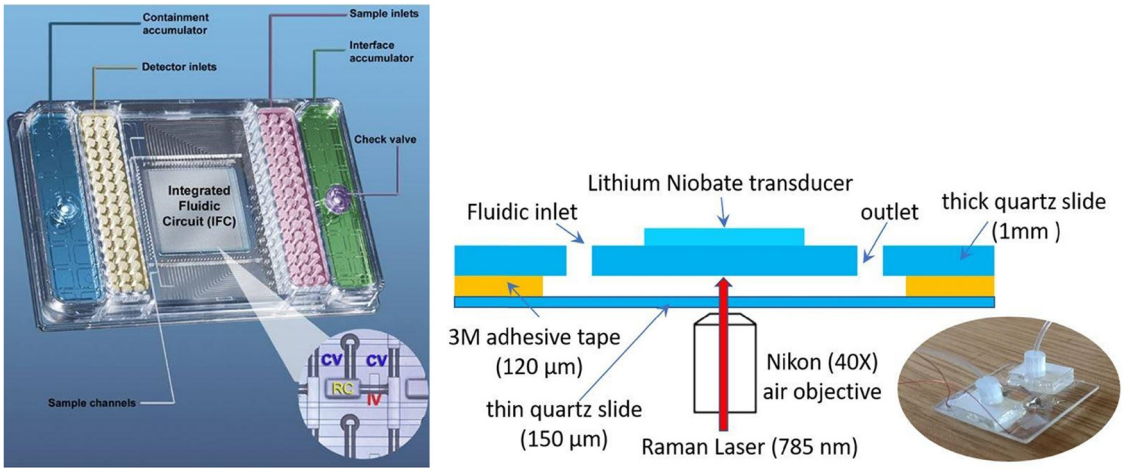

Figure 3: Left: Picture of the high-throughput (48 × 48 array) microfluidic device used in ref. [32] (developed in ref. [33]) for the transcriptomic profiling for speeding drug development programs. The sample and reagents are introduced through the inlets, which are pressure-driven through valves to the integrated fluidic circuit, composed of fluid lines, valves, and microchambers in which the PCR takes place. Right: Cross-sectional view and picture of the chamber of the Raman platform developed in ref. [34]. Cells go through silicone tubes into a trapping chamber of quartz. Then, acoustic pressure is applied through a lithium transducer to make them levitate and aggregate to determine the Raman spectra. Images reproduced with permission from PLoS (left, [33]) and Communications Biology (right, [34]) journals.

2.3 Other methods for drug development

Time-lapse microscopy combined with microdevices for bacterial culture is the primary technique used in drug testing applications and basic research. Still, other methods are used in the field of drug development. Instead of focusing on the quantification of death rates and characterization of the persistent cells, this research analyzed the transcriptome and other markers to determine the response of the bacterial cells to the antibiotics applied.

Murima *et al.* [32] utilized a high-throughput microfluidic array developed in ref. [33] to obtain biomarker genes that could improve and shorten the traditional anti-TB drug-discovery schemes. This device contains an integrated fluidic circuit chip containing valves and 2304 chambers where the real-time polymerase chain reaction (PCR) occurs (Figure 3 left). After the fluids are pressure-driven in the corresponding chambers, the chip is placed inside the instrument, where the thermal cycling occurs. The high number of wells allows performing many simultaneous reactions, which were used to analyze transcriptomics of *M. tuberculosis* and *M. bovis* in the presence and absence of several growth inhibitors.

Raman spectroscopy was used to analyze the effect of isoniazid in the lipidic expression of *M. smegmatis* [34]. The device consists of a flow cell with a trapping chamber, formed by laser-cutting the channel between quartz slides and sealed with transfer tape, as can be observed in Figure 3, right. The inlet and outlet are made with silicone tubes. The transparent lithium transducer allowed the acoustic pressure required for the cells to levitate and aggregate in the channel and to be trapped in the lateral direction to obtain the wavelength-modulated Raman spectra. The live-cell monitoring was carried out by acoustic trapping after 7 days of preculture.

3 Microfluidic applications in basic research

Thus far, the focus has been primarily on developing microfluidic technologies for diverse and specific purposes, mainly motivated by the novelty of the techniques, which had to be tested and characterized before being integrated into the laboratory and the scientific know-how. However, different research groups and facilities have also applied these technologies to better understand disease mechanisms. This knowledge ranges from changes in morphology and cell interactions to death rates and antibiotic exposure patterns.

Research groups dedicated to investigating *Mtb* and TB disease often use either already characterized or commercial microfluidic platforms for their experiments without developing a device themselves. Additionally, the investigations tend to be more focused on understanding a particular phenomenon rather than testing the global feasibility of a technique or drug. This section

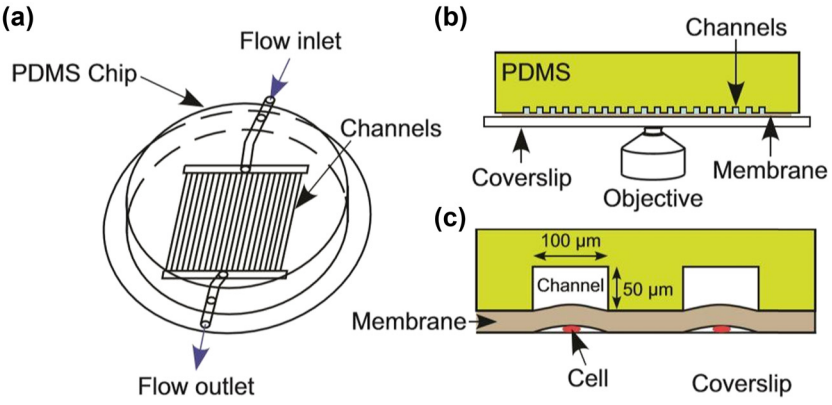

Figure 4: Schematic of the PDMS microfluidic chip mounted on a cellulose membrane and a glass coverslip for time-lapse imaging. (a) Top view depicting inlet, outlet, and parallel channels. (b) Cross-sectional view of the channels in the PDMS chip. (c) Closeup to the cells growing in the microchambers, covered by a membrane to allow media diffusion from the channels. Image reproduced with permission from Science [29].

summarizes the main applications of commonly used imaging devices.

Many researchers use variations of different platforms developed in the School of Life Sciences of the Swiss Federal Institute of Technology, particularly the one presented by Wakamoto *et al.* [29]. This microdevice consists of 100 µm × 50 µm parallel microchannels patterned on a PDMS chip, covered by a semipermeable membrane, then mounted on a glass coverslip (Figure 4). Cells get trapped between the membrane and the coverslip, continuously supplied with nutrients by diffusion from the flow channels across the membrane. The whole system is sealed in a sandwich configuration by a base adapter with screws. This enclosure allows 2D growth while restraining cells in the plane of focus for long imaging periods. In combination with time-lapse microscopy, this setup was initially used to study the enzymatic expression of persistent mycobacterial cells and their effect on cell survival.

The research group used microfluidics and time-lapse setup to study different parameters, characteristics, and behaviors of *Mycobacterium* cells. For example, in ref. [35], they measured chromosome division and replication to gain insights into the dynamics of *Mycobacterium* cellular growth. Other studies used the platform to assess antibiotics' effects on intracellular adenosine triphosphate (ATP) levels [36] or to study the role of ribosomal activity and its associated gene expression in the latent state after chemotherapy [37]. It was also used to study the response to isoniazid of *M. smegmatis* penicillin-binding protein mutant [38] and *NADH pyrophosphatase* mutant [39]. More recently, this combination of microfluidic devices and time-lapse optical microscopy was used to measure single-cell pole growing to better understand the *Mtb* cell division process [40].

The same group developed other chips with different microchannel configurations, as the protocol presented in ref. [41]. Similarities are shared, like the channel dimensions, using a semipermeable membrane between the cells and the PDMS chip, or the mechanical clamping with screws. However, this second configuration is a multiplexed version that allows testing different bacterial strains (Figure 5). With this platform, the cell responses to DNA damage and its relationship with drug resistance were analyzed [42] or the characterization of the cellular response against a new molecular drug target [43].

Another custom-made device was proposed by Aldridge *et al.* [44]. In this case, the PDMS chip with the patterned microchannels is attached to the glass coverslip and sealed by plasma bonding treatment, avoiding the difficulties entailed by the mechanical clamp presented in the previous devices. The other main difference resides in the lack of membranes between the cells and the PDMS chip. In this device, the cells are pumped and grown directly in the channels, not patterned chambers. These channels for cellular growth connect with the main channel through which media is flowed, transporting nutrients. Bacterial cells are thus fed by diffusion between the main channel and the shallow ones, as can be observed in Figure 6, left.

This microdevice was used to analyze the asymmetric division of *Mtb* cells and its associated antibiotic resistance (particularly to Rifampicin) in ref. [48]. The media and the antibiotic were mixed and injected into the viewing device for the morphological analysis of the polarized division. The Morphological Evaluation and Understanding of Stress (MorphEUS) project also used

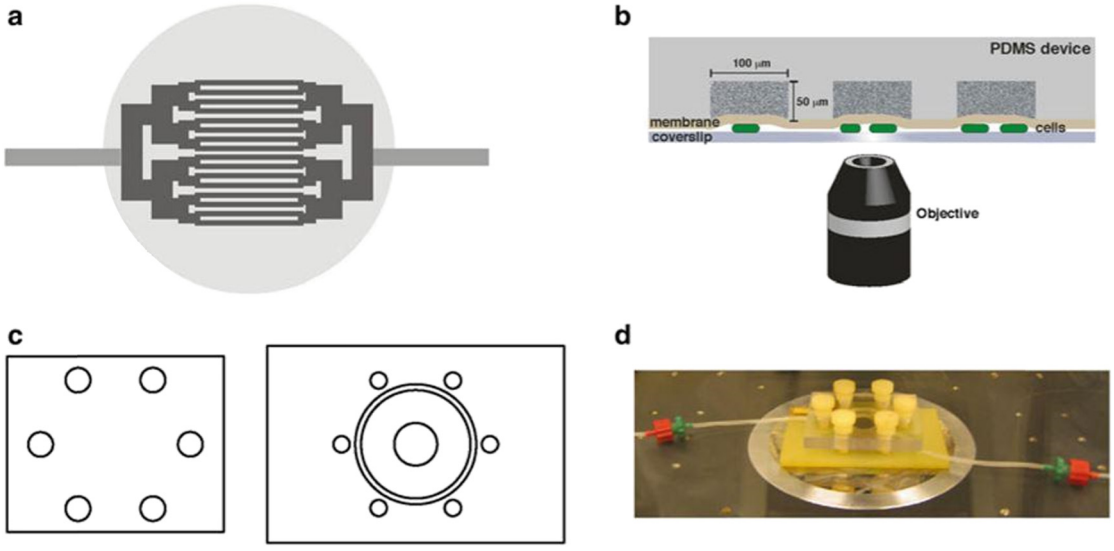

Figure 5: Representation of the PDMS microfluidic device presented in ref. [41]. (a) Top view of the microdevice, depicting the channel layout and the inlets and outlets. (b) Cross section of the setup for its visualization. The cells get trapped between the coverslip and a semi-permeable membrane for media to diffuse from the channels. (c) Acrylic frames of the clamp system, fixed with screws. (d) Picture of the device, once assembled. Image reproduced with permission from Methods in Molecular Biology [41].

this platform to classify tested drugs into different categories according to their modes of action, characterized in ref. [49], continuing with the idea of the utility of morphological profiling to elucidate bacterial response to drugs.

Another use of the device includes measuring the polar growth and response of single cells in the presence of stressors such as antibiotics. Remarkably, the link between the asymmetrical division of physiological subpopulations and resistance is analyzed and compared with other bacteria such as *E. coli*. The platform was also used to study the changes produced in the host–pathogen interaction and bacterial burden upon vaccination [50].

Studying the biological processes of *Mycobacteria* can also benefit from commercial microfluidic platforms. For example, the CellASIC ONIX2 Microfluidic Systems (Millipore Sigma) are plastic plates with multiple channels and microfluidic chambers for cell culture (Figure 6, right), allowing tight control of the flow, temperature, and gas exchange. These plates were used [45] to characterize and elucidate the role of transpeptidase

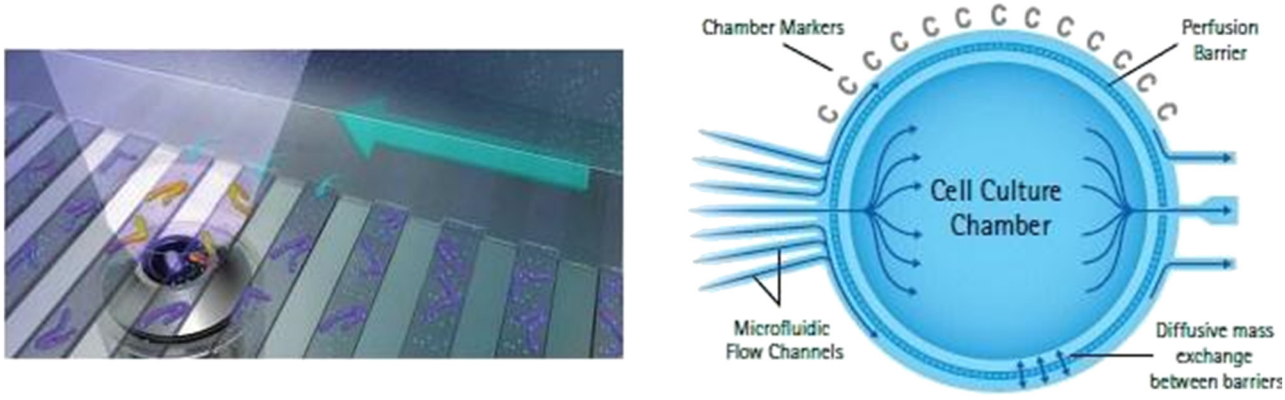

Figure 6: Left: Representation of the microfluidic device developed in ref. [44] for long-term imaging of bacteria, consisting of the main flow channel (big blue arrow) that diffuses into the cell channels. Right: Scheme of the microfluidic chambers present in the CellASIC ONIX2 Microfluidic plate used in refs [45–47]. A membrane covers the cell culture chamber allowing mass diffusion of liquids from the microfluidic channels. Images reproduced with permission from Science (left, [44]) and from Merkmillipore webpage (right, http://merckmillipore.com/).

crosslinks in cell shape and death and the possibilities for drug targeting against them. In ref. [46], the same microfluidic plate was used to follow the replication of bacteria and the chromosome dynamics after adding drugs altering the cell division mechanisms. It was also used more recently in ref. [47] to investigate ultra-short peptides and their effect on membrane activity of mycobacterial cells to exploit their antimicrobial properties.

Other methods to characterize and visualize bacteria to retrieve basic biological information include the dielectrophoresis technique proposed in ref. [51]. In this work, a microdevice containing a single-cell suspension of *M. smegmatis* in buffer was prepared and then high frequencies were applied. Cell movement was recorded to analyze cell death and drug tolerance mechanisms.

4 Other applications

Microfluidic technology has shown utility in the fight against *Mtb* for different diagnostic methodologies, the advancement of drug candidates' selection in its various forms, or enhancing the general knowledge about the disease through basic research. This section will focus on other contributions of these devices to the management of TB.

4.1 Production of anti-TB vaccines

Vaccination programs can be essential for disease management by preventing active and latent TB. The first (and to the day, only) vaccine against TB is the Bacille Calmette Guérin (BCG), introduced in 1921 [52]. It prevents extrapulmonary TB in infants by newborn vaccination; however, it fails to stop the pulmonary form of the disease [53]. Due to these limitations, new vaccine candidates are continuously being proposed and reviewed. Some of the leading candidates and their clinical phases are evaluated in detail in refs [54,55].

Particularly, cationic liposomes were formulated as a gene delivery system that could be used in gene therapy and vaccine due to their ability to form complexes with DNAhsp65 [56–58]. The low yield production obtained with conventional methods and the escalation required led to the use of microfluidic devices to generate high lipid concentrations. Two PDMS devices were proposed based on single and double hydrodynamic focusing (SHF and DHF, respectively) to enhance vesicle formation and mixing [59], as depicted in Figure 7. These devices facilitate optimizing parameters affecting liposome production for the development and production of anti-TB vaccines. This process led to the high yield production of liposomes, suitable for gene delivery and vaccination due to their unilamellar nature and particle size.

This study demonstrates the possibility of taking advantage of microfluidic devices not only for low-scale analysis or diagnostics but also for scaled-up production of pharmaceutical compounds, especially in the cases of vaccines or antibiotics.

4.2 Diagnosis of coinfection of TB and HIV

TB is the leading cause of death in HIV-co-infected patients, with a proportion of approximately 8.6% of TB patients co-infected with HIV in 2019 [1]. Additionally, HIV infection in TB patients increments the probability of activating a latent TB state between 80- and 100-fold [60]. Nevertheless, its diagnosis remains challenging in HIV co-infected individuals due to a high frequency of smear-negative disease, high rates of extrapulmonary TB, and reduced pulmonary load [18]. Traditional techniques do not provide rapid and accurate results to manage HIV co-infected patients effectively [61]. Despite its importance, there are almost no microfluidic detection methods to detect both diseases simultaneously.

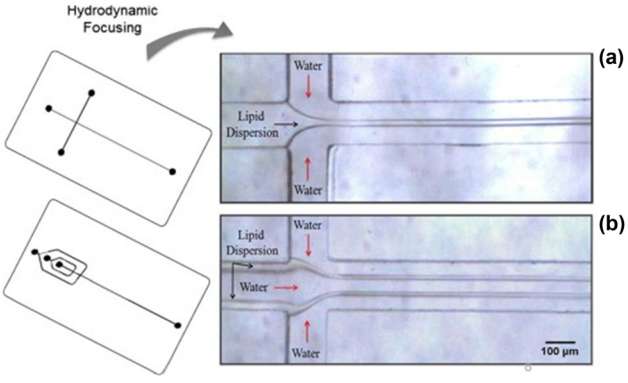

Figure 7: Schematic and photography of the microfluidic devices developed in ref. [59] depicting the single (a) and double (b) hydrodynamic focusing. Lipids are diluted in ethanol and injected in the center channel with a flow velocity of 143 mm/s. Deionized water flows through the exterior channels to hydrodynamically compress the central flow. In the case of double focusing, another flow-focusing stream is added to each side. Image reproduced with permission from Chemical Engineering Journal [59].

A multiplexed nucleic acid amplification test (NAAT) was developed by the Fudan University of Shangai, portraying a microdevice for simultaneous detection of HIV, TB, and *Pneumocystis carinii pneumonia* (PCP) using a loop-mediated isothermal amplification strategy [62]. The PDMS device consists of a multiple loop-mediated isothermal amplification (LAMP) with the shape of a five-pointed star (Figure 8). The sample is loaded in the middle of the chip and distributed to the pools located at the points of the star by capillary forces. Three of the amplification pools are coated with LAMP probes against HIV, *Mtb*, and PCP. The fourth one serves as a positive control (coated with β-actin LAMP probe) and the last one as negative control (uncoated). Once the amplification pools fill with the nucleic acid sample and LAMP buffer, the inlet is sealed using uncured PDMS that requires on-site incubation. Results can be checked within 90 min with the naked eye (supporting its utility in low resources areas) by the appearance of white residue generated during the LAMP reaction.

Additionally, green fluorescence is produced by SYBR green I and can be detected using agarose gel electrophoresis. The detection limit of the multiplexed chip is 50 copies/μL for HIV and 100/μL copies for *Mtb* and PCP, respectively. Moreover, no nonspecific amplification or cross-reactivity was detected when samples of the diseases were tested using nucleic acids of different viruses as templates.

This platform is presented as a proof-of-concept for resource-poor countries. However, the accuracy of the primers and the rates of false positives and negatives needs to be characterized.

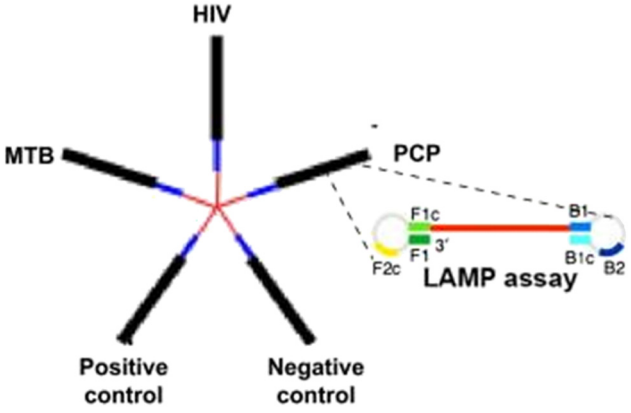

Figure 8: Schematic representation of the five-channel μLAMP-based microfluidic device. The amplification pools in the channels are filled with the sample and the LAMP reaction buffer, then sealed. Positive samples form a white magnesium pyrophosphate precipitate that can be assessed with the naked eye or using agarose gel electrophoresis. Image reproduced with permission from Talanta [62].

5 Future perspectives

Although many significant contributions have been made to the field of TB regarding mechanisms of action and several detection strategies, further work is required to understand the behavior of the disease entirely. In particular, it is still necessary to comprehend and bridge the gaps that limit the use of these technologies at the POC. Moreover, different perspectives are always open for investigation since perfect devices (*i.e.*, affordable, rapid, specific, sensitive, user-friendly) have not been deciphered. Some fields could strongly benefit from the advantages offered by microfluidic devices, especially those with translational capabilities or related to comorbidities. Notably, the reproduction and development of *ex vivo* tissues can significantly benefit from the use of microfluidic technologies. During the last decade, there has been an important advancement in the encapsulation and recreation of living tissues inside chips through organ-on-chip technology.

5.1 Organ-on-a-chip and organoids

Advancing towards the elimination of TB requires improving the diagnosis and identification of drug resistance and a deeper understanding of the interactions between the pathogen and the host cells in the pulmonary tract during infection and drug treatment [63]. *Mtb* presents a sustained interaction over time with host cells [64], which displays a spatial organization of the host–pathogen interaction [65]. Moreover, the extracellular matrix influences the survivability of the infected host cells [66]. Additionally, interactions with the host cells and environment affect bacterial physiology, inducing stress-mediated variations in their genetic expression [67]. These factors disclose the importance of developing novel models representing the diversity of microenvironments and elements that mediate the interaction between pulmonary cells and *Mtb* [68]. Animal models or human 2D cellular models have been primarily used for drug development and testing, pharmacokinetics, and research in general. Basic 3D structures of lung tissues have also served to model the spatiotemporal features of the diseases [69,70].

Organoids and organ-on-a-chip are more complex models featuring 3D cellular organization that has

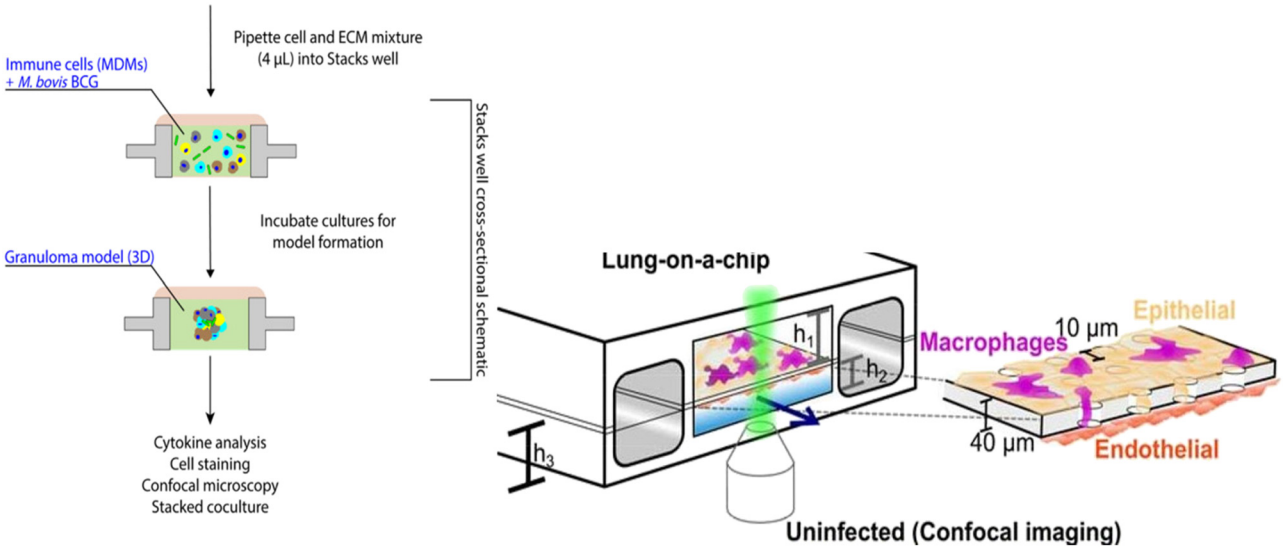

Figure 9: 3D organ-on-a-chip models for *Mtb* interaction with lung tissue. Left: Workflow showing the setup of a microfluidic device containing granuloma microspheres grown in a suspended microfluidics "Stack system" from ref. [76]. Immune and Mycobacteria cells arrange in collagen matrix into a 3D granuloma model. Right: Schematic representation of the lung-on-a-chip from ref. [77]. Alveolar epithelial cells and macrophages cover the upper face of the membrane in the air-filled alveolar channel, while endothelial cells reside in the lower, liquid-filled channel. The tissues able to maintain this interface are infected with *Mtb* and imaged with confocal microscopy. Images adapted with permission from Front Bioeng Biotechnol. (left, [76]) and eLife (right, [77]).

emerged in the last decade to bridge this gap between 2D cell cultures, animal models, and the actual 3D microenvironments. These technologies and their potential applications in the pharmaceutical analysis have been extensively reviewed elsewhere [63,71–74]. However, despite their strong potential for TB research, barely any endeavors have been dedicated to this field yet. Most lung-on-chip models will require adaptation for infection and security requirements in general, particularly TB-specific conditions, while organoids have not been used to analyze *Mtb* infections to date [71]. On a related note, a microsphere system combined with a microfluidic plate has been developed to model the effect of dynamic antibiotic concentration on *Mtb* [75]. A suspended microfluidic platform has also been used as a granuloma infection model [76]. Another example is the use of a commercial lung-on-a-chip model to analyze the interactions between *Mtb* and lung at the early infection stage [77].

Regarding the former [75], 3D microspheres were prepared by bioelectrospray methodology [78] using primary human cells (monocytes and T cells), active *Mtb*, and type I collagen on the microfluidic device, leading to the formation of human granulomas after 14 days. The system modulated the drug concentrations over time to replicate the pharmacokinetics observed *in vivo* during patient treatment. The granuloma's genetic expression and the pharmacokinetics of antibiotics were measured and compared with the liquid culture, demonstrating the differences in the treatment outcome. The microfluidic devices were manufactured from a plexiglass sheet by micromilling, with inlet and outlet channels, and were then introduced in the pits of a 24-well plate before microsphere seeding.

The granuloma environment was also mimicked in ref. [76] using an *in vitro* suspended microfluidic platform adapted from the previously described Stacks system [79]. This system is based on capillary pinning to enable the stacking of layers with different microenvironments and uses open culture wells, which also allow easy imaging. Furthermore, the system does not require pumps (as traditionally occurs in microfluidic devices) because it functions based on surface tension and capillary forces. In this study, the Stacks system was used to create an infection model to study signaling microenvironments, combining human monocyte-derived macrophages and *M. bovis* BCG. The stacks were suspended in a 3D collagen extracellular matrix (ECM) and cultured to initiate paracrine signaling (Figure 9 left).

Another model using spheroids is presented in ref. [80], generated first by the combination of human monocyte and macrophage spheroids, subjected to different Mycobacteria strains. Then, the complexity of the model increased by incorporating peripheral blood mononuclear cells and lung fibroblasts. Finally, these 3D models were used to analyze the co-culture response to drugs such as Isoniazid and Rifampicin.

Table 1: Characterization of microdevices: their types, techniques, purposes, and areas of use

Type of microdevice	Main technique	Purpose	Area of use	Reference
PDMS flow channel filled with alginate hydrogel	Time-lapse microscopy	Phenotypic variations observation	Drug testing and development	[21]
Disc agarose channel: plexiglass chip filled with agarose hydrogel	Time-lapse microscopy	Automatic tracking of single cells for drug susceptibility	Drug testing and development	[18,22,25]
InfectChip: cellulose membrane between micropatterned chambers in a coverslip and a PDMS chip	Time-lapse microscopy	Dynamics between *Mtb* and phagocytes, testing of antibiotics and drug resistance	Drug testing and development, basic research	[26,30,31]
PDMS patterned with grooves	Time-lapse microscopy	Effect of drugs on dormant cells	Drug testing and development	[27,28]
High-throughput microfluidic array	Transcriptomic profiling using RT-PCR	Obtain biomarker genes	Drug testing and development	[32,33]
Flow cell with a trapping chamber between quartz slides	Raman spectroscopy	Effect of isoniazid in the lipidic expression	Drug testing and development	[34]
PDMS chip with microchannels covered by semipermeable membrane mounted on a glass slip	Time-lapse microscopy	Varied: chromosome division, ribosomal activity, ATP levels, etc.	Basic research	[29,35–41]
PDMS chip with patterned microchannels mounted on glass slip without a membrane	Time-lapse microscopy	Asymmetric division, morphological evaluation, and understanding of stress (MorphEUS)	Basic research	[44,48–50]
CellASIC ONIX2	Time-lapse microscopy	Role of transpeptidase crosslinks on cell shape, chromosome dynamics, and membrane activity	Basic research	[45–47]
Single-cell suspension in buffer	Dielectrophoresis	Cell death and drug tolerance	Basic research	[51]
PDMS chip with straight channels	Hydrodynamic focusing	To enhance vesicle formation and mixing	Production of anti-TB vaccines	[59]
Five-pointed star chambers and channels in PDMS	Nucleic acid amplification test through LAMP	Simultaneous detection of HIV, TB, and PCP	Diagnosis of coinfection of TB and HIV	
Microspheres seeded in micromilled plexiglass sheet	Bioelectrospray	Formation of human granuloma to replicate pharmacokinetics	Organ-on-chip and organoids	[75]
Stack system: suspended microfluidic platform	Capillary pinning for stacking preconditioned layers	Infection model for signaling analysis	Organ-on-chip and organoids	[76]
Spheroids	Time-lapse microscopy	Response to drugs	Organ-on-chip and organoids, drug testing, and development	[80]
Commercial lung-on-chip: PDMS seeded with cells on different channels	Time-lapse microscopy	Dynamics of early infection, the role of surfactant	Organ-on-chip and organoids	[77,81]

Concerning the lung-on-chip [77], commercial lung chips were manufactured in PDMS from Emulate (Boston, MA), then preactivated and coated with ECM solution according to the manufacturer's instructions. Then, endothelial cells were seeded in the different channels on the bottom face of the porous membrane (the vascular channel) and alveolar epithelial cells (AECs) on the epithelial channel (Figure 9 right). The use of two different cell types allows recreating the air–liquid interface present at the alveoli. After organ maturation, macrophages are introduced to the epithelial channel and allowed to attach to the epithelium. The lung tissues seeded on the chip capable of maintaining the interface are then infected with *Mtb*, allowing the analysis of its development in macrophages and AECs. Time-lapse fluorescence microscopy is used to study the dynamics of early infection and quantify the intracellular progress of the bacteria. This lung chip was also used in ref. [81] with murine cells and time-lapse microscopy to elucidate the role of surfactants produced by the alveolar epithelial cells in reducing bacterial growth.

These platforms that are still being developed and optimized present a broad range of applications. In the field of TB, they offer the possibility to study pathogen behavior in a more realistic environment and strongly increase the capacities of DSTs. In this way, the effect of antibiotics on bacterial populations and pulmonary tissue grown in the chip or organoid can be quantified. Different research groups worldwide continue their research in these areas. Furthermore, these systems grant the opportunity to analyze and model the interactions between drugs, pathogens, and tissues. They could also be helpful for the study of coinfections, especially those of the respiratory airway.

Studying these coinfections has growing importance, as respiratory tract infections (*i.e.*, avian influenza, coronavirus) have resulted in pandemic situations during the last decade. Although different meta-analyses have found connections between susceptibility to COVID-19 and the presence of TB [82,83], further studies will be required to investigate the correlations between the diseases to advance the fight against them. Thus, the development of these platforms (organ-on-a-chip and organoids) can significantly enhance the knowledge about the interactions between the diseases and speed up the research pipeline.

5.2 Conclusion

Microfluidic devices have been present in the biomedical field for some decades now, enabling the advance and development of multiple techniques and platforms due to their numerous benefits over traditional methods. They have roles in the basic research field and at the translational and clinical stage in diagnosis and drug testing and development.

In this manuscript, we have dissected the potential applications of microfluidics to improve treatment prescription or to increment our knowledge about TB disease. Microdevices can provide an advantage over traditional diagnosis methods due to their simplicity and ease of use without medical facilities. They can also be used to detect different resistant strains of the bacteria, allowing more personalized treatment for each patient.

Moreover, many of the reviewed platforms have POC testing potential, some of them optimized in terms of cost for their use in underdeveloped or resource-constrained countries. There is a growing tendency to reduce the prices, materials, and laboratory setting requirements, as can be observed in the development of lab-on-paper devices in combination with smartphones compared to more traditional PDMS chips. In this way, microdevices position themselves at the forefront of biomedical research, with promising potential for future developments and opportunities.

Several research groups over the globe have routinely incorporated microfluidic devices in their laboratories as a daily tool, constituting an important source of knowledge about the basic forms of the disease and their relationship with other illnesses that entail threats to humanity. Moreover, through the development of new organ-on-chip and other techniques, insights into the behavior of the bacteria within the human body and the host reactions can significantly improve our understanding of infection mechanisms. Microdevices also present new opportunities in therapeutic and drug development testing. They not only enhance the diagnosis and treatment guidance but also facilitate basic research and drug development (Table 1).

Funding information: This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No. 853989. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and Global Alliance for TB Drug Development non-profit organisation, Bill & Melinda Gates Foundation and University of Dundee. This work was partially funded by Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación, under grant PID2019-109820RB-I00, MCIN/AEI/10.13039/501100011033/, co-finance by European Regional Development Fund (ERDF), "A way of making Europe."

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

References

[1] World Health Organization. Global tuberculosis report 2019; 2019.

[2] Adigun R, Singh R. Tuberculosis. StatPearls. 2020, Accessed: Sep. 08, 2021. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/28722945.

[3] Mani V, Wang S, Inci F, De Libero G, Singhal A, Demirci U. Emerging technologies for monitoring drug-resistant tuberculosis at the point-of-care. Adv Drug Deliv Rev. 2014;78:105–17.

[4] Wang S, Inci F, De Libero G, Singhal A, Demirci U. Point-of-care assays for tuberculosis: role of nanotechnology/microfluidics. Biotechnol Adv. 2013;31(4):438–49.

[5] Dheda K, Ruhwald M, Theron G, Peter J, Yam WC. Point-of-care diagnosis of tuberculosis: past, present and future. Respirol Carlton Vic. 2013;18(2):217–32.

[6] Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T Peer-Rev. J Formul Manag. 2015;40(4):277–83.

[7] Eker B, Ortmann J, Migliori GB, Sotgiu G, Muetterlein R, Centis R, et al. German TBNET G. Multidrug- and extensively drug-resistant tuberculosis, Germany. Emerg Infect Dis. 2008;14(11):1700–6.

[8] Young DB, Perkins MD, Duncan K, Barry CE. Confronting the scientific obstacles to global control of tuberculosis. J Clin Invest. 2008;118(4):1255–65.

[9] Blöndal K. Barriers to reaching the targets for tuberculosis control: multidrug-resistant tuberculosis. Bull World Health Organ. 2007;85(5):387–90.

[10] Yang TW, Park HO, Jang HN, Yang JH, Kim SH, Moon SH, et al. Side effects associated with the treatment of multidrug-resistant tuberculosis at a tuberculosis referral hospital in South Korea: A retrospective study. Medicine (Baltimore). 2017;96(28):e7482.

[11] Ahn CH, Choi JW. Microfluidic devices and their applications to lab-on-a-chip. springer handbook of nanotechnology. In: Bhushan B, editor. Berlin, Heidelberg: Springer Berlin Heidelberg; 2010. p. 503–30.

[12] Polla DL, Erdman AG, Robbins WP, Markus DT, Diaz-Diaz J, Rizq R, et al. Microdevices in medicine. Annu Rev Biomed Eng. 2000;2(1):551–76.

[13] Hauck TS, Giri S, Gao Y, Chan WCW. Nanotechnology diagnostics for infectious diseases prevalent in developing countries. Adv Drug Deliv Rev. 2010;62(4–5):438–48.

[14] Albert-Smet I, Marcos-Vidal A, Vaquero JJ, Desco M, Muñoz-Barrutia A, Ripoll J. Applications of light-sheet microscopy in microdevices. Front Neuroanat. 2019;13:1.

[15] Cañadas-Ortega M, Gómez-Cruz C, Muñoz-Barrutia A, Vaquero JJ. The role of diagnostic microdevices in the crisis of tuberculosis. In: Rezaei N, editor. Integrated Science - Science without Borders. Springer Nature Switzerland AG; 2021.

[16] Kim SJ. Drug-susceptibility testing in tuberculosis: methods and reliability of results. Eur Respir J. 2005;25(3):564.

[17] Richter E, Rüsch-Gerdes S, Hillemann D. Drug-susceptibility testing in TB: current status and future prospects. Expert Rev Respir Med. 2009;3(5):497–510.

[18] Jung YG, Kim H, Lee S, Kim S, Jo E, Kim EG, et al. A rapid culture system uninfluenced by an inoculum effect increases reliability and convenience for drug susceptibility testing of Mycobacterium tuberculosis. Sci Rep. 2018;8:8.

[19] Rusconi R, Garren M, Stocker R. Microfluidics expanding the frontiers of microbial ecology. Annu Rev Biophys. 2014;43(1):65.

[20] Chiu DT, deMello AJ, Di Carlo D, Doyle PS, Hansen C, Maceiczyk RM, et al. Small but perfectly formed? Successes, challenges, and opportunities for microfluidics in the chemical and biological sciences. Chem. 2017;2(2):201–23.

[21] Golchin SA, Stratford J, Curry RJ, McFadden J. A microfluidic system for long-term time-lapse microscopy studies of mycobacteria. Tuberc Edinb Scotl. 2012;92(6):489–96.

[22] Choi J, Yoo J, Kim KJ, Kim EG, Park KO, Kim H, et al. Rapid drug susceptibility test of Mycobacterium tuberculosis using microscopic time-lapse imaging in an agarose matrix. Appl Microbiol Biotechnol. 2016;100(5):2355–65.

[23] Brook I. Inoculum effect. Rev Infect Dis. 1989;11(3):361–8.

[24] Mitchison DA. Drug resistance in tuberculosis. Eur Respir J. 2005;25(2):376–9.

[25] Kim H, Lee S, Jo E, Kim S, Kim H, Kim EG, et al. Performance of quantamatrix microfluidic agarose channel system integrated with mycobacteria growth indicator tube liquid culture. Appl Microbiol Biotechnol. 2021;105(14–15):6059–72.

[26] Delincé MJ, Bureau JB, López-Jiménez AT, Cosson P, Soldati T, McKinney JD. A microfluidic cell-trapping device for single-cell tracking of host–microbe interactions. Lab Chip. 2016;16(17):3276–85.

[27] Sala C, Dhar N, Hartkoorn RC, Zhang M, Ha YH, Schneider P, et al. Simple model for testing drugs against nonreplicating mycobacterium tuberculosis. Antimicrob Agents Chemother. 2010;54(10):4150–8.

[28] Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Bacterial persistence as a phenotypic switch. Science. 2004;305(5690):1622–5.

[29] Wakamoto Y, Dhar N, Chait R, Schneider K, Signorino-Gelo F, Leibler S, et al. Dynamic persistence of antibiotic-stressed mycobacteria. Science. 2013;339(6115):91–5.

[30] Dhar N, Dubée V, Ballell L, Cuinet G, Hugonnet JE, Signorino-Gelo F, et al. Rapid cytolysis of Mycobacterium tuberculosis by faropenem, an orally bioavailable β-lactam antibiotic. Antimicrob Agents Chemother. 2015;59(2):1308–19.

[31] Martínez-Hoyos M, Perez-Herran E, Gulten G, Encinas L, Álvarez-Gómez D, Alvarez E, et al. Antitubercular drugs for an old target: GSK693 as a promising InhA direct inhibitor. EBioMedicine. 2016;8:291–301.

[32] Murima P, de Sessions PF, Lim V, Naim AN, Bifani P, Boshoff HI, et al. Exploring the mode of action of bioactive compounds by microfluidic transcriptional profiling in mycobacteria. PloS One. 2013;8(7):e69191.

[33] Spurgeon SL, Jones RC, Ramakrishnan R. High throughput gene expression measurement with real time PCR in a microfluidic dynamic array. PloS One. 2008;3(2):e1662.

[34] Baron VO, Chen M, Hammarstrom B, Hammond R, Glynne-Jones P, Gillespie SH, et al. Real-time monitoring of live mycobacteria with a microfluidic acoustic-Raman platform. Commun Biol. 2020;3(1):236.
[35] Santi I, Dhar N, Bousbaine D, Wakamoto Y, McKinney JD. Single-cell dynamics of the chromosome replication and cell division cycles in mycobacteria. Nat Commun. 2013;4:2470.
[36] Maglica Z, Özdemir E, McKinney JD. Single-cell tracking reveals antibiotic-induced changes in mycobacterial energy metabolism. mBio. 2015;6(1):e02236-14.
[37] Manina G, Dhar N, McKinney JD. Stress and host immunity amplify Mycobacterium tuberculosis phenotypic heterogeneity and induce nongrowing metabolically active forms. Cell Host Microbe. 2015;17(1):32–46.
[38] Elitas M. On-chip isoniazid exposure of mycobacterium smegmatis penicillin-binding protein (PBP) mutant using time-lapse fluorescent microscopy. Micromachines. 2018;9(11):561.
[39] Elitas M. Isoniazid killing of mycobacterium smegmatis NADH pyrophosphatase mutant at single-cell level using microfluidics and time-lapse microscopy. Sci Rep. 2017;7:10770.
[40] Hannebelle M, Ven J, Toniolo C, Eskandarian HA, Vuaridel-Thurre G, McKinney JD, et al. A biphasic growth model for cell pole elongation in mycobacteria. Nat Commun. 2020;11:452.
[41] Dhar N, Manina G. Single-cell analysis of mycobacteria using microfluidics and time-lapse microscopy. Methods Mol Biol Clifton NJ. 2015;1285:241–56.
[42] Manina G, Griego A, Singh LK, McKinney JD, Dhar N. Preexisting variation in DNA damage response predicts the fate of single mycobacteria under stress. EMBO J. 2019;38(22):e101876.
[43] Singh V, Donini S, Pacitto A, Sala C, Hartkoorn RC, Dhar N, et al. The inosine monophosphate dehydrogenase, GuaB2, Is a vulnerable new bactericidal drug target for tuberculosis. ACS Infect Dis. 2017;3(1):5–17.
[44] Aldridge BB, Fernandez-Suarez M, Heller D, Ambravaneswaran V, Irimia D, Toner M, et al. Asymmetry and aging of mycobacterial cells lead to variable growth and antibiotic susceptibility. Science. 2012;335(6064):100–4.
[45] Baranowski C, Welsh MA, Sham LT, Eskandarian HA, Lim HC, Kieser KJ, et al. Maturing Mycobacterium smegmatis peptidoglycan requires non-canonical crosslinks to maintain shape. eLife. 2018;7:e37516.
[46] Trojanowski D, Kołodziej M, Hołówka J, Müller R, Zakrzewska-Czerwińska J. Watching DNA replication inhibitors in action: exploiting time-lapse microfluidic microscopy as a tool for target-drug interaction studies in mycobacterium. Antimicrob Agents Chemother. 2019;63(10):e00739–19.
[47] Khara JS, Mojsoska B, Mukherjee D, Langford PR, Robertson BD, Jenssen H, et al. Ultra-short antimicrobial peptoids show propensity for membrane activity against multidrug resistant Mycobacterium tuberculosis. Front Microbiol. 2020;11:417.
[48] Richardson K, Bennion OT, Tan S, Hoang AN, Cokol M, Aldridge BB. Temporal and intrinsic factors of rifampicin tolerance in mycobacteria. Proc Natl Acad Sci. 2016;113(29):8302–7.
[49] Smith TC, Pullen KM, Olson MC, McNellis ME, Richardson I, Hu S, et al. Morphological profiling of tubercle bacilli identifies drug pathways of action. Proc Natl Acad Sci U S A. 2020;117(31):18744–53.
[50] Sukumar N, Tan S, Aldridge BB, Russell DG. Exploitation of Mycobacterium tuberculosis reporter strains to probe the impact of vaccination at sites of infection. PLoS Pathog. 2014;10(9):e1004394.
[51] Elitas M, Dhar N, Schneider K, Valero A, Braschler T, McKinney JD, et al. Dielectrophoresis as a single cell characterization method for bacteria. Biomed Phys Eng Express. 2017;3:015005.
[52] Ravenel MP. La vaccination préventive contre la tuberculose par le 'BCG. Am J Public Heal Nations Heal. 1928;18(8):1075.
[53] Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA. 1994;271(9):698–702.
[54] Kaufmann SHE. Vaccination against tuberculosis: revamping bcg by molecular genetics guided by immunology. Front Immunol. 2020;11:316.
[55] Kaufmann SHE, Weiner J, von Reyn CF. Novel approaches to tuberculosis vaccine development. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. 2017;56:263–7.
[56] Rosada RS, de la Torre LG, Frantz FG, Trombone AP, Zárate-Bladés CR, Fonseca DM, et al. Protection against tuberculosis by a single intranasal administration of DNA-hsp65 vaccine complexed with cationic liposomes. BMC Immunol. 2008;9:38.
[57] Rosada R, Silva C, Santana MH, Nakaie C, Torre L. Effectiveness, against tuberculosis, of pseudo-ternary complexes: peptide-DNA-cationic liposome. J Colloid Interface Sci. Sep. 2011;373:102–9. doi: 10.1016/j.jcis.2011.09.040
[58] Zárate-Bladés CR, Bonato VL, da Silveira EL, Oliveira e Paula M, Junta CM, Sandrin-Garcia P, et al. Comprehensive gene expression profiling in lungs of mice infected with Mycobacterium tuberculosis following DNAhsp65 immunotherapy. J Gene Med. 2009;11(1):66–78.
[59] Balbino TA, Aoki NT, Gasperini AAM, Oliveira CLP, Azzoni AR, Cavalcanti LP, et al. Continuous flow production of cationic liposomes at high lipid concentration in microfluidic devices for gene delivery applications. Chem Eng J. 2013;226:423–33.
[60] Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev. 2011;24(2):314–50.
[61] Méndez-Samperio P. Diagnosis of tuberculosis in HIV Co-infected individuals: current status, challenges and opportunities for the future. Scand J Immunol. 2017;86(2):76–82.
[62] Xu L, Kong J. A multiplexed nucleic acid microsystem for point-of-care detection of HIV coinfection with MTB and PCP. Talanta. 2013;117C:532–5.
[63] 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(8):e1006421.
[64] Russell DG. Mycobacterium tuberculosis and the intimate discourse of a chronic infection. Immunol Rev. 2011;240(1):252–68.
[65] Marakalala MJ, Raju RM, Sharma K, Zhang YJ, Eugenin EA, Prideaux B, et al. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nat Med. 2016;22(5):531–8.
[66] Al Shammari B, Shiomi T, Tezera L, Bielecka MK, Workman V, Sathyamoorthy T, et al. The extracellular matrix regulates

granuloma necrosis in tuberculosis. J Infect Dis. 2015;212(3):463–73.
[67] Westermann AJ, Gorski SA, Vogel J. Dual RNA-seq of pathogen and host. Nat Rev Microbiol. 2012;10(9):618–30.
[68] Lenaerts A, Barry 3rd CE, Dartois V. Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. Immunol Rev. 2015;264(1):288–307.
[69] Braian C, Svensson M, Brighenti S, Lerm M, Parasa VR. A 3D human lung tissue model for functional studies on mycobacterium tuberculosis infection. J Vis Exp JoVE. 2015;104:53084.
[70] Tezera LB, Bielecka MK, Chancellor A, Reichmann MT, Shammari BA, Brace P, et al. Dissection of the host-pathogen interaction in human tuberculosis using a bioengineered 3-dimensional model. eLife. 2017;6:e21283.
[71] Bielecka MK, Elkington P. Advanced cellular systems to study tuberculosis treatment. Curr Opin Pharmacol. 2018;42:16–21.
[72] Konar D, Devarasetty M, Yildiz DV, Atala A, Murphy SV. Lung-on-a-chip technologies for disease modeling and drug development. Biomed Eng Comput Biol. 2016;7(Suppl 1):17–27.
[73] Cui P, Wang S. Application of microfluidic chip technology in pharmaceutical analysis: a review. J Pharm Anal. 2019;9(4):238–47.
[74] Li Y, Wu Q, Sun X, Shen J, Chen A. Organoids as a powerful model for respiratory diseases. Stem Cells Int. 2020;2020:5847876.
[75] Bielecka MK, Tezera LB, Zmijan R, Drobniewski F, Zhang X, Jayasinghe S, et al. A bioengineered three-dimensional cell culture platform integrated with microfluidics to address antimicrobial resistance in tuberculosis. mBio. 2017;8(1):e02073–16.
[76] Berry SB, Gower MS, Su X, Seshadri C, Theberge AB. A modular microscale granuloma model for immune-microenvironment signaling studies in vitro. Front Bioeng Biotechnol. 2020;8:931.
[77] Thacker VV, Dhar N, Sharma K, Barrile R, Karalis K, McKinney JD. A lung-on-chip model of early Mycobacterium tuberculosis infection reveals an essential role for alveolar epithelial cells in controlling bacterial growth. Elife. 2020;9:e59961.
[78] Neuži P, Giselbrecht S, Länge K, Huang TJ, Manz A. Revisiting lab-on-a-chip technology for drug discovery. Nat Rev Drug Discov. 2012;11(8):620–32.
[79] Yu J, Berthier E, Craig A, de Groot TE, Sparks S, Ingram PN, et al. Reconfigurable open microfluidics for studying the spatiotemporal dynamics of paracrine signalling. Nat Biomed Eng. 2019;3(10):830–41.
[80] Mukundan S, Singh P, Shah A, Kumar R, O'Neill KC, Carter CL, et al. In vitro miniaturized tuberculosis spheroid model. Biomed. 2021;9(9):1209.
[81] Thacker VV, Dhar N, Sharma K, Barrile R, Karalis K, McKinney JD. A lung-on-chip model of early Mycobacterium tuberculosis infection reveals an essential role for alveolar epithelial cells in controlling bacterial growth. Elife. 2020;9:1–73.
[82] Chen Y, Wang Y, Fleming J, Yu Y, Gu Y, Liu C, et al. Active or latent tuberculosis increases susceptibility to COVID-19 and disease severity. medRxiv. 2020. doi: 2020.03.10.20033795
[83] Tadolini M, Codecasa LR, García-García JM, Blanc FX, Borisov S, Alffenaar JW, et al. Active tuberculosis, sequelae and COVID-19 coinfection: first cohort of 49 cases. Eur Respir J. 2020;56(1):2001398.