

Harnessing microfluidics for Microbiology: from bacteria-host interactions to emerging cancer therapies

Corresponding Author: Dr Stephanie Descroix

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This review article—focusing on recent advances in microfluidic systems for studying bacteria–host interactions and cancer therapy—is overall well-structured and clearly written. The manuscript begins with three representative microfluidic platforms (Mother Machines, Droplet Microfluidics, and Microchambers), comprehensively summarizing their applications and progress in single-bacterium phenotyping and omics studies. It then moves on to bacterial collective behaviors, including quorum sensing, biofilm formation, and mechanical confinement.

The second major section focuses on advances in Organ-on-Chip technologies for in vitro modeling of host microenvironments, covering key aspects such as three-dimensional architectures, fluid flow and shear stress, mechanical stretching, substrate and extracellular matrix stiffness, and oxygen level control. The final section highlights recent progress in studying interactions between bacteria and the tumor microenvironment, as well as intervention strategies, including suppression of pathogenic or harmful bacteria, enhancement of beneficial strains, and fecal microbiota transplantation.

The reviewers recommend publication of the manuscript in Communications Biology after the comments have been addressed.

Major Comments

The conclusion and outlook section is currently organized into three parts: advantages of microfluidic systems, future perspectives, and limitations and controversies. The reviewers suggest swapping the order of the latter two sections. Presenting advantages first, followed by limitations, and then future directions would better align with general reader expectations and conventional logical flow.

Specific Points

1. Limitations and Controversies – Sample Biomass and Throughput Challenges

In the limitations and controversies section, the authors first highlight the comparative advantages and disadvantages of microfluidic systems versus animal models, as well as the need for their combined use under current research standards. The manuscript then emphasizes the challenge posed by low bacterial biomass in tumor microenvironments during sample preparation and analysis.

The reviewers suggest adding and emphasizing another important point: bacterial and host-cell samples obtained from Organ-on-Chip or Tumor-on-Chip platforms also often suffer from low biomass. This significantly limits the acquisition of system-level biological data, ranging from phenotypic characterization to omics analyses. One potential solution is to increase the parallelization and throughput of Organ-on-Chip or Tumor-on-Chip devices. However, achieving orders-of-magnitude increases in throughput is not realistically feasible through manual operation alone and would require the integration of laboratory automation technologies.

2. Limitations and Controversies – Data Bias, Artifacts, and Reproducibility

The authors further point out that data obtained from in vitro microfluidic systems may be distorted by operator-induced experimental bias, system artifacts, and inter-device inconsistency. Their proposed solution—validating in vitro findings using “controllable physiological systems”—is reasonable and well taken. The reviewers agree with this approach but recommends adding discussion of more upstream, root-cause solutions. As mentioned above, laboratory automation enabling high-throughput parallel device operation could substantially improve system consistency and data reliability. In addition, data analysis workflows should minimize “non-holistic” human intervention, such as cherry-picking results that favor hypothesis validation or excessive data polishing.

While it is difficult to eliminate human bias in data interpretation, potential mitigation strategies include the use of AI-assisted, high-throughput automated data analysis tools, the application of holistic analysis parameters, and transparent reporting of parameter choices and settings. This would allow third-party researchers to reproduce the same conclusions using identical raw data, analysis tools, and parameter configurations.

3. Limitations and Controversies – Adoption Barriers

The reviewer further suggests adding a discussion on adoption barriers. Microfluidic systems—especially highly complex Organ-on-Chip and Tumor-on-Chip platforms—often involve significant engineering complexity (e.g., closed-system configurations, microfabrication and fluidic control requirements). Combined with throughput limitations, system artifacts, and consistency issues discussed above, these factors create high barriers to adoption, particularly for researchers in biology and biomedical sciences.

As a result, in practice, microfluidic technologies remain largely confined to engineering-oriented research groups and laboratories, with relatively limited uptake in traditional biology and biomedical laboratories. Taken together, improving fabrication throughput, system consistency, and unbiased data analysis will be critical for enabling true partial standardization of microfluidic systems and for achieving their broad acceptance and routine use by the biological and biomedical research communities.

Minor Comments

>line 54 “...skin, nasal passages, lungs are oxygen-rich...” It would be more accurate to specify that the surface of skin is oxygen-rich. Other layers of the skin (e.g., epidermis, dermis, and hypodermis) are largely anaerobic, with pO₂ levels below 5%.

>line 331 “...in liquid media surrounded by mineral oil impermeable to O₂...” The statement that mineral oil is “impermeable to O₂” lacks accuracy. Mineral oil is a low oxygen-permeability medium and may be approximately considered “impermeable to O₂” in short-term experiments (e.g., a few hours). However, in long-term experiments, oxygen can effectively permeate through mineral oil and alter anaerobic conditions.

>line 353 uses AST, but then defines the acronym later in line 360

>line 514-529 - the section on biofilms seems sparse given the broad diversity of techniques we available to study "crosstalk between physiochemical signals and bacterial collective behaviors". The reviewers would like to suggest the inclusion of these papers as a starting point for a more comprehensive review of biofilm formation: A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation, O'Loughlin et al., 2013; Spatially structured exchange of metabolites enhances bacterial survival and resilience in biofilms, Zhang et al., 2024; Bacterial conjugation can restructure biofilms and increase their resilience while constraining host cell dispersal, Winans et al., 2025

>line 578 “...which condis-erably facilitate live imaging...” Typo: “condiserably” should be corrected to “considerably.”

>line 643 - would have liked a sentence to reference more/all current organ on chip model. Currently, only lung and gut are mentioned. Could the authors provide more examples of organ on chip model, such as skin, vagina, etc.?

>line 748 - again I am interested of the total extent of this kind of device. It is easy to imagine a heart or lung model would also benefit from stretching forces

>line 811-814 - should they include sources for this claim?

>line 820 - Would like to know if there is Organ-on-Chip or Tumor-on-Chip that investigates the unique interface between medical implants and tissue? Understanding biofilm growth on implanted devices is clinically relevant, and it would be good to acknowledge the systems that allow for testing of surfaces with distinct rigidities (hard, abiotic surfaces that interface with softer tissues).

>Line 1038 - would like at bare minimum citations for the seven cancer types

>line 1291-1293 - Do you feel they supported this claim? From my reading the previous section was mostly discussion of

biology rather than an investigation of current technology

>line 1469 - ulcerative colitis is not a cancer?

>line 1469-1482 - These technologies are cool but would like more explicit speculation about their application to tumor-on-chip models. This is my comment more broadly for the latter half of this section. The authors deviate from reviewing bacteria and cancer to current advances in host-microbe interactions. The reviewers would like a little more connection to the section header.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

The main claims of the review are the applications of microfluidics ranging from mother machine, microchamber, and droplet-based designs used in traditional microbiology studies to organ-on-chip and tumour-on-chip technologies used to investigate bacterial-host and bacterial-cancer interactions. The authors made a substantial review of the literature to provide a solid foundation to the perspective they want to present to the reader. They tap on cutting-edge research that is novel and frequently ground-breaking for the fields of clinical microbiology and oncology. This review will definitely influence thinking and the development of future research projects.

I only have a few minor comments:

Lines 158-167: mention the capacity of microfluidics to be used as a tool for antimicrobial susceptibility testing (AST), to set the scene for what comes later in the review.

Lines 268-271: lacking a reference for the mentioned study of bacterial phenotypes and persisters.

In this context, the authors may wish to consider these works:

Łapińska, U. et al. Fast bacterial growth reduces antibiotic accumulation and efficacy. *Elife* 11, (2022). DOI: 10.7554/eLife.74062

Goode, O. et al. Persister *Escherichia coli* cells have a lower intracellular pH than susceptible cells but maintain their pH in response to antibiotic treatment. *mBio* 12, (2021). DOI: 10.1128/mBio.00909-21

In the 'Antibiotic susceptibility' subsection (starting at line 341), they should include studies that use mother machine for AST.

For this, the authors may wish to consider the following reference:

Cama, J. et al. Single-cell microfluidics facilitates the rapid quantification of antibiotic accumulation in Gram-negative bacteria. *Lab Chip* 20, 2765–2775 (2020). DOI: 10.1039/D0LC00242A

Line 435 – typo, 'continute' instead of contribute)

Table 1 should be referred to somewhere in the text.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have satisfactorily addressed all concerns. We recommend publication of the revised manuscript in *Communications Biology*.

Reviewer #3

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Reviewer #4

(Remarks to the Author)

The authors have appropriately incorporated the suggested references, I have no further comments or concerns.

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Responses to Reviewers

Manuscript number: COMMSBIO-26-0972-T

Corresponding authors: Maria Rescigno, Maria Carla Parrini & Stéphanie Descroix

We thank all the reviewers for their time and effort in helping us improve our scientific article. Below, we provide our responses to the reviewers' questions and suggestions. Our responses in this document are presented in **blue**, and the modified text is highlighted in **red** in the revised manuscript, for enhanced clarity.

Reviewer #1 (Remarks to the Author):

This review article—focusing on recent advances in microfluidic systems for studying bacteria–host interactions and cancer therapy—is overall well-structured and clearly written. The manuscript begins with three representative microfluidic platforms (Mother Machines, Droplet Microfluidics, and Microchambers), comprehensively summarizing their applications and progress in single-bacterium phenotyping and omics studies. It then moves on to bacterial collective behaviors, including quorum sensing, biofilm formation, and mechanical confinement.

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We thank Reviewers #1 and #3 for this suggestion. We have indeed switched the two paragraphs, corrected Figure 7, and adjusted the references to the Figure 7C and 7D, accordingly.

Specific Points:

1. Limitations and Controversies – Sample Biomass and Throughput Challenges

In the limitations and controversies section, the authors first highlight the comparative advantages and disadvantages of microfluidic systems versus animal models, as well as the need for their combined use under current research standards. The manuscript then emphasizes the challenge posed by low bacterial biomass in tumor microenvironments during sample preparation and analysis.

The reviewers suggest adding and emphasizing another important point: bacterial and host-cell samples obtained from Organ-on-Chip or Tumor-on-Chip platforms also often suffer from low biomass. This significantly limits the acquisition of system-level biological data, ranging from phenotypic characterization to omics analyses. One potential solution is to increase the parallelization and throughput of Organ-on-Chip or Tumor-on-Chip devices. However, achieving orders-of-magnitude increases in throughput is not realistically feasible through manual operation alone and would require the integration of laboratory automation technologies.

We thank the Reviewers #1 and #3 for highlighting this important point. Indeed, the low biomass of Organ-on-Chip and Tumor-on-Chip platforms is a limitation that may oblige to parallelize experiments and to pool extracts from

multiple chips. However, on the other hand, the low number of cells is crucial to analyse rare and precious samples such as patient-derived primary cells. For instance, the commercially available Idexx chip contains up to 20,000-40,000 cells. This on-chip low biomass did not prevent multi-omics investigation (e.g., transcriptomics, proteomics, or metabolomics), as omics analyses on cells isolated from chips have already been described in the literature (1). This information has now been added to the discussion lines 1516-1525.

2. Limitations and Controversies – Data Bias, Artifacts, and Reproducibility

The authors further point out that data obtained from in vitro microfluidic systems may be distorted by operator-induced experimental bias, system artifacts, and inter-device inconsistency. Their proposed solution—validating in vitro findings using “controllable physiological systems”—is reasonable and well taken. The reviewers agree with this approach but recommends adding discussion of more upstream, root-cause solutions. As mentioned above, laboratory automation enabling high-throughput parallel device operation could substantially improve system consistency and data reliability. In addition, data analysis workflows should minimize “non-holistic” human intervention, such as cherry-picking results that favor hypothesis validation or excessive data polishing.

While it is difficult to eliminate human bias in data interpretation, potential mitigation strategies include the use of AI-assisted, high-throughput automated data analysis tools, the application of holistic analysis parameters, and transparent reporting of parameter choices and settings. This would allow third-party researchers to reproduce the same conclusions using identical raw data, analysis tools, and parameter configurations.

We thank the Reviewers #1 and #3 for this insightful comment and fully agree that, in addition to validating on-chip findings with controllable physiological systems, upstream solutions addressing the root causes of variability and bias are essential. In this regard, we would like to highlight that significant efforts are currently underway in the field to standardize and automate both image acquisition and analysis workflows. Recent studies have demonstrated the development of automated, high-throughput imaging platforms and standardized analysis pipelines, which substantially reduce operator-dependent variability and improve inter-device consistency (2, 3). Furthermore, we agree that minimizing non-holistic human intervention in data analysis is critical. As suggested, AI-assisted tools represent a promising avenue to reduce subjective bias, such as selective data inclusion or excessive data processing. In addition to improving consistency, such approaches can facilitate the establishment of reproducible, third-party-accessible workflows by enabling the use of shared raw datasets, analysis pipelines, and parameter settings (4).

We have incorporated these points into the manuscript to emphasize that standardization, automation, and AI-assisted analysis together represent important complementary strategies to enhance data reliability and reproducibility in microfluidic systems (lines 1551-1558).

3. Limitations and Controversies – Adoption Barriers

The reviewer further suggests adding a discussion on adoption barriers. Microfluidic systems—especially highly complex Organ-on-Chip and Tumor-on-Chip platforms—often involve significant engineering complexity (e.g., closed-system configurations, microfabrication and fluidic control requirements). Combined with throughput limitations, system artifacts, and consistency issues discussed above, these factors create high barriers to adoption, particularly for researchers in biology and biomedical sciences.

As a result, in practice, microfluidic technologies remain largely confined to engineering-oriented research groups and laboratories, with relatively limited uptake in traditional biology and biomedical laboratories. Taken together, improving fabrication throughput, system consistency, and unbiased data analysis will be critical for enabling true partial standardization of microfluidic systems and for achieving their broad acceptance and routine use by the biological and biomedical research communities.

We thank the reviewer for this comment. While complex microfluidic systems, such as Organ-on-Chip and Tumor-on-Chip platforms, require specialized expertise for design and microfabrication, once fabricated, the devices can be adapted for use in biological and biomedical labs, enabling researchers without microengineering expertise to integrate them into their workflows. Collaborative efforts between engineering-focused and biological laboratories have been further supported by national and international funding initiatives, which promote translational research and provide a “techno-society” push toward making microfluidic technologies more accessible.

Similarly, industrial partners have recognized these challenges and now provide robust, easy-to-handle microfluidic platforms designed specifically for biological applications. These companies not only supply standardized devices but also offer maintenance and guidance to support non-engineers in their use. Additionally, many platforms include modular flow systems that integrate seamlessly with standard biological instruments and can be adapted to common laboratory devices such as microscopes, cytometers, and plate readers. By combining standardized fabrication,

adaptive device design, collaborative support, and industry-backed solutions, microfluidic systems are becoming increasingly accessible and practical for routine use in biological and biomedical research.

This information has been added in the discussion – *Limitation and debates* section – of the revised manuscript (lines 1559-1572).

Minor Comments:

- line 54 "...skin, nasal passages, lungs are oxygen-rich..." It would be more accurate to specify that the surface of skin is oxygen-rich. Other layers of the skin (e.g., epidermis, dermis, and hypodermis) are largely anaerobic, with pO₂ levels below 5%.

We thank Reviewers #1 and #3 for this suggestion. We have indeed corrected the sentence in the revised manuscript (lines 53-54).

- line 331 "...in liquid media surrounded by mineral oil impermeable to O₂..." The statement that mineral oil is "impermeable to O₂" lacks accuracy. Mineral oil is a low oxygen-permeability medium and may be approximately considered "impermeable to O₂" in short-term experiments (e.g., a few hours). However, in long-term experiments, oxygen can effectively permeate through mineral oil and alter anaerobic conditions.

The statement has been modified to be more nuanced in line 331 of the revised manuscript.

- line 353 uses AST, but then defines the acronym later in line 360

The acronym AST line 349 has been defined in the revised manuscript, as suggested by the Reviewers, before the second mention of the word line 363.

- line 514-529 - the section on biofilms seems sparse given the broad diversity of techniques we available to study "crosstalk between physiochemical signals and bacterial collective behaviors". The reviewers would like to suggest the inclusion of these papers as a starting point for a more comprehensive review of biofilm formation: A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation, O'Loughlin et al., 2013; Spatially structured exchange of metabolites enhances bacterial survival and resilience in biofilms, Zhang et al., 2024; Bacterial conjugation can restructure biofilms and increase their resilience while constraining host cell dispersal, Winans et al., 2025.

We thank the Reviewers #2 and #3 for suggesting these three references that will improve the section related to the contribution of molecular cues on biofilm formation. The reference O'Loughlin et al. (2013) (5) has been added in the first sentence of the paragraph (line 517). The two other references (Zhang et al., 2024 (6) and Winans et al., 2025 (7)) have been detailed in the subsection "*Biofilm formation*" (lines 517-536) of the revised manuscript.

- line 578 "...which condiserably facilitate live imaging..." Typo: "condiserably" should be corrected to "considerably."

This typo has been corrected line 596 of the revised manuscript.

- line 643 - would have liked a sentence to reference more/all current organ on chip model. Currently, only lung and gut are mentioned. Could the authors provide more examples of organ on chip model, such as skin, vagina, etc.?

Vagina-on-chips are already referenced line 859 of the main manuscript. Also, we have added new references to discuss the current Organ-on-Chip modeling the female reproductive system of the revised manuscript (8) (lines 618-625).

- line 748 - again I am interested of the total extent of this kind of device. It is easy to imagine a heart or lung model would also benefit from stretching forces

We thank the Reviewers #1 and #3 for this insightful point. We have added a new reference in the revised manuscript to highlight how stretch can be used in Heart-on-chip platforms (9–11) (lines 715-716).

- line 811-814 - should they include sources for this claim?

We initially referred to the publications mentioned in the previous paragraph. For the sake of clarity, we have added these references in the revised manuscript (line 839).

- line 820 - Would like to know if there is Organ-on-Chip or Tumor-on-Chip that investigates the unique interface between medical implants and tissue? Understanding biofilm growth on implanted devices is clinically relevant, and it would be good to acknowledge the systems that allow for testing of surfaces with distinct rigidities (hard, abiotic surfaces that interface with softer tissues).

We thank the reviewer for this insightful question. Direct Organ-on-Chip models specifically dedicated to the interface between medical implants and surrounding tissue remain relatively limited. One publication displays a stent-on-chip microfluidic platform to model bacterial attachment (*Pseudomonas fluorescens*) on a stented ureter under realistic flow conditions (12). The authors demonstrate that bacterial colonization preferentially occurs in low shear stress regions of the stent. This example has been added in the revised manuscript, lines 687-693.

- Line 1038 - would like at bare minimum citations for the seven cancer types
The reference Nejman *et al.* was initially applied to the whole of the sentence, not only the glioblastoma claim. The reference has been moved earlier in the sentence for the sake of clarity, following the Reviewers' comment (lines 1062-1065).
- line 1291-1293 - Do you feel they supported this claim? From my reading the previous section was mostly discussion of biology rather than an investigation of current technology. We agree that the sentence highlighted by the Reviewers #1 and #3, related to the section *Applying Tumor-on-Chips (ToC) to investigate cancer–bacteria interactions and tumor responses*, might be confusing. We therefore revised this sentence by "Overall, microfluidics devices allow the recapitulation of the tumoral microenvironment, and enable quantitative analysis of molecular and cellular responses" (lines 1291) of the revised manuscript.
- line 1469 - ulcerative colitis is not a cancer?
We thank the Reviewers #1 and #3 for pointing this unclear statement. Indeed, ulcerative colitis is not a cancer. However, it is highly associated to colorectal cancer. Following the next comment, we have decided to remove the whole paragraph from the manuscript.
- line 1469-1482 - These technologies are cool but would like more explicit speculation about their application to tumor-on-chip models. This is my comment more broadly for the latter half of this section. The authors deviate from reviewing bacteria and cancer to current advances in host-microbe interactions. The reviewers would like a little more connection to the section header.
We agree with the Reviewers #1 and #3 that this paragraph is decorrelated from the main topic. We have, therefore, considered removing it from the manuscript.

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank the Reviewer #3 for his/her contribution to improving our manuscript. We have answered his/her questions in the previous section.

Reviewer #4 (Remarks to the Author):

The main claims of the review are the applications of microfluidics ranging from mother machine, microchamber, and droplet-based designs used in traditional microbiology studies to organ-on-chip and tumour-on-chip technologies used to investigate bacterial-host and bacterial-cancer interactions. The authors made a substantial review of the literature to provide a solid foundation to the perspective they want to present to the reader. They tap on cutting-edge research that is novel and frequently ground-breaking for the fields of clinical microbiology and oncology. This review will definitely influence thinking and the development of future research projects.

I only have a **few minor comments**:

- Lines 158-167: mention the capacity of microfluidics to be used as a tool for antimicrobial susceptibility testing (AST), to set the scene for what comes later in the review. We thank the Reviewer #4 for pointing this mistake. Indeed, we have defined the term lines 349 and mentioned the acronym later in line 363.
- Lines 268-271: lacking a reference for the mentioned study of bacterial phenotypes and persisters. In this context, the authors may wish to consider these works: Łapińska, U. *et al.* Fast bacterial growth reduces antibiotic accumulation and efficacy. *Elife* 11, (2022). DOI: 10.7554/eLife.74062, Goode, O. *et al.* Persister *Escherichia coli* cells have a lower intracellular pH than susceptible cells but maintain their pH in response to antibiotic treatment. *mBio* 12, (2021). DOI: 10.1128/mBio.00909-21 As suggested, we have added the references Łapińska *et al.* (2022) (13) and Goode *et al.* (2021) (14) line 271 of the revised manuscript.
- In the 'Antibiotic susceptibility' subsection (starting at line 341), they should include studies that use mother machine for AST. For this, the authors may wish to consider the following reference: Cama, J. *et al.* Single-cell microfluidics facilitates the rapid quantification of antibiotic accumulation in Gram-negative bacteria. *Lab Chip* 20, 2765–2775 (2020). DOI: 10.1039/D0LC00242A

We thank te Reviewer #4 for suggesting this work. We have indeed added the reference Cama *et al.* (2020) (15) line 353-354 of the revised manuscript.

- Line 435 – typo, ‘continute’ instead of contribute)

This type has been corrected in line 436 of the revised manuscript.

- Table 1 should be referred to somewhere in the text.

We thank the Reviewer #4 for this suggestion. We have added the reference to Table 1 in the conclusion section of the revised manuscript (line 1502).

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15. Jehangir Cama, Margaritis Voliotis, Jeremy Metz, Ashley Smith, Jari Iannucci, Ulrich F. Keyser, Krasimira Tsaneva-Atanasova, and Stefano Pagliara. Single-cell microfluidics facilitates the rapid quantification of antibiotic accumulation in gram-negative bacteria. *Lab Chip*, 20:2765–2775, 2020. doi: 10.1039/D0LC00242A.