Chapter 10

PermeaSense: Rethinking How We Test Antimicrobials in the Fight Against Biofilm Infections

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Abstract

Antibiotic resistance is a growing global health crisis, projected to cause 10 million deaths annually by 2050. A central driver is the persistence of biofilms, which are structured bacterial communities encased in a protective matrix that make bacteria up to 1000 times more tolerant to antimicrobials. This resilience complicates infection treatment and industrial decontamination, contributing to economic losses estimated at $1.2 trillion annually.

Current antimicrobial testing methods do not fully capture the spatial complexity of biofilms. They rely on bulk averages that mask tolerant sub-regions and do not resolve the matrix's contribution to limited penetration. This lack of resolution hinders the rational design of anti-biofilm therapies.

To overcome these limitations, we developed *PermeaSense*, a microfluidic platform that cultivates biofilms on engineered membranes and integrates mechanical sensing with optical microscopy to quantify drug penetration and matrix disruption in real time. Unlike conventional assays, *PermeaSense* generates spatial susceptibility profiles, revealing where treatments fail within biofilms.

We validated the system across diverse bacterial species and environmental conditions relevant to healthcare and industry. Results show that biofilm composition, architecture, and environment collectively shape permeability and antimicrobial efficacy. By providing mechanistic, reproducible insights into biofilm resilience, *PermeaSense* is designed to accelerate the development of next-generation therapies and optimised decontamination strategies. It offers a critical tool for bridging the gap between discovery and application, advancing efforts to mitigate antibiotic resistance and improve public health.

Keywords: *Biofilm, Antimicrobial tolerance, Drug testing platform, Spatial mapping*

Introduction

Bacterial biofilms, high density cell populations within a self-secreted polymer matrix, are often portrayed as impermeable fortresses, but their contribution to antimicrobial resistance is more nuanced. Many antibiotics can diffuse through the extracellular matrix, yet their effective concentration is reduced by binding or inactivation within the matrix polymers that also generates sharp gradients in oxygen and nutrients, creating metabolically inactive zones where drugs that rely on active growth, such

as β-lactams or fluoroquinolones, are inherently less effective[1]. Coupled with stress responses, persister formation, and enzyme-mediated collective defences, biofilms form reservoirs of tolerant cells that sustain chronic infections and recurrent industrial contamination[2]. While the oft-cited "100–1000× tolerance" is context-dependent, the practical reality is clear: biofilms increase treatment failure, relapse, and control costs.

Measuring drug penetration into biofilms is therefore vital, not because penetration alone explains tolerance, but because it separates chemical exclusion from physiological tolerance as causes of failure. This distinction is critical for rational therapy design[3]. For small-molecule antibiotics, mapping penetration clarifies whether poor outcomes arise from diffusion barriers or metabolic heterogeneity. For newer modalities such as peptides, enzymes, nanoparticles, and phages, penetration is often the dominant bottleneck[4]. Quantitative penetration profiling also enables rational design of combination strategies, such as pairing matrix-disrupting agents with antibiotics, replacing trial-and-error with evidence-based approaches[5].

Despite advances, antibiotic discovery faces a persistent translation gap: compounds that succeed in vitro often fail in vivo, especially in biofilm-associated infections[6]. Gold-standard minimum inhibitory concentration (MIC) and minimum biofilm concentration (MBC) assays measure activity in planktonic cultures but offer no insight into structured environments[7]. Commercial biofilm assays, such as MBEC plates, capture tolerance but reduce complex biology to a single endpoint[8]. Advanced flow cells and confocal imaging provide mechanistic detail, yet they are slow, operator-dependent, and too low-throughput for iterative pipelines[9]. Developers are left choosing between speed without inference or depth without scalability.

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[1]Jeanyoung Jo, Alexa Price-Whelan, and Lars E.P. Dietrich, "Gradients and Consequences of Heterogeneity in Biofilms," *Nature Reviews Microbiology* 20 (2022): 593–607, https://doi.org/10.1038/s41579-022-00692-2

[2]Ho Yu Liu, Emma L. Prentice, and Mark A. Webber, "Mechanisms of Antimicrobial Resistance in Biofilms," *Npj Antimicrobials and Resistance* 2, no. 27 (2024), https://doi.org/10.1038/s44259-024-00046-3

[3]Andy Y. An et al., "An Overview of Biological and Computational Methods for Designing Mechanism-Informed Anti-Biofilm Agents," *Frontiers in Microbiology* 12 (2021): 640787, https://doi.org/10.3389/fmicb.2021.640787

[4]Cesar Augusto Roque-Borda et al., "Antimicrobial Peptides: A Promising Alternative to Conventional Antimicrobials for Combating Polymicrobial Biofilms," *Advanced Science* 12, no. 1 (2024): e2410893, https://doi.org/https://doi.org/10.1002/advs.202410893

[5]Ergun Akturk et al., "Combining Phages and Antibiotic to Enhance Antibiofilm Efficacy against an in Vitro Dual Species Wound Biofilm," *Biofilm* 6 (2023): 100147, https://doi.org/10.1016/j.bioflm.2023.100147

[6]Tom Coenye et al., "Global Challenges and Microbial Biofilms: Identification of Priority Questions in Biofilm Research, Innovation and Policy," *Biofilm* 8 (2024): 100210, https://doi.org/10.1016/j.bioflm.2024.100210

[7]Amber De Bleeckere et al., "High Throughput Determination of the Biofilm Prevention Concentration for Pseudomonas Aeruginosa Biofilms Using a Synthetic Cystic Fibrosis Sputum Medium," *Biofilm* 5 (2023): 100106, https://doi.org/10.1016/j.bioflm.2023.100106

[8]Kristina Ivanova et al., "Integrated Biofilm Dispersion and Virulence Responsiveness for Targeted Treatment of Pseudomonas Aeruginosa Infection in Lungs," *Advanced Functional Materials* 34, no. 46 (2024), https://doi.org/10.1002/adfm.202402868

[9]Yuzhen Zhang et al., "A Microfluidic Approach for Quantitative Study of Spatial Heterogeneity in Bacterial Biofilms," *Small Science* 2, no. 10 (2022): 2200047, https://doi.org/10.1002/smsc.202200047

This mismatch creates bottlenecks: lack of mechanistic feedback during optimisation, poor reproducibility from heterogeneous growth, and a persistent throughput–realism trade-off. Without tools to resolve whether drug failure stems from penetration limits or physiology, optimisation remains empirical, and regulatory progress slows.

PermeaSense aims to address this gap by leveraging a microfluidic platform that delivers real-time, spatially resolved data on penetration and efficacy. By standardising biofilm growth on engineered membranes, it produces predictable architectures and gradients that mimic clinically relevant states. Integrated flow control and optical sensing enable label-free monitoring, distinguishing penetration-limited from physiology-driven failure. Positioned as a mid-pipeline tool, *PermeaSense* complements rather than replaces existing assays, accelerating lead optimisation, guiding rational combination design, and generating mechanistic datasets suitable for AI-driven pipelines.

Results and Discussion

We developed a tuneable microfluidic membrane platform that enables reproducible biofilm growth, continuous measurement of permeability, and subsequent assessment of compound penetration within the same system (Figure **1**a). The system integrates an optically transparent microchannel with an embedded porous membrane that supports uniform biofilm growth while allowing continuous in-plane imaging. A dynamic flow environment, simulating both constant and fluctuating conditions, is controlled via a pressure modulator, while an array of flow sensors records membrane flux to calculate permeability. Together, these features eliminate the need for confocal sectioning and enable simultaneous monitoring of biofilm structure and transport dynamics, overcoming the reproducibility challenges that limit conventional assays.

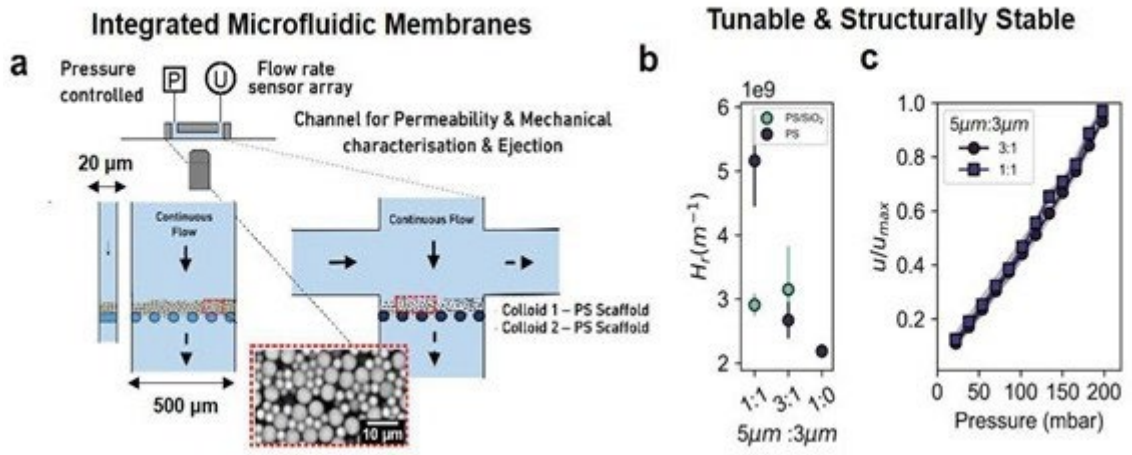

Figure 1: Development of Permeasense microfluidics incorporating in-situ flow assembled colloidal membranes. (a) Top view of the key microfluidic innovation. After membrane formation (inset) the device is sintered to produce a mechanically stable membrane. Black arrows indicate the flow direction. (b) The hydraulic resistance () of the microfluidic membranes tuned by adjusting the volume ratio of colloids and their material composition. (c) Linear pressure ramps with outlet velocity indicates compliance with Darcy's law.

The membranes are fabricated by trapping and sintering colloidal particles within a microstructured pillar array, producing stable porous substrates whose geometry and hydraulic resistance can be precisely tuned (Figure **1**b). By selecting particle size and composition, we generated pore structures

that exclude bacterial penetration. Validation experiments confirmed mechanical robustness (Figure **1**c). These tests confirmed the membranes provide a reliable reference material for biofilm permeability studies.

When inoculated with bacteria, the membranes reproducibly supported biofilm attachment and expansion (Figure **2**a). In *Staphylococcus epidermidis*, biofilm growth produced a gradual decline in flux, culminating in complete channel occlusion after approximately 6.5 hours. Time-lapse imaging and segmentation enabled direct correlation of strsuctural growth with hydraulic resistance (Figures **2**b, c). We imposed various pressure regimes that can mimic physiological fluid dynamic environments, and showed biofilm hydraulic resistance was invariant across physiologically relevant pressures (Figure **2**d).

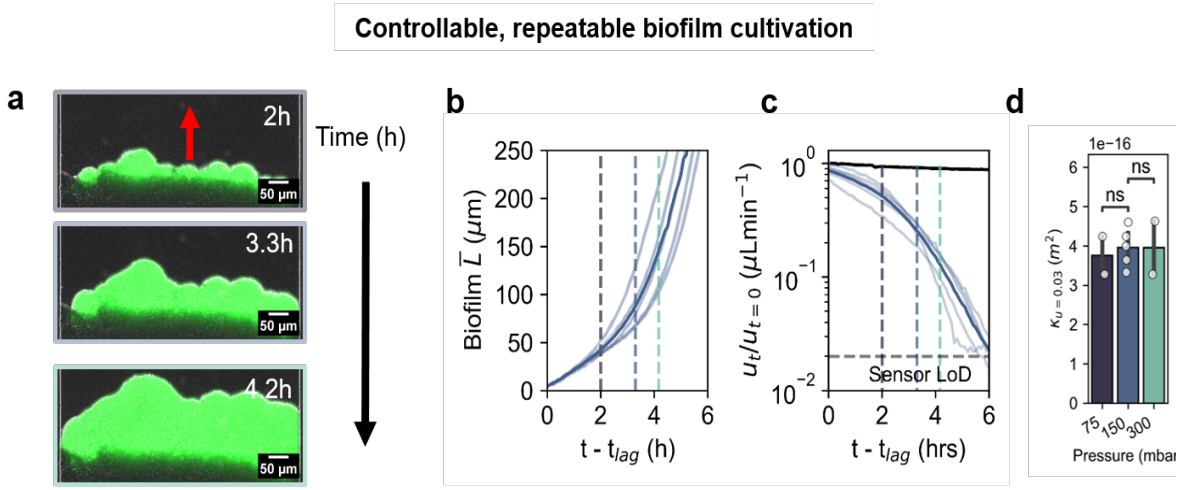

Figure 2: Quantifying real-time biofilm hydraulic permeability using Permeasense (a) Representative epi-fluorescent micrographs S. epidermidis 1457 biofilm growth upon the membrane substrate. The coloured frames denote the time slice from the subsequent dashed lines plots. (b) Biofilm development is tracked by measuring the biofilm thickness using time lapse microscopy. (c) Microfluidic flow sensors measure the flow rate (u) at the channel outlet, enabling the calculation of permeability. The black curve denotes control experiments performed with sterile media (d) Biofilm hydraulic resistance (H_r) and permeability (κ) are invariant under varying hydrostatic.

The platform enabled matrix compositional and environmental perturbations to be directly linked to permeability (Figure **3**a). In *S. epidermidis*, *Escherichia coli*, and *Bacillus subtilis* mutants lacking key matrix polysaccharide components produced significantly more permeable biofilms (Figure **3**b). Nutrient composition further modulated structure: substrate-limited conditions significantly reduced biofilm permeability, underscoring how global metabolic state shapes matrix transport properties. Extending this approach to multiple species, including *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, revealed that increasingly pathogenic biofilms have lower matrix permeability (Figure **3**c). These comparisons highlight the platform's utility as a standardised assay for cross-species benchmarking of biofilm permeability and structure.

Building on this foundation, we developed an antibiotic assay that leverages the membrane architecture to provide spatially resolved efficacy profiles. Biofilms are first cultivated to predefined thicknesses, while optical flow analysis continuously tracks biofilm expansion across its depth (Figure **4**a). Then, antimicrobial compounds are administered. Local shifts in expansion rates reveal how efficacy varies with biofilm thickness, while complementary staining with extracellular DNA markers captures the

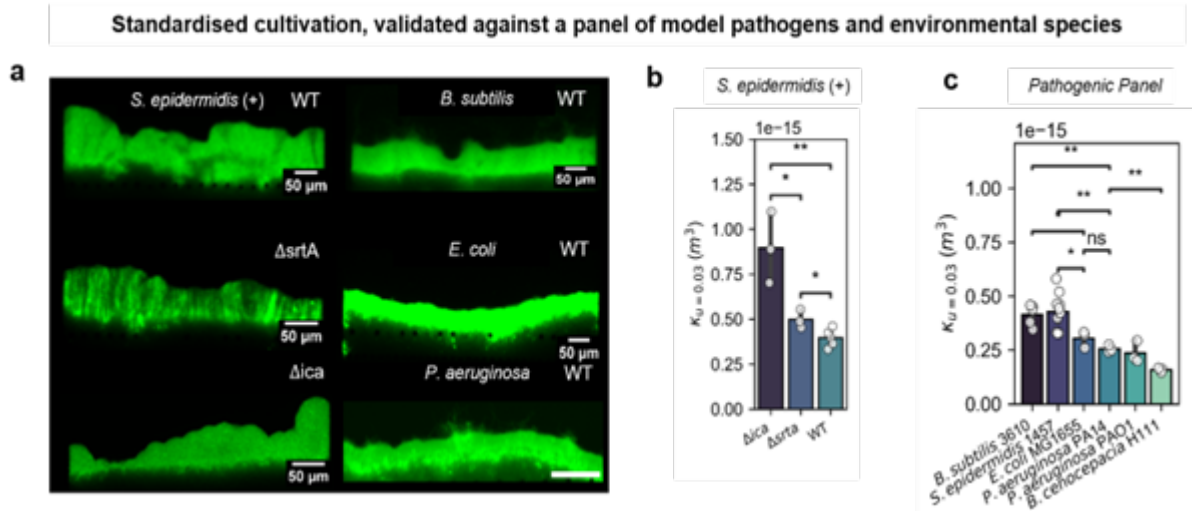

Figure 3: Permeasense is species agnostic and reveals that polysaccharide extracellular matrix components significantly contribute to biofilm permeability across multiple model biofilm species (a) Representative epi-fluorescence images of environmental and pathogenic biofilms (b) Permeability plots of S. epidermidis biofilm grown in kinetically limiting conditions. (c) Permeability plot overview of pertinent pathogens.

progression of cell death through successive layers (Figure **4**b). The combined readout produces a quantitative penetration-depth metric that directly links compound activity within the biofilm architecture, distinguishing penetration-limited effects from physiological tolerance. This approach moves beyond endpoint survival measurements by providing a mechanistic, real-time view of how antimicrobials act within structured microbial communities.

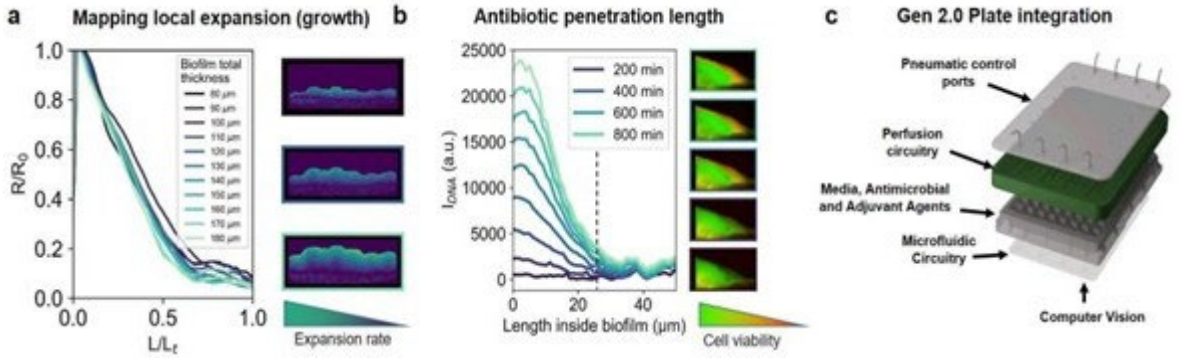

Figure 4: Parallelising the Permeasense technology for high throughput antibiotic penetration profiling (a) Optical flow analysis determines localised growth rates through the biofilm thickness (b) Integrated fluorescence imaging of eDNA release maps the activity and penetration upon antibiotic exposure (vancomycin 3 µg/ml vs S. epidermidis). (c) Schematic of Gen 2.0 platform integrated into a microtitre plate configuration.

To enhance throughput and usability, the platform is being scaled to incorporate 20 parallel channels via pneumatic microfluidic switches (Figure **4**c). Our 2.0 design preserves the analytical power of the original device while enabling parallel testing of compound panels. A further iteration is focused on minimising operator expertise. By embedding the microfluidic circuitry into a standard microtitre plate format, the system will align with familiar laboratory workflows: users will load wells with growth media, inoculum, and candidate antimicrobials, while an integrated manifold manages flow control and optical sensing. This format not only lowers the barrier to adoption but also positions the system for integration into automated discovery pipelines.

Conclusion

Collectively, these results demonstrate a platform that provides a robust, tuneable, and reproducible method for quantifying biofilm permeability and antimicrobial penetration in situ. Unlike conventional assays that yield only average endpoint values, the platform delivers continuous, mechanistic datasets that resolve where and why treatment fails. Its reproducibility makes it suitable for standardised use, while its mechanistic richness provides the kind of labels suitable for AI-driven design. Positioned as a mid-pipeline tool, *PermeaSense* bridges the translational gap that often causes promising compounds to fail in vivo. By providing clarity on compound penetration, tolerance, and combination effects in a scalable and user-friendly format, the platform offers a pathway for accelerating next-generation antimicrobial discovery and regulatory validation.

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References

Akturk, Ergun, Luís D. R. Melo, Hugo Oliveira, Aurélie Crabbé, Tom Coenye, and Joana Azeredo. "Combining Phages and Antibiotic to Enhance Antibiofilm Efficacy Against an in Vitro Dual Species Wound Biofilm." *Biofilm* 6 (2023): 100147. https://doi.org/10.1016/j.bioflm.2023.100147

An, Andy Y., Ka-Yee Grace Choi, Arjun S. Baghela, and Robert E. W. Hancock. "An Overview of Biological and Computational Methods for Designing Mechanism-informed Anti-biofilm Agents." *Frontiers in Microbiology* 12 (Apr 2021): 640787. https://doi.org/10.3389/fmicb.2021.640787

De Bleeckere, Amber, Sara Van den Bossche, Pieter-Jan De Sutter, Tine Beirens, Aurélie Crabbé, and Tom Coenye. "High Throughput Determination of the Biofilm Prevention Concentration for *Pseudomonas aeruginosa* Biofilms Using a Synthetic Cystic Fibrosis Sputum Medium." *Biofilm* 5 (Dec 2023): 100106. https://doi.org/10.1016/j.bioflm.2023.100106

Coenye, Tom, Merja Ahonen, Skip Anderson, Miguel Cámara, Parvathi Chundi, Matthew Fields, Ines Foidl, et al. "Global Challenges and Microbial Biofilms: Identification of Priority Questions in Biofilm Research, Innovation and Policy." *Biofilm* 8 (Dec 2024): 100210. https://doi.org/10.1016/j.bioflm.2024.100210

Ivanova, Kristina, Eva Ramon, Urszula Wnorowska, Katerina Todorova, Aleksandra Ivanova, Julio Bastos-Arrieta, Antonio Puertas-Segura, et al. "Integrated Biofilm Dispersion and Virulence Responsiveness for Targeted Treatment of Pseudomonas Aeruginosa Infection in Lungs." *Advanced Functional Materials* 34, no. 46 (Oct 2024). https://doi.org/10.1002/adfm.202402868

Jo, Jeanyoung, Alexa Price-Whelan, and Lars E. P. Dietrich. "Gradients and Consequences of Heterogeneity in Biofilms." *Nature Reviews Microbiology* 20 (Feb 2022): 593–607. https://doi.org/10.1038/s41579-022-00692-2

Liu, Ho Yu, Emma L. Prentice, and Mark A. Webber. "Mechanisms of Antimicrobial Resistance in Biofilms." *Npj Antimicrobials and Resistance* 2, no. 27 (Oct 2024). https://doi.org/10.1038/s44259-024-00046-3

Roque-Borda, Cesar Augusto, Laura Maria Duran Gleriani Primo, Kaila Petronila Medina-Alarcón, Isabella C. Campos, Camila De Fátima Nascimento, Mauro M. S. Saraiva, Angelo Berchieri Junior, et al. "Antimicrobial Peptides: A Promising Alternative to Conventional Antimicrobials for Combating Polymicrobial Biofilms." *Advanced Science* 12, no. 1 (Nov 2024): e2410893. https://doi.org/10.1002/advs.202410893

Zhang, Yuzhen, Yumin Cai, Lingbin Zeng, Peng Liu, Luyan Z. Ma, and Jintao Liu. "A Microfluidic Approach for Quantitative Study of Spatial Heterogeneity in Bacterial Biofilms." *Small Science* 2, no. 10 (Sep 2022): 2200047. https://doi.org/10.1002/smsc.202200047