

A simple diagnostic platform reveals shear stress as critical in biofilm development

Núria Blanco-Cabra^{a,b,*}, José Huguet^{c,d}, Josep Samitier^{c,d,e}, Romén Rodríguez-Trujillo^{c,d,e}, Eduard Torrents^{a,b,*}

^a Bacterial Infections and Antimicrobial Therapies Group (BIAT), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

^b Microbiology Section, Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona (UB), Barcelona, Spain

^c Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

^d Department of Electronics and Biomedical Engineering, Faculty of Physics, University of Barcelona (UB), Barcelona, Spain

^e Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Av. Monforte de Lemos, 3-5, Pabellón 11, Planta 0, Madrid, Spain

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ABSTRACT

Biofilms are complex microbial communities that exhibit increased tolerance to antibiotics, posing significant challenges in treating chronic infections and highlighting the need for more accurate and personalized diagnostic approaches, as well as simple and accessible platforms for growing biofilms under controlled flow conditions that better mimic in vivo environments. The XpertBiofilm is a novel platform designed to reproduce dynamic flow conditions by applying controlled shear stress, enabling a more realistic simulation of biofilm formation while allowing straightforward downstream analysis. Using this platform, we demonstrated a direct relationship between shear stress and biofilm biomass formation in *Pseudomonas aeruginosa*. Moreover, the XpertBiofilm was successfully validated for diagnostic applications using both clinical *P. aeruginosa* strains and direct sputum samples from cystic fibrosis patients, accurately reflecting their known susceptibility profiles and supporting the platform's potential to enable better patient treatment selection. Overall, the XpertBiofilm provides a reliable, user-friendly, and physiologically relevant platform for studying biofilms and evaluating therapeutic strategies, offering strong potential for both basic research and personalized clinical diagnostics.

1. Introduction

Biofilms develop when bacteria stick to a surface or to each other and produce an extracellular matrix that embeds them [1,2]. This matrix, which varies among different biofilm types, consists of polysaccharides, lipids, proteins, and extracellular DNA (eDNA) [3], acting as a barrier that shields from antibiotics and the immune system. As a result, bacteria in biofilms are naturally more resistant to antibiotics than their free-floating, planktonic counterparts, a phenomenon called tolerance, which contributes to the chronic nature of biofilm infections [4]. Besides the inherent and passive antibiotic resistance of biofilms, antimicrobial resistance (AMR) in bacteria is currently increasing worldwide, posing a serious public health threat due to the misuse and overuse of antibiotics [5]. Today, roughly 60–80% of bacterial infections are caused by

biofilms [6,7]. This, combined with the rise of AMR, emphasizes the urgent need to find better and more personalized treatments for biofilm infections and to enhance the effectiveness of existing antibiofilm therapies [8].

Several devices have been designed to grow biofilms in vitro for research purposes and for testing potential antibiofilm treatments [9–11]. These systems aim to replicate the environmental conditions that biofilms encounter and can be classified as static or dynamic, depending on how the culture media are supplied to the biofilm. In static systems, the medium remains stationary, limiting nutrient availability. In contrast, dynamic systems continuously provide fresh media maintaining steady nutrient availability, while removing planktonic cells that do not form and develop a biofilm [10,12,13]. In some dynamic models, biofilms are formed on coupons, enabling growth on different surface

* Corresponding authors at: Bacterial Infections and Antimicrobial Therapies Group (BIAT), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.

E-mail addresses: nblanco@ibecbarcelona.eu (N. Blanco-Cabra), etorrents@ibecbarcelona.eu (E. Torrents).

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materials. Known examples of these coupon-based systems include the CDC Biofilm Reactor® [14], the Drip Flow Biofilm Reactor® [15], the Bio-inLine® Biofilm Reactor [16] and the Coupon Evaluation Flow Cell (BioSurface Technologies, USA).

Additionally, in dynamic systems, flowing media expose biofilm cells to shear stress, as it naturally occurs in the body [17]. This shear stress, a frictional force acting parallel to the biofilm surface, is known to influence biofilm formation [18]. Therefore, biofilms grown under shear stress tend to adhere better, develop a stronger extracellular matrix [19] and exhibit increased antibiotic tolerance [20,21]. Specifically, *Pseudomonas aeruginosa*, an opportunistic pathogen that commonly forms biofilms in chronic conditions such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), or chronic wound infections [22], shows enhanced adhesion to the substrate when forming biofilms under certain shear stress levels [23–25]. However, the exact role of shear stress remains poorly understood, as some studies report varying effects across species and conditions [18,26–30].

These biofilm dynamic systems that can mimic shear stress within the body could accurately reproduce in vivo biofilm conditions and thus better predict antibiotic resistance [31]. However, they are less commonly used than static models due to their technical complexity, especially in a clinical setting. Analyzing biofilm parameters in these dynamic systems usually requires confocal microscopy and specialized personnel, making the process tedious, complicated, and difficult to be implemented in routine laboratories.

To address these challenges, the XpertBiofilm platform was designed to simulate physiologically relevant shear stress conditions in a straightforward way. The XpertBiofilm provides a quick, simple, and cost-effective method to grow and analyze biofilms without needing specialized equipment or highly trained personnel. By fine-tuning the shear stress conditions, the system produces uniform and reproducible bacterial biofilms, and results show that biofilm formation increases proportionally with higher shear stress. Additionally, the XpertBiofilm system has been validated using bacterial clinical isolates and sputum samples from CF patients with an easy and effective method for measuring antibiotic susceptibility, eliminating the need for advanced microscopy techniques or complex equipment. In summary, the XpertBiofilm offers a reliable, user-friendly, and straightforward platform to grow biofilms and evaluate the effectiveness of potential personalized treatments for biofilm infections.

2. Materials and methods

2.1. Bacterial strains

Pseudomonas aeruginosa PAO1 wild type (CECT 4122, ATCC 15692) and *P. aeruginosa* PAO1 expressing green fluorescence protein (eGFP) [11] are laboratory strains susceptible to ciprofloxacin (MIC < 0.5 µg/mL). *P. aeruginosa* PAET1 is a clinical strain resistant to ciprofloxacin (MIC 1–2 µg/mL), isolated from a CF patient with a chronic infection [32], and *P. aeruginosa* PA166 is an extensively drug-resistant (XDR) strain [32] resistant to ciprofloxacin (MIC > 10 µg/mL). All strains were grown in Luria Bertani broth (LB) (Scharlab S.L., Spain) at 37 °C with agitation until reaching an optical density (OD_{550 nm}) of 0.1 (lag phase), then used to inoculate the setup for static or continuous biofilm formation.

2.2. Clinical sputum samples

Sputum samples from CF patients were collected at the Microbiology Department of Hospital Universitari Vall d'Hebron, where its antibiotic sensitivity in planktonic conditions was determined. Sputum I was classified as ciprofloxacin-susceptible and ampicillin-resistant, whereas sputum II was determined ciprofloxacin-resistant and ampicillin-susceptible. Ethical approval for human sample collection and handling was granted by the Clinical Research Ethics Committee

(Comitè Ètic d'Investigació Clínica, CEIC) under approval number PR (AG)275/2019. To process the samples, 20 µL of sputum was dissolved in 800 µL of 0.1% DTT in PBS 1X (Thermo Scientific, USA). The sputum was then incubated at 37 °C with agitation for 1 h and vortexed for at least 2 min before inoculation into the system.

2.3. Device fabrication

The different prototypes, including the XpertBiofilm, were fabricated from polycarbonate stock using a Genos L200E-M CNC milling system (Okuma, Germany) at Centre CIM, Universitat Politècnica de Catalunya (CIM UPC, Spain). Precision drilling was used to create the openings required for fluidic access.

2.4. Shear stress quantification

The shear stress distribution in the different prototypes, including the XpertBiofilm was simulated using the finite element method with COMSOL Multiphysics 6.2 software. The culture medium was modeled as an incompressible Newtonian fluid with the same physical properties as water. No-slip boundary conditions were applied to the walls. The inlet was set to a fully developed, steady flow. To optimize the reliability of the simulation, an appropriate mesh was used in the model.

2.5. Static biofilm formation and treatment in a 24-well plate

Coupons (12 mm diameter round cover glasses) (Eprexia, Netherlands) were placed vertically in each of the wells of a 24-well plate (SPL Life Sciences Co., Ltd, Korea) and held in place using a piece of silicone (area of 1 cm², thickness: 1.5 mm ± 0.3 mm) (Merefsa, Spain). Wells were filled with 1 mL of the bacterial culture supplemented with 0.2% glucose (Thermo Scientific, USA). After 72 h at 37 °C, mature biofilms were washed twice with 1 mL of phosphate-buffered saline (PBS) (Fisher Scientific, Spain) and treated with different concentrations of ciprofloxacin (Cayman Chemical, USA) for 5 h.

2.6. Continuous biofilm formation and treatment inside the different prototypes

The dynamic biofilm formation setup consisted of a fresh medium reservoir connected to a high-precision Ismatec IPC16 ISM933 multi-channel peristaltic pump (Ismatec, Switzerland). From the pump, 1.5 mm diameter Tygon tubing (E-3603, dD BioLab, Spain) directed the medium into each prototype, which was subsequently connected to a waste collection bottle. To prevent air bubble introduction into the system, bubble traps (DTU, Denmark) were installed upstream of the device.

For biofilm growth, coupons (12 mm diameter round cover glasses) (Eprexia, Netherlands) were placed inside each prototype. The assembled setup was sterilized by circulating 0.5% (v/v) sodium hypochlorite through the tubing and prototypes, followed by rinsing with sterile water. LB broth medium supplemented with 0.2% glucose was then pumped through the device with the peristaltic pump as previously described [11]. Bacterial cultures or patient-derived sputum samples were inoculated into the system using a 26 G needle (Terumo Europe, Belgium). In the Prototype 2 and 3 (XpertBiofilm), inoculation was performed through the dedicated injection port (WHEATON® COMPLETEPak Stopper, Sigma-Aldrich, USA) whereas in Prototype 1 inoculation was carried out directly into the Tygon tubing. After bacterial inoculation, the flow was paused for 2 h to allow bacterial attachment to the coupon. After this attachment period, continuous flow was initiated at the desired rate (2.5, 10, 25, 45 or 100 µL/min) and maintained for 72 h at room temperature (RT) (22–24 °C) in a room with temperature-controlled environment to allow the development of mature biofilms. Ciprofloxacin (Cayman Chemical, USA) and ampicillin (Sigma-Aldrich, USA) treatments were introduced into the system in the

same manner as the bacteria, and flow was halted during the 16-hour treatment.

2.7. Biofilm quantification with microscopy

To determine the biofilm biomass and thickness of the *P. aeruginosa* PAO1 expressing eGFP by microscopy, the coupon where the biofilm formed was visualized under a confocal microscope (Zeiss LSM 800, Germany) with a 488 nm laser. FIJI, ImageJ, and COMSTAT2 software were used for biomass calculation.

2.8. Biofilm quantification with dyes

Coupons (12 mm diameter round cover glasses) (Epreidia, Netherlands) containing the biofilm were transferred to a 48-well plate (Nunc, Thermo Fisher Scientific, USA), and the biofilm was stained with 300 μ L of different dyes, then incubated in the dark for one hour at 37 °C. Fluorescence readings were taken using the Spark Multimode Microplate Reader (Tecan, Switzerland). Fluorescein diacetate (FDA) (Sigma-Aldrich, USA) was used at 8 μ g/mL in PBS and measured at 490/526 nm for excitation and emission. Resazurin (PrestoBlue® Cell viability reagent, Thermo Fisher Scientific, USA) was diluted 1:10 in PBS according to the manufacturer's guidelines, and fluorescence was measured at 560/590 nm (excitation/emission). DAPI dilactate (Thermo Fisher Scientific, USA) was used at 300 nM, with fluorescence measured at 358/461 nm (excitation/emission). The LIVE/DEAD™ kit was tested with Syto9 at 5 μ M and Propidium iodide at 30 μ M (BacLight™, Life Technologies, USA), with fluorescence measured at 480/500 nm and 490/635 nm (excitation/emission), respectively. Fluorescence measurements were acquired using dye-specific gain settings. For each fluorophore, the gain was optimized using the untreated control and subsequently kept constant for all experiments with the same fluorophore.

2.9. Biofilm quantification with CFU counting

To quantify the CFU forming the biofilm, bacteria were detached from the coupon (cover glasses) by sonicating them for 5 min in 500 μ L of PBS + 5% TWEEN® 20 (Sigma-Aldrich, USA) and vortexing for 30 s at high speed. Serial dilutions were prepared and plated on LB agar (Scharlab S.L., Spain) and incubated at 37 °C to count the colonies formed.

2.10. Statistical analysis

Results were statistically analyzed and plotted using GraphPad Prism 10.6.1. The specific statistical tests applied are indicated in the corresponding figure legends.

3. Results

3.1. The XpertBiofilm enables biofilm growth under shear stress and straightforward biofilm analysis

Previous work in our laboratory with dynamic biofilm devices, such as flow cells [33–35] and the FlowShear TriCell [36], highlighted the need for a user-friendly platform to analyze biofilms. Our aim in this work was to design a platform that preserved the advantages of these established systems while improving practicality and broadening usability. Specifically, we sought to develop a chamber in which biofilms could develop on a removable coupon, enabling both standard microscopy and straightforward quantitative biofilm analysis without requiring specialized equipment or advanced technical expertise, while also minimizing culture medium consumption in each biofilm experiment.

Building on our previous microfluidic biofilm device development

[11] and drawing on design features from established and widely used coupon-based systems [14–16], we aimed to incorporate the most advantageous aspects of these platforms, while addressing their major limitations. These include the large volumes of medium typically required [37], the heterogeneous shear-stress profiles they generate [19], and the inability to treat individual coupons independently [38]. In addition, in our experience, many of these systems offer limited accessibility to the growth surface, restrict options for inoculation or treatment, and rely almost exclusively on confocal microscopy for downstream analysis. Together, these factors complicate reproducible biofilm formation and hinder antibiotic susceptibility testing.

Motivated by these constraints, we designed a first prototype (Fig. 1a, Prototype 1 and Fig. S1a), consisting of a compact growth chamber that allowed insertion of a removable circular coupon. This design aimed to maintain controlled shear stress during biofilm growth while improving usability, reducing medium consumption, and enabling flexible downstream analysis, including both microscopy and plate-reader-based assays. Biofilm formation inside this prototype was first evaluated at two standard flow rates (45 and 100 μ L/min) and quantified using confocal microscopy with a *P. aeruginosa* PAO1 laboratory strain encoding a constitutive green fluorescent protein (eGFP). In this first version, the resulting biofilms grew homogeneously, with biomass values of 10.19 and 16.18 μ m²/ μ m³ at flow rates of 45 and 100 μ L/min, respectively (Fig. 1a, Prototype 1). However, an important limitation remained unresolved, since the growth chamber was not easily accessible, as it was sealed with a cover glass fixed with liquid silicone [39], which limited accessibility and ease of use.

To address this limitation, a second prototype was developed with improved accessibility of the growth chamber, incorporating a screw cap for sealing and facilitating coupon access (Fig. 1a, Prototype 2 and Fig. S1b). Nevertheless, this design yielded poor biofilm formation, especially at the lowest flow rate (45 μ L/min), where a non-homogeneous biofilm with biomass below 1 μ m³/ μ m² was observed (Fig. 1a, Prototype 2). Increasing the flow rate to 100 μ L/min modestly improved biofilm biomass (6.55 μ m³/ μ m²). These results suggested that flow dynamics may have influenced biofilm development, since shear stress (τ) increases directly with flow rate (Q), as described in the following equation:

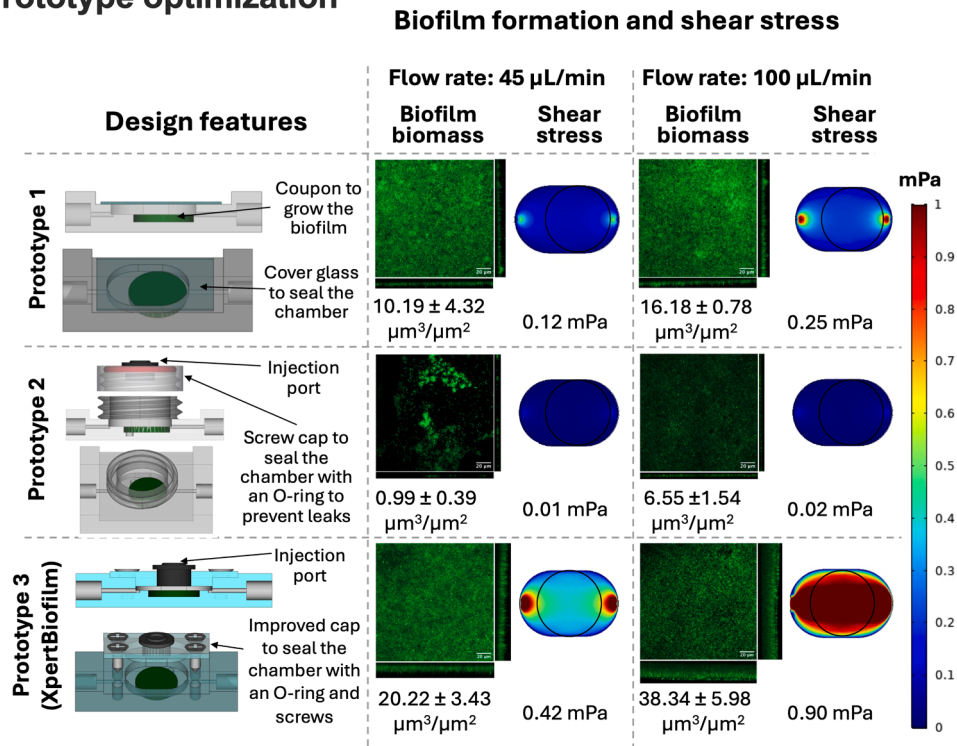
$$\tau = (6\mu Q)/(h^2 w)$$

where (μ) is the viscosity of the growth media and (h) and (w) are the height and the width of the chamber, respectively.

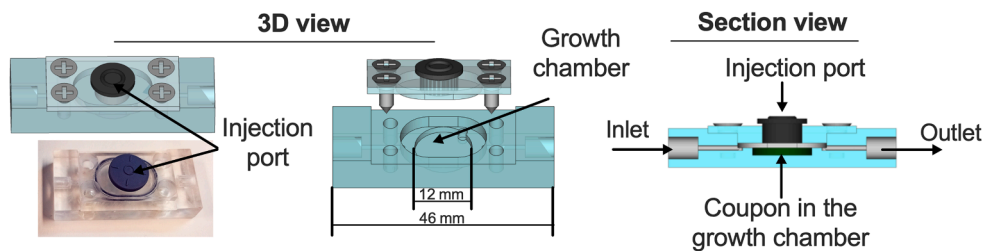
Thus, computational fluid dynamics simulations using COMSOL Multiphysics were performed to analyze the shear stress formed inside each biofilm growth chamber (see Materials and Methods). The analyses revealed that the increased chamber height (h) required by the screw-cap design led to an overall 10-fold reduction in shear stress (from 0.12 to 0.01 mPa) (Fig. 1a, Prototype 2 vs. Prototype 1), which likely explains the impaired biofilm formation observed at lower flow rates. Consequently, a third prototype was developed to optimize the chamber height and restore adequate shear stress levels (Fig. 1a, Prototype 3 and Fig. S1c). Simulations of this version indicated higher shear stress values (0.42 and 0.90 mPa at 45 and 100 μ L/min, respectively), and biofilm formation remained robust, with increased biomass values of 20.22 and 38.34 μ m²/ μ m³ at 45 and 100 μ L/min, respectively (Fig. 1a, Prototype 3). This optimized prototype version was selected as the final design and designated as the XpertBiofilm.

Thus, in the Prototype 3 design (XpertBiofilm), the growth chamber allows the insertion of a 12-mm-diameter coupon onto which the biofilm forms (Fig. 1b and Fig. S1c). Importantly, these coupons can be fabricated from a wide range of materials, including plastic, silicone, titanium, hydroxyapatite, or glass, making the device versatile for studying biofilm formation on clinically relevant surfaces. The device also includes strategically placed apertures for inlet and outlet tubing, enabling continuous media flow. Thus, the inlet tubing connects to a bottle of

a) Prototype optimization



b) Prototype 3 design (XpertBiofilm)



c) Setup

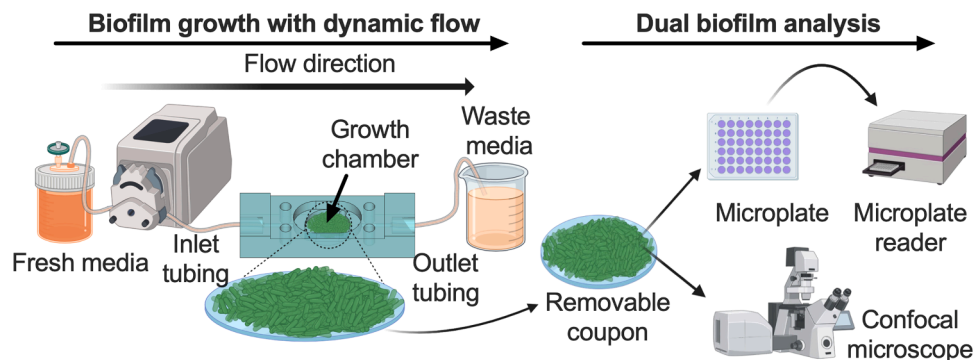


Fig. 1. Prototype optimization and system setup. a) Features of the different prototypes, together with biofilm biomass values ($\mu\text{m}^3/\mu\text{m}^2$) obtained from *P. aeruginosa* PAO1 eGFP biofilms grown in each prototype at two flow rates (45 and 100 $\mu\text{L}/\text{min}$). Values represent the mean $\pm$ standard deviation (SD) from three independent biological replicates. For each prototype and flow rate, shear stress simulations and mean shear stress values within the growth chamber are shown, generated using COMSOL Multiphysics. Color scale for shear stress is displayed on the right (in mPa). Detailed measurements and design specifications for each prototype are provided in [Supplementary Figure 1](#). b) Three-dimensional rendering, photograph, and detailed cross-sectional view of the Prototype 3 (XpertBiofilm). Detailed measurements and design specifications are provided in [Supplementary Figure 1](#). c) Schematic overview of the system setup and workflow for biofilm growth and analysis in Prototype 3 (XpertBiofilm).

fresh culture medium, while the outlet tubing brings waste medium into a separate container (Fig. 1c and Fig. S2). For practical usability, the XpertBiofilm also includes an injection port (Fig. 1b), accessible with a standard needle, allowing easy inoculation of bacterial cultures as well as administration of antimicrobial treatments or other supplements.

Following the setup scheme in Fig. 1c, once the bacteria are inoculated, fresh culture medium is pumped into the growth chamber at the desired flow rate using a peristaltic pump. This setup maintains a continuous flow of medium, which supplies nutrients and generates shear stress on the biofilm. Inside the growth chamber, the biofilm grows on the coupon under a consistent shear stress determined by the selected flow rate. The flowing media also removes planktonic bacteria that do not form part of the biofilm, directing them to the waste container (Fig. 1c and Fig. S2).

Once the biofilm is formed on the coupon (typically after ~3 days, depending on the microorganism), it can be analyzed using various methods. The removable coupon design enables high-resolution visualization using confocal or other advanced microscopy techniques. Moreover, the round coupon can also be placed directly into a 48-well microplate for straightforward biofilm quantification using indirect staining in a microplate reader (see Materials and Methods and Fig. 1c).

3.2. The XpertBiofilm achieves consistent biofilm formation and provides insight into shear stress-biomass dynamics

Continuous flow is a crucial feature of dynamic biofilm systems, as it

directly influences nutrient availability and shear stress. Since shear stress proved to be a critical factor influencing biofilm formation during the prototype optimization phase (Fig. 1a), its role in promoting biofilm growth was further investigated. The XpertBiofilm device was tested at increasing flow rates (2.5, 10, 25, 45 and 100 $\mu\text{L}/\text{min}$), and biofilm biomass and thickness were quantified using standard confocal microscopy, as previously described [11,33–36]. Both parameters increased with the flow rate, reaching an average biomass of $38.34 \pm 5.98 \mu\text{m}^3/\mu\text{m}^2$ and a thickness of $55.32 \pm 14.89 \mu\text{m}$ at the highest flow rate (100 $\mu\text{L}/\text{min}$) (Fig. 2a). To better understand this relationship, shear stress levels inside the growth chamber were computationally simulated across the same flow rates using COMSOL Multiphysics, and the calculated values were then correlated with the experimentally measured biomass (Fig. 2b). The analysis revealed a strong positive and significant correlation ($p < 0.0001$ and $R^2 = 0.91$), indicating that higher shear stress contributes directly to increased biofilm biomass. This finding highlights the importance of controlling the flow rate in the growth chamber design and, consequently, the shear stress in dynamic-flow devices, as these parameters play a crucial role in optimizing biofilm development.

To complete the system optimization, the optimal flow rate for achieving homogeneous and reproducible biofilm formation in the XpertBiofilm was determined by assessing variability in biomass and thickness across biological replicates using both standard deviation (SD) and coefficient of variation (CV). Since the lowest variability in both biomass and thickness was consistently observed at 45 $\mu\text{L}/\text{min}$ (Fig. 2a),

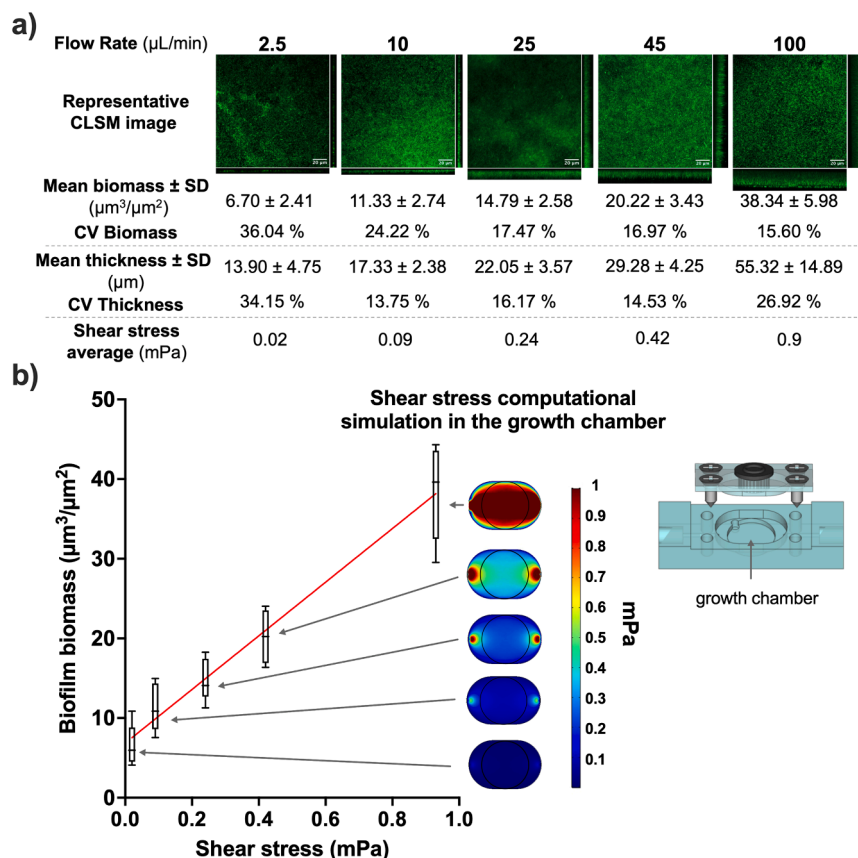


Fig. 2. Effect of flow rate and shear stress on biofilm development in the XpertBiofilm a) Representative confocal microscopy images (sum and orthogonal views) of biofilms formed under different flow rates, obtained from at least three independent biological experiments. Mean $\pm$ standard deviation (SD) values for biofilm biomass and thickness, along with their coefficients of variation (CV) are shown alongside with the average shear stress values inside the growth chamber for each condition. b) Correlation between average shear stress in the growth chamber and biofilm biomass, using the values obtained in a). Correlation is represented as min-max plots and analyzed by simple linear regression ($R^2 = 0.91$ and p -value < 0.0001 , red line). Computational simulations of shear stress distribution inside the growth chamber at the different flow rates, with the corresponding color scale in mPa, are shown. Average shear stress values in a) and shear stress simulations in b) were generated using COMSOL Multiphysics 6.2.

this flow rate was chosen as optimal flow for reproducible biofilm formation while maintaining reasonable medium consumption throughout the experiment.

To further confirm the robustness of the system, homogeneity was also assessed within a single biofilm at the optimized flow rate of 45 $\mu\text{L}/\text{min}$. Different regions of the same *P. aeruginosa* PAO1 biofilm were imaged, and their biomass and thickness were quantified. As shown in Fig. 3, the biofilm formed was homogeneous across different areas of the same coupon, with CV values of only 8.73% for biomass and 12.23% for thickness (Fig. 3b and c), demonstrating consistent biofilm formation within the coupon. Together, these results demonstrate that the Xpert-Biofilm platform not only supports robust biofilm growth at optimized flow rates but also ensures reproducible and homogeneous biofilm development.

3.3. Optimization of straightforward biofilm analysis and antimicrobial assessment

Following optimization of the device architecture and flow conditions, we next focused on refining the biofilm analysis. As shown in Fig. 1c, the XpertBiofilm provides a versatile platform for downstream analyses. Because the coupon on which the biofilm develops can be easily removed, biofilms can be examined either by confocal microscopy (as shown in Fig. 1a, 2a and 3a) or by rapid, indirect quantitative measurements of biofilm biomass using a microplate reader. This latter approach offers a simple and accessible method that could be implemented in clinical laboratories or hospitals, as it does not require advanced microscopy imaging or specialized image analysis software (e. g., ImageJ, COMSTAT or BiofilmQ). To enable this simplified workflow, however, it was necessary to identify and optimize a fluorescence-based staining method capable of reliably quantifying biofilm biomass growth and viability before its integration into the XpertBiofilm platform.

Several fluorescent dyes have been reported in the literature for biofilm biomass quantification [40]. In this work, some of these dyes were evaluated to identify the most suitable option for the XpertBiofilm platform. For this assessment, *P. aeruginosa* biofilms were grown statically on 12 mm round glass coupons placed vertically in a 24-well plate, to maximize biofilm formation at the air-liquid interface (see Materials and Methods and Fig. 4a). As a proof of concept for antibiotic treatment, after 72 h of incubation, the biofilms were treated with 10 $\mu\text{g}/\text{mL}$ ciprofloxacin for 5 h. Following treatment, coupons were gently rinsed with PBS to remove detached or dead bacteria from the biofilm and then transferred to a new microtiter plate for fluorescence measurements in a microplate reader using dye-appropriate excitation/emission parameters (see Materials and Methods and Fig. 4a).

Four different fluorescent dyes were evaluated: two enzymatic dyes (Fluorescein Diacetate (FDA) and resazurin) and two DNA-staining dyes (DAPI and the LIVE/DEAD™ kit (SYTO9 and PI)). As expected, each fluorophore yielded different relative fluorescence unit (RFU) values

and therefore required dye-specific gain settings (see Materials and Methods and Supplementary Table 1). Nevertheless, for all dyes, ciprofloxacin treatment reduced the normalized RFU values compared with non-treated coupons, reflecting a decrease in biofilm biomass. This RFU reduction was more pronounced with the enzymatic dyes FDA and resazurin (Fig. 4b, yellow and purple bars), compared to the non-enzymatic dyes DAPI and the LIVE/DEAD™ kit (Fig. 4b, blue and green-red bars). Among the enzymatic dyes, FDA showed the most pronounced RFU reduction (Fig. 4b, yellow bars); however, its high variability reduced statistical significance, making it less sensitive to treatment than the other enzymatic dye resazurin, which showed more consistent and reproducible results (Fig. 4b, purple bars).

To verify that the observed fluorescence reduction after ciprofloxacin treatment corresponded to a reduction in biofilm biomass, bacteria were recovered from the treated and non-treated coupons and plated onto LB agar for colony-forming unit (CFU) quantification (see Materials and Methods). This analysis confirmed a significant reduction of more than 4 logs of viable bacteria in the biofilm treated with 10 $\mu\text{g}/\text{mL}$ of ciprofloxacin (Supplementary Table 2), validating the fluorescent reduction seen in Fig. 4b.

The three dyes showing significant fluorescence differences between treated and non-treated biofilms (FDA, resazurin and DAPI) (Fig. 4b, yellow, purple and blue bars) were subsequently tested across a range of ciprofloxacin concentrations to establish their response curves and sensitivity, using the same statically grown biofilms (Fig. 4c-e). Among them, the enzymatic dye resazurin displayed the clearest and most consistent dose-dependent response (Fig. 4d), identifying it as the most suitable dye for assessing biofilm susceptibility to antibiotics. Therefore, resazurin was selected for subsequent antimicrobial testing within the XpertBiofilm continuous-flow system.

3.4. XpertBiofilm allows direct antibiotic susceptibility testing of biofilms from bacterial clinical isolates and patient samples

With the system conditions fully optimized in terms of growth chamber design, experimental setup, flow rate and fluorescence-based quantification analysis method, the XpertBiofilm platform was validated for its usability in assessing antibiotic susceptibility in biofilms. Validation was performed using both reference and clinical *P. aeruginosa* isolates, as well as samples from chronically infected patients, specifically CF sputum samples directly inoculated into the XpertBiofilm platform (Fig. 5a).

In both conditions, using bacterial isolates or CF sputum samples, biofilms were developed on a glass coupon inside the XpertBiofilm platform under a continuous flow (45 $\mu\text{L}/\text{min}$) for 72 h, followed by a 16-hour antibiotic treatment (Fig. 5a). Each condition was tested in three independent experiments, and biofilm quantification was performed using the enzymatic dye resazurin, previously identified as the most robust fluorescent readout (Fig. 4). Fluorescence was measured by

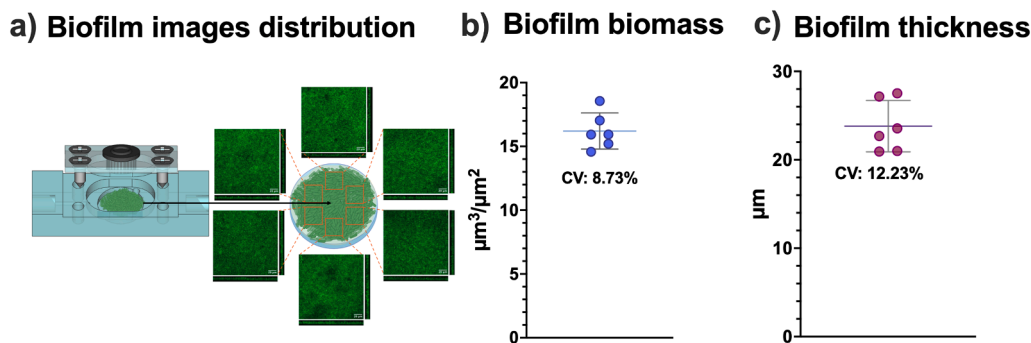


Fig. 3. Homogeneity within a single biofilm in the XpertBiofilm platform: a) Confocal microscopy images (sum and orthogonal views) of *P. aeruginosa* PAO1 eGFP biofilms formed in different regions of the same coupon at 45 $\mu\text{L}/\text{min}$ for 72 h at RT. b) and c) Quantified biofilm biomass and thickness for each region, and the corresponding coefficients of variation (CV) values. Created with BioRender.com.

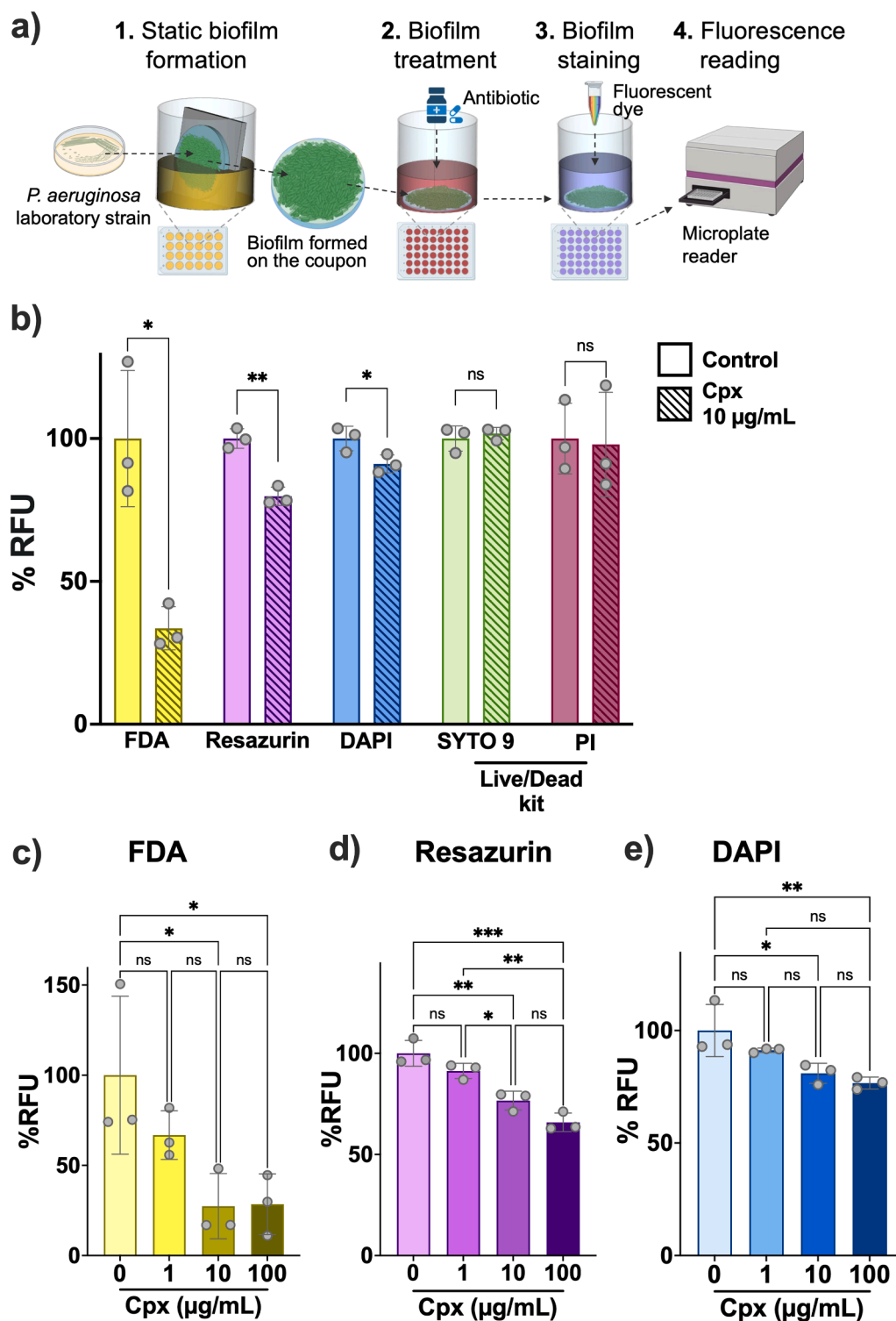


Fig. 4. Validation of fluorescent dyes in biofilms grown under static conditions in a 24-well plate: a) Schematic illustration of the workflow. b) Percentage of Relative Fluorescence Units (RFU) of the non-treated biofilm (solid bars) and the biofilm treated with 10 µg/mL of ciprofloxacin (stripped bars) dyed with Fluorescein Diacetate (FDA, yellow bars), resazurin (purple bars), DAPI (blue bars) and the LIVE/DEAD™ kit (Syto9, green bars and Propidium Iodide, PI, red bars). c-e) Percentage of Relative Fluorescence Units (RFU) of the biofilm treated with increasing concentrations of ciprofloxacin (Cpx) and dyed with Fluorescein Diacetate (FDA), resazurin, and DAPI. Data are presented as mean values and SD from three independent biological experiments. Data was compared with a Student Unpaired *t*-test in the b) and c) graphs and with a one-way ANOVA analysis with a Tukey's multiple tests in the c-e) graphs (*, *p*-value < 0.05; **, *p*-value < 0.01; ***, *p*-value < 0.001, ns, not significant). Created with Biorender.com.

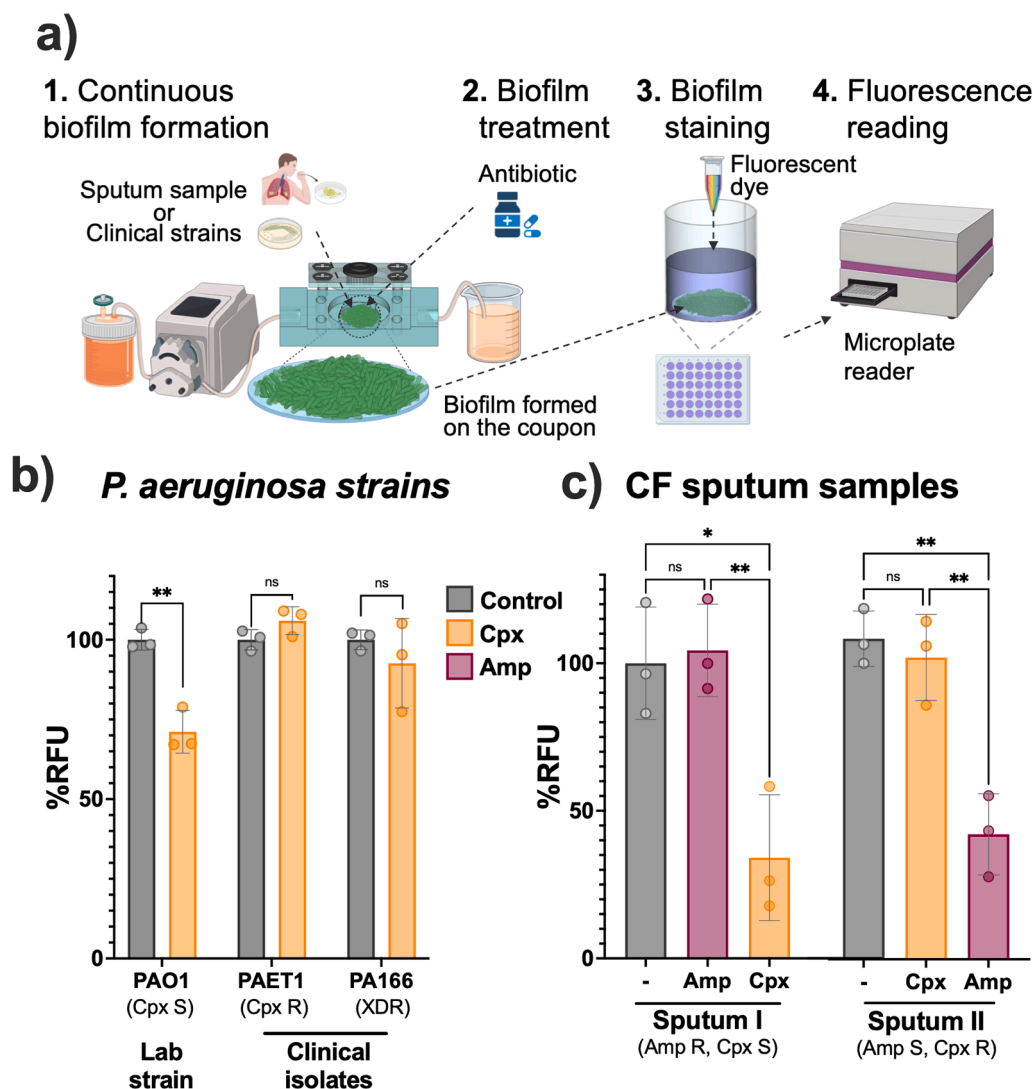


Fig. 5. XpertBiofilm validation with clinical strains and CF sputum samples: a) Schematic illustration of the workflow. b-c) Percentage of Relative Fluorescence Units (RFU) of biofilms grown in the XpertBiofilm from *P. aeruginosa* strains (b) and CF sputum samples (c), treated with 100 µg/mL of ciprofloxacin (Cpx) or ampicillin (Amp) and dyed with resazurin. Data represent the mean and SD from three independent experiments. Data was compared with a Student Unpaired *t*-test in the b) graph and with a one-way ANOVA analysis with a Tukey's multiple tests in the c) (*, *p*-value < 0.05; **, *p*-value < 0.01; ns, not significant). PAO1: *P. aeruginosa* PAO1 lab strain; PAET1: *P. aeruginosa* chronic clinical isolate; PA166: *P. aeruginosa* acute clinical isolate; XDR: extensively drug-resistant strain; Cpx S: Ciprofloxacin sensitive; Cpx R: Ciprofloxacin resistant; XDR: extensively drug-resistant strain; Amp S: Ampicillin sensitive; Amp R: Ampicillin resistant. Created with Biorender.com.

removing the coupon and reading the signal in a standard microplate reader (see Materials and Methods) (Fig. 5a).

For strain-based validation, three *P. aeruginosa* strains with distinct resistance profiles were tested: the laboratory strain PAO1, the chronic CF clinical isolate PAET1, and PA166, an extensively drug-resistant (XDR) strain from an acute infection (see Materials and Methods). As expected, the biofilm formed by the ciprofloxacin-susceptible PAO1 strain was significantly reduced with the antibiotic treatment (Fig. 5b). In contrast, the PAET1 strain biofilm, which has an intermediate resistance level (10-fold higher MIC than PAO1), exhibited a mild, non-significant increase in fluorescence, corresponding to an slight increase in biofilm biomass, suggesting that the ciprofloxacin concentration used was sub-optimal for this strain [41]. Meanwhile, the biofilm produced by the XDR strain PA166, known to be highly resistant to ciprofloxacin, showed no significant response to the treatment but a higher variability (Fig. 5b). These results demonstrate that the XpertBiofilm provides a reliable and accessible platform for antibiotic susceptibility testing in biofilms, without the need for specialized imaging

equipment.

To validate clinical applicability, two CF sputum samples with previously characterized antibiotic susceptibility profiles (see Materials and Methods) were directly inoculated into the XpertBiofilm and cultured under the same conditions (see Materials and Methods and Fig. 5a). The resulting biofilms were treated with ampicillin, ciprofloxacin, or left untreated. The biofilm formed from sputum sample I, which was resistant to ampicillin and sensitive to ciprofloxacin, showed a reduction in fluorescence only after ciprofloxacin treatment. Conversely, sputum sample II, which was sensitive to ampicillin and resistant to ciprofloxacin, responded only to ampicillin treatment (Fig. 5c). This demonstrates the XpertBiofilm's clinical relevance and its potential as a practical tool for directly assessing the antibiotic susceptibility of biofilms in a routine clinical practice for a specific patient.

4. Discussion

The XpertBiofilm was designed as a simple, versatile and

physiologically relevant platform for growing and analyzing biofilms under controlled flow conditions. After some prototype optimization (Fig. 1a), the final device was engineered with an optimized growth chamber geometry that ensures stable flow and uniform shear stress distribution, critical parameters for achieving homogeneous biofilm development. Moreover, it allows for the easy insertion and removal of coupons made of different substrates (Fig. 1b and Fig. S1c). This versatility enables biofilm growth on a wide range of materials, such as plastic, silicone, titanium, hydroxyapatite or glass, beyond others, making the platform ideal for studying biofilm formation across a wide range of bacterial or fungal species. This parameter is especially important, as the diverse physicochemical properties of each material affect bacterial attachment and biofilm development in species-specific ways [42–44]. Thus, the XpertBiofilm can be used to evaluate biofilm-resistant surfaces or materials designed to inhibit bacterial colonization, making it a valuable tool for new antibiofilm strategies [45,46]. Importantly, biofilms formed in the XpertBiofilm exhibited high homogeneity both across biological replicates at different flow rates (especially at 45 $\mu\text{L}/\text{min}$) and also across the entire coupon surface (Fig. 2a and 3), reducing spatial variability and enhancing the reliability of antibiotic susceptibility measurements.

A key step in optimizing the functionality of the XpertBiofilm was hydrodynamic improvement. Early prototypes of the device employed different sealing mechanisms, which altered internal flow patterns (Fig. 1a, Prototype 2), resulting in biofilm biomass values below those typically reported for *P. aeruginosa* biofilms grown under dynamic conditions [11,47,48]. Thus, after some computational fluid dynamics analysis, it was found that an increased chamber height significantly reduced shear stress, which explains the impaired biofilm development in Prototype 2. Restoring the appropriate chamber height by modifying the chamber size corrected the issue, yielding higher shear stress values and producing more robust biofilms (Fig. 1a, XpertBiofilm (Prototype 3)).

Computational fluid dynamics simulations further demonstrated a strong positive correlation between shear stress and biofilm biomass formation (Fig. 2b), highlighting the critical role of shear stress in promoting biofilm formation. This positive correlation aligns with several previous studies, in which increased shear stress has been shown to strengthen surface adhesin-mediated adhesion [49] and enhance biofilm growth [17,27,50–53]. Moreover, some bacteria can sense shear stress and modulate their gene expression to improve bacterial fitness [54]. Notably, *P. aeruginosa*, the organism used in this study, responds to shear stress by activating biofilm-related genes [55]. However, it's essential to consider the variability in bacterial strains and, therefore, the shear stresses applied. Some studies have also shown that low shear stress actually promotes biofilm formation [18,28,56], because up to a certain shear stress rate, there is inhibition of the biofilm development [26,57,58]. Altogether, these findings highlight that the effect of shear stress on biofilm formation is highly dependent on its magnitude and the biological context, emphasizing the need to define the optimal shear stress range for each bacterium and model system.

In particular, within the lung environment, shear stress generated by mucus transport is a key factor in pathogen clearance, with shear stress values around 0.8 dynes/cm² (80 mPa) [59]. Moreover, under healthy conditions, coughing can generate shear stresses as high as 1700 dynes/cm² (170 Pa) to eliminate bacteria [60]. However, in CF disease, where *P. aeruginosa* biofilms are a major cause of morbidity and mortality, shear stress is believed to be lower due to the thick and viscous sputum that hampers pathogen cleaning [61]. This suggests that while the CF lung environment has enough shear stress to facilitate robust biofilm formation, it lacks the necessary force to remove the attached bacteria and prevent chronic infections. The XpertBiofilm allows precise control over shear stress levels to mimic these *in vivo* conditions. Notably, the optimal shear stress range identified for homogeneous biofilm formation inside the XpertBiofilm was approximately 10–300 times lower than that associated with healthy conditions,

supporting its utility for modeling the CF lung environment and generating robust biofilms for testing.

In further optimizing the system to make it suitable for use beyond specialized biofilm research laboratories, biofilm quantification and antibiotic testing workflows were simplified. Conventional dynamic systems like flow cells often depend on confocal microscopy for the analysis, requiring specialized equipment, technical expertise, and complex data processing [62,63]. Although confocal microscopy is invaluable for in-depth studies [3,64], it is impractical for routine antibiotic susceptibility testing in clinical settings. The XpertBiofilm eliminates this dependency by enabling a more accessible, streamlined workflow: the biofilm is grown on a detachable coupon that can be transferred to a standard microplate reader for analysis using fluorescent dye-based assays. Among the various dyes reported for biofilm quantification [10,65], four were evaluated to be used in the XpertBiofilm: two enzymatic dyes, (FDA and resazurin) along with two DNA-staining dyes (DAPI and the LIVE/DEAD viability kit) (Fig. 4). Above all, within the conditions tested, resazurin showed the clearest and most consistent dose-dependent response, making it the most suitable option to use with XpertBiofilm (Fig. 4). This result aligns with previous reports, as this enzymatic dye showed good correlation with colony-forming units (CFUs) in biofilms [12,65,66]. This approach not only simplifies analysis but also provides functional information about biofilm viability, unlike crystal violet staining, which cannot distinguish between live and dead cells [67]. Moreover, XpertBiofilm can be readily adapted in the future to newly developed dyes for biofilm biomass assessment.

Finally, the XpertBiofilm was validated as a functional platform for antibiotic susceptibility testing in both laboratory and clinical contexts. This was demonstrated using three different *P. aeruginosa* strains and two sputum samples from patients suffering from CF with known antibiotic susceptibility profiles. After treatment with an antibiotic, a significant reduction in biofilm biomass was observed in antibiotic-susceptible strains or in sputum samples, whereas no significant effects were observed in the resistant strains. These results were fully consistent with the known susceptibility profiles, further confirming the reliability and clinical relevance of the XpertBiofilm system. Importantly, the XpertBiofilm also supported direct biofilm formation from patient-derived CF sputum samples, enabling *ex vivo* modeling of biofilm infections without prior bacterial isolation. This capability highlights the system's clinical relevance and its potential as a rapid, practical tool for guiding clinicians for antibiotic selection in chronic infections and personalizing treatment.

5. Conclusion

In this work, we developed the XpertBiofilm, a biofilm growth platform that enables controlled investigation of biofilm development under defined hydrodynamic conditions. By modulating shear stress, we demonstrate that this parameter plays a key role in governing *P. aeruginosa* biofilm formation. The XpertBiofilm combines physiological relevance with experimental accessibility, supporting multiple substrates for biofilm growth as well as both microscopy and straightforward fluorescence-based analysis. Moreover, the successful validation of the XpertBiofilm using both *P. aeruginosa* clinical strains and CF-patient-derived sputum samples demonstrates its versatility as a platform for studying biofilm biology and evaluating antimicrobial treatments under conditions that closely mimic real infections.

Ethics approval

Human sample collection and handling was performed in accordance with the principles of the Declaration of Helsinki, with ethical approval granted by the Clinical Research Ethics Committee (Comitè Ètic d'Investigació Clínica, CEIC) under approval number PR(AG)275/2019. The requirement for informed consent was waived by the ethics committee due to the retrospective use of fully anonymized samples.

CRediT authorship contribution statement

Núria Blanco-Cabra: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José Huguet:** Writing – review & editing, Software, Methodology. **Josep Samitier:** Writing – review & editing, Validation, Supervision, Software. **Romén Rodríguez-Trujillo:** Writing – review & editing, Supervision, Software. **Eduard Torrents:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eduard Torrents reports financial support and administrative support were provided by Institute for Bioengineering of Catalonia. Eduard Torrents has patent #BIOFILM FORMATION (PCT/EP2026/060925) pending to Not licenced. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.colsurfb.2026.116013](https://doi.org/10.1016/j.colsurfb.2026.116013).

Data availability

All data generated or analyzed during this study are included in this published article and are available from the corresponding author on reasonable request.

References

- H.-C. Flemming, et al., Biofilms: an emergent form of bacterial life, *Nat. Rev. Microbiol.* 14 (2016) 563–575, <https://doi.org/10.1038/nrmicro.2016.94>.
- K. Sauer, et al., The biofilm life cycle: expanding the conceptual model of biofilm formation, *Nat. Rev. Microbiol.* 20 (2022) 608–620, <https://doi.org/10.1038/s41579-022-00767-0>.
- H.C. Flemming, J. Wingender, The biofilm matrix, *Nat. Rev. Microbiol.* 8 (2010) 623–633, <https://doi.org/10.1038/nrmicro2415>.
- P.S. Stewart, Antimicrobial tolerance in biofilms, *Microbiol. Spectr.* 3 (2015), <https://doi.org/10.1128/microbiolspec.MB-0010-2014>.
- C.E. Flynn, J. Guarner, Emerging antimicrobial resistance, *Mod. Pathol.* 36 (2023) 100249, <https://doi.org/10.1016/j.modpat.2023.100249>.
- S. Yang, et al., Biofilm tolerance, resistance and infections increasing threat of public health, *Microb. Cell.* 10 (2023) 233–247, <https://doi.org/10.15698/mic2023.11.807>.
- D. Fleming, K. Rumbaugh, The consequences of biofilm dispersal on the host, *Sci. Rep.* 8 (2018) 10738, <https://doi.org/10.1038/s41598-018-29121-2>.
- C.-L. Moo, et al., Mechanisms of antimicrobial resistance (AMR) and alternative approaches to overcome AMR, *Curr. Drug. Discov. Technol.* 17 (2020) 430–447, <https://doi.org/10.2174/1570163816666190304122219>.
- J. Alcácer-Almansa, et al., Chapter 7 - Methods for studying biofilms: Microfluidics and translation in the clinical context, in: V. Gurtler, M. Patrauchan (Eds.), *Methods in Microbiology*, Academic Press, 2023, pp. 195–233.
- J. Azeredo, et al., Critical review on biofilm methods, *Crit. Rev. Microbiol.* 43 (2017) 313–351, <https://doi.org/10.1080/1040841x.2016.1208146>.
- N. Blanco-Cabra, et al., A new BiofilmChip device for testing biofilm formation and antibiotic susceptibility, *NPJ Biofilms Micro* 7 (2021) 62, <https://doi.org/10.1038/s41522-021-00236-1>.
- T.F. Bahamondez-Canas, L.A. Heersema, H.D.C. Smyth, Current Status of In Vitro Models and Assays for Susceptibility Testing for Wound Biofilm Infections, *Biomedicine* 7 (2019), <https://doi.org/10.3390/biomedicine7020034>.
- D. Lebeaux, et al., From in vitro to in vivo Models of Bacterial Biofilm-Related Infections, *Pathogens* 2 (2013) 288–356, <https://doi.org/10.3390/pathogens2020288>.
- D.M. Goeres, et al., Statistical assessment of a laboratory method for growing biofilms, *Microbiology* 151 (2005) 757–762, <https://doi.org/10.1099/mic.0.27709-0>.
- D.M. Goeres, et al., A method for growing a biofilm under low shear at the air–liquid interface using the drip flow biofilm reactor, *Nat. Protoc.* 4 (2009) 783–788, <https://doi.org/10.1038/nprot.2009.59>.
- L. Hall-Stoodley, et al., *Establishment of Experimental Biofilms Using the Modified Robbins Device and Flow Cells*, in: C. Edwards (Ed.), *Environmental Monitoring of Bacteria*, Humana Press, Totowa, NJ, 1999, pp. 307–319.
- F. Biagini, et al., A Millifluidic Chamber for Controlled Shear Stress Testing: Application to Microbial Cultures, *Ann. Biomed. Eng.* 51 (2023) 2923–2933, <https://doi.org/10.1007/s10439-023-03361-4>.
- A.L.M. Chun, et al., Early biofilm and streamer formation is mediated by wall shear stress and surface wettability: A multifactorial microfluidic study, *Microbiologyopen* 11 (2022) e1310, <https://doi.org/10.1002/mbo3.1310>.
- E. Johnson, T. Petersen, D.M. Goeres, Characterizing the Shearing Stresses within the CDC Biofilm Reactor Using Computational Fluid Dynamics, *Microorganisms* 9 (2021) 1709, <https://doi.org/10.3390/microorganisms9081709>.
- Y. Zhang, et al., Understanding the effects of aerodynamic and hydrodynamic shear forces on *Pseudomonas aeruginosa* biofilm growth, *Biotechnol. Bioeng.* 119 (2022) 1483–1497, <https://doi.org/10.1002/bit.28077>.
- V. Kostenko, et al., *Staphylococcus aureus* biofilm formation and tolerance to antibiotics in response to oscillatory shear stresses of physiological levels, *FEMS Immunol. & Med. Microbiol.* 59 (2010) 421–431, <https://doi.org/10.1111/j.1574-695x.2010.00694.x>.
- M.T.T. Thi, D. Wibowo, B.H.A. Rehm, *Pseudomonas aeruginosa* Biofilms, *Int. J. Mol. Sci.* 21 (2020) 8671, <https://doi.org/10.3390/ijms21228671>.
- S. Lecuyer, et al., Shear Stress Increases the Residence Time of Adhesion of *Pseudomonas aeruginosa*, *Biophys. J.* 100 (2011) 341–350, <https://doi.org/10.1016/j.bpj.2010.11.078>.
- A.V. Nguyen, et al., A high-throughput integrated biofilm-on-a-chip platform for the investigation of combinatory physicochemical responses to chemical and fluid shear stress, *PLoS. One* 17 (2022) e0272294, <https://doi.org/10.1371/journal.pone.0272294>.
- G. Savorana, et al., The influence of bodily fluid rheology on biofilm formation: known facts and open questions, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 383 (2025) 20240267, <https://doi.org/10.1098/rsta.2024.0267>.
- A. Park, et al., Effect of shear stress on the formation of bacterial biofilm in a microfluidic channel, *BioChip J.* 5 (2011) 236–241, <https://doi.org/10.1007/s13206-011-5307-9>.
- A. Rochex, et al., Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities, *Water Res.* 42 (2008) 4915–4922, <https://doi.org/10.1016/j.watres.2008.09.015>.
- A. Godain, et al., Influence of shear stress on electroactive biofilm characteristics and performance in microbial fuel cells, *Biosens. Bioelectron.* 244 (2024) 115806, <https://doi.org/10.1016/j.bios.2023.115806>.
- A.A.D. Jones, C.R. Buie, Continuous shear stress alters metabolism, mass-transport, and growth in electroactive biofilms independent of surface substrate transport, *Sci. Rep.* 9 (2019) 2602, <https://doi.org/10.1038/s41598-019-39267-2>.
- M. Krsmanovic, et al., Hydrodynamics and surface properties influence biofilm proliferation, *Adv. Colloid Interface Sci.* 288 (2021) 102336, <https://doi.org/10.1016/j.cis.2020.102336>.
- A.P. Fonseca, J.C. Sousa, Effect of shear stress on growth, adhesion and biofilm formation of *Pseudomonas aeruginosa* with antibiotic-induced morphological changes, *Int. J. Antimicrob. Agents* 30 (2007) 236–241, <https://doi.org/10.1016/j.ijantimicag.2007.04.011>.
- A. Crespo, et al., A single point mutation in class III ribonucleotide reductase promoter renders *Pseudomonas aeruginosa* PAO1 inefficient for anaerobic growth and infection, *Sci. Rep.* 7 (2017) 13350, <https://doi.org/10.1038/s41598-017-14051-2>.
- N. Blanco-Cabra, et al., Neutralization of ionic interactions by dextran-based single-chain nanoparticles improves tobramycin diffusion into a mature biofilm, *NPJ Biofilms Micro* 8 (2022) 52, <https://doi.org/10.1038/s41522-022-00317-9>.
- N. Blanco-Cabra, et al., Characterization of different alginate lyases for dissolving *Pseudomonas aeruginosa* biofilms, *Sci. Rep.* 10 (2020) 9390, <https://doi.org/10.1038/s41598-020-66293-2>.
- M. Cendra, et al., Optimal environmental and culture conditions allow the *in vitro* coexistence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in stable biofilms, *Sci. Rep.* 9 (2019) 1–17, <https://doi.org/10.1038/s41598-019-52726-0>.

- [36] J. Alcácer-Almansa, N. Blanco-Cabra, E. Torrents, *Burkholderia cenocepacia* and *Pseudomonas aeruginosa* in dual-species models: Insights into population distribution, antibiotic susceptibility, and virulence, *Virulence* 16 (2025) 2494039, <https://doi.org/10.1080/21505594.2025.2494039>.
- [37] I.B. Gomes, et al., Standardized reactors for the study of medical biofilms: a review of the principles and latest modifications, *Crit. Rev. Biotechnol.* 38 (2018) 657–670, <https://doi.org/10.1080/07388551.2017.1380601>.
- [38] D. Shi, et al., In vitro and ex vivo systems at the forefront of infection modeling and drug discovery, *Biomaterials* 198 (2019) 228–249, <https://doi.org/10.1016/j.biomaterials.2018.10.030>.
- [39] C. Sternberg, T. Tolker-Nielsen, Growing and Analyzing Biofilms in Flow Cells, *Curr. Protoc. Microbiol.* 00 (2006) 1B.2.1–1B.2.15, <https://doi.org/10.1002/9780471729259.mc01b02s00>.
- [40] J. Allkja, et al., Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates, *Biofilm* 2 (2020) 100010, <https://doi.org/10.1016/j.biofilm.2019.100010>.
- [41] J.B. Kaplan, Antibiotic-induced biofilm formation, *Int. J. Artif. Organs* 34 (2011) 737–751, <https://doi.org/10.5301/ijao.5000027>.
- [42] R.M. Donlan, Biofilms: microbial life on surfaces, *Emerg. Infect. Dis.* 8 (2002) 881–890, <https://doi.org/10.3201/eid0809.020063>.
- [43] K. Schilcher, A.R. Horswill, Staphylococcal Biofilm Development: Structure, Regulation, and Treatment Strategies, *Microbiol. Mol. Biol. Rev.* 84 (2020), <https://doi.org/10.1128/mmb.00026-19>.
- [44] T.R. Garrett, M. Bhakoo, Z. Zhang, Bacterial adhesion and biofilms on surfaces, *Progress. Nat. Sci.* 18 (2008) 1049–1056, <https://doi.org/10.1016/j.pnsc.2008.04.001>.
- [45] A. Banerjee, et al., Inhibition and eradication of bacterial biofilm using polymeric materials, *Biomater. Sci.* 11 (2022) 11–36, <https://doi.org/10.1039/d2bm01276f>.
- [46] W. Li, et al., Surface Design for Antibacterial Materials: From Fundamentals to Advanced Strategies, *Adv. Sci. (Weinh.)* 8 (2021) e2100368, <https://doi.org/10.1002/adv.202100368>.
- [47] A.R. Varadarajan, et al., An integrated model system to gain mechanistic insights into biofilm-associated antimicrobial resistance in *Pseudomonas aeruginosa* MPAO1, *npj Biofilms Micro* 6 (2020) 46, <https://doi.org/10.1038/s41522-020-00154-8>.
- [48] S.M. Daboor, et al., Marine Bacteria, A Source for Alginolytic Enzyme to Disrupt *Pseudomonas aeruginosa* Biofilms, *Mar. Drugs* 17 (2019), <https://doi.org/10.3390/md17050307>.
- [49] S.L. Arias, I.L. Brito, Biophysical determinants of biofilm formation in the gut, *Curr. Opin. Biomed. Eng.* 18 (2021) 100275, <https://doi.org/10.1016/j.cobme.2021.100275>.
- [50] A. David, et al., cmpX overexpression in *Pseudomonas aeruginosa* affects biofilm formation and cell morphology in response to shear stress, *Biofilm* 7 (2024) 100191, <https://doi.org/10.1016/j.biofilm.2024.100191>.
- [51] A. Fanesi, et al., Shear stress affects the architecture and cohesion of *Chlorella vulgaris* biofilms, *Sci. Rep.* 11 (2021) 4002, <https://doi.org/10.1038/s41598-021-83523-3>.
- [52] D. McLeod, L. Wei, Z. Li, A standard 96-well based high throughput microfluidic perfusion biofilm reactor for in situ optical analysis, *Biomed. Micro* 25 (2023) 26, <https://doi.org/10.1007/s10544-023-00668-w>.
- [53] S. Wang, et al., Dynamic Changes in Biofilm Structures under Dynamic Flow Conditions, *Appl. Environ. Microbiol.* 88 (2022), <https://doi.org/10.1128/aem.01072-22>.
- [54] A. Ramachandran, H.A. Stone, Z. Gitai, Free-swimming bacteria transcriptionally respond to shear flow, *Proc. Natl. Acad. Sci. USA* 121 (2024) e2406688121, <https://doi.org/10.1073/pnas.2406688121>.
- [55] E.K. Perry, M.W. Tan, Bacterial biofilms in the human body: prevalence and impacts on health and disease, *Front. Cell. Infect. Microbiol.* 13 (2023) 1237164, <https://doi.org/10.3389/fcimb.2023.1237164>.
- [56] G. Wei, J.Q. Yang, Impacts of hydrodynamic conditions and microscale surface roughness on the critical shear stress to develop and thickness of early-stage *Pseudomonas putida* biofilms, *Biotechnol. Bioeng.* 120 (2023) 1797–1808, <https://doi.org/10.1002/bit.28409>.
- [57] P. Thomen, et al., Bacterial biofilm under flow: First a physical struggle to stay, then a matter of breathing, *PLoS. One* 12 (2017) e0175197, <https://doi.org/10.1371/journal.pone.0175197>.
- [58] D. Marra, M. Rizzo, S. Caserta, Microfluidics unveils role of gravity and shear stress on *Pseudomonas fluorescens* motility and biofilm growth, *npj Biofilms Micro* 11 (2025) 122, <https://doi.org/10.1038/s41522-025-00744-4>.
- [59] A. Crabbé, et al., Response of *Pseudomonas aeruginosa* to Spaceflight and Spaceflight Analogue Culture: Implications for Astronaut Health and the Clinic. Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells, Springer New York, 2016, pp. 237–257.
- [60] R. Tarran, et al., Normal and cystic fibrosis airway surface liquid homeostasis, Eff. phasic Shear. Stress. *Viral Infect. J. Biol. Chem.* 280 (2005) 35751–35759, <https://doi.org/10.1074/jbc.M505832200>.
- [61] J. Dingemans, et al., Effect of Shear Stress on *Pseudomonas aeruginosa* Isolated from the Cystic Fibrosis Lung, *mBio* 7 (2016) e00813-16, <https://doi.org/10.1128/mbio.00813-16>.
- [62] T. Tolker-Nielsen, C. Sternberg, Methods for studying biofilm formation: flow cells and confocal laser scanning microscopy, *Methods Mol. Biol.* 1149 (2014) 615–629, https://doi.org/10.1007/978-1-4939-0473-0_47.
- [63] A.J. Morris, et al., Quantifying the Effects of Antimicrobials on In vitro Biofilm Architecture using COMSTAT Software, *J. Vis. Exp.* (2020), <https://doi.org/10.3791/61759>.
- [64] C. Reichhardt, M.R. Parsek, Confocal Laser Scanning Microscopy for Analysis of *Pseudomonas aeruginosa* Biofilm Architecture and Matrix Localization, *Front. Microbiol.* 10 (2019) 677, <https://doi.org/10.3389/fmicb.2019.00677>.
- [65] E. Peeters, H.J. Nelis, T. Coenye, Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates, *J. Microbiol. Methods* 72 (2008) 157–165, <https://doi.org/10.1016/j.mimet.2007.11.010>.
- [66] D.L. Wannigama, et al., Simple fluorometric-based assay of antibiotic effectiveness for *Acinetobacter baumannii* biofilms, *Sci. Rep.* 9 (2019) 6300, <https://doi.org/10.1038/s41598-019-42353-0>.
- [67] K. Doll, et al., Quantifying implant-associated biofilms: Comparison of microscopic, microbiologic and biochemical methods, *J. Microbiol. Methods* 130 (2016) 61–68, <https://doi.org/10.1016/j.mimet.2016.07.016>.