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A Microfluidics Platform for the High-throughput Study of Pseudomonas Aeruginosa Biofilm Formation Under Antibiotic Stress

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Bacterial biofilms, microbial communities encased in an extracellular matrix, drive persistent infections and hinder antibiotic treatments due to limited drug penetration and slow bacterial metabolism. Sub-minimal inhibitory concentrations of antibiotics can enhance biofilm formation by inducing stress responses, necessitating alternative therapeutic approaches and a comprehensive understanding of biofilm dynamics. Our study developed a droplet microfluidics-based platform for high-throughput screening of biofilm formation. We used Pseudomonas aeruginosa PAO1 to assess biofilm formation under varying nutrient conditions and exposure to multiple dilutions of sub-MIC tobramycin (<2 μg/mL). Time-lapse monitoring of bacterial aggregation in picoliter droplets was done via Z-stack fluorescence microscopy. We observed that P. aeruginosa PAO1 forms robust biofilms, particularly in nutrient-rich Luria-Bertani media, with sub-MIC tobramycin enhancing biofilm growth. Fluorescence microscopy of P. aeruginosa PAO1 cells further showed bacterial aggregation predominantly at the water-oil droplet interface, suggesting a preference for surface-associated growth and the method's suitability for large-scale antimicrobial screening. Future work will use microfluidics to explore sub-MIC bacterial phenotypes, phage-biofilm interactions, antibiotic-biofilm effects and phage-antibiotic synergy.