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A Simple and Cost-effective Method for Studying Bacterial Biofilm Formation

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Introduction

Bacterial biofilm formation has captured the attention of microbiology researchers due to the wide range of infections that it can be associated with, as well as its involvement in food spoilage, industrial biofouling, and possibly sewage treatment. However, due to the lack of standardisation of existing methods and the expensive equipment required, BBF remains difficult to study. We want to describe a new low-cost, easy-to-replicate protocol for a 3D-printed microfluidic device that can be used to study BBF dynamically. Methods: We designed the device with SolidWorks 3D CAD software and printed it with the Creality3D Ender 5 printer. We grew enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa strains [1].

Description

Bacterial biofilm formation is typically a multi-step process that includes the bacteria attaching to the substrate, forming microcolonies, growing and maturing the microcolonies into the mature biofilm, and finally dispersing the mature biofilm. New research has revealed that biofilms are frequently involved in human pathologies. Endocarditis, prosthetic device infections, catheter-related urinary tract infections, and bacteremia are all examples of biofilm-mediated infections. Unsurprisingly, there is a brand-new global trend to investigate BBF, its role in human infections, and methods of eradication. Existing data show how difficult it can be to remove a mature biofilm from various substrates, and with the emerging threat of antimicrobial resistance, there is a chance that biofilm eradication will play a critical role in the future of medicine [2].

There are numerous approaches to studying BBF, each with its own set of advantages and disadvantages. BBF research can be easily organised in both static and dynamic formats. Some of the most common methods for studying BBF are static, with the microtiter plate technique taking centre stage. This technique has some limitations, including poor reproducibility, nutrient exhaustion, and difficult direct inspection. However, because it is inexpensive and does not require any special equipment, it can be used successfully for screening biofilm formation capacity. There are many devices available that can provide a wide range of information for the dynamic study of biofilm formation, such as the Calgary device, the Robbins device, the Drip Flow Biofilm Reactor or Flow Chamber [3].

Then, using low-cost starting materials, the device could serve as a simple alternative to conventional methods for BBF studies. Several attempts have already been made to create such a device; however, the lack of uniformity in the data they produce makes this approach questionable. Previously, wet etching, reactive ion etching, conventional machining, photolithography, soft lithography, hot embossing, injection molding, laser ablation, in situ construction, and plasma etching were used to fabricate microfluidic devices. The presented design has a significant advantage in that it is H-type structured, with the double input allowing for the cultivation of polymicrobial biofilms as well as testing for the antibiofilm effect of various substances. This provides a great deal of flexibility in terms of the types of research protocols that can be used.

The absence of visible deposition lines suggests the formation of a thicker biofilm layer, or multiple layers deposited on the printed plates, a structure that is favoured by the experimental parameters. SEM images of the biofilm formed inside the microfluidic devices show more disorganised structures with defined colonies but no identifiable single cells. The experimental conditions, such as the sheer stress and flow rate present inside the microfluidic channels, could explain this phenomenon. Another notable feature is the arrangement of the colonies for the Enterococcus and Klebsiella biofilms along the deposition lines, with cervices between the filaments and small connection areas between colonies. The biofilms of Staphylococcus and Pseudomonas appear to be more uniformly distributed along the filament deposition lines [4].

The quantitative evaluation of the biofilm produced by the wild strains analysed revealed several similarities with the results of other studies, highlighting the accuracy of the results and confirming the utility of this working protocol. However, when we compared the Gram-positive bacteria from our study, Enterococcus faecalis and Staphylococcus aureus, we found that Enterococcus faecalis produced more biofilm, which appears to contradict the findings. who discovered that there is no significant difference in biofilm production between Enterococcus and Staphylococcus. A study of dual species biofilm, on the other hand, found that heme released respiration, increasing growth and the overall biomass of the biofilm [5].

Conclusion

Bacterial biofilm formation in vivo is a significant cause of mortality and morbidity, particularly in the hospital setting. Biofilms are known to be involved in many types of infections, usually involving the introduction of an external medical device into the human body, such as urinary catheters, central venous catheters, orthopaedic prostheses, cardiac prostheses, and many others. Although significant progress has been made in the study of BBF, there are still some unresolved issues, most notably the lack of standardization, the prohibitive cost of materials required for these studies, and the difficulties in interpretation. This paper presents a low-cost and simple method for studying BBF in both static and dynamic modes.

Acknowledgement

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Conflict of Interest

None.

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