INNOVATIVE MODEL SYSTEMS FOR CF-RELATED BIOFILM RESEARCH USING SYMCEL CALSCREENER AND SYNTHETIC CF SPUTUM MEDIA

INTRODUCTION

Investigating cystic fibrosis (CF)-related biofilm infec-tions presents a significant challenge due to the limitations of conventional biofilm models. Traditional methods, such as microtiter assays, are outdated and widely recognized for their lack of clinical relevance[1]. Although high-throughput models like MBEC assays offer accessibility and speed, their reductionistic nature often results in data that fails to translate into clinical success, leading to a substantial waste of resources. One critical issue with these models is the discrepancy between clinically relevant chemical and atmospheric environments inhibiting the development of correct pathogenic phenotypes.

The Symcel calScreener, when used in conjunction with synthetic CF Sputum Media (SCFM), offers a sophisticated alternative for studying CF biofilms[2]. This approach models cystic fibrosis infections within a chemically complex environment, effectively mimicking the "messy" infections observed in patients. This system allows for the investigation of both model strains and clinical isolates, maintaining clinically relevant biofilm aggregates[3]. One of the key advantages of the calScreener method is its ability to provide in situ analysis with rich kinetic data, even from cells deep within biofilms. This approach minimizes the need to remove cells from their natural environment, thereby reducing the risk of false positives due to misleading evaluation steps.

Furthermore, the calScreener enables real-time data acquisition and testing of treatments directly within the model, eliminating additional evaluation steps such as sonication and plating. Its versatility allows for experiments under anaerobic or micro-aerophilic conditions, providing a more comprehensive under-standing of CF-related biofilm infections and enhancing the potential for successful therapeutic interventions.

PROCEDURE

Clinical Isolates from long-time *Pseudomonas aeruginosa* infected patients with cystic fibrosis were grown overnight in either LB or SCFM1. The culture was diluted in either SCFM5 or 50°C warm semi-solid 0.5% LB-agar, and 200 µL was transferred to each calVial. The samples were incubated for 6 hours inside the calScreener at 37°C.

After the end of incubation, samples were treated with saline or either 4 or 8 µg/mL of tobramycin and incubated for 40 hours in the calScreener at 37°C (Fig. 1) .

Biofilm in scfm survives high tobramycin concentrations with clear subpopulations.

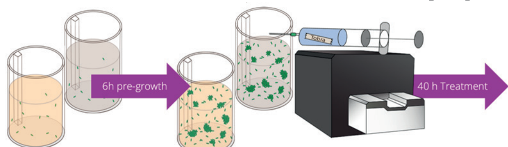

Figure 1 Schematic workflow of SCFM and LB agar being inoculated with single cells for them to grow into biofilm aggregates ready for treatment evaluation in the calScreener.

RESULTS

The phenotype of each of the three strains varied significantly between the two media used. All strains investigated showed substantial metabolic output during the six-hour pre-growth period, as well as post-treatment. In both LB semi-agar and SCFM, tobramycin treatments resulted in a concentration-dependent reduction of metabolic activity and a delay in peaking consistent with biofilm behavior (Figs. 2 and 3). Notably, the clinical CF strains exhibited a decreased response to treatments in SCFM compared to LB-agar, an effect not observed with lab strains such as PA14 (Fig. 3). This study successfully detected the kinetic impact of treatments on biofilm aggregates in situ within SCFM, highlighting its profound influence on treatment efficacy.

Biofilm aggregates grown in lb-agar are being eradicated, producing misleading results.

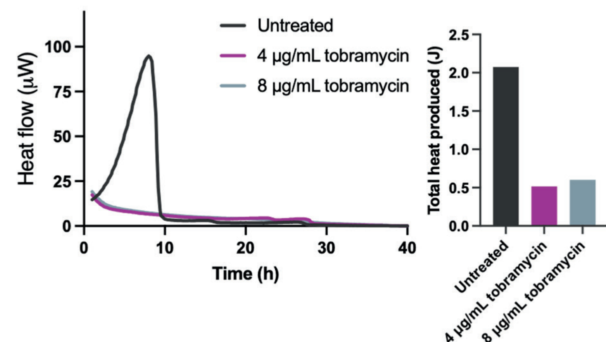

Figure 2 Biofilm aggregate grown in LB-based agar could be completely eradicated by both 4 and 8 µg/mL tobramycin as the clinical strain did not exhibit tolerant pathogenesis in the clinically irrelevant media. The calScreener detected the decline in metabolic activity of all the cells in the aggregates as the antibiotic killed the cells.

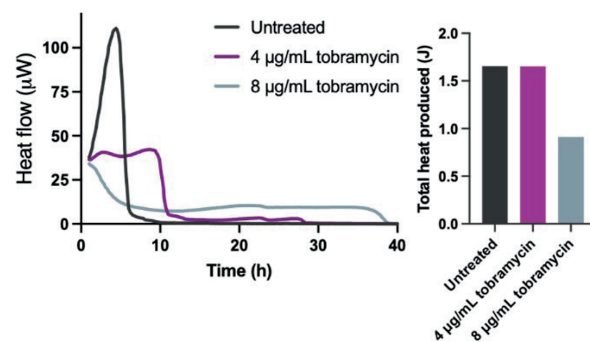

Figure 3 Subpopulations in biofilm aggregate grown in SCFM survived both 4 and 8 µg/mL tobramycin. Although 4 µg/mL did affect the aggregated population the total metabolism was not altered from the untreated sample – it merely lowered the population output. The calScreener detected the surviving metabolic slow subpopulation in aggregates treated with 8 µg/mL, seen as a prolonged, low metabolic signal.

CONCLUSION

The Symcel calScreener, used with synthetic CF Sputum Media, has the potential to significantly enhance the study of cystic fibrosis-related biofilm infections. The calScreener addresses this by mimicking complex CF infection conditions, providing real-time kinetic data and *in situ analysis* without disrupting biofilms.

This method accurately detects treatment impacts on biofilms in SCFM, highlighting clinically relevant responses. For instance, biofilms in SCFM show reduced response to tobramycin compared to those in LB agar, underscoring the importance of using appropriate media. By offering a realistic CF model, the calScreener improves research accuracy, optimizes resource use, and enhances the potential for successful clinical interventions.

The need for in vivo-like assays is not limited to CF research. With the use of microcalorimetry, models are not limited by their complexity but rather the imagination of the researchers. The importance of bridging the gap between *in vitro* and *in vivo* will not decrease.

References

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