On-Demand Chemically Reconfigurable Biofilm Microball Hydrogels for Antibiotic Assay

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Abstract

- Device-associated infections are responsible for more than 50,000 deaths per year and are linked to Center for Disease Control and Prevention (CDC) priority antibiotic-resistant bacteria. These infections involve bacterial biofilms, which are difficult to treat with antimicrobials, in some cases exceeding 10,000 times the minimal inhibitory concentration (MIC)
- Most pharmaceutical interventions do not take into account the impact and role of biofilm, biomaterials colonization or necrotic tissue in their performance testing methodology. Currently there are no established methods to study the difference between genetic resistance of bacteria to antibiotics and phenotypic resistance of bacteria living in biofilm communities to antibiotics
- In this work we develop a method to study biofilm in alginate hydrogels that can be dissolved, allowing for measurement of both resistance and persistence to antibiotics, with the goal of developing more effective interventions for device associated infections

Introduction

- Antibiotic resistance threatens to usher in a return to the pre-penicillin era of unchecked infections
- Carbapenem-resistant *Enterobacteriaceae*, Methicillin-resistant *Staphylococcus aureus*, drug-resistant *Streptococcus pneumoniae*, and multidrug-resistant *Pseudomonas aeruginosa*, all are CDC urgent or serious threats, are found in infections associated with medical device use
- Current testing is based on planktonic bacterial susceptibility models

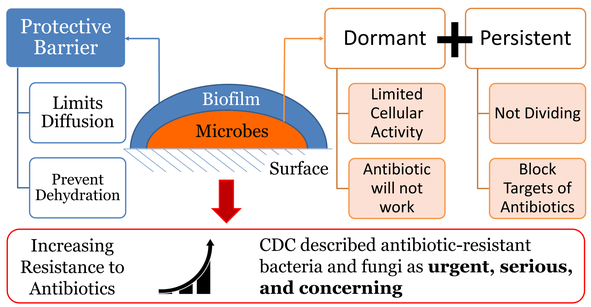

Figure 1. Schematics of several characteristics of biofilm's protective barrier and the embedded microbes leading to resistance to antibiotics

Table 1. Strains of bacteria used in this study

Escherichia coli	**RP437/pRSH103**	Red Fluorescence
	XEN14	Bioluminescence
Pseudomonas aeruginosa	**PAO1/pTdK-GFP**	Green Fluorescence
Staphylococcus aureus	**AH2547/pCM29**	Green Fluorescence

Materials and Methods

- Bacterial strains (Table 1) inoculated into 2% w/v alginate + 2× Mueller Hinton (MHB) broth (5×105 CFU per hydrogel)
- Bacterial alginate hydrogel was formed using spraying technique of CaCl2 (Fig. 2A)
- Viability assessment using 1000× antibiotics was quantified by microplate reader and verified by dissolving the gel for agar plating
- Confocal microscopy to assess the formation of biofilm balls
- Ruby Biofilm Stain to verify the biofilm matrix

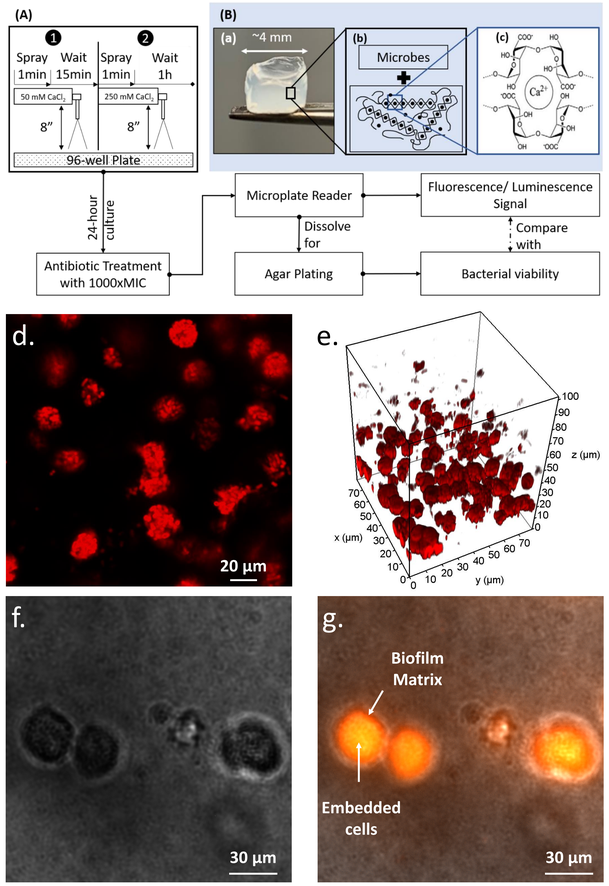

Figure 2. (A) Schematics of formation of alginate biofilm gel and experiment setup. (B) Molecular structure of alginate polysaccharide chains crosslinked by calcium ions. (d) – (e) Confocal images of biofilm balls and 3D reconstruction in alginate gel. (f) – (g) Biomatrix and cell staining

Results and Discussion

- Confocal microscopy shows the formation of biofilm balls (Fig. 2d-e)
- Stains show biofilm matrix enveloping the cells within (Fig. 2f- g)
- MIC testing was performed to obtain a baseline for the interaction of each strain with antibiotics (Table 2)
- Fig. 3a-c, fluorescence signals from hydrogels cultured with MHB, confirmed with plating viability, showed the bacterial biofilms stayed viable over 3 days

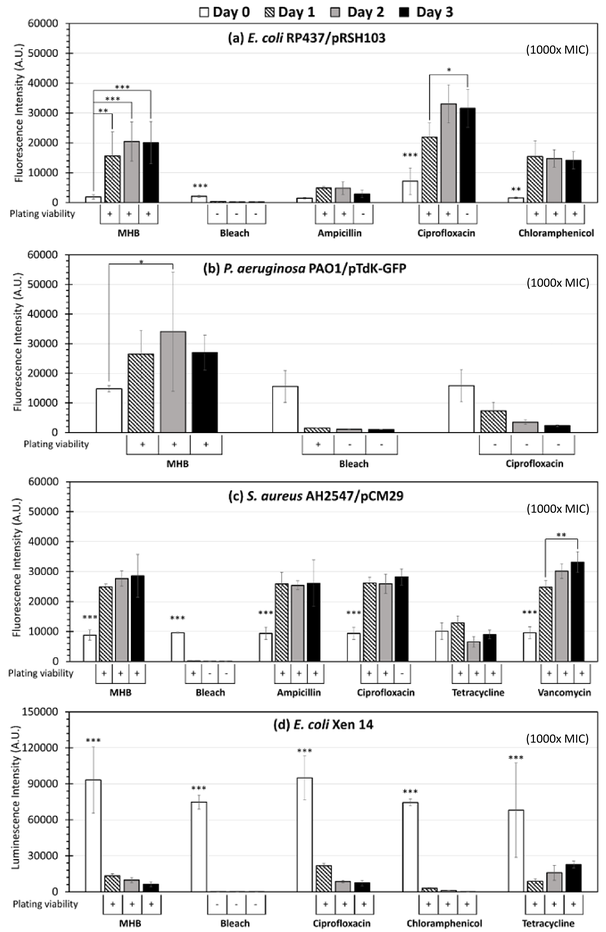

Figure 3. Fluorescence/Bioluminescence signals of (a) *E. coli* RP437/pRSH103, (b) *E. coli* Xen14, (c) *P. aeruginosa* PAO1/pTdK-GFP, (c) *S. aureus* AH2547/pCM29, and (d) *E. coli* Xen14 treated with MHB (negative control), bleach (positive control), and different antibiotics from day 0 to day 3. Plating viability is presented as (+) for live and (-) for dead

- Fig. 3a, inconsistency between the high fluorescence signal and the no viability at day three is of particular concern
- Fig. 3b – c, in bleach treatment, vital signs were detected on day 1 suggesting the strong persistent nature of biofilm in the healthcare environment and thus the need for rigorous cleaning with extended exposure times
- Fig. 3c, fluorescence signal detected with biofilms treated with ciprofloxacin on day 3, suggests there is a lag between loss of bacterial culturability and the loss of fluorescent signal. This could be due to slow degradation of fluorophores or due to viable but non-culturable bacteria- further investigation is needed
- Fig. 3d, persistent bacteria in biofilm may be metabolically inactive, resulting in poor expression of the luciferin-luciferase system; this raises concerns about the reliability of bioluminescence for quantification of culturable bacteria

Table 2. MIC obtained antibiotics tested with bacterial strains and the reference range for each drug with related ATCC bacterial strains

	Antibiotic		Ampicillin	Ciprofloxacin	Chloramphenicol	Tetracycline	Vancomycin
E. coli	**RP437/pRSH103**	MIC (μg/mL)	4	0.035	4	128	128
	XEN14	MIC (μg/mL)	128	0.035	2	0.5	128
	ATCC25922[1]	Range[1] (μg/mL)	8 – 32	0.25 – 1	8 – 32	4 – 16	N/A
P. aeruginosa	**PAO1/pTdK-GFP**	MIC (μg/mL)	512	1	>512	128	>512
	ATCC27856[1]	Range[1] (μg/mL)	N/A	0.5 – 2	N/A	N/A	N/A
S. aureus	**AH2547/pCM29**	MIC (μg/mL)	0.5	0.125	64	0.5	1
	ATCC29213[1]	Range[1] (μg/mL)	N/A	1 – 4	8 – 32	4 – 16	2 – 16

[1]Information of reference strains and MIC ranges obtained from CLSI M100, 29th.
N/A: the antibiotic is not considered for treatment

Conclusion

- Developed and characterized a hydrogel assay that was able to form a robust bacterial biofilm
- Found several anomalies between the culture results (gold standard) and the spectroscopic results
- Future works will focus on DNA sequencing and gene expression with in-depth antibiotic resistance studies

Acknowledgement

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Disclaimer