Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Development of mechanism-based antibacterial synergy of Fmoc-phenylalanine hydrogel with aztreonam

Avinash Yashwant Gahane, Virender Singh, Ashok Kumar, Ashwani Kumar Thakur*

[a]Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, Kanpur 208016, India

*Corresponding Author:

Ashwani K Thakur, Email: akthakur@iitk.ac.in

Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, Kanpur 208016, India

Contents

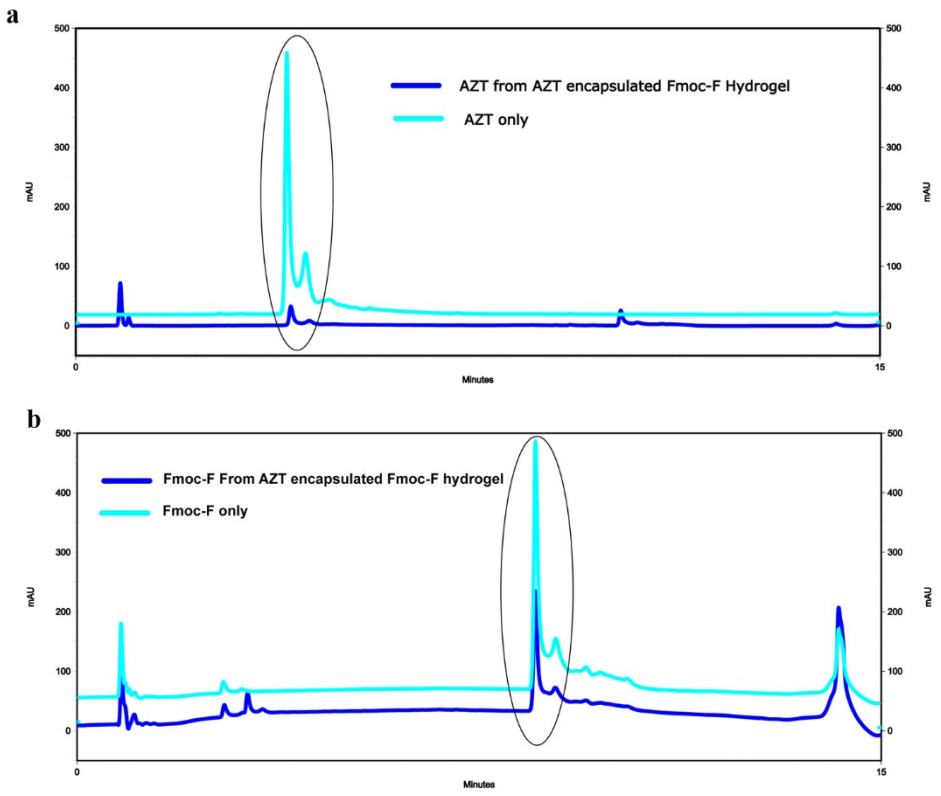

Figure S1: a) RP-HPLC profile of AZT from AZT encapsulated Fmoc-F hydrogel. b) HPLC profile of Fmoc-F from AZT encapsulated Fmoc-F hydrogel.

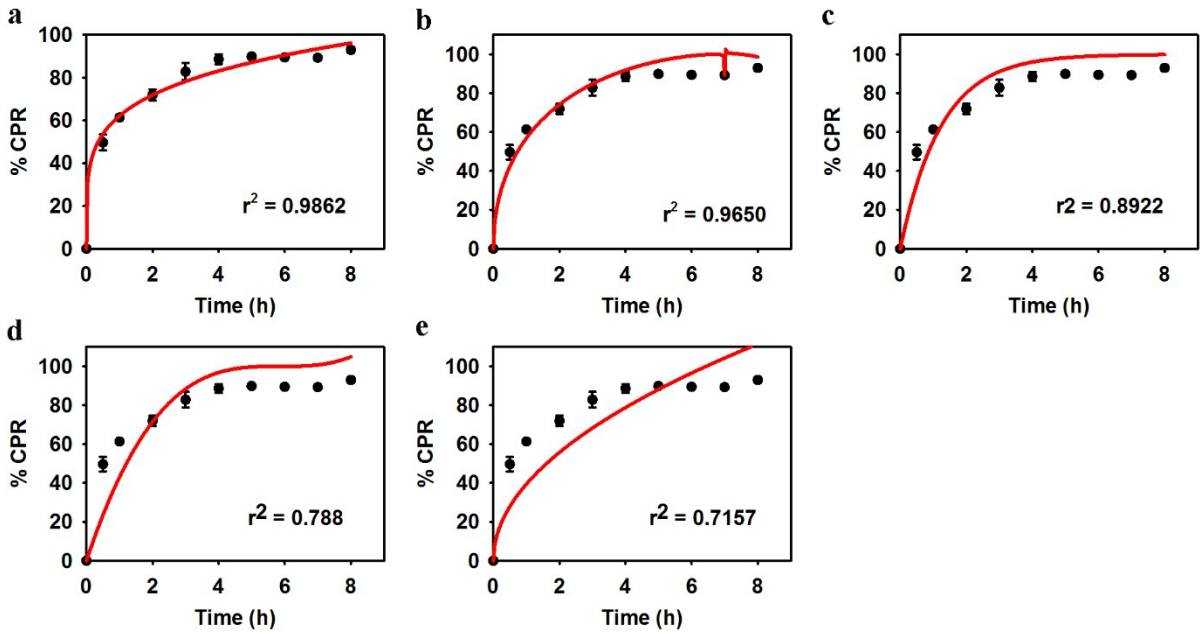

Figure S2: Different release kinetic models for simultaneous release profile of AZT from AZT encapsulated Fmoc-F hydrogel a) Kormeryer-Peppas model b) Baker and Lonsdale model c) First order kinetics model d) Hixon and Crowel model e) Higuchi Square Root Time model.

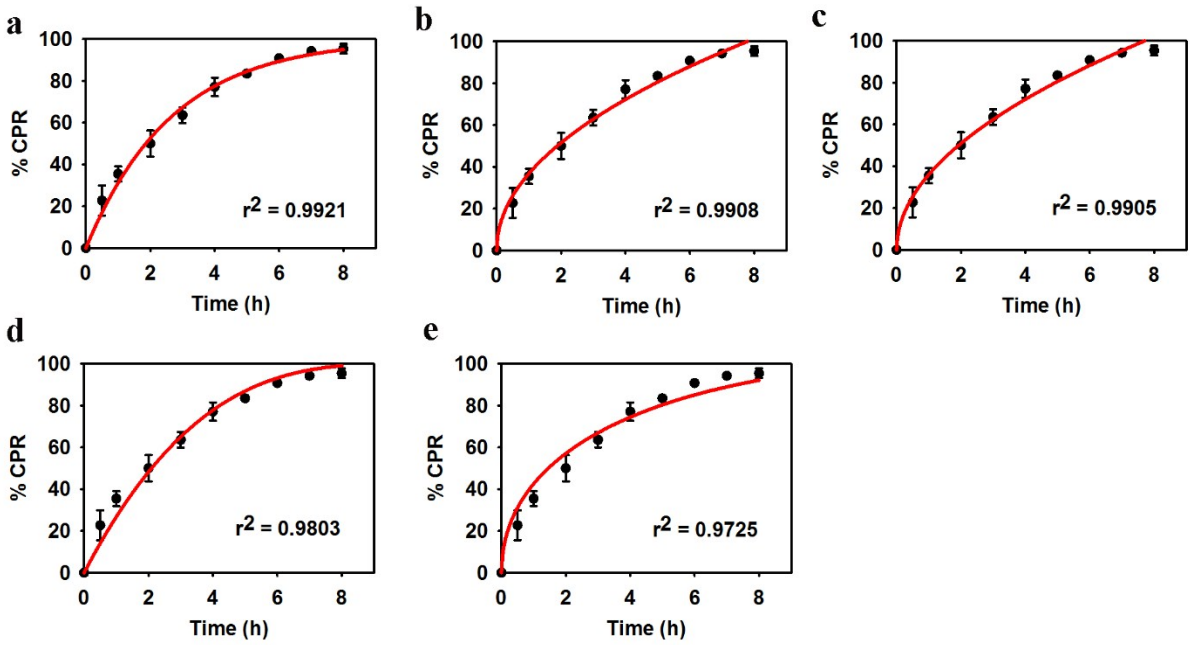

Figure S3: Simultaneous release profile of Fmoc-F from AZT encapsulated Fmoc-F hydrogel and different release kinetic models. a) First order kinetics model b) Kormeryer-Peppas model c) Higuchi Square Root Time model d) Hixon and Crowel model e) Baker and Lonsdale model.

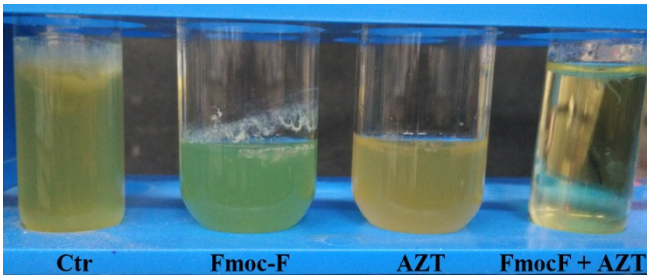

Figure S4: Effect of AZT encapsulated Fmoc-F hydrogel against *S. aureus* and *P. aeruginosa* co-culture.

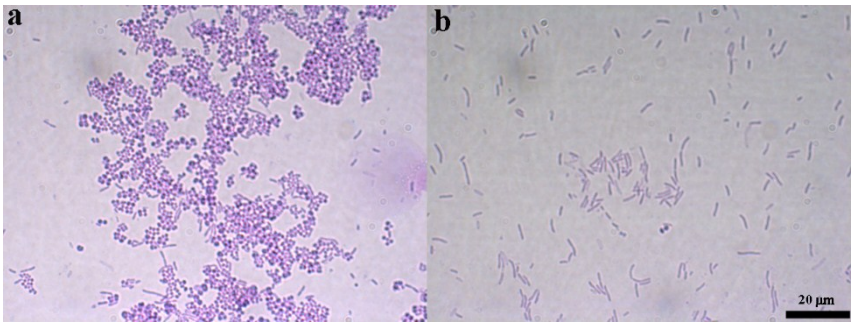

Figure S5: Gram-staining of *S. aureus* and *P. aeruginosa* co-culture treated with AZT (a) or Fmoc-F hydrogel (b) alone.

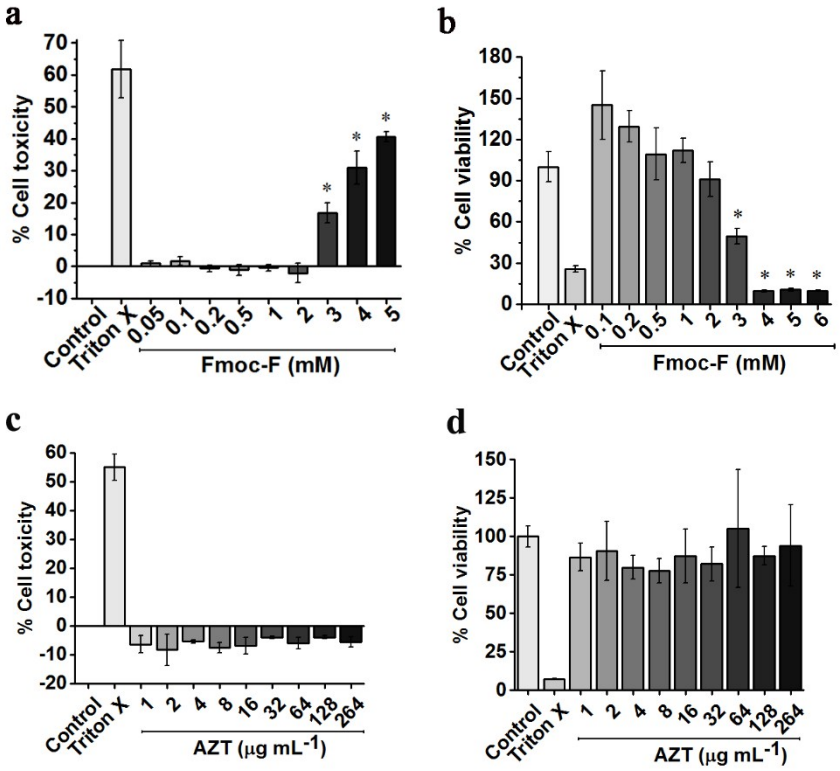

Figure S6: Cytotoxic effect of various concentrations of Fmoc-F (0.05 – 6 mM) on MDCK cells by LDH assay (a) or MTT assay (b). Cytotoxic effect of various concentrations of AZT (1 – 264 µg mL^{-1}) on MDCK cells by LDH assay (c) or MTT assay (d). Triton X (1%) was used as positive

control. *$p < 0.05$ *vs* control, One-way ANOVA followed by Dunnett's multiple comparison test.

Table S1: Result parameters obtained after data analysis by Gompertz equation to determine MIC of AZT or AZT in Fmoc-F hydrogel or AZT + Fmoc-F solution.

	AZT	**AZT in Fmoc-F hydrogel**	**AZT + Fmoc-F solution**
Best-fit values			
logMIC	1.767	1.031	-0.2489
Slope	3.279	7.514	2.219
Bottom	1.644	0.05348	1.091
Span	97.66	50.79	250.9
MIC	**58.53**	**10.74**	**0.5638**
Std. Error			
logMIC	0.09443	0.003513	0.2938
Slope	0.8979	0.1299	1.803
Bottom	4.464	0.05348	0.5696
Span	10.08	0.1753	431.2
95% Confidence Intervals			
logMIC	1.467 to 2.068	0.9864 to 1.076	-1.513 to 1.015
Slope	0.4216 to 6.136	5.863 to 9.164	-5.540 to 9.979
Bottom	-12.56 to 15.85	-0.6260 to 0.7329	-1.360 to 3.542
Span	65.59 to 129.7	48.57 to 53.02	-1605 to 2106
MIC	29.30 to 116.9	9.692 to 11.90	0.03068 to 10.36
Goodness of Fit			
Degrees of Freedom	3	1	2
R square	0.9891	1.000	0.9992
Absolute Sum of Squares	111.2	0.005719	1.924
Sy.x	6.088	0.07563	0.9809
Number of points			
Analyzed	7	5	6

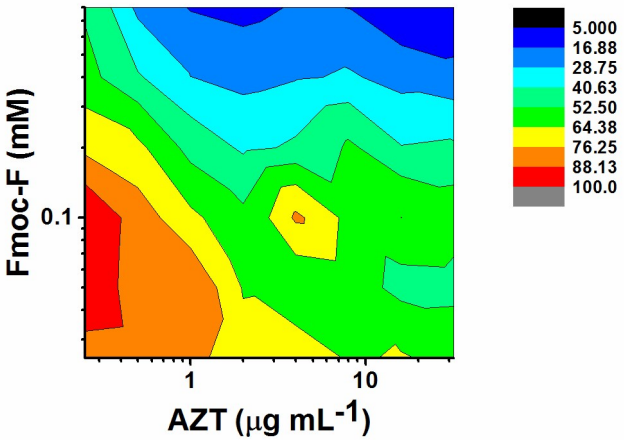

Figure S7: Heat plot showing the effect of AZT and Fmoc-F solution combination on *S. aureus*.

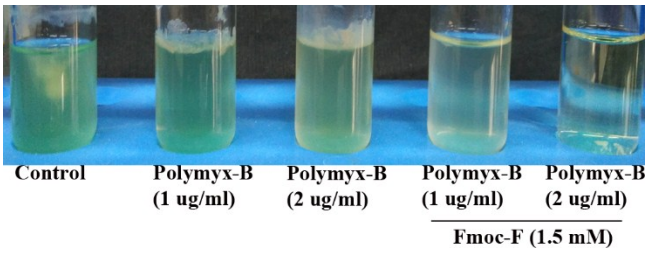

Figure S8: Effect of polymyxin B (1 or 2 µg mL^{-1}) + Fmoc-F (1.5 mM) combination or polymyxin B alone against *P. aeruginosa* using broth micro-dilution assay.

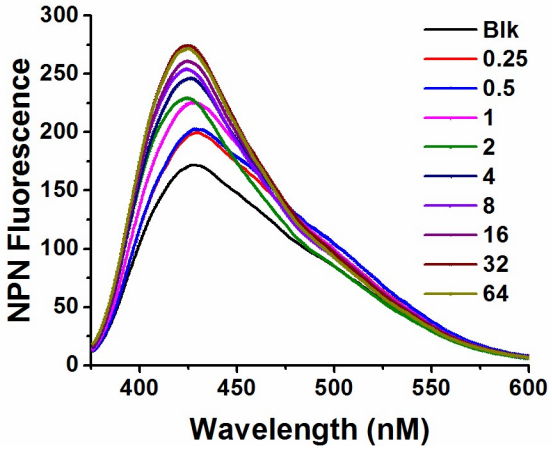

Figure S9: Effect of different concentrations (0.25 – 64 µg mL^{-1}) of polymyxin B on NPN fluorescence.

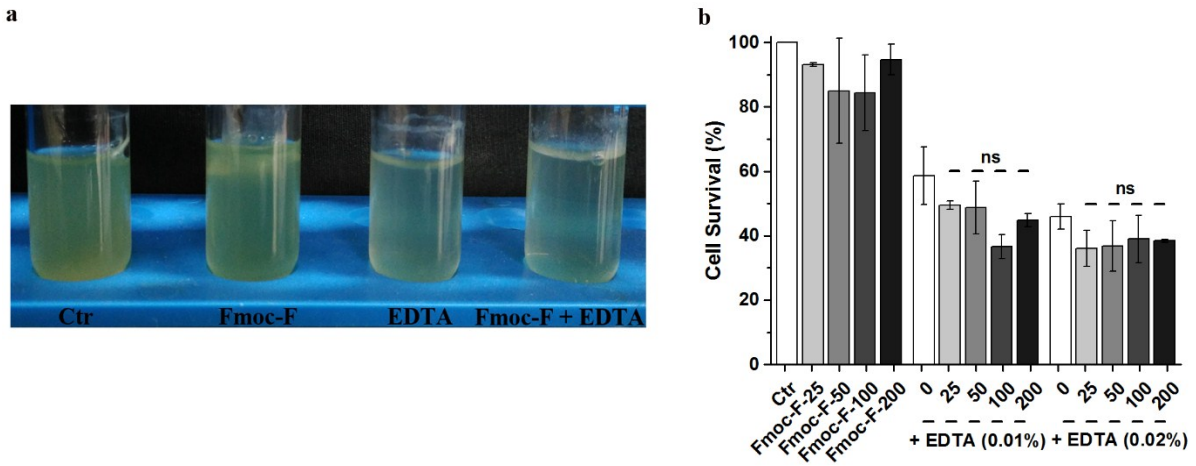

Figure S10: a) Effect of EDTA (0.02 %) + Fmoc-F (1.5 mM) combination or EDTA (0.02 %) alone against *P. aeruginosa* using broth micro-dilution assay. b) Effect of various concentrations of EDTA (0.01 or 0.02 %) + Fmoc-F (25 – 200 µM) combination or EDTA (0.01 or 0.02 %) alone against *P. aeruginosa* using broth micro-dilution assay. ns – nonsignificant, One-way ANOVA followed by Newman-Keuls multiple comparison test.