

Supporting Information

Ciprofloxacin poly(β -amino ester) conjugates enhance antibiofilm activity and slow the development of resistance.

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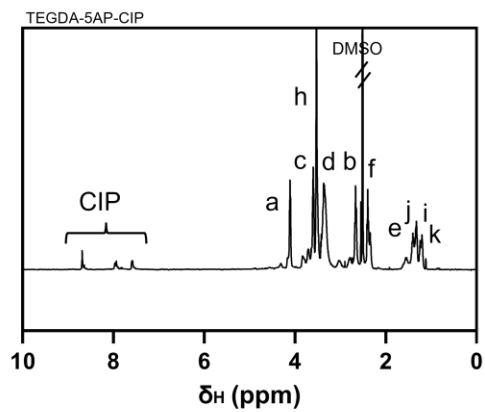
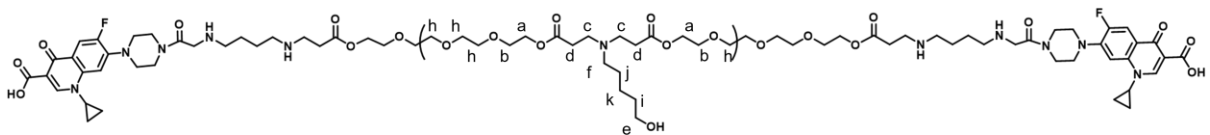
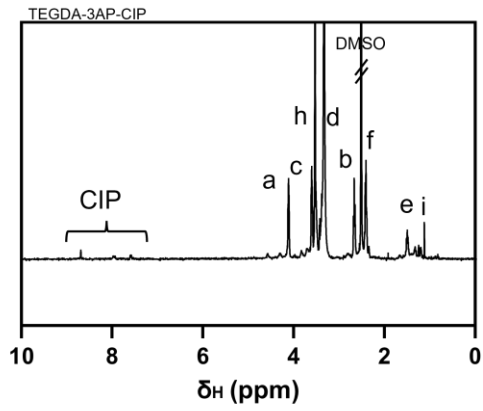
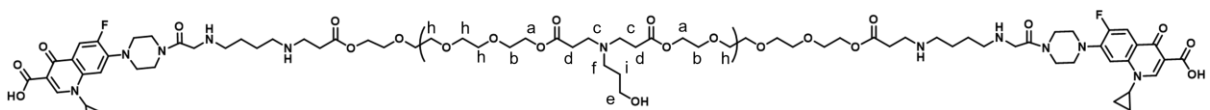
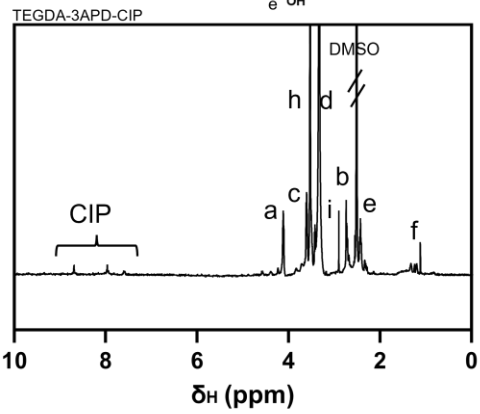
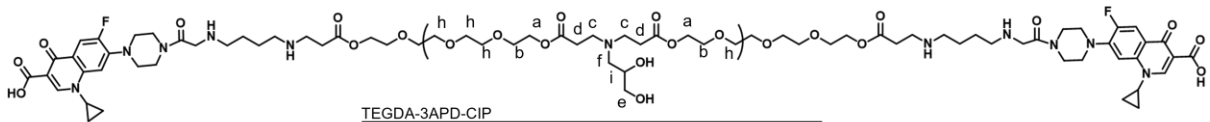
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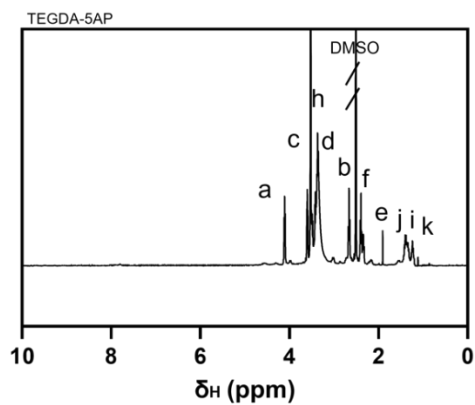
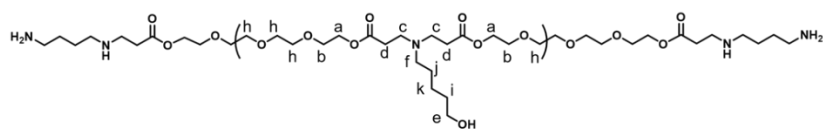
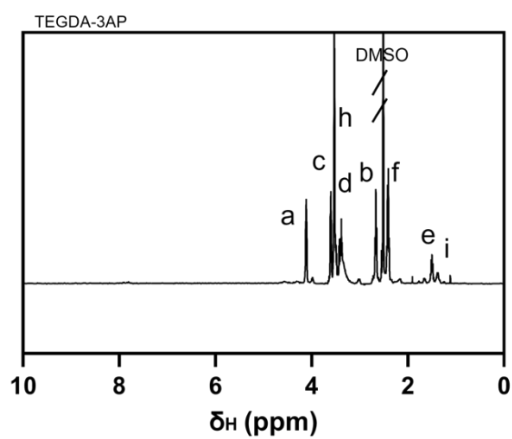
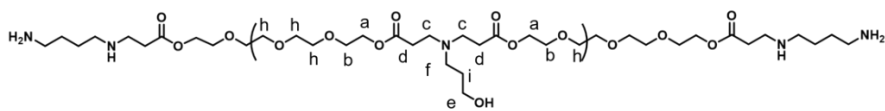
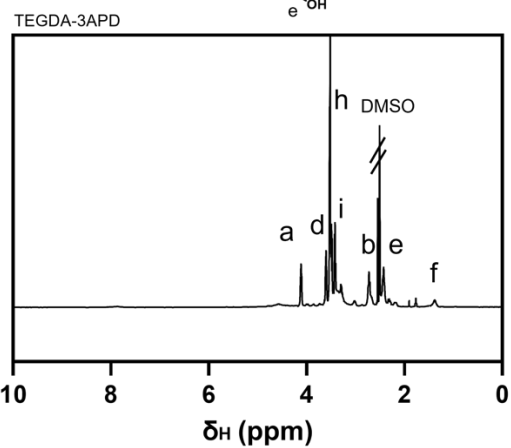
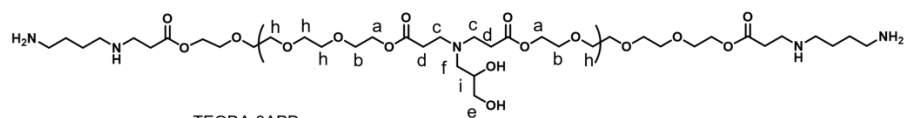


Figure S1: ^1H NMR spectra of polymers in DMSO-d_6 recorded at 25°C with a Bruker NMR400 MHz.

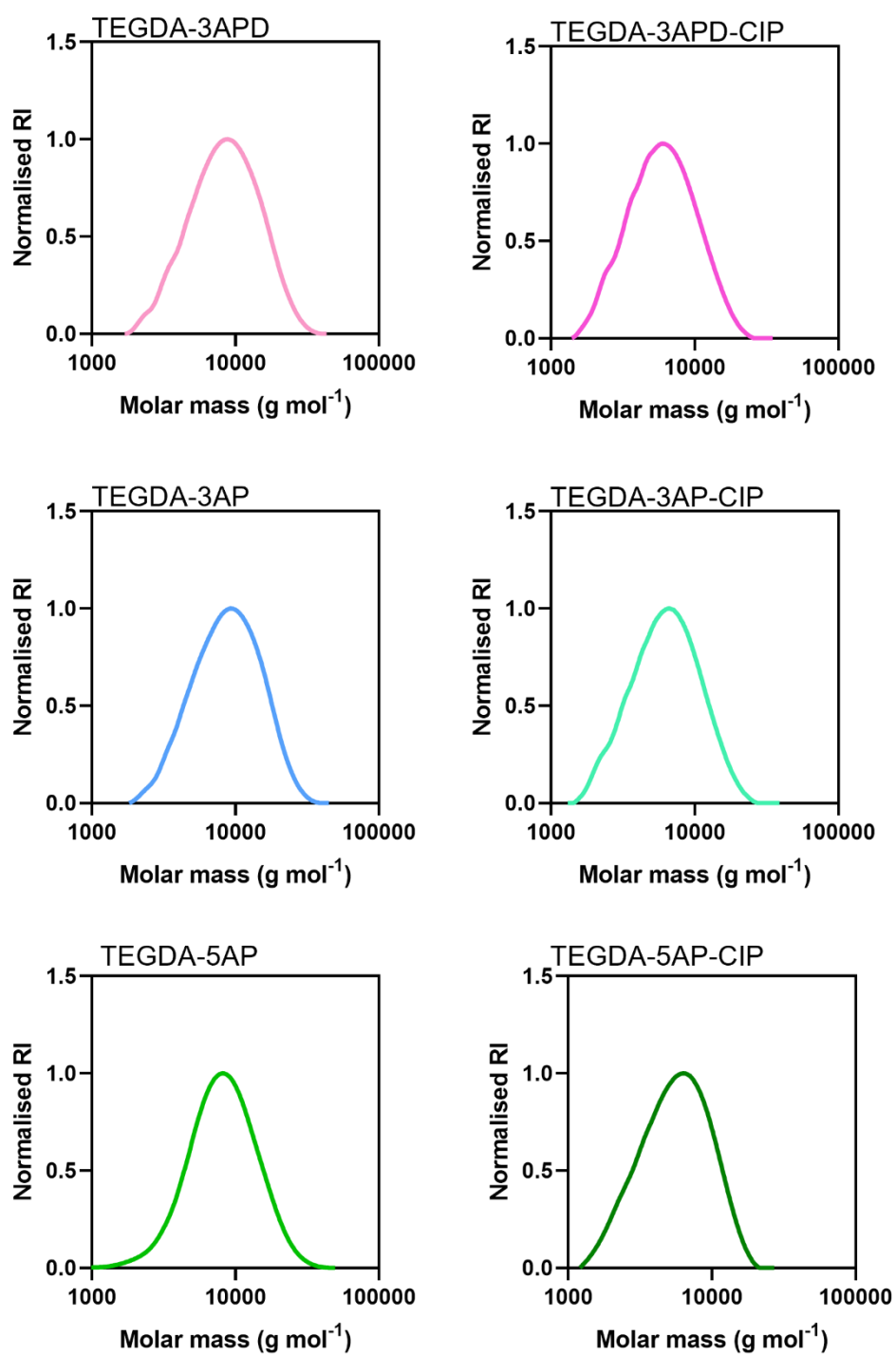


Figure S2: DMF-SEC chromatograms of prepared polymers.

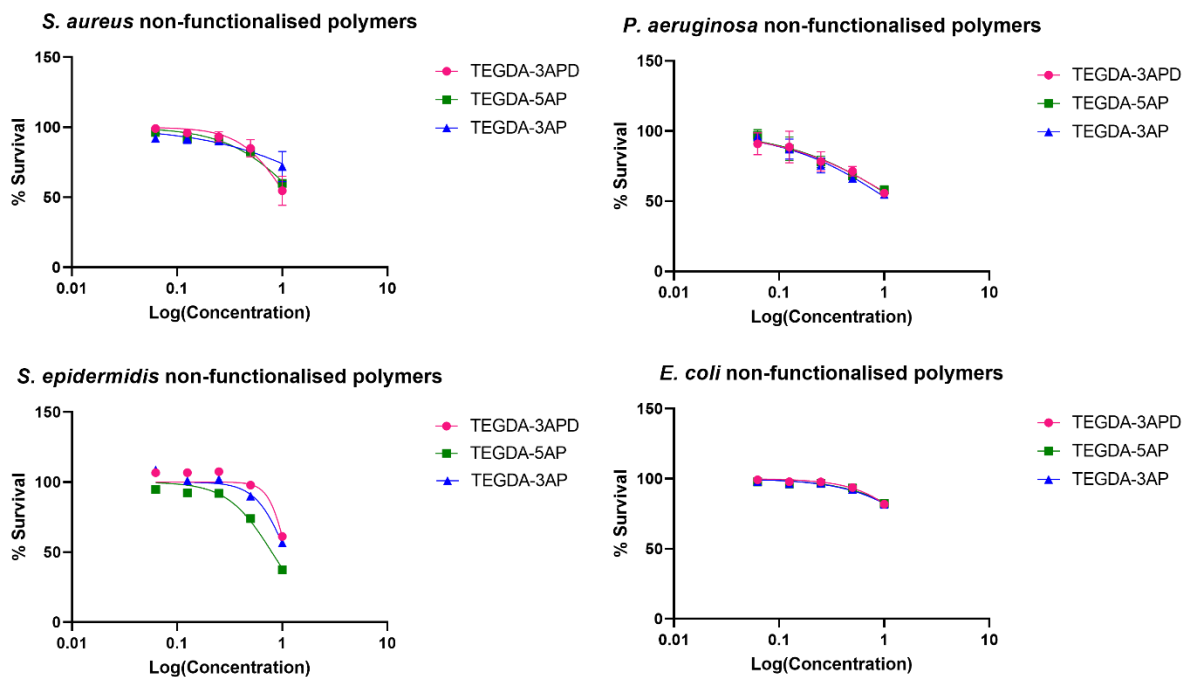


Figure S3: Concentration curves for TEGDA-3APD (pink), TEGDA-5AP (green) and TEGDA-3AP (blue) polymers with no CIP conjugated, against Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) and Gram-negative bacteria: *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) All measurements were performed in duplicate, using biologically independent replicates and the error bars represent the mean \pm standard deviation.

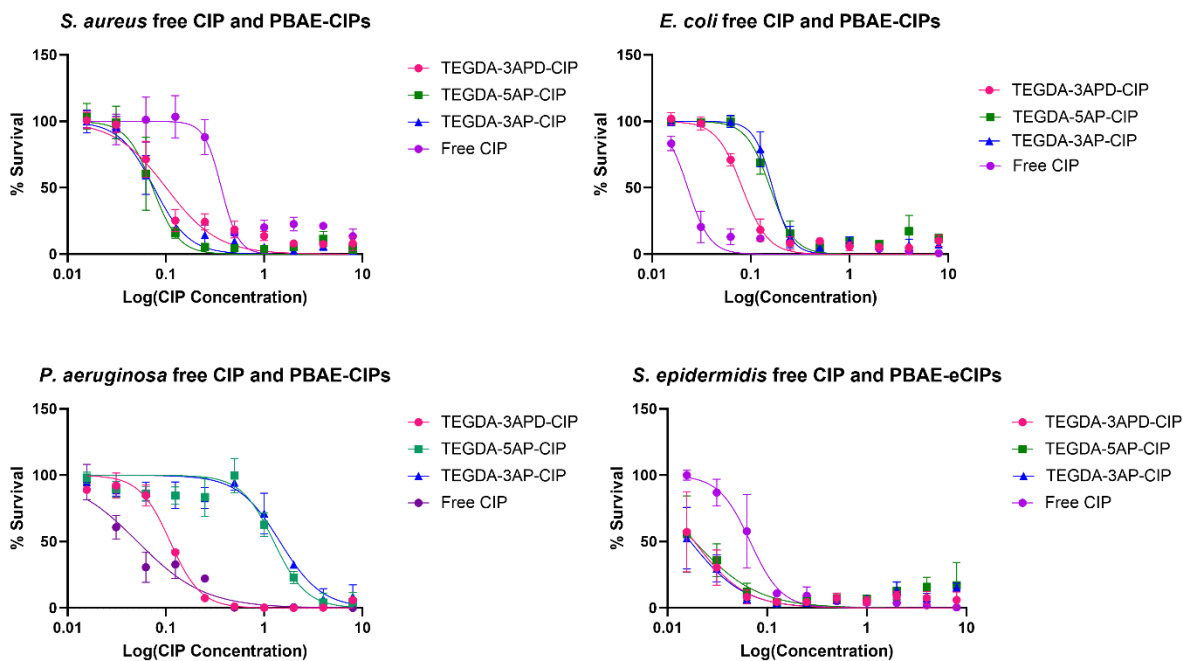


Figure S4: Concentration curves for TEGDA-3APD-CIP (pink), TEGDA-5AP-CIP (green), TEGDA-3AP-CIP (blue) and CIP (purple), against Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) and Gram-negative bacteria: *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) All measurements were performed in duplicate, using biologically independent replicates and the error bars represent the mean \pm standard deviation.

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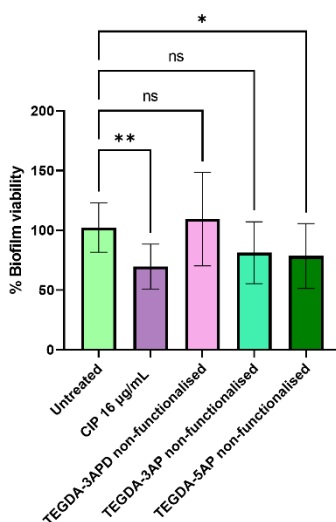


Figure S5: Bar charts showing viability in mature *P. aeruginosa* biofilms grown in a rolling bioreactor, quantified after treatment with TEGDA-3APD (pink), TEGDA-5AP (green) and TEGDA-3AP (blue), at a polymer concentration of 2 mg mL⁻¹. All measurements were performed in triplicate, using biologically independent replicates and the error bars represent the mean \pm standard deviation. Statistical testing was performed with a one-way ANOVA followed by a post-hoc Tukey test to identify individual comparisons. Statistical significance is represented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

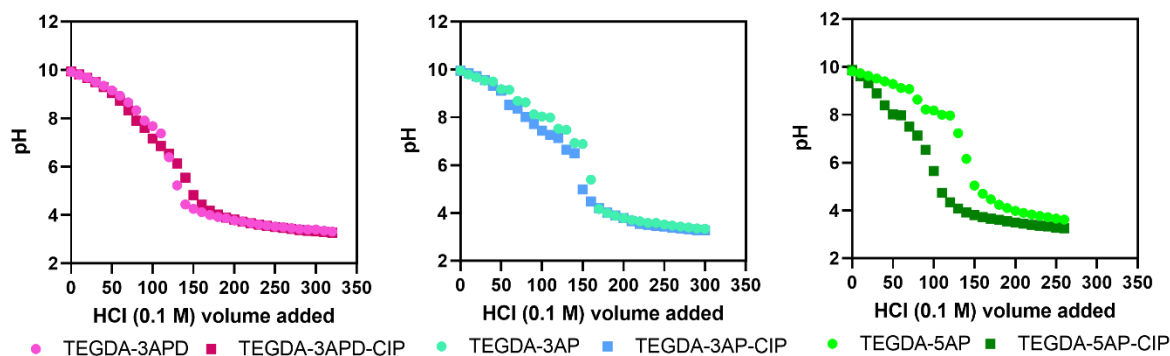


Figure S6: Titration curves obtained by adding HCl (0.1 M) to TEGDA-3APD (light pink circle), TEGDA-3APD-CIP (dark pink square), TEGDA-3AP (light blue circle), TEGDA-3AP-CIP (dark blue square), TEGDA-5AP (light green circle) and TEGDA-5AP-CIP (dark green square) polymers.

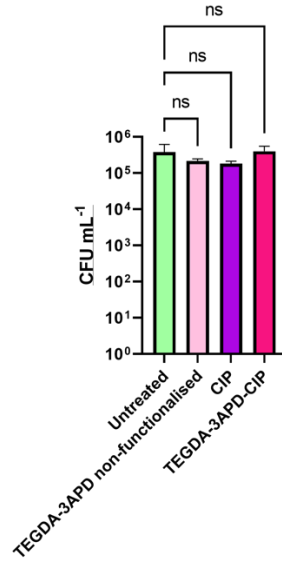


Figure S7: Bar charts showing susceptibility (CFU mL⁻¹) of planktonic *C. albicans* to TEGDA-3APD-CIP (pink), free CIP (purple) and non-functionalised TEGDA-3APD (light pink) at concentrations equivalent to 0.5 µg mL⁻¹ free CIP (67 mg non-functionalised polymer). All measurements were performed in triplicate, using biologically independent replicates and the error bars represent the mean ± standard deviation. Statistical testing was performed with a one-way ANOVA followed by a post-hoc Tukey test to identify individual comparisons. Statistical significance is represented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

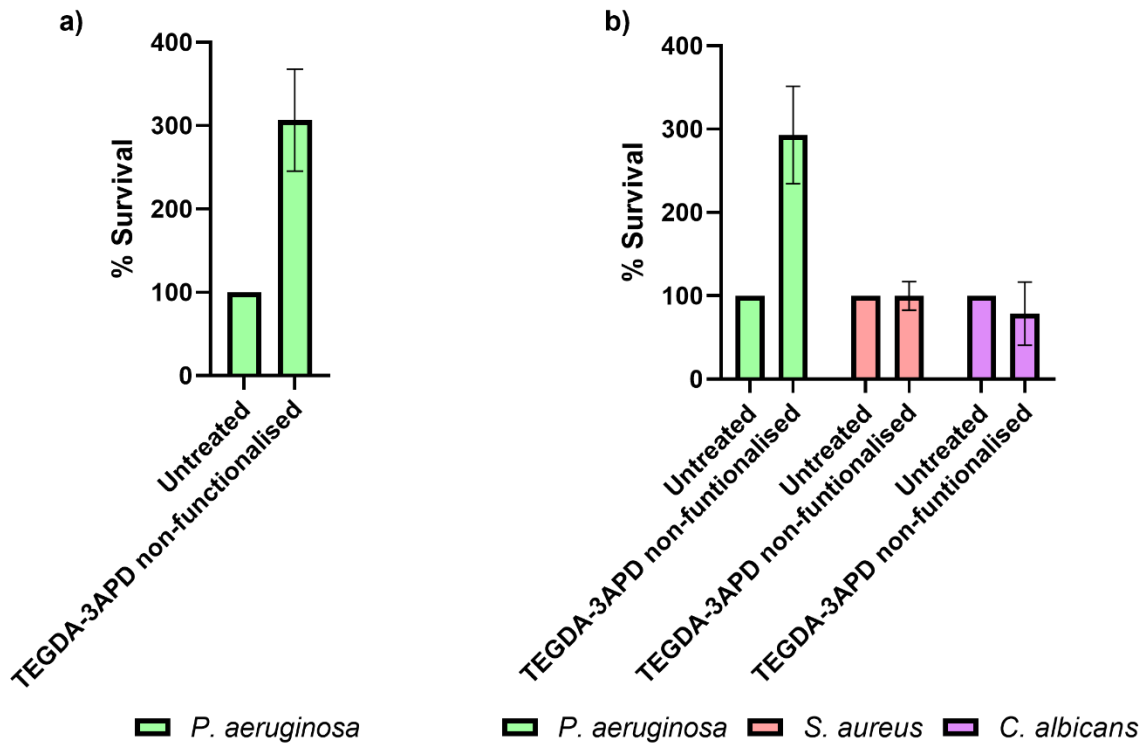


Figure S8: a) Bar charts showing percentage survival determined using CFU mL⁻¹ values in static monospecies biofilms composed of *P. aeruginosa* (light green) quantified after treatment with 0.068 µg mL⁻¹ TEGDA-3APD. b) Bar charts showing percentage survival determined

using CFU mL^{-1} values in static multispecies biofilms composed of *P. aeruginosa* (light green), *S. aureus* (light red) and *C. albicans* (violet) quantified after treatment with 0.068 $\mu\text{g mL}^{-1}$ TEGDA-3APD. All measurements were performed in triplicate, using biologically independent replicates and the error bars represent the mean \pm standard deviation.

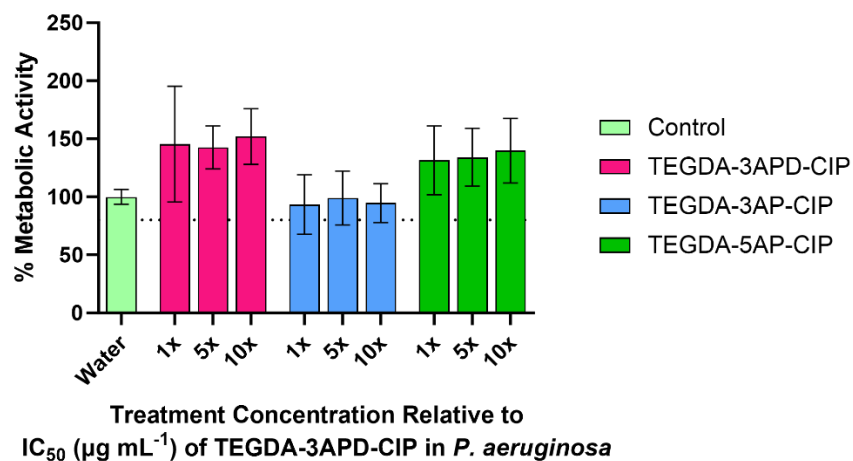


Figure S9: Metabolic activity of A549 cells treated with polymers for 48 h, normalised against killed (0%) and untreated ('medium', 100%) controls, measured using PrestoBlue assay. Each bar represents the mean and average of two biological replicates each with three technical replicates.

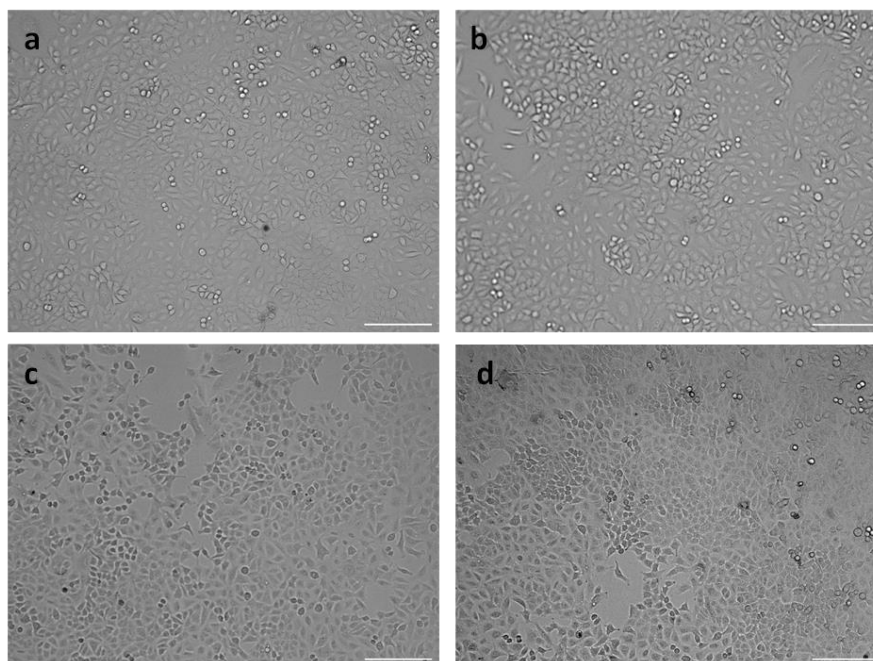


Figure S10: Brightfield microscope images showing minimal density/shape differences between A549 cells following 48h in medium (a) or 10x concentration polymer (b=TEGDA-3APD-CIP, c=TEGDA-3AP-CIP, d=TEGDA-5AP-CIP). Scale bar is 200 μm .