

## Polymyxin B Stabilized DNA Micelles for Sustained Antibacterial and Antibiofilm Activity against *P. aeruginosa*

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### Supplementary Information

**Table S1: Sequences of ssDNA designed for the fabrication of DNA micelles and ssDNA used in agarose gel electrophoresis.**

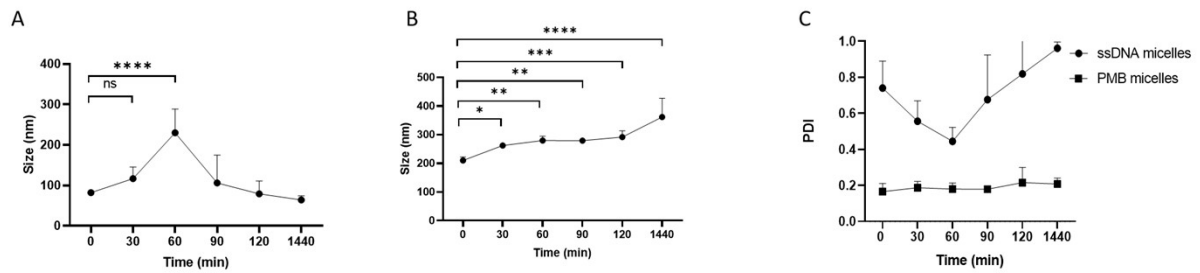
Detailed sequences and modifications of single stranded DNA (ssDNA) used in micelle preparation.

Name	Sequence
15bp_ssDNA (unmodified ssDNA)	5'-ATCGGTAGGGTGTCA-3'
15bp_chol_ssDNA	5'-ATCGGTAGGGTGTCA/3CholTEG/-3'
Fluorescent_15bp_chol_ssDNA	5'-RhoR-XN-ATCGGTAGGGTGTCA-3CholTEG-3'
Peptide nucleic acid (PNA)	5'Ac-O- GCC ATG TGA TTG - 3'NH <sub>2</sub>
ssDNA sequence (agarose gel)	5'-A GTC TTC GTC CTT ATC GGT AG A GGT GCT GAG CGG AAT CCT GA A AGT GTA CAA GGT ATC TCG AC-3'

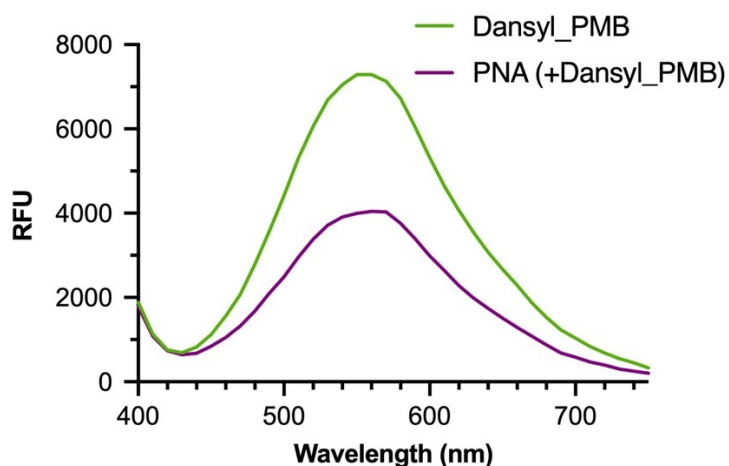
**Figure S1 – Characterization of ssDNA micelles using TEM imaging, showing the poorly formed micellar structures with uneven shape and size.**



**Figure S2 – Stability of ssDNA and PMB micelles over time. Size of (A) ssDNA micelles and (B) PMB micelles over time; (C) PDI of ssDNA and PMB micelles over time.**



**Figure S3 – Fluorescence emission scan of dansyl\_PMB with and without the presence of PNA.**



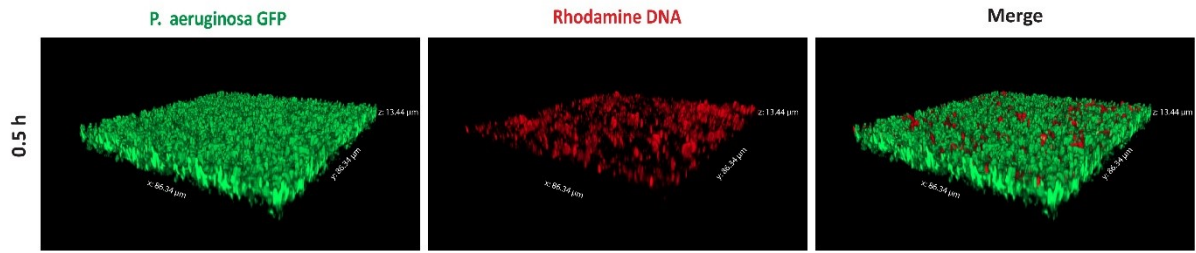
**Table S2: MIC of *P. aeruginosa* incubated with free PMB and PMB micelles.**

Minimal inhibitory concentration (MIC) of free polymyxin B or polymyxin B micelles against *P. aeruginosa*.

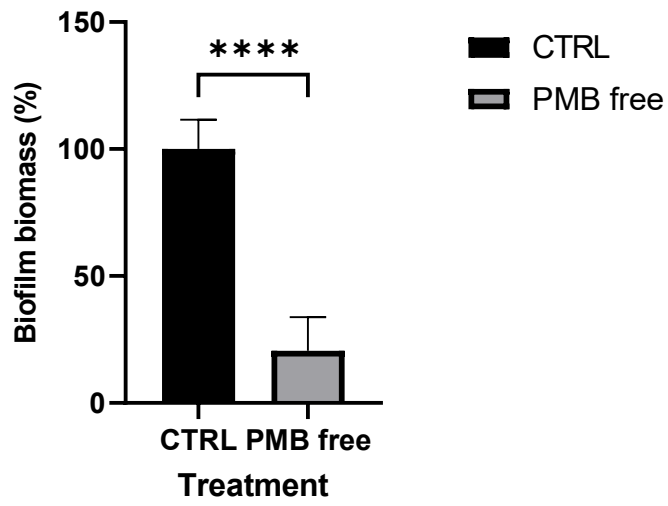
Formulation	Species	Strain	MIC ( $\mu\text{g}/\text{mL}$ )
Free polymyxin B	<i>P. aeruginosa</i>	PAO1	2
Polymyxin B micelles	<i>P. aeruginosa</i>	PAO1	2

**Figure S4 – Z-stack images with confocal laser scanning microscopy (CLSM) of *P. aeruginosa* biofilms incubated with PMB micelles after 30 minutes.**

Z-stack images obtained via confocal laser scanning microscopy (CLSM) of mature *P. aeruginosa* biofilms incubated with rhodamine labelled PMB micelles. The images show rapid penetration of the PMB micelles after 30 minutes of incubation, however deep penetration was not achieved within this time frame.



**Figure S5 – Biofilm inhibition of free polymyxin B.**



**Figure S6 – Cell viability of skin cells (HDF) incubated with free polymyxin B.**

Results show the low toxicity of free polymyxin B (PMB) in human dermal fibroblasts at the reported concentrations used in PMB micelle preparation.

