Supporting Information

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Polymyxin B-Triggered Assembly of Peptide Hydrogels for Localized and Sustained Release of Combined Antimicrobial Therapy

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Figure S2. Characterization of EPA (C_{15}H_{31}CONH-EEE-CNH_{2}): (A) analytical RP-HPLC trace under the gradient of 98% to 0% H_{2}O (2% to 100% ACN) with 0.1% NH_{4}OH from 5 to 35 min showing high purity; (B) ESI-MS spectrum showing the expected molecular mass (C_{31}H_{54}N_{4}O_{10}, Mw: 642.78 g/mol).
Figure S3. Characterization of BPA (C$_{15}$H$_{31}$CONH-VVVAAEEE-COH$_{2}$): (A) analytical RP-HPLC trace under the gradient of 98% to 0% H$_2$O (2% to 100% ACN) with 0.1% NH$_4$OH from 5 to 35 min showing high purity; (B) ESI-MS spectrum showing the expected molecular mass (C$_{55}$H$_{96}$N$_{10}$O$_{16}$, Mw: 1153.41 g/mol).

Figure S4. Characterization of the commercially purchased PMB: (A) analytical RP-HPLC trace of PMB under the gradient of 98% to 0% H$_2$O (0.1% TFA) from 5 to 35 min, showing two peaks and indicating that the purchased PMB contains a mixture of PMB1 (labeled with green circle) and PMB2 (labeled with red circle); (B) ESI-MS spectrum of PMB showing the expected molecular mass and confirming the presence of both PMB1 (C$_{55}$H$_{96}$N$_{16}$O$_{13}$, Mw: 1203.50 g/mol) and PMB2 (C$_{55}$H$_{96}$N$_{16}$O$_{12}$, Mw: 1189.47 g/mol) in the commercial PMB product.
Figure S5. CAC determination for TPA and BPA: maximum fluorescence emission wavelength and intensity of Nile red as function of PA concentration to determine the CAC. (A) CAC of TPA at 0.02 mg/mL; (B) CAC of BPA at 0.002 mM (0.002 mg/mL).
Figure S6. Standard curve of PMB: fluorescence intensity of fluorescamine measured as function of the concentration of PMB.
Figure S7. Mechanical stiffness of the three representative PA hydrogels: amplitude sweep of the PMB Gel (A), PMB+Ca Gel (C), and Ca Gel (E); frequency sweep of the PMB Gel (B), PMB+Ca Gel (D), and Ca Gel (F).
Figure S8. Images of the hydrogels over the release period (120 h, 5 days): The PMB-triggered PA hydrogels were prepared at the bottom of the 15 mL centrifuge tubes with 1 mL PBS added on top for performing the release experiments.

Figure S9. Bacterial inhibition activity of PMB solutions and hydrogels against *P. aeruginosa* PA14: pictured agar plates showing antimicrobial activities of 1-PMB Gel, 2-PMB+Ca Gel, 3-Ca Gel, 4-PMB Solution, 5-PMB+Ca Solution, 6-
PBS with their loaded discs being diffused and transferred onto *P. aeruginosa* PA14 suspension covered agar plate every 1.5 h over a 24 hour period (visible zone shown for 6-PBS at 24 h is due to the removal of the disc at the end of the experiment).

**Figure S10. Bacterial inhibition activity of PMB solutions and hydrogels against *E. coli*:** (A) pictured agar plates showing susceptibility of *E. coli* to PMB and PMB+Ca solutions examined by dose response experiments; (B) Inhibition zone changes in response to the different concentrations of PMB; (C) pictured agar plates showing antimicrobial activities of 1-PMB Gel, 2-PMB+Ca Gel, 3-Ca Gel, 4-PMB Solution, 5-PMB+Ca Solution, 6-PBS with their loaded discs being diffused and transferred onto *E. coli* suspension swabbed agar plate every 1.5 h over a 24 hour period (visible zone shown for 6-PBS at 24 h is due to the removal of the disc at the end of the experiment); (D) Inhibition zone changes over time for the different formulations of PMB. Ca Gel and PBS displayed no bacterial inhibition activity against *E. coli* and no inhibition zones were observed. Data are given as mean ± SD (n = 3).
Figure S11. Bacterial inhibition activity of PMB solutions and hydrogels against *A. baumannii*: (A) pictured agar plates showing susceptibility of *A. baumannii* to PMB and PMB+Ca solutions examined by dose response experiments; (B) Inhibition zone changes in response to the different concentrations of PMB; (C) pictured agar plates showing antimicrobial activities of 1-PMB Gel, 2-PMB+Ca Gel, 3-Ca Gel, 4-PMB Solution, 5-PMB+Ca Solution, 6-PBS with their loaded discs being diffused and transferred onto *A. baumannii* suspension covered agar plate every 1.5 h over a 24 hour period (visible zone shown for 6-PBS at 24 h is due to the removal of the disc at the end of the experiment); (D) Inhibition zone changes over time for the different formulations of PMB. Ca Gel and PBS displayed no bacterial inhibition activity against *E. coli* and no inhibition zones were observed. Data are given as mean ± SD (n = 3).
Figure S12. Safety profile of TPA and PMB solutions on *Galleria Mellonella*: Images taken at different periods of time after injection of $10 \mu$L TPA (20 mg/mL) and PMB (2 mg/mL) solutions into the *Galleria Mellonella*, using the BD Micro-Fine Insulin Syringe (0.3 mL) with Needle (30 G), with no obvious toxicity observed at day 1 and day 5.
Figure S13. Representative images of the *Galleria Mellonella* immediately after the various procedures: (A) after burn; (B) after burn and bacteria inoculation; (C) with hydrogel onto the infected burn wound.
Table S1. Ability of various polyanionic solutions with high viscosity to form hydrogels when mixed with PMB: the names and chemical structures of different polyanions tested; pictures showing the initial viscosity of different polyanionic solutions (20 mg/mL), when they are mixed with equal volume of 2 mg/mL PMB solution, and after being incubated at 37 °C overnight.

| Polyanions       | Chemical Structure | Polyanionic Solution (20 mg/mL) | + PMB Solution (2 mg/mL) | Overnight Incubation (37 °C) | Observations                                                                 |
|------------------|--------------------|---------------------------------|--------------------------|------------------------------|------------------------------------------------------------------------------|
| Alginate         | ![ Alginate Structure ] | ![ Alginate Solution ] | ![ Alginate + PMB Solution ] | ![ Alginate Overnight Incubation ] | Opaque interface appeared with addition of PMB solution, but disappeared after overnight incubation; viscosity decreased. |
| Hyaluronic Acid  | ![ Hyaluronic Acid Structure ] | ![ Hyaluronic Acid Solution ] | ![ Hyaluronic Acid + PMB Solution ] | ![ Hyaluronic Acid Overnight Incubation ] | No significant changes were observed with addition of PMB solution. |
| Polyacrylic Acid | ![ Polyacrylic Acid Structure ] | ![ Polyacrylic Acid Solution ] | ![ Polyacrylic Acid + PMB Solution ] | ![ Polyacrylic Acid Overnight Incubation ] | Opaque interface appeared with addition of PMB solution, but disappeared after overnight incubation; viscosity decreased. |