Supporting Information

Biocide loaded shear-thinning hydrogel with anti-biofilm efficacy cures topical infection

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Table of Contents:

Experimental section

Materials and instrumentation. Bromoacetyl bromide, 1,12-dibromododecane, Gelatin (Mw = 40-50 kDa), and Gelzan™ CM, commonly known as gellan were purchased from Sigma-Aldrich and used. L-Phenylalanine, ethanol, methylene chloride, potassium hydroxide, sodium sulphate, and *N,N*, dimethylamine, were purchased from Spectrochem, India. Nuclear magnetic resonance (NMR) spectra were recorded using Bruker (AV-400) 400 MHz spectrometer in deuterated solvents. High-performance liquid chromatography (HPLC) was performed in an SPD-M20A Shimadzu instrument. Viscoelastic properties of the gels were measured in TA instrument (DHR-3 rheometer). Methicillin-resistant *S. aureus* (MRSA) ATCC33591, were obtained from the American Type Culture Collection (ATCC). *S. epidermidis* MTCC3615, *E. coli* MTCC443, *A. baumannii* MTCC1425, and *P. aeruginosa* MTCC424 were obtained from MTCC, India. All three clinical isolates, MRSA R3545, MRSA R3889, and MRSA R3890 were obtained from the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India. VRSA 1, VRSA 4, and VRSA 12 were obtained from Central Drug Research Institute (CDRI), India. *S. epidermidis* L-1292 was obtained from L. V. Prasad Eye Hospital, Hyderabad, India. All the bacterial strains were preserved in nutrient broth (NB) at -80 ºC supplemented with 15-50 % (v/v) glycerol. The frozen stocks were grown overnight on NB agar plate incubated at 37 ˚C for 24 h. Bacterial growth media (NB and NB agar) and any other materials for microbiology experiments were obtained from HiMedia. Tecan Infinite Pro series M200 Microplate Reader was used for optical density (O.D.) measurement or fluorescence measurement.

Synthesis procedure of ethyl (2-bromoacetyl) phenylalaninate (1a). Ethyl ester bromides were synthesized by following our previously published protocol. Briefly, thionyl chloride (659 µL, 9.1 mmol) was added dropwise at 0-5 ˚C to 15-20 mL suspension of L-Phenylalanine (0.5 g, 3 mmol) in methanol and the entire reaction mixture was refluxed for 12h. Next, the excess methanol and thionyl chloride was evaporated by a rotary evaporator. The solid residue was washed with dry diethyl ether, which resulted in a white solid crude product. This crude white solid was dissolved in 10 mL of dichloromethane and potassium carbonate (1 g, 7.6 mmol) was added to the organic solution after dissolving it in 10 mL of distilled water. A solution of bromoacetyl bromide (0.87 mL, 10 mmol) in dichloromethane (10 mL) was then added dropwise to the reaction mixture at 5 °C for 1h. The reaction mixture was stirred at room temperature for another 12h. The aqueous solution was separated and washed with dichloromethane. The organic solution was washed with water and passed over the anhydrous Na_2SO_4 and concentrated to yield a white or yellowish white solid product with 94% yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm. 1.24 (t, *J* = 7.1 Hz, -COOCH$_2$C*H*$_3$, 3H), 3.09-3.20 (m, -C*H*$_2$Ph, 2H), 3.85 (s, BrC*H*$_2$CONH, 2H), 4.2 (q, *J* = 7.1 Hz, -COOC*H*$_2$CH$_3$, 2H), 4.78-4.83 (App quint, -C*H*$_{(Phe)}$, 1H), 6.86-6.87 (br., -CON*H*, 1H), 7.13-7.30 (m, *H*$_{Ph,}$ 5H). HRMS (m/z): 314.0384, 316.0360 [(M+H)$^+$] (observed), 314.0386, 316.0366 [(M+H)$^+$] (calculated).

Synthetic procedure of *N,N,N′,N′,* tetramethyldodecane-1,12-diamine (1b). At first, $NHMe_2$ gas was collected into chloroform solution of 1,12-dibromododecane in a sealed tube at 0 ˚C till the volume of the resulting solution was roughly doubled. Then, the reaction mixture was allowed to stir for 24 h at room temperature. Next, the reaction mixture was cooled and transferred into a RB. The solution was then kept in water bath to remove excess $NHMe_2$ followed by solvent evaporation by using rotary evaporator. Reaction mixture was then washed with 2(M) aqueous KOH solution after dissolving it in $CHCl_3$. Finally, $CHCl_3$ layer was collected and evaporated to dryness to get a yellow gummy liquid, **1b** with quantitative yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm. .1.23-1.24 (br. s, -(CH_2)$_8$$CH_2CH_2N(CH_3)_2$, 16H), 1.40-1.42 (br. m, -$CH_2CH_2N(CH_3)_2$, 4H), 2.19-2.22 (br. m, -$CH_2CH_2N(CH_3)_2$, 16H). HRMS (m/z): 257.2950 [$(M+H)^+$] (observed), 257.2951 [$(M+H)^+$] (calculated).

Synthetic procedure of amino acid conjugated small antibacterial molecules (ASAM-10). Individually, ethyl ester bromide intermediate **1a** (2.8 equiv.) were reacted with *N, N, N′, N′,* tetramethyldodecane 1,12-diamines, **1b** (0.25g, 1 equiv.) in dry $CHCl_3$ (8-10 mL) at 65 ˚C. At the end of 24 h, the reaction mixture was evaporated by using rotary-evaporator and the residue was dissolved in minimum amount of $CHCl_3$. The product was then precipitated by adding excess dry diethyl ether and the white residue was washed repeatedly with dry diethyl ether to remove the excess amount of activated ethyl ester bromides. The entire exercise resulted in the generation of amino acid conjugated small antibacterial molecules-10 (ASAM-**10**) with a quantitative yield.

FT-IR (cm^{-1}): 3182 (amide N-H str.), 2920 (CH_2 assym. str.), 2854 (CH_2 sym. str.), 1740 (ester C=O str.), 1674 (amide I C=O str.), 1542 (amide II N-H ben.), 1460 (CH_2 scissor), 1197 (C-O str). ^{1}H-NMR (400MHz, D_2O): δ/ppm. 1.24-1.31 (s, –$(CH_3)_2N^+(CH_2)_2$(CH_2)$_8$$(CH_2)_2N^+(CH_3)_2$-, 16H and t, terminal –$CH_3$, 6H), 1.59-1.70 (m, –$(CH_3)_2N^+CH_2CH_2$- 4H), 3.00-3.39 (m, -$CH_2N^+(CH_3)_2$, -$CH_2$Ph, 20H), 3.95-4.1 (m, –NHCO$CH_2N^+(CH_3)_2$-, 4H), 4.20-4.25 (q, -OCH_2CH_3, 4H), 4.80-4.83 (App quint. –$CH_{(Phe)}$, 2H), 7.28-7.40 (m, -$H_{(Ph)}$, 10H). HRMS (m/z): 362.2558 [(M-2Br)/2 $^+$] (observed), 362.2564 [(M-2Br)/2 $^+$] (calculated).

Bactericidal kinetics. A similar protocol was followed like antibacterial assay, described under experimental section in the main article. Herein, the only difference was that the bacterial cell viability was determined at different time points, such as 1 h, 2 h, 4 h, and 6 h.

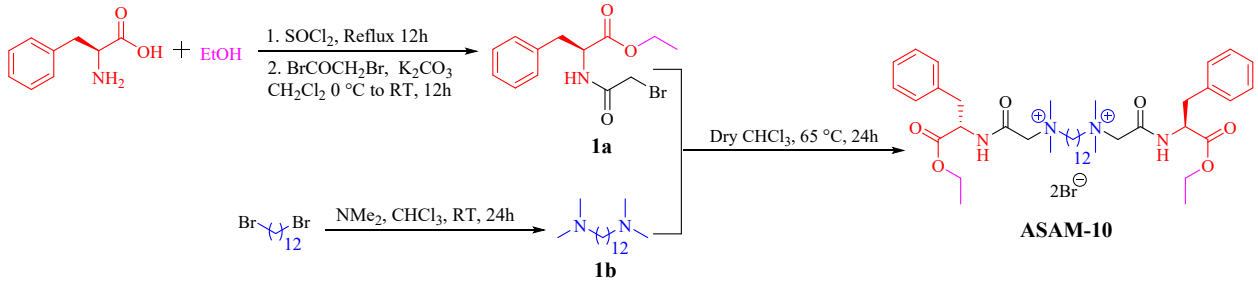
Activity against stationary phase bacteria. A mid-log phase (6 h grown culture) bacterial culture was diluted to a 1:1000 ratio in nutrient broth and incubated at 37 ˚C for 16 h in shaking conditions to achieve stationary cells. Later on, the bacterial suspension was centrifuged (9000 rpm, 2 min) and resuspended in normal saline. On the other hand, the persister cells were generated from the stationary cells upon specific antibiotic exposure for 3 h. 1 mL of stationary phase culture was treated with 100 µg/mL (for MRSA) and 300 µg/mL (for *E. coli*) of ampicillin sodium for 3 h at 37 ˚C. Next, the bacteria were centrifuged, washed

3-4 times, and resuspended in normal saline to remove the traces of the antibiotic. Then these stationary and persister phase cells were treated with hydrogels similarly to the antibacterial assay.

Bacterial live/dead assay. Briefly, 200 µL of ~10^8 CFU/mL midlog phase bacterial suspension in normal saline was added over 100 µL of GGH-0.1 and incubated at 37 ˚C. After 4h of incubation, bacterial suspension was centrifuged to remove the released biocide completely. Then, the bacterial palate was resuspended with normal saline followed by the addition of 5 µL of Syto-9 (3 µM) and PI (15 µM) mixture and incubated for half an hour. Further, the dye-containing bacterial suspension was centrifuged to remove the excess unbound dye and the palate was resuspended with 50 µL of normal saline. Finally, 5 µL of bacterial suspension was taken into a glass slide and processed for confocal microscopy at 63 X resolution.

Supplementary Figures

Scheme S1. Synthesis of ASAM-10



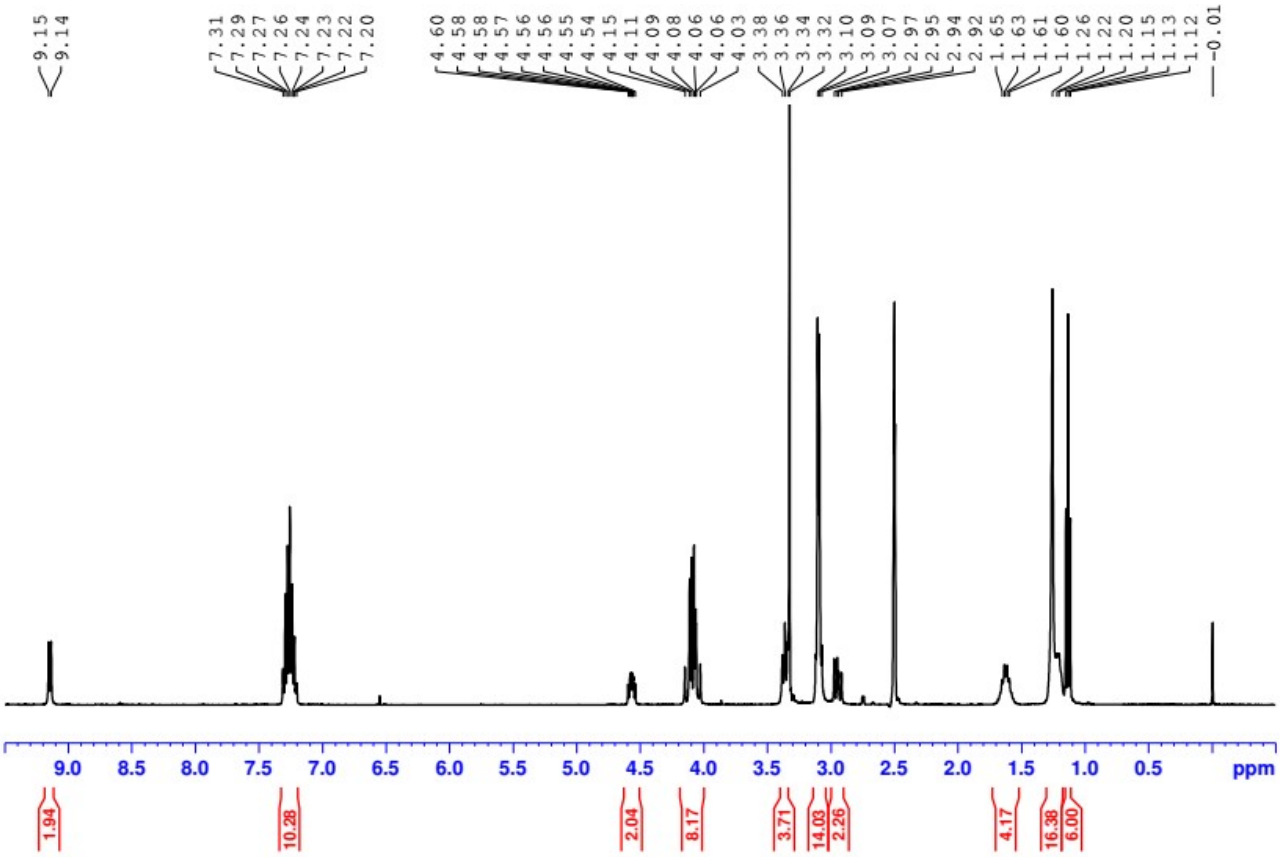


Figure S1. ^{1}H-NMR of ASAM-10. The NMR was taken in DMSO-d_6 and the solvent peak was calibrated at the δ value of 2.5 ppm.

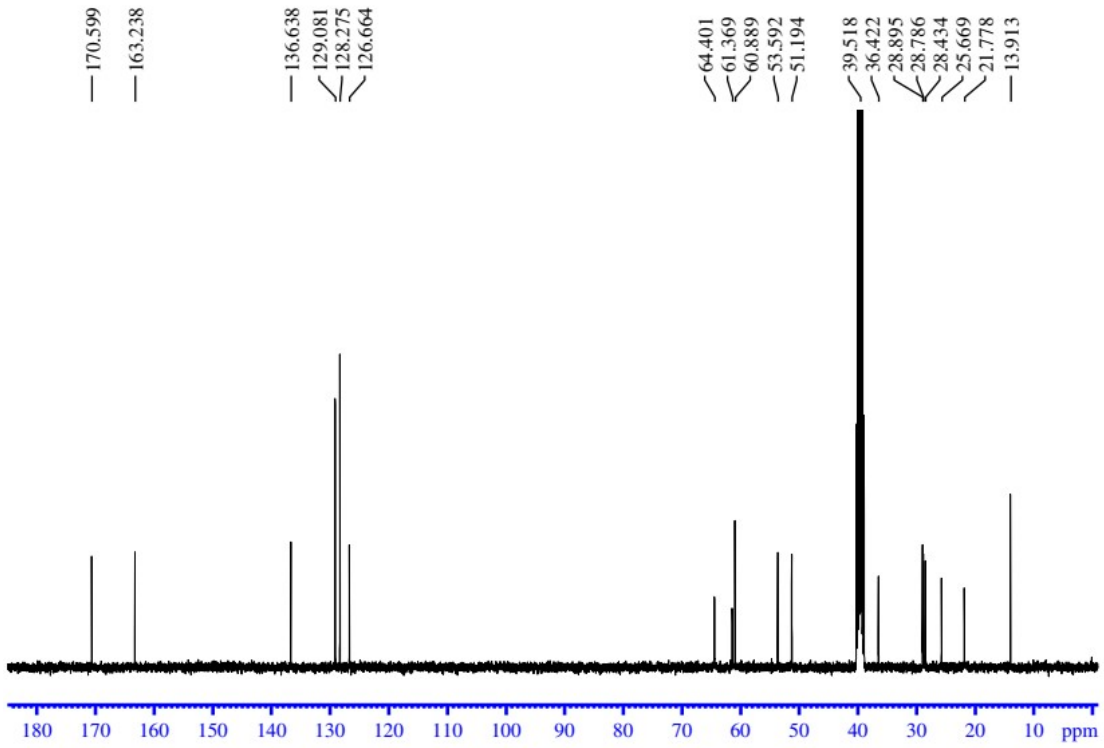


Figure S2. ^{13}C-NMR of ASAM-10**.** The NMR was taken in DMSO-d_6 and the solvent peak was calibrated at the δ value of 39.52 ppm.

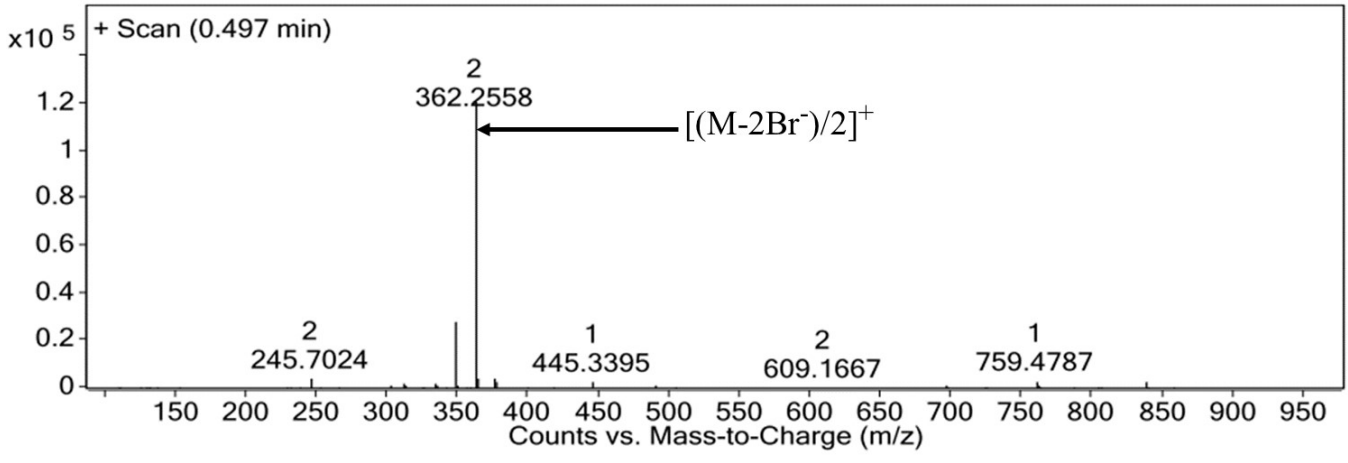


Figure S3. HR-MS of ASAM-10.

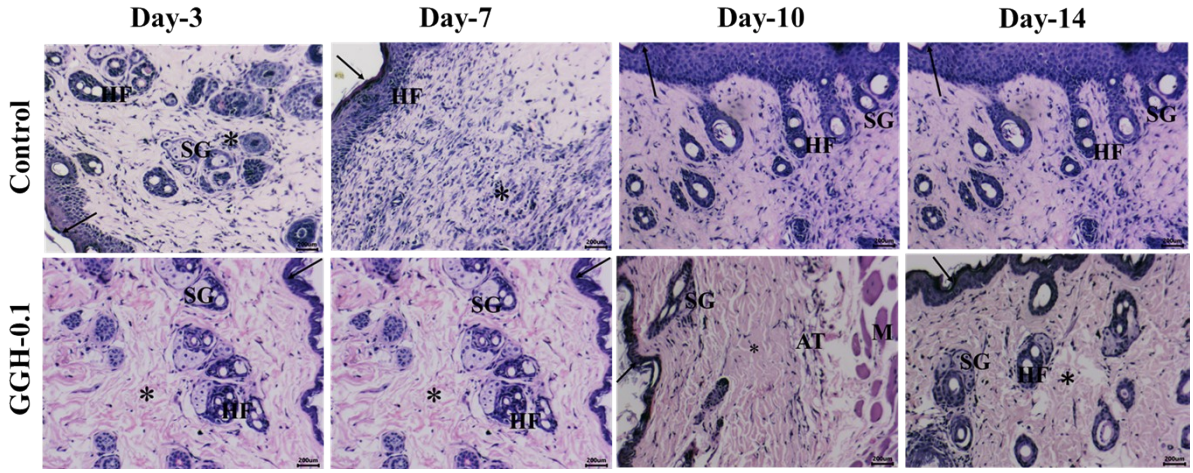


Figure S4. Histopathological analysis (H and E staining) of the tissue samples collected from the wound site at different days. The scale bar was 200 µm.