Supplementary information

Highly Active Nanoparticles Enhanced Rapid Adsorption-killing Mechanism to Combat Multidrug-resistant Bacteria

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Contents

1 Experimental Section

1.1 Materials

Methyl methacrylate (MMA, 99%), methacrylatoethyl trimethyl ammonium chloride, ammonium persulfate (APS), 2,2-azobisisobutyronitrile (AIBN), n-lauryl acrylate, N, N-dimethyl formamide (DMF), isopropanol (IPA), ethyl acrylate (EA, 99%), disodium phosphate, albumin bovine serum, fetal bovine serum, and potassium dihydrogen phosphate, were supplied by Aladdin (China), Cienry Biotechnology Co., Ltd (China), Sinopharm (China) respectively, and purified by recrystallization before use. Luria-bertani (LB) broth, LB agar, minimum essential medium (MEM) culture, and sterile saline (0.9% NaCl) were purchased from Sinopharm (Shanghai, China), Qingdao Hope Bio-Technology Co., Ltd (China) and Shanghaiyuanye Bio-Technology Co., Ltd (China), respectively. Polypropylene (PP) non-woven fabric was supplied by Chaomei (China). Ultrapure water (18 MΩ cm^{-1}, Millipore Milli-Q, USA) was used. Phosphate-buffered saline (PBS, pH 7.4, 0.03 mol/L) were prepared and sterilized before use.

1.2 Characterization and Instruments.

The size distribution and zeta potential of CME and ANPQs were determined by dynamic light scattering system (DLS, MALVERN, Zetasizer Nano ZSP, UK). All measurements were repeated three times. Fourier transform infrared spectroscopy (FTIR, BRUKER, Vector-22, Germany) and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR, BRUKER, Avance III 500 MHz, Germany) were used to characterize the chemical composition. Neon-substituted chloroform ($CDCl_3$) was used as the solvent and $Si(CH_3)_4$ as the internal standard. XPS measurements were tested using the Thermo Scientific K-alpha XPS system. Prepare samples for XPS by dripping 20 μL of the diluted sample onto a silicon wafer. XPS data were analyzed using the Casa XPS software and energy referenced to the C 1s peak with a binding energy of 284.8 eV. The emulsification performance of CME was mainly characterized by critical micelle concentration (CMC). The CMC was tested with a conductivity meter (CM, DDS, 11A, China). The electrical conductivity of emulsifier aqueous solutions with

different concentration was tested and the logarithm curve of the electrical conductivity and the concentration was plotted. The inflection point on the graph is the critical micelle concentration of the emulsifier. The specific surface area of the fabric was measured by specific surface area micropore pore size analyzer (AUTOSORB-IQ-MP, Konta Technology, Hong Kong). The morphology of ANPQ series was examined by field emission scanning electron microscopy (FESEM, S-4800, Japan) at 2.0 kV and transmission electron microscope (TEM, JEM-2100, JEOL, Japan). The ANPQs was dripped on the copper grids and then dried at room temperature. After drying, copper grids were used for TEM characterization.

1.3 Scanning Electron Microscopy (SEM).

Briefly, the bacterial suspensions (~10^8 CFU/ml) were incubated with ANPQ-11% for 1 h at 37 °C, the mixtures were collected by centrifuged (10000 rpm for 5 min). The bacterial pellets were resuspended in PBS. Twenty microliters of the resulting suspensions were directly placed onto clean silicon slices. And then, the naturally dried samples were fixed with paraformaldehyde (4%) for 45 min at 4 °C. The fixed samples were washed with PBS three times and dehydrated for 30(0.5 h), 50(0.5 h), 70(0.5 h), 90(0.5 h), 100 % (0.5 h) ethanol. Finally, the samples were dried and coated with platinum for FESEM observation.

1.4 Transmission Electron Microscopy (TEM).

After exposed to ANPQ-11%, the bacteria were washed in PBS (0.1 M, pH 7.4). The exposed bacterial suspension was centrifuged down to a pellet and fixed in 2.5% glutaraldehyde overnight. Removed the glutaraldehyde solution, washed three times with PBS for 15 min, and fixed with 1% osmium acid solution for 1.5h. Fixed cell pellets were washed with PBS for 15 min, dehydrated with 30%, 50%, 70%, 80%, 90%, 100%, and 100% v/v (EtOH in water) for 30 min, respectively. And then rinsed with EtOH/acetone (1:1) for 30 min and with pure acetone 30 min for two times. The bacterial samples were then soaked in 1:1 acetone/epoxy for 2 hours and 1:3

acetone/epoxy overnight. It was then soaked in fresh epoxy for an additional 8 hours and finally fixed with neat resin in a vacuum oven. Finally, the fixed samples were sectioned and stained with uranyl acetate and lead citrate for TEM imaging.

1.5Antiviral Activity Assays [1].

1.5.1 Cell Culture and Virus Propagation.

Madin-Daby canine kidney (MDCK) cells was used for virus cultivation and antiviral activity studies. The cells were cultured in sterilized cell culture flasks supplemented with Dulbecco's modified Eagle's medium (DMEM, containing 1% penicillin, 1% streptomycin and 10% fetal bovine serum) at 37 °C (5% CO2, 95% air). An envelope negative sense single-stranded virus (H1N1) was selected as a model for respiratory infections. Viruses were cultivated in the MDCK cell line and the TCID50 (Tissue Culture Infectious Dose 50) virus titer was determined.

1.5.2 Evaluation of Antiviral Activity.

The antiviral activity test was carried out according ISO18184-2019. Cells were seeded into 96-well plates and incubated at 37 °C and 5% CO^2 for 24 h. The sample was prepared by cutting in a size of 3 × 3 cm^2. Prepare test samples and control samples with a weight of 0.4g, which will be used after cleaning. The virus suspension was added dropwise to the test and control samples for 0.5, 1 and 2 hours at room temperature. The virus was recovered by repeated pipetting with SCDLP neutralizer. The recovery solutions of the above groups were serially diluted, inoculated into MDCK cells, and cultured at 37°C in 5% CO^2 for 2-3 days. Cell lesions were observed, and the lesions were recorded. Tests were repeated 3 times. According to the Reed-Muench formula [2], the half-tissue infection dose TCID50 was calculated. The antiviral activity of $PP_{ANPQ\text{-}11\%}$ was evaluated by the following Eq. (S1):

$$Mv = log(Vb) - log(Vc) \quad \text{(S1)}$$

where *Mv* represents antiviral activity, *Vb* represents TCID50 titer of the control sample, and *Vc* is the TCID50 titer of the sample. The values were recorded in **Table S1.**

1.6 Hemolysis Assay.

The hemolysis assay was performed according to GB/T 16886.5-2003 and GB/T 14233.2-2005 Table 2. The fresh pig blood with sodium citrate was supplied by Beijing Bersee Science and Technology Co. Ltd (Beijing, China). Firstly, the samples were immersed in 0.9% NaCl (aq) at a ratio of 6 cm^2/mL and then incubated for 24 h in a shaker at 37 °C and 200 rpm. The resulting solutions were stored at 4 °C for use. And then 5 mL of test solutions were added in a centrifuge tube. Tubes with distilled water as positive group. Tubes with 0.9% NaCl (aq) as negative group. 4.9 mL of 0.9% NaCl(aq) was added and incubated for 30 min at 37 °C. After incubation, 100 μl of fresh pig blood was added and incubated for 1 h at 37 °C. Finally, the point tubes were centrifuged at 3500 rpm for 5 min and supernatant was transferred and the absorbance was measured at a wavelength of 545 nm by a micro-plate reader (Thermo Scientific Multiskan FC, America). The hemolysis rate (HR) was calculated by following Eq. (S2):

$$HR = (B - B_0)/(B_1 - B_0) * 100 \quad \text{(S2)}$$

Where: B, B_0 and B_1 is the test group absorbance, negative control group absorbance and positive control group absorbance, respectively. At least three biologically independent replicates were performed per sample, each using two technical replicates.

1.7 In Vitro Cell viability Assays [3].

Mouse embryonic fibroblast cell line L929 cells obtained from Chinese Academy of Sciences Cell Bank, were cultured in sterilized cell culture flasks supplemented with MEM (containing 1% penicillin, 1% streptomycin and 10% fetal bovine serum) at 37 °C (5% CO_2, 95% air). Before confluence, these L929 cells were passaged after trypsinization. After several passages, the cultured cells were used for the following experiments. The Cell Counting Kit-8 (Bosterbio, USA)) was used for revealing the cytocompatibility of the antibacterial nonwoven fabric. After autoclave (121 °C, 20 min), the samples were immersed in fresh MEM at a ratio of 6 cm^2/mL, and then

incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 24 h and the resulting solution was for use. For testing, the L929 cells were seeded into 96-well plates with 200 μL of MEM at an initial cell density of 1×10^4 per well. Cultured 1 day, each well of 96-well plates was replaced with 200 μL of the resulting solution as test group. Incubated another day under the same culture condition, the cell viability of difference samples was evaluated by the CCK8 kit at the absorbance of 450 nm. Blank group only incubated with CCK8 solution. The Cell Viability (%) was calculated by following Eq. (S3):

$$Cell\ Viability\ (\%) = (C_1 - C_0)/(C_2 - C_0) * 100 \quad \text{(S3)}$$

Where: C_1 - for the test group absorbance; C_2 - negative control group absorbance; C_0 - blank control group absorbance. At least three biologically independent replicates were performed per sample, each using two technical replicates.

Cell Morphology. L929 cells were directly seeded onto the test pellets in 24-well plates with 1 mL of MEM at a density of 5×10^4 cells per well. After 1 day, each well was replaced by 1 mL of above resulting solutions. Positive groups were incubated with phenol solution (in fresh MEM at a ratio of 0.03 g/mL). The cells were incubated another 24 h, and then every sample was observed by inverted microscopy (Olympus IX2-UCB, Japan). Each image of samples was chosen from over 10 images on different locations.

1.8 In Vitro Irritation Test.

The animal skin irritation test was conducted to assess the potential of the material producing irritation. This study was conducted based on the International Organization for Standardization GB/T 16886.10-2017: Biological evaluation of medical devices-part 10: Tests for irritation and delayed-typehypersensitivity; GB/T 16886.10-2017: Biological evaluation of medical devices-part 12: Sample preparation and reference materials. The test samples were cut into a piece of 2.5 cm×2.5 cm. Each test group and the negative control (absorbent gauze) groups were contacted on animal skin directly. Each test group tested 3 times on 3 male, young adult, 2.0~3.0 kg New Zealand White

rabbits (Yizheng Animal Biotechnology Co., Ltd. 20200313-1). Observations for erythema and edema were conducted at 24, 48 and 72 hours after contact. On the day before the test, the fur on the backs of rabbits (approximately 10 cm × 15 cm) were clipped and both sides of the spinal for application and observation. A 25 mm × 25 mm section of absorbent guaze patch was saturated with freshly sterile water and then covered on the test sites. The test samples and the reagent control were applied to the region, as shown in **Fig. S1**.

The test site was covered with a gauze patch and wrapped with a semi-occlusive bandaged for 24h. At the end of the contact time, the dressing was removed. A natural lighting was used to visualize the skin reactions. The skin reaction for erythema and oedema were described and scored at 1, 24, 48, 72 hours. The tissue reaction for erythema and oedema were graded according to the classification system given below, and the results were recorded in **Table S2**.

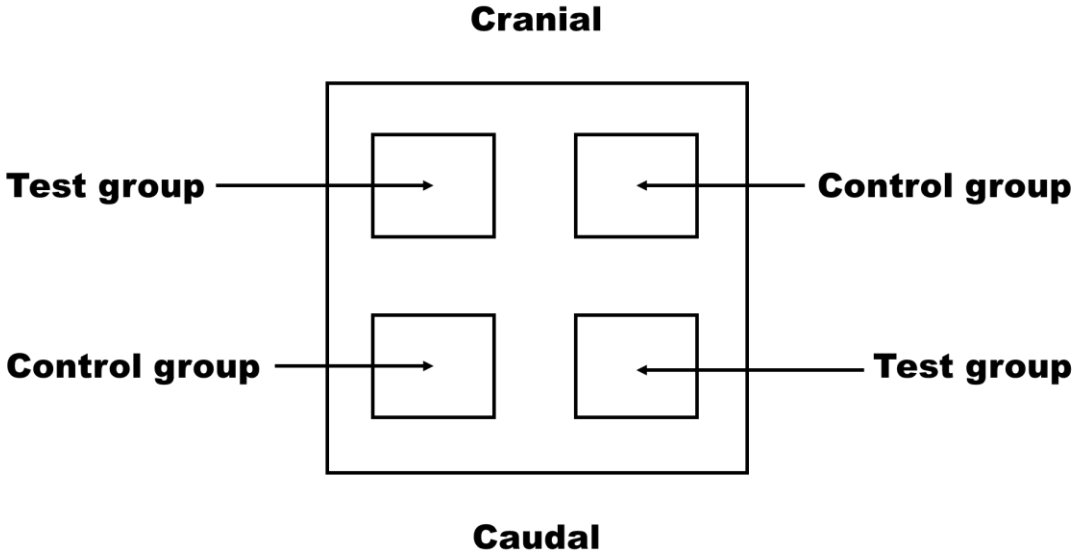

Fig. S1 Region illustration of the test samples and the reagent control.

1.9 Guinea Pigs' Maximization Test.

Guinea pig is the most commonly accepted model and has been used to study photoallergy since late 1960s [4]. This study was conducted based on the International Organization for Standardization GB/T 16886-2017. 15 adult male albino guinea pigs

(10 used for test group and 5 used for control group) were supplied by Yizheng Animal Biotechnology Co., Ltd (2020.03.11-2). The test samples were cut into a piece of 2.5 cm*2.5 cm. Clipped and shaved the fur on all treatment sites before test. Test samples and absorbent gauze patch were saturated with freshly sterile water and covered on the test sites and then wrapped with a semi-occlusive bandaged for 6h. Repeated this step for three consecutive days within a week and repeated the same method for three weeks. Control groups were treated with blank liquid in the same way. 14 days after the last induction, the test samples fully humidified with sterile water were locally applied to the hairless abdomen of all test animals and control animals. Removed the occlusive dressings and patches after 24 h and assessed the application sites for erythema and oedema using the Magnusson and Kligman grading scale given in **Table S3**. And then assessed again after removing the elicitation patch for 48 hours. The results were shown in **Table S4**.

2 Results and Discussion

Table S1 Antivirus values of $PP_{ANPQ-11\%}$ against H1N1.

Virus	Time (h)	Groups	$lg(TCID_{50}/mL)$	Mv
H1N1	0.5	PP	5.61	0.61
		$PP_{ACNP-11\%}$	6.22	
	1	PP	5.56	0.77
		$PP_{ACNP-11\%}$	6.33	
	2	PP	5.39	1.02
		$PP_{ACNP-11\%}$	6.41	

Table S2 Results of the animal skin irritation test.

		Erythema			**Oedema**		
Time (h)		24	48	72	24	48	72
Test groups	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
Control groups	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
PII		Test groups: 0.00			Control groups: 0.00		

Note: Each test article was tested three times on three rabbits; the numbers of erythema and oedema were counted and recorded.

Table S3 Magnusson and Kligman scale.

Patch test reaction	Grading scale
No obvious change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

Table S4 Results of Guinea pigs' maximization test.

Group	No	Weight (g)	24h after excitation		48h after excitation	
			Skin reaction rating	Other abnormal reactions	Skin reaction rating	Other abnormal reactions
Test groups	6	388	0	N	0	N
	7	405	0	N	0	N
	8	412	0	N	0	N
	9	342	0	N	0	N
	10	432	0	N	0	N
	11	388	0	N	0	N
	12	362	0	N	0	N
	13	392	0	N	0	N
	14	418	0	N	0	N
	15	358	0	N	0	N
Control groups	1	358	0	N	0	N
	2	389	0	N	0	N
	3	402	0	N	0	N
	4	377	0	N	0	N
	5	412	0	N	0	N

Note: N means no abnormal system signs were found.

1 Synthesis and characterization of macromolecular emulsifiers.

The structures of macromolecular emulsifiers were verified by FTIR and ^{1}H NMR spectra. The spectrum of the polymer is mainly characterized by bands at 2998 and 2951 cm^{-1} (νas-CH_3 and νs -CH_3, respectively), 1727 cm^{-1}(ν C=O) (**Fig. S2**a). In 1H NMR spectra of P(MMA-DMC) (**Fig. S2**b), it showed that the polymer had peaks at 3.58 ppm for -O-CH_2-CH_2-N- group, 3.38 ppm for-O-CH_3 group, 2.51 ppm for -C-CH_2-N- group and -N(CH_3)- group in addition to the peaks from 0.75 to 0.93 ppm for the -C-CH_3 groups. The results demonstrated that the macromolecular emulsifier was successfully synthesized. As is shown in **Fig. S2**c, the diameter of macromolecular emulsifier ranged from 200 to 300 nm. Besides, the lg (κ/(mS/cm))-lg (concentration (mg.mL^{-1})) curve (**Fig. S2**d) showed that the CMC of macromolecular emulsifiers was 0.0168 g/mL at 25 ℃, which indicated that the macromolecular emulsifier we synthesized possessed good emulsifying properties.

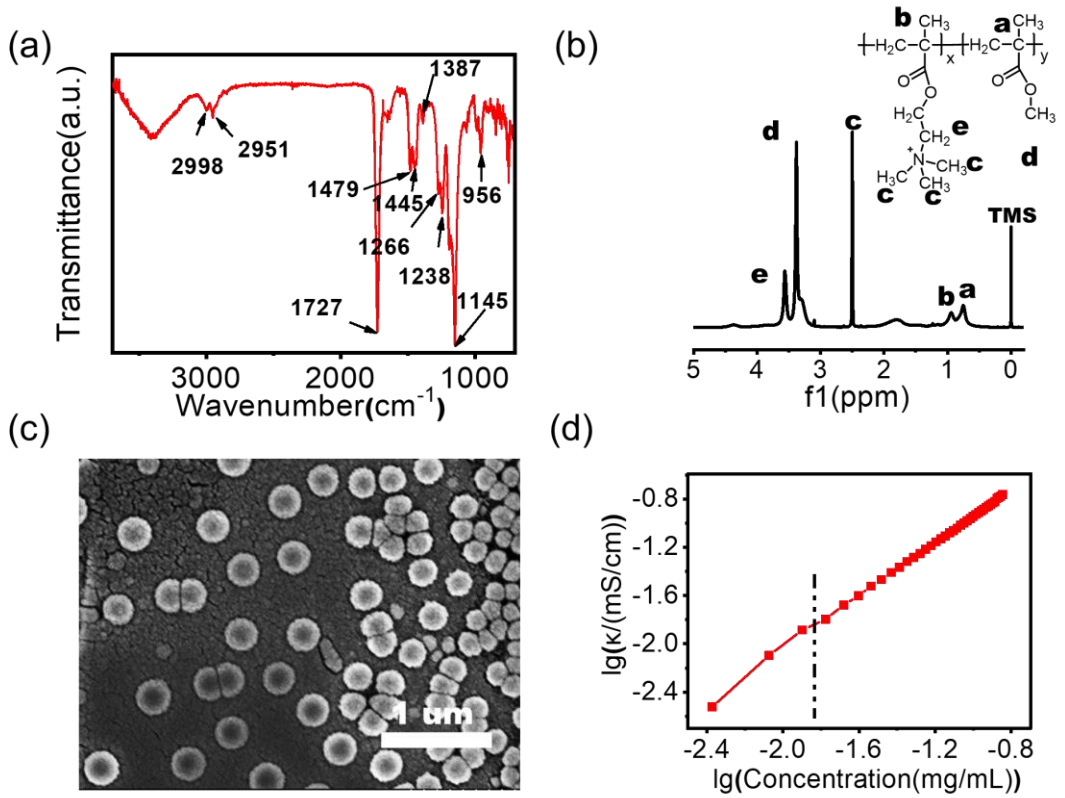

Fig. S2 Structure and characterization of macromolecular emulsifiers. (a) FTIR. (b) ^{1}H NMR. (c) Morphology of macromolecular emulsifiers. (d) CMC of macromolecular emulsifiers.

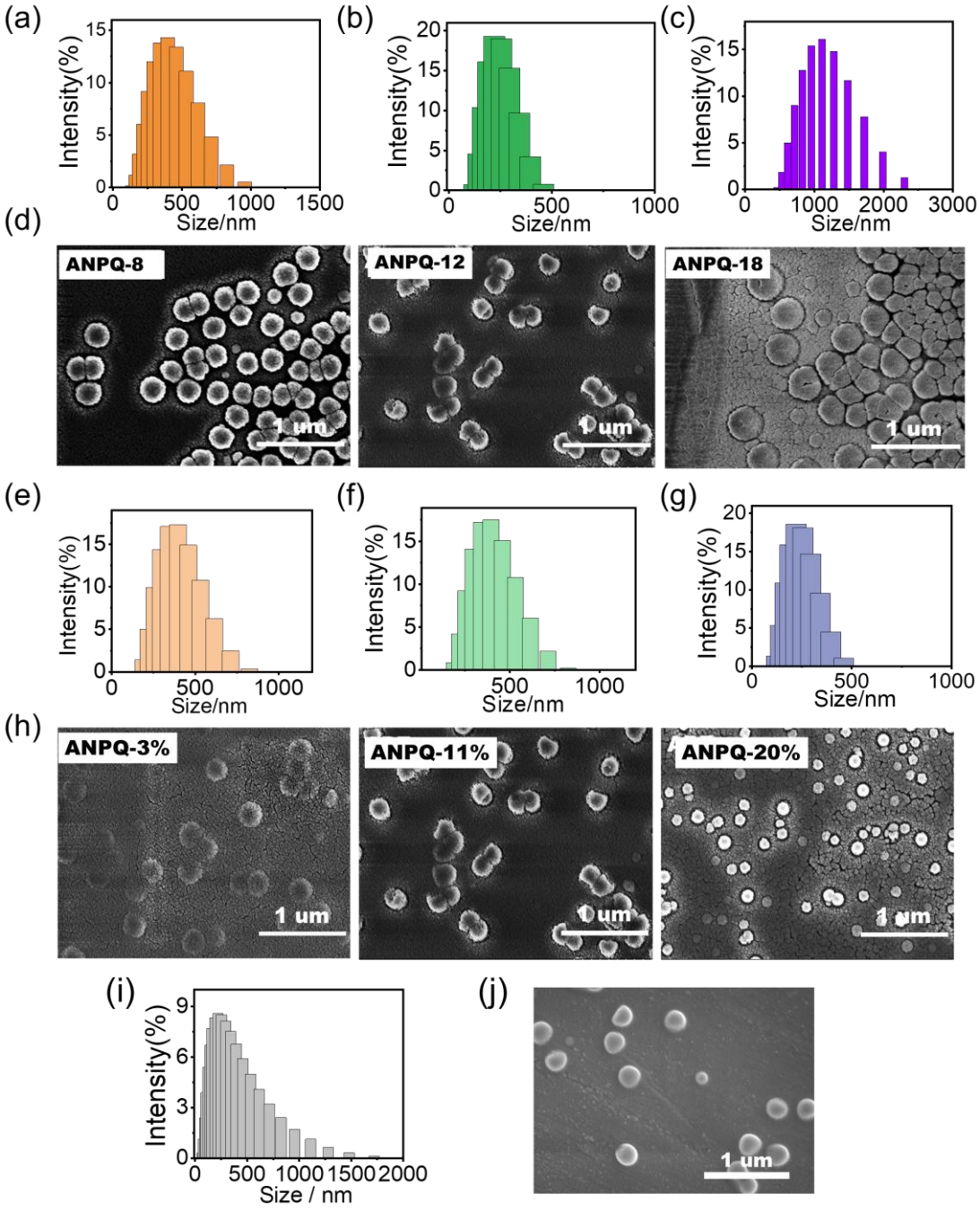

Fig. S3 Size distribution of (a) ANPQ-6, (b) ANPQ-10 and (c) ANPQ-16, respectively. (d) Morphology of ANPQ with various long aliphatic chains. Size distribution of (e) ANPQ-3%, (f) ANPQ-11% and (g) ANPQ-20%, respectively. (h) Morphology of ANPQ with different cationic domain contents. (i) Size distribution of ANPQ-SC. (j) SEM results of ANPQ-SC.

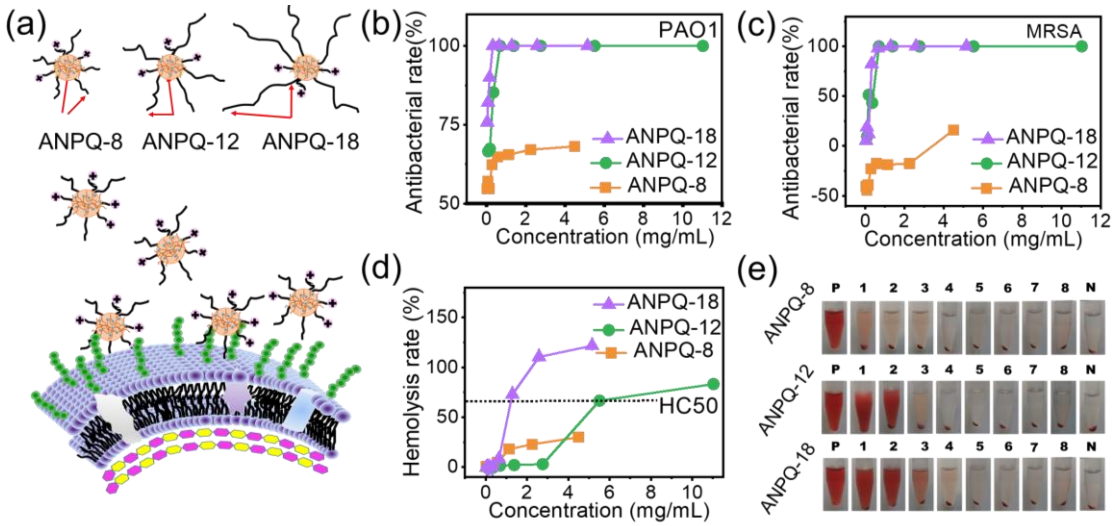

Fig. S4 (a) Scheme of ANPQs with different alkyl chain lengths against bacteria. (b, c) Bactericidal activity of ANPQs with different alkyl chain lengths. (d, e) Hemolysis rate (%) of ANPQ with different alkyl chain lengths.

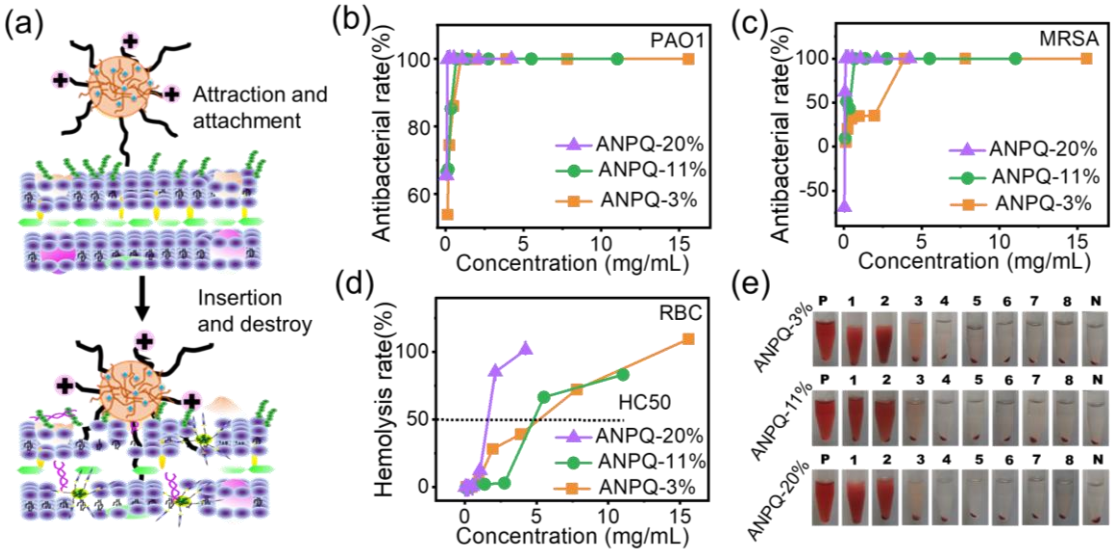

Fig. S5 (a) Scheme of ANPQs with different cationic domain contents against bacteria. (b, c) Bactericidal activity of ANPQ with different cationic domain contents. (d, e) Hemolysis rate (%) of ANPQ with different cationic domain contents.

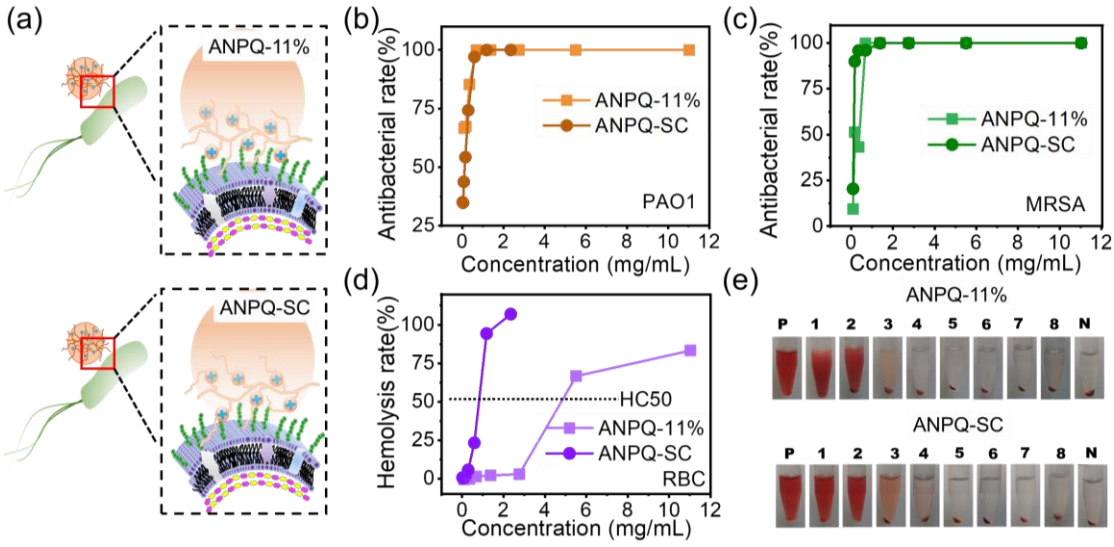

Fig. S6 (a) Scheme of ANPQ-SC against bacteria. (b, c) Bactericidal activity of ANPQ-SC. (d, e) Hemolysis rate (%) of ANPQ-SC.

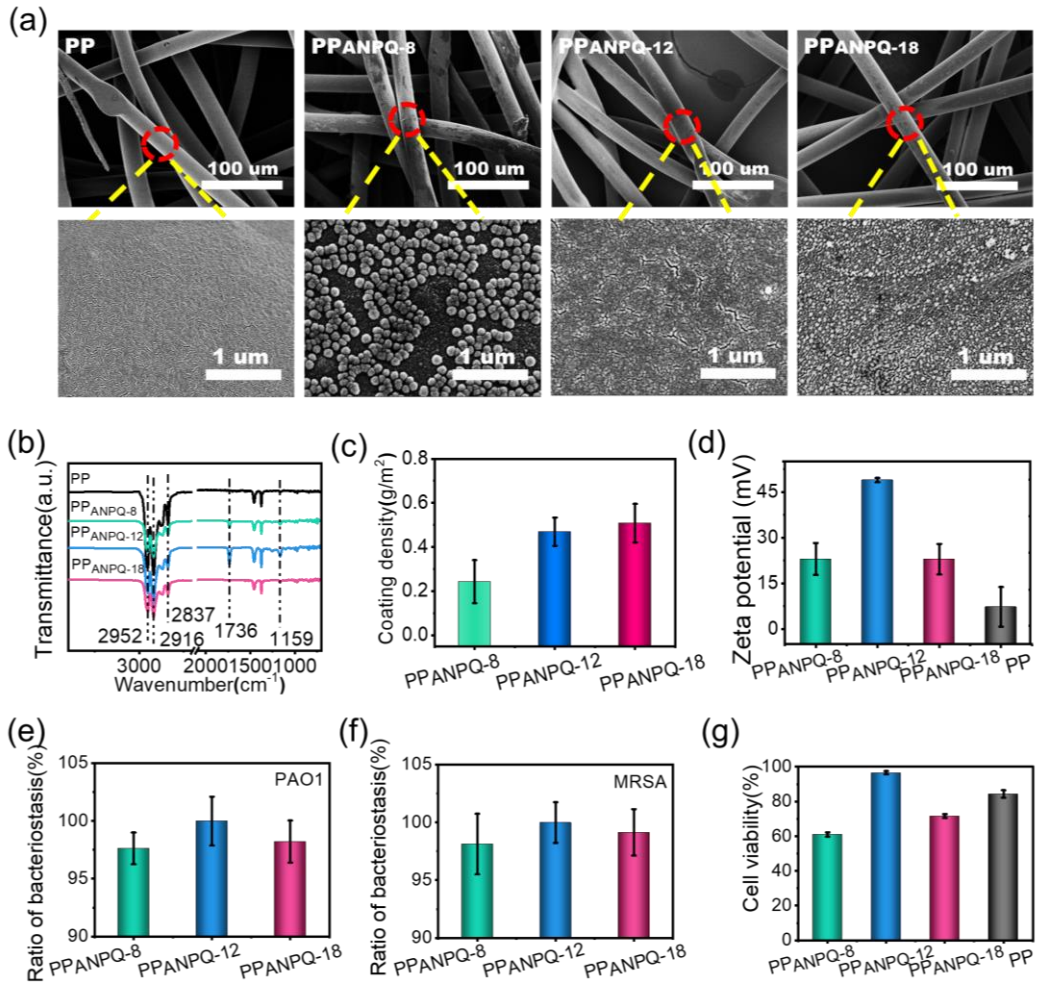

Fig. S7 (a) SEM, (b) FTIR (c) Coating density, and (d) Zeta potential of ANPQ-6,

ANPQ-10 and ANPQ-16, respectively. Bactericidal activity against (e) PAO1 and (f) MRSA of ANPQ with different alkyl chain lengths, respectively. (g) Cell viability of L929 cells of ANPQ with different alkyl chain lengths.

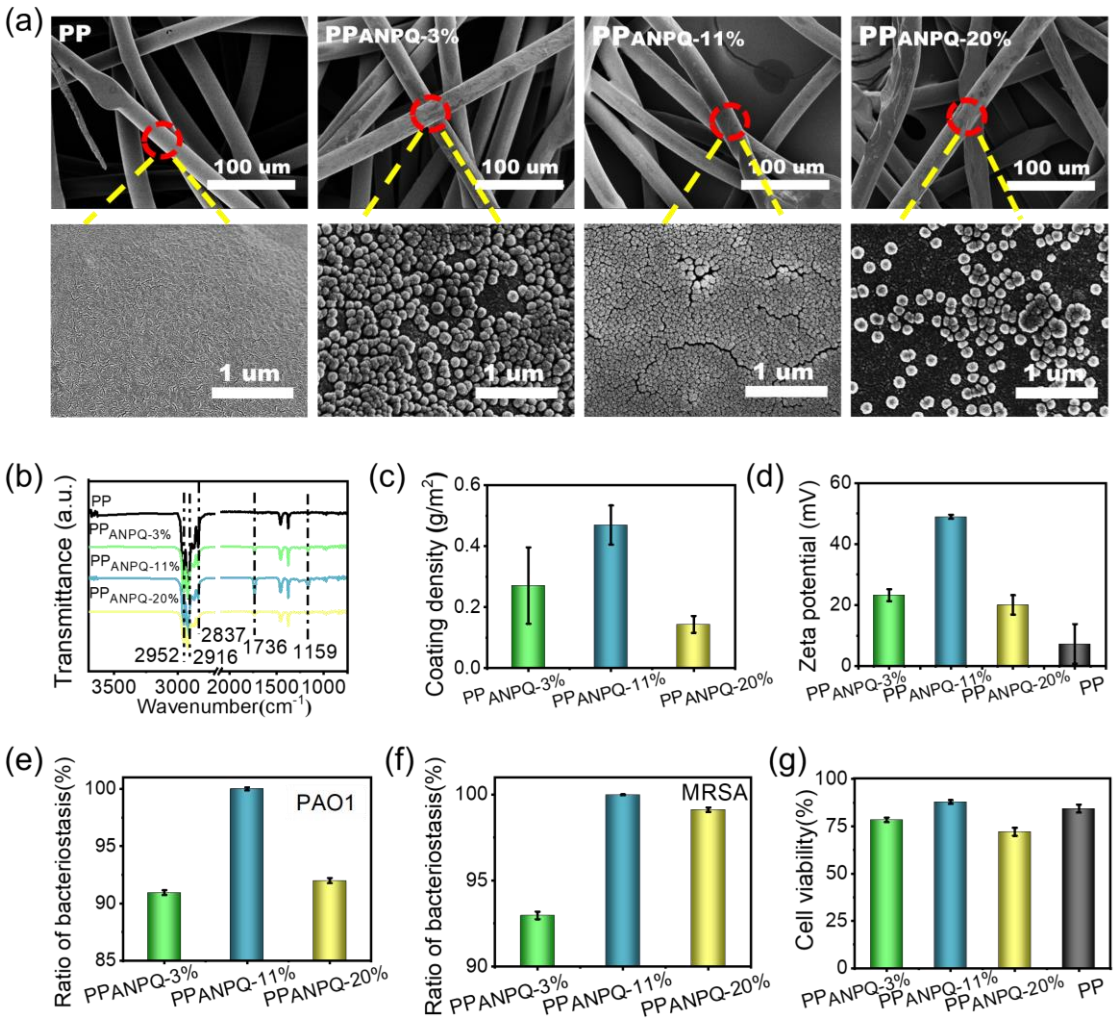

Fig. S8 (a) SEM, (b) FTIR (c) Coating density, and (d) Zeta potential of ANPQ-3%, ANPQ-11% and ANPQ-20%, respectively. Bactericidal activity against (e) PAO1 and (f) MRSA of ANPQ with different cationic domain contents, respectively. (g) Cell viability of L929 cells of ANPQ with different cationic domain contents.

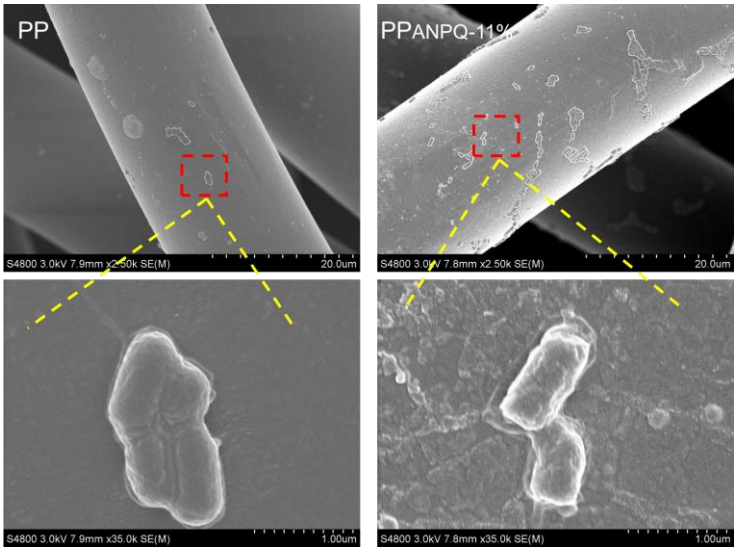

Fig. S9 SEM images of PAO1 cells after exposure to the uncoated surfaces and coated surfaces.

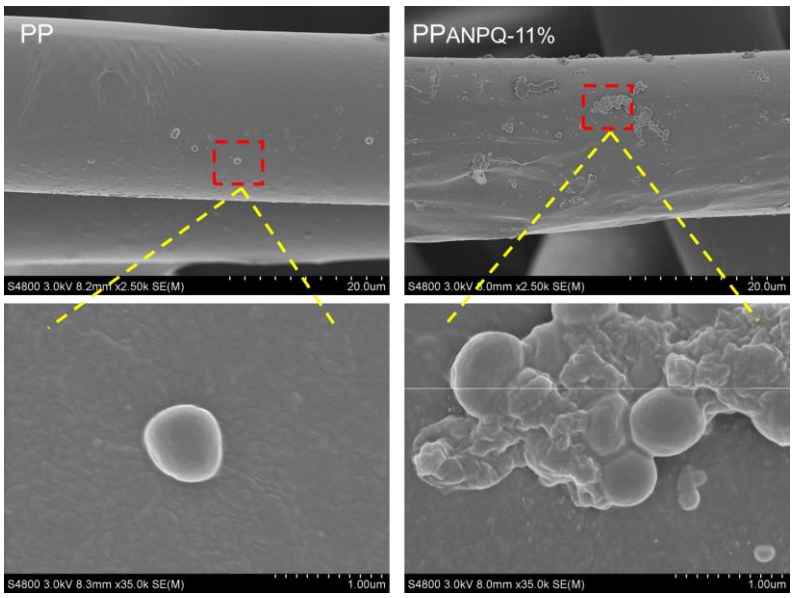

Fig. S10 SEM images of MRSA cells after exposure to the uncoated surfaces and coated surfaces.

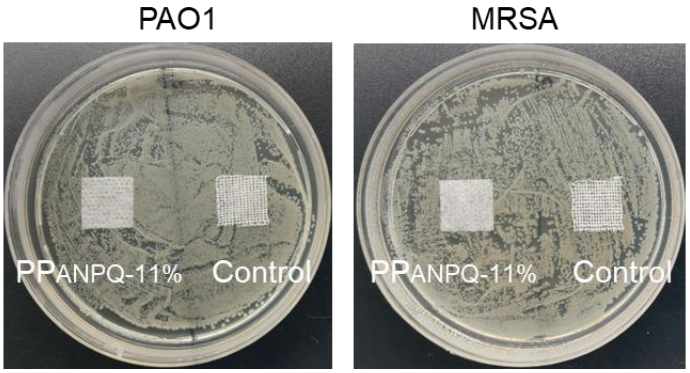

Fig. S11 Inhibition zone test of ANPQ-11%.

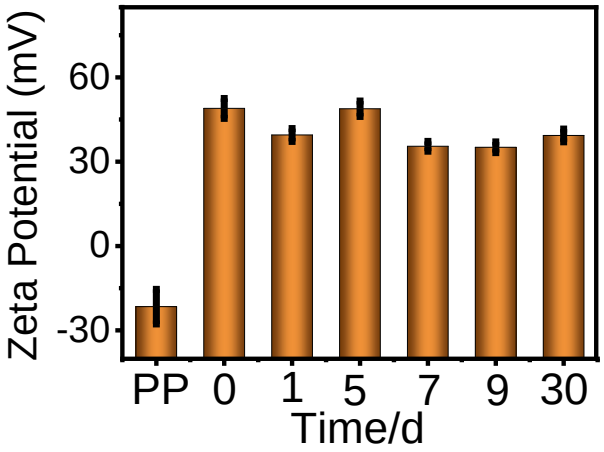

Fig. S12 Zeta potential of $PP_{ANPQ-11\%}$ after 30-day continuous treatment.

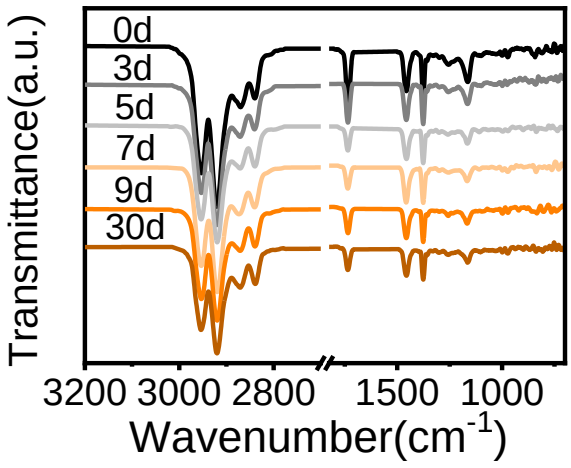

Fig. S13 ATR-FTIR of $PP_{ANPQ-11\%}$ after 30-day continuous treatment.

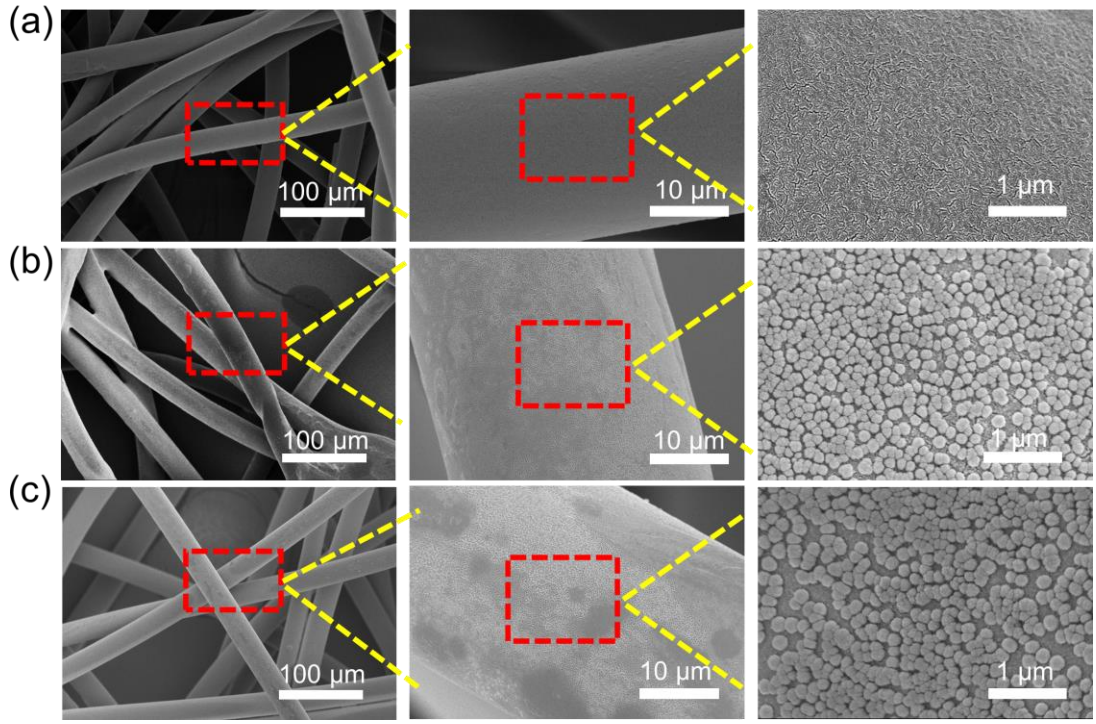

Fig. S14 SEM of (a) PP, $PP_{ANPQ-11\%}$ (b) before and (c) after 30-day continuous treatment.

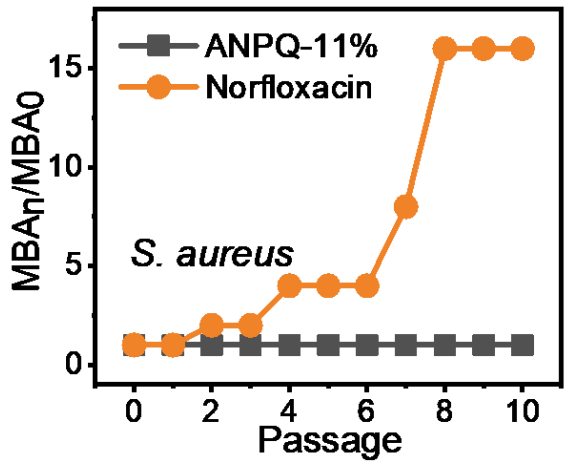

Fig. S15 Antibacterial resistance test on ANPQ-11% and Norfloxacin against *S. aureus* ATCC6538.

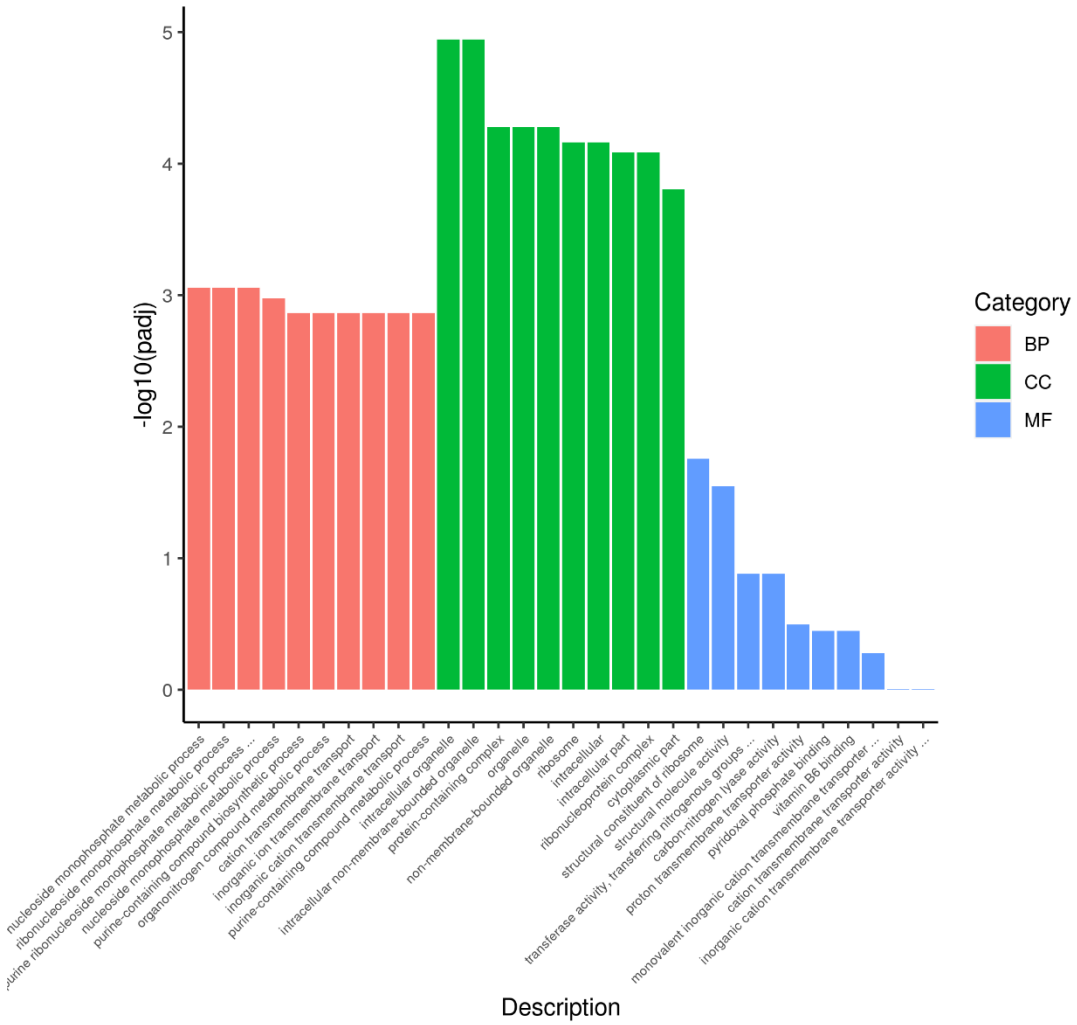

Fig. S16 Enrichment Analysis Workflow of Gene Ontology (GO) processes for altered genes after treated with ANPQ-11% compared with untreated control.

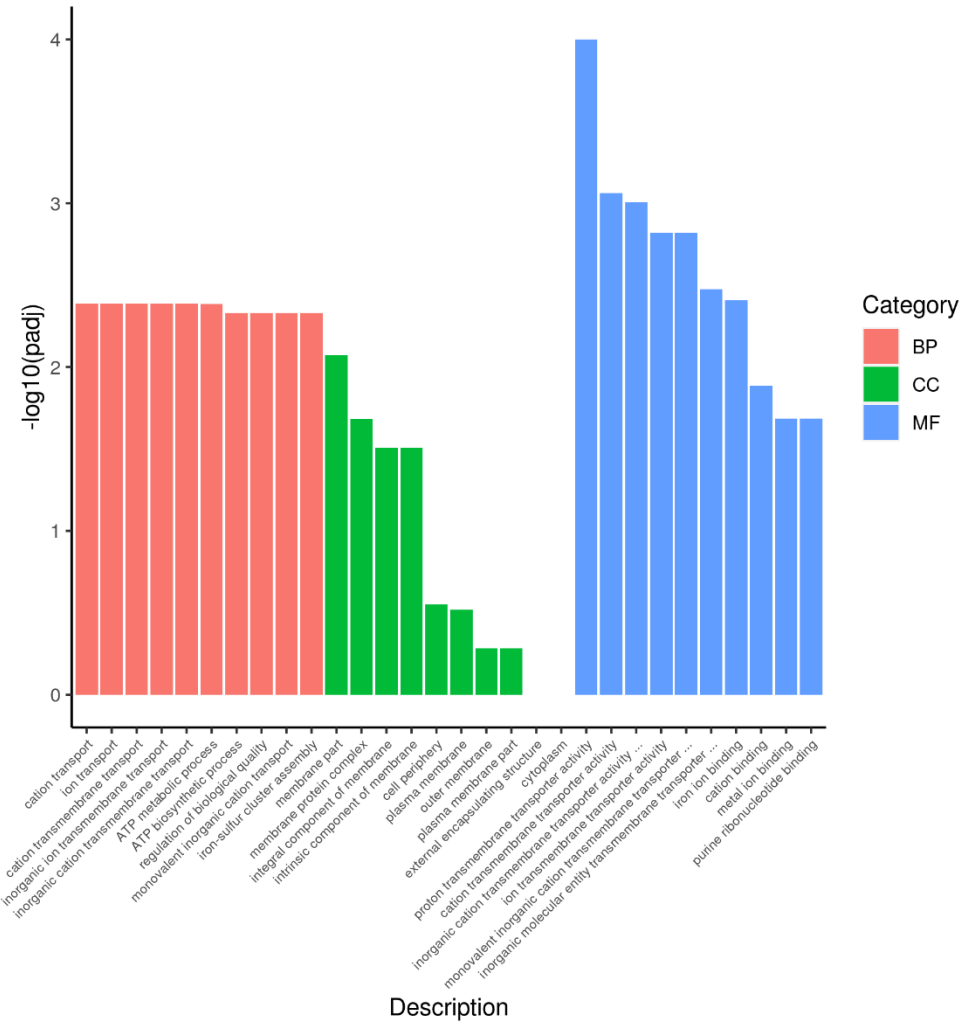

Fig. S17 Enrichment Analysis Workflow of Gene Ontology (GO) processes for altered genes after treated with levofloxacin compared with untreated control.

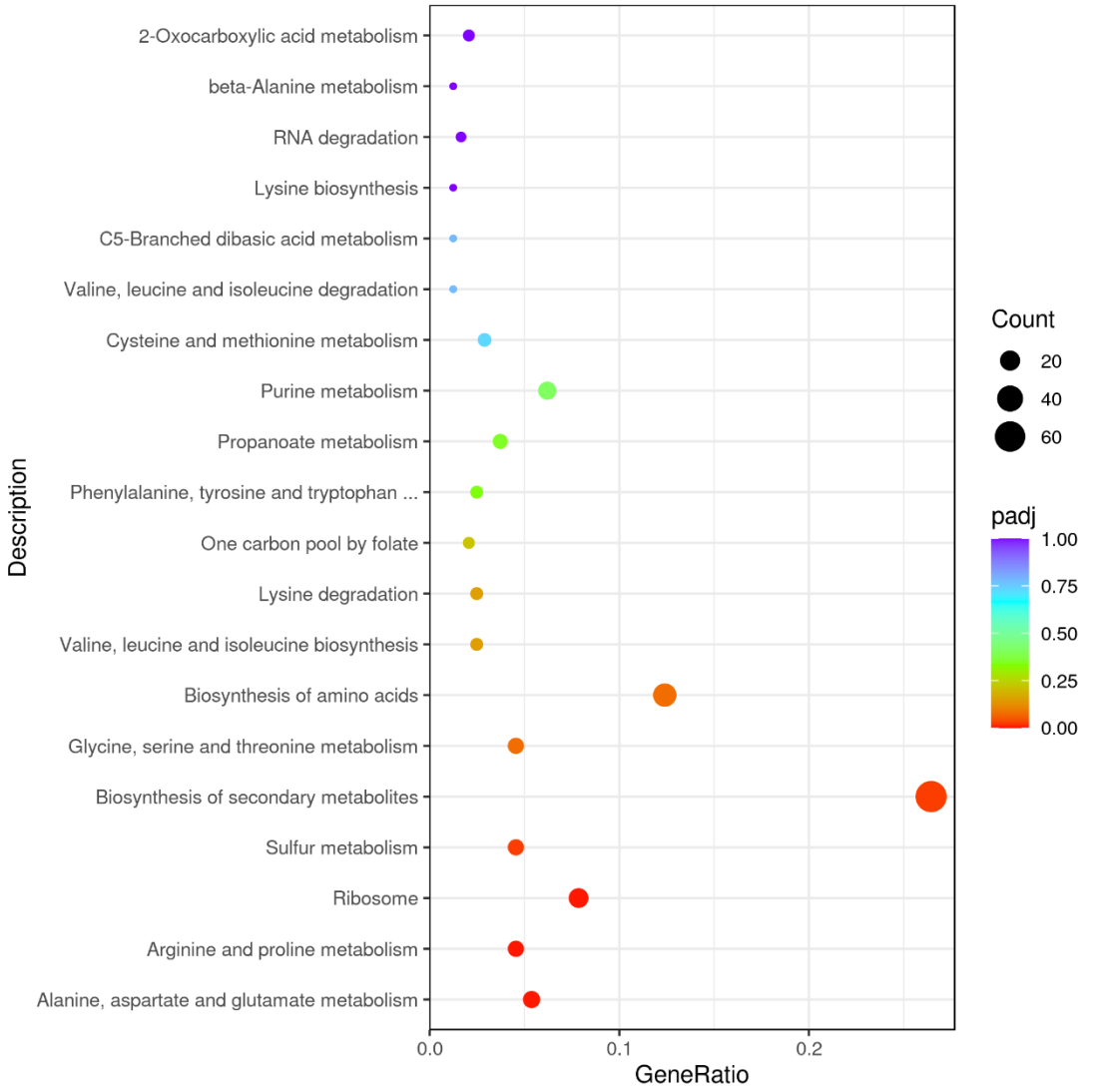

Fig. S18 Enrichment Analysis Workflow of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for altered genes after treated with ANPQ-11% compared with untreated control.

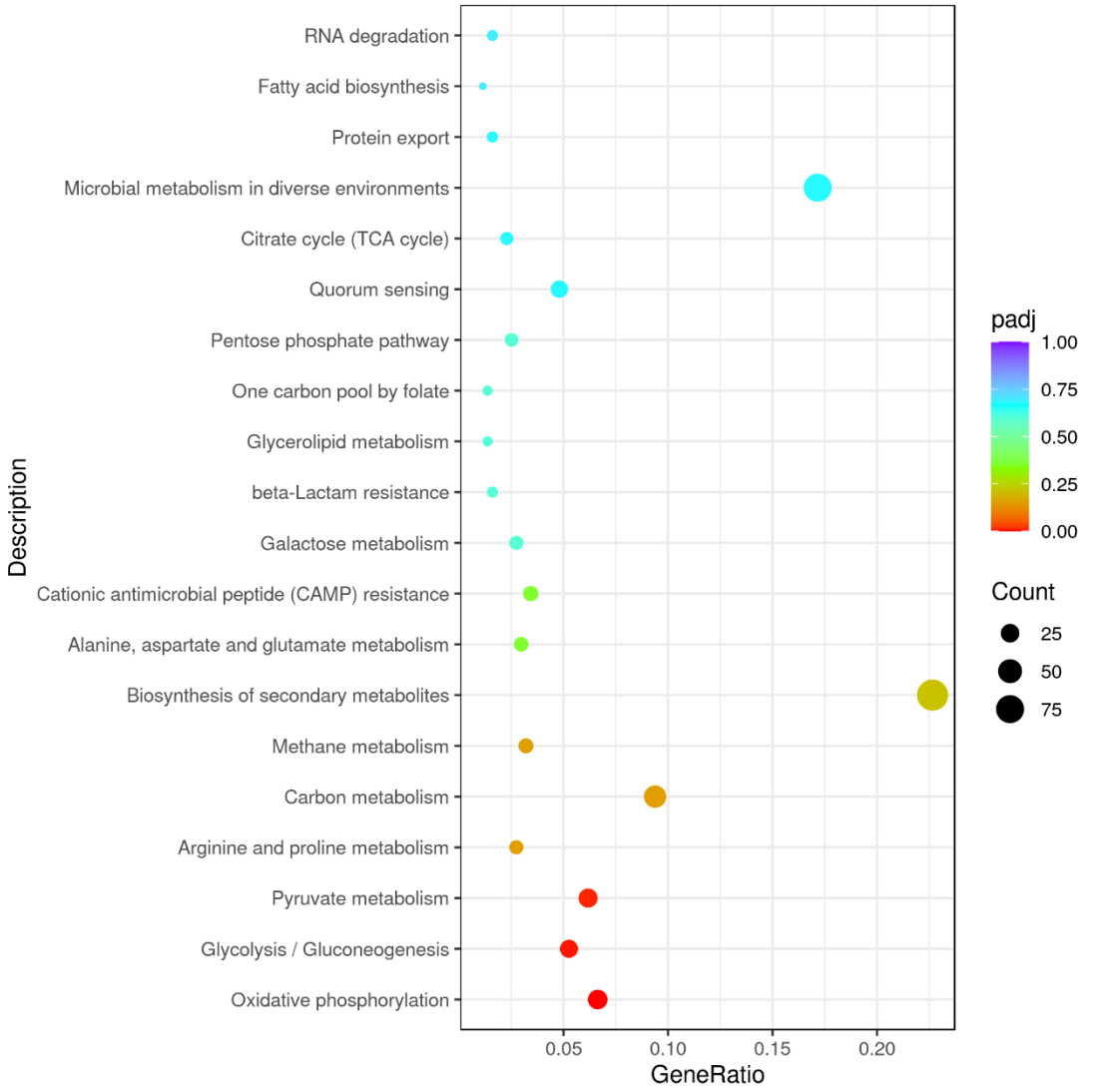

Fig. S19 Enrichment Analysis Workflow of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for altered genes after treated with levofloxacin compared with untreated control.

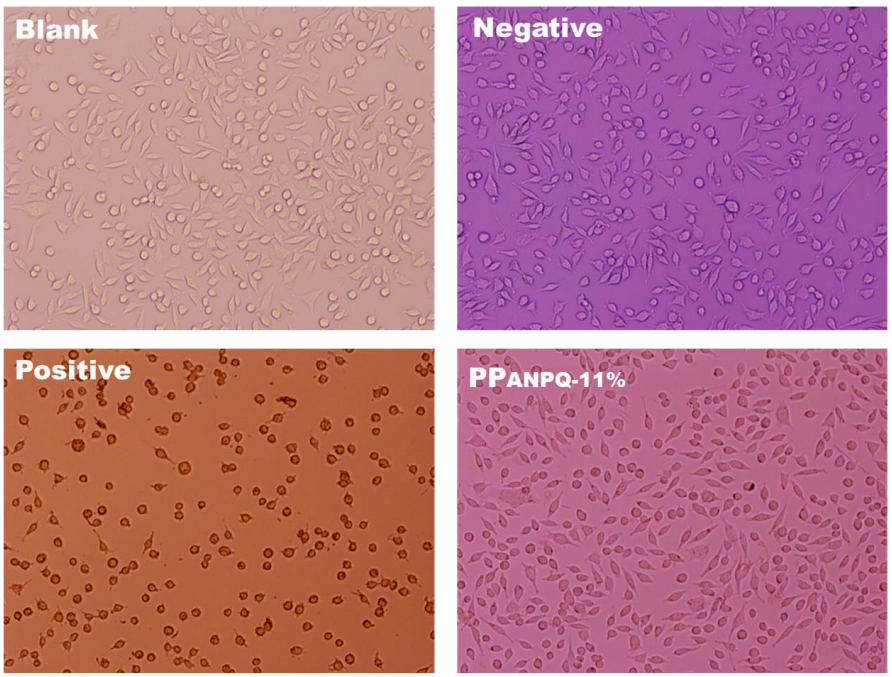

Fig. S20 Cell Morphology of L929 cells.

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