Supplementary Information

Succinylated casein-coated peptide-mesoporous silica nanoparticles as an antibiotic against intestinal bacterial infection

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Table S1. Primer sequences of TNF-α, IL-1β, MMP-9, and β-actin[a] used in quantitative RT-PCR[b]

Target Molecule		Sequence
TNF-α	F	5'-GGAACTGGCAGAAGAGGC-3'
	R	5'-CACTTGGTGGTTGCTACG-3'
IL-1β	F	5'-GAAATGCCACCTTTTGACAGTG-3'
	R	5'-TGGATGCTCTCATCAGGACAG-3'
MMP-9	F	5'-GACATCTTCCAGTACCAAGACA-3'
	R	5'-TCACCTCATGGTCCACCTTGTT-3'
β-actin	F	5'-TGTTACCAACTGGGACGACA-3'
	R	5'-GGGGTGTTGAAGGTCTCAAA-3'

[a] The primers were synthesized by Beijing Genomics Institute (BGI, Beijing, CHN).

[b] RNA was extracted using the TaKaRa (Dalian, CHN) RNAiso Plus reagent. A total of 900 ng of RNA was reverse-transcribed by TaKaRa PrimeScript TM RT-PCR kit (DRR014A). Data were obtained with Bio-Rad iQ5 standard edition optical system software (version 2.1) and analyzed for statistical significance using a Student-Newman-Keuls multiple comparisons test.

Fig S1. BCA protein determination calculating the loading efficiency of T7E21R-HD5 on MSN at different mass ratios. The prepared MSN was incubated with T7E21R-HD5 at mass ratios of 1:1, 1:2, 1:3, 2:1, and 3:1 at 4 °C overnight. MSN@T7E21R-HD5 was collected by high speed centrifugation at 12,000 rpm for 15 min. The remaining content of T7E21R-HD5 in the supernatant was detected by BCA and denoted as C_{rm}. The total concentration of T7E21R-HD5 was denoted as C_{tot}. The loading efficiency was determined as $(C_{tot}-C_{rm})/C_{tot} \times 100\%$. This experiment was conducted in triplicate and repeated twice.

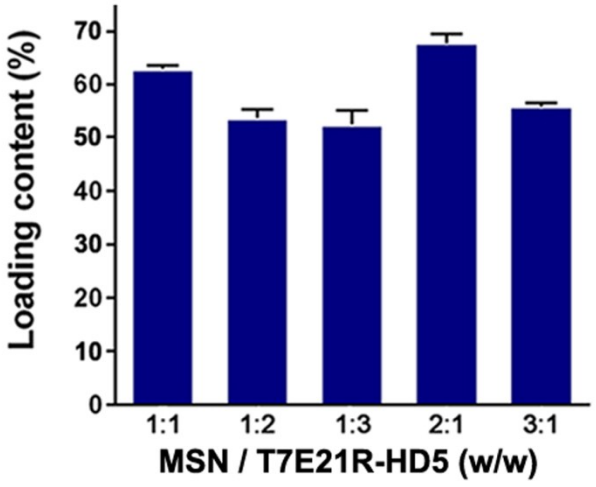


Fig S2. MALDI-TOF demonstrating the presence of T7E21R-HD5 on the prepared nanoparticle. Mass spectrometric analysis was performed in the linear mode on a time-of-flight mass spectrometer (MALDI 7090, Shimadzu, Kyoto, JPN). A 1-μL aliquot of T7E21R-HD5 (1.0 mg mL^{-1}), 2.0 mg mL^{-1} of MSN, and the MSN@T7E21R-HD5 mixture were mixed with sinapinic acid (v/v, 1:1) and were cocrystallized at room temperature. Measured molecular mass of T7E21R-HD5 is 3664.0 Da, close to the theoretical mass (3664.4 Da).

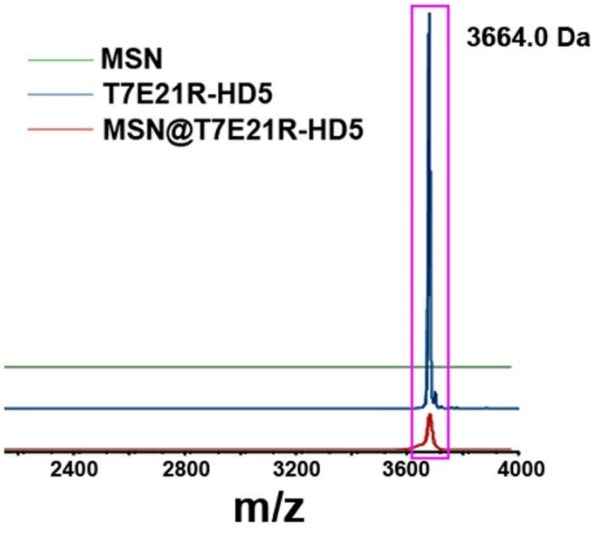


Fig S3. Circular dichroism spectra revealing the secondary structure of T7E21R-HD5 absorbed onto MSNs. T7E21R-HD5 were prepared in sterile water at a concentration of 100 μg mL^{-1} and incubated with 62.5 μg mL^{-1} of MSNs at room temperature overnight. After centrifugation, the precipitate was resuspended with equivoluminal sterile water. The secondary structure was analyzed using an Applied Photophysics Chirascan instrument (Leatherhead, Surrey, UK) at 27°C. A cell with a 1-mm path length was used. The spectral data were obtained from 190 to 260 nm at 1-nm intervals. The time per point was 0.5 s, and the scanning time was approximately 65 s. T7E21R-HD5 maintained the □-sheet structure, manifested by a minima around 200 nm.

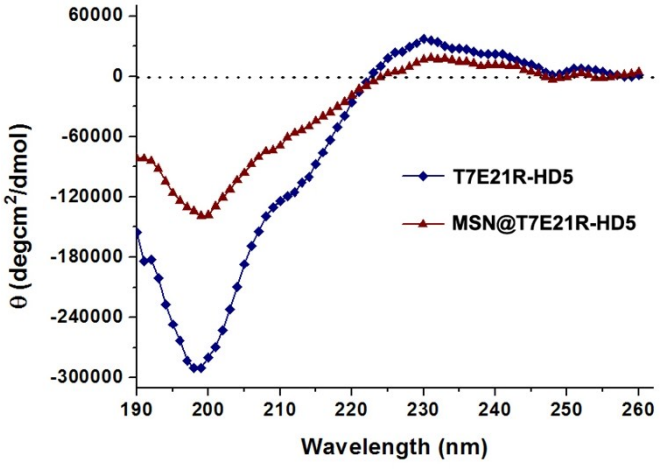


Fig S4. 16S rDNA sequencing confirming microbial species and Kirby-Bauer method determining the antibiotic resistance. (A) Bacterial genomic DNA was extracted and sequenced by Beijing Genomics Institute. The data was analyzed by the Basic Local Alignment Search Tool of NCBI, demonstrating that the isolate is *E. coli*. (B) The isolated *E. coli* cultured overnight was coated on the MHB plate, which was then dried at room temperature. Nine OXOID antibiotic tablets, specifically, IMP, AMP, LEV, CXM, CTX, ATM, TE, CIP, and AX were subsequently attached. Bacteria were incubated at 37 ^{o}C for 16 h. The multi-drug resistance was confirmed by the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards.

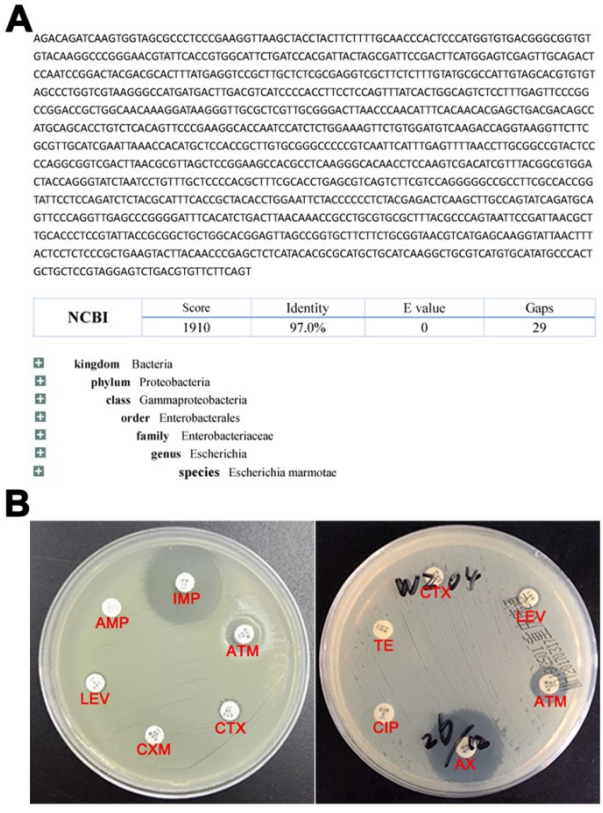


Fig S5. Body weights of MDR *E. coli*-infected mice treated with T7E21R-HD5, MSN@T7E21R-HD5, MSN@T7E21R-HD5@SCN, and CIP. The female 8-week-old BALB/c mice (18-22 g) were infected with 2.5 × 10^9 CFU of MDR *E. coli*. T7E21R-HD5, MSN@T7E21R-HD5, and MSN@T7E21R-HD5@SCN were given after 12 h, and CIP was the negative control. The body weight was recorded once a day and consecutively monitored for five days. The results are shown as the means ± SD.

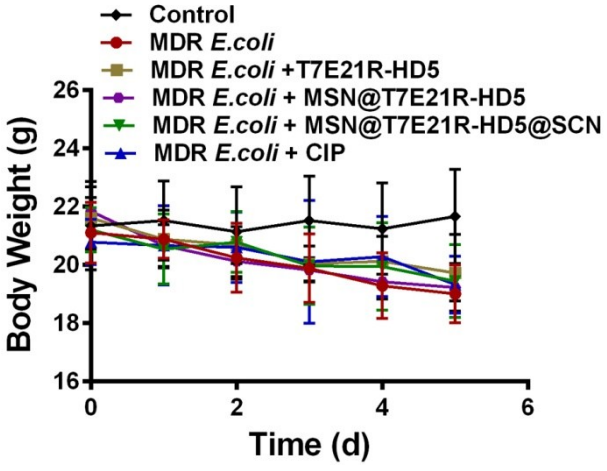
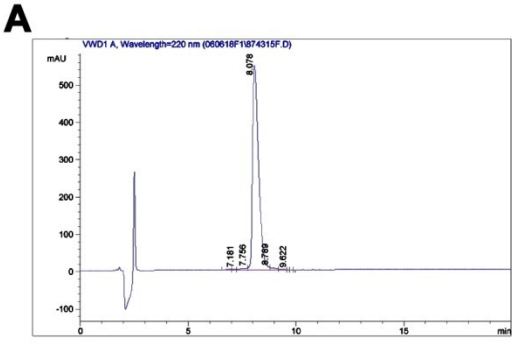


Fig S6. The amino acid sequence, purity, and molecular mass of T7E21R-HD5. Peptide was prepared using the solid phase chemical synthesis. (A) The amino acid sequence is ATCYCRRGRCATRESLSGVCRISGRLYRLCCR (Cys3-Cys31/Cys5-Cys20/Cys10-Cys30). The purity was 97.8%, which was measured by reverse-phase high performance liquid chromatography. The chromatographic data were obtained at 40 °C on a Phenomenex/Luna C18 (2) column (5 µm, 4.6 × 150 mm) applying a linear gradient of 20-40% buffer B (buffer A: 0.1% trifluoroacetic acid in water; buffer B: 0.09% trifluoroacetic acid in (80% acetonitrile plus 20% water)) at a flow rate of 1 mL min^{-1} over 20 min. (B) The molecular mass (3664.0 Da) was determined by electrospray ionization mass spectrometry (Chiron Mimotopes, Victoria, Australia).



Signal 1:VWD1 A, Wavelength=220 nm

Peak #	RT [min]	Type	Height	Width [min]	Area	Area %
1	6.892	BV	0.768	0.182	11.048	0.103
2	7.181	VV	1.070	0.149	11.222	0.104
3	7.756	MF	4.718	0.294	83.132	0.774
4	8.078	MF	546.668	0.320	10510.101	97.845
5	8.789	MF	6.405	0.195	101.293	0.943
6	9.177	FM	1.872	0.179	20.094	0.187
7	9.622	VV	0.338	0.081	2.090	0.019
8	9.761	VV	0.255	0.096	1.781	0.017
9	9.921	VP	0.237	0.053	0.777	0.007