Supporting Information

Novel Multifunctional Silver Nanocomposite Serves as a Resistance-Reversal Agent to Synergistically Combat Carbapenem-Resistant *Acinetobacter baumannii*

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1. Additional Experimental Section

1.1. Screening methods of carbapenem-resistant *Acinetobacter baumannii.*

0.5 McFarland *A. baumannii* suspension was smeared on the Mueller-Hinton (MH) agar plate (Oxoid, Basingstoke, England). Imipenem disk and imipenem disk added with 10 μL of 0.05 mol/L EDTA were on the MH agar plate. Additionally, imipenem disk and EDTA (0.5 mol/L, 10 μL) disk were aligned about 1 cm apart. The samples were incubated at 37℃ for 18 h.

1.2 PCR assay.

According to published papers and Primer3 software (http://bioinfo.ut.ee/primer3/), we confirmed the bla_{NDM}, bla_{VIM}, and bla_{IMP} primers for PCR assays[1-4]. We extracted bacterial DNA using a MolPure Bacterial DNA kit (Yeasen Biotechnology, China). The DNA was stored at -20℃ for the next steps. The PCR master mix was as follows: 12.5 μL of GoTap Green Master Mix (2X), 2 μL of upstream primer (10 μM), 2 μL of downstream primer (10 μM), 5 μL of DNA template and 3.5 μL of nuclease-free water. Using different temperatures, the procedure consisted of 35 cycles. The primers used for the PCR assay are given in **Table S1**.

1.3. SH-PEG2000-NOTA preparation.

First, we dissolved 0.5 g of NH_2-PEG_{2000}-SH in 5 mL of trichloromethane. This mixed solution was combined with triethylamine (1.0 eq.) and DMF solution containing a NOTA-NHS (1.0 eq.) volume of 0.5 mL during the 2 h reaction at room temperature. After reducing pressure and concentrating the reaction fluid, the reaction was stopped by pouring into ice ethyl ether, and the filtration product was collected. We carried out vacuum drying to achieve the final by repeating the above method.

1.4. Observation of carbapenem-resistant *A. baumannii* morphology by SEM.

After incubation of carbapenem-resistant *A. baumannii* and nanocomposites at 37°C for 12 hours, the bacteria were resuspended in PBS three times (10000 rpm, 3 min). Samples were fixed with 2.5% glutaraldehyde and 0.1 mol/L coconut oleic acid ester buffer (pH=7.4) at 4°C for 24 h. Then, the samples were incubated with 0.2 mol/L coconut oleic acid ester buffer (pH=7.4) and washed with PBS three times. The samples were dehydrated in a gradient with different concentrations of ethyl alcohol (30%, 50%, 70%, 90%). After air drying (24 h), a layer of gold platinum (7 nm) was applied to the samples for SEM analysis.

According to the imipenem concentration, the dosage was 60 μg/mL for the respective materials except AgNPs and AgNPs-PEG-NOTA (based on the AgNPs concentration, the dosage was 50 μg/mL).

2. Additional Results

Figure S1. Screening of carbapenem-resistant *A. baumannii*. (A) Disk diffusion assay of imipenem alone. (B) Disk diffusion assay of imipenem and EDTA. (C) Modified imipenem-EDTA disk potentiation test.

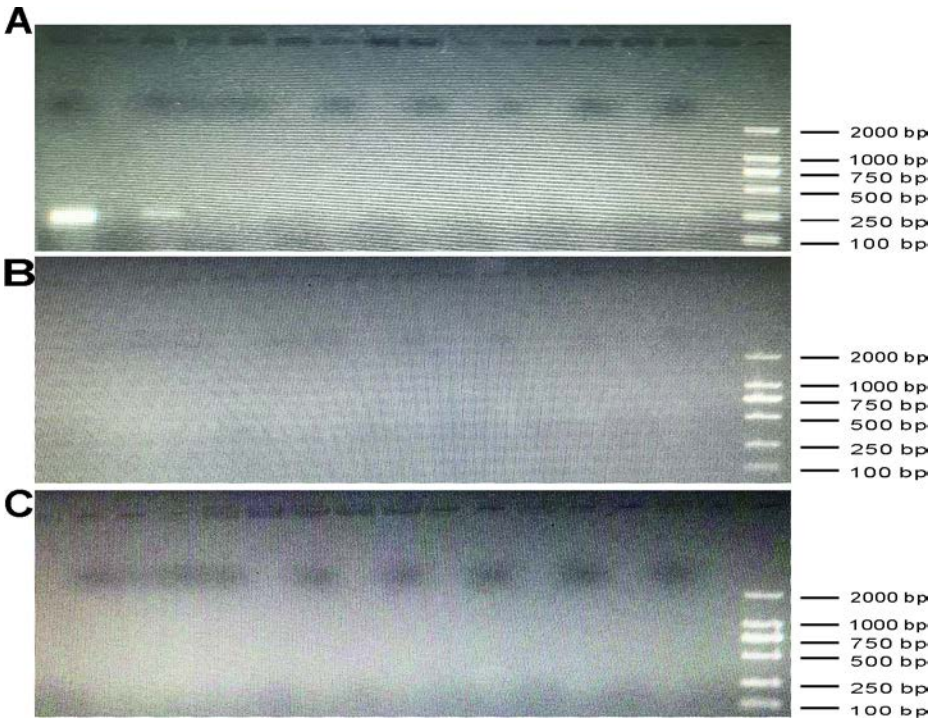


Figure S2. Screening of resistance-associated genes by PCR assay. (A) bla$_{NDM}$ gene. (B) bla$_{IMP}$ gene. (C) bla$_{VIM}$ gene.

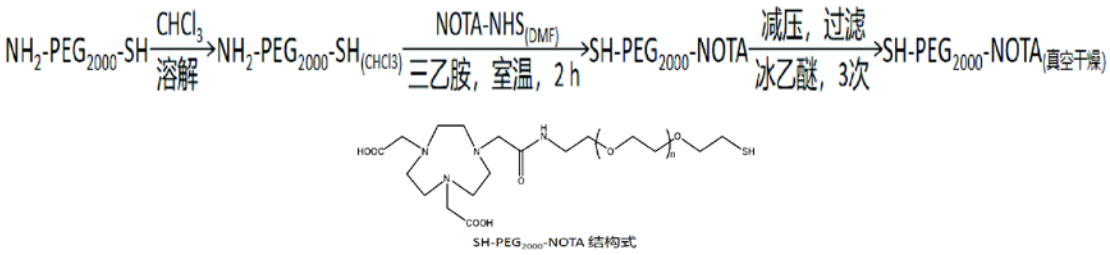
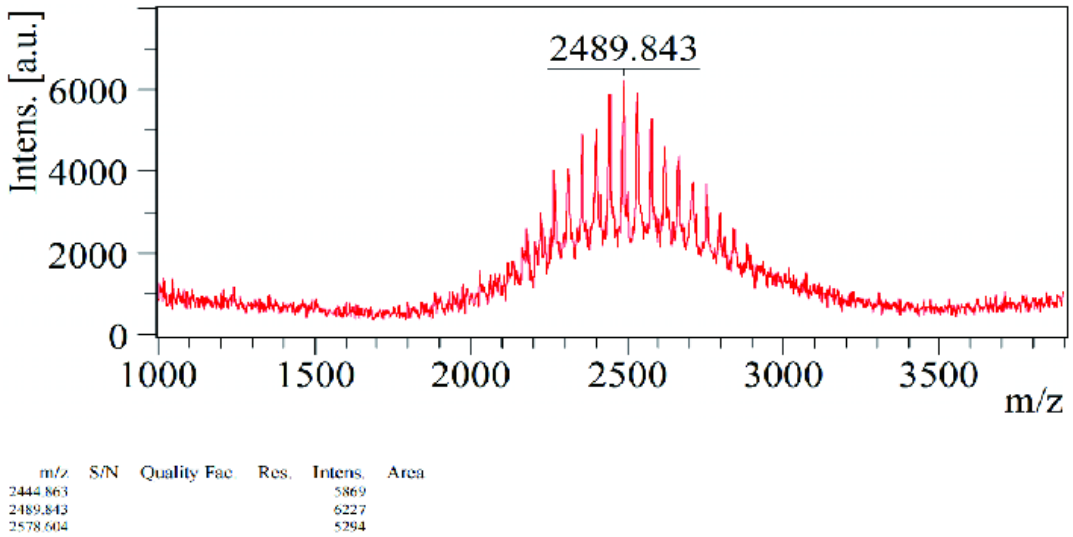


Figure S3. The flow diagram of SH-PEG2000-NOTA synthesis.



m/z	S/N	Quality Fac.	Res.	Intens.	Area
2444.863				5869	
2489.843				6227	
2578.604				5294	

Figure S4. Mass spectrogram of SH-PEG$_{2000}$-NOTA.

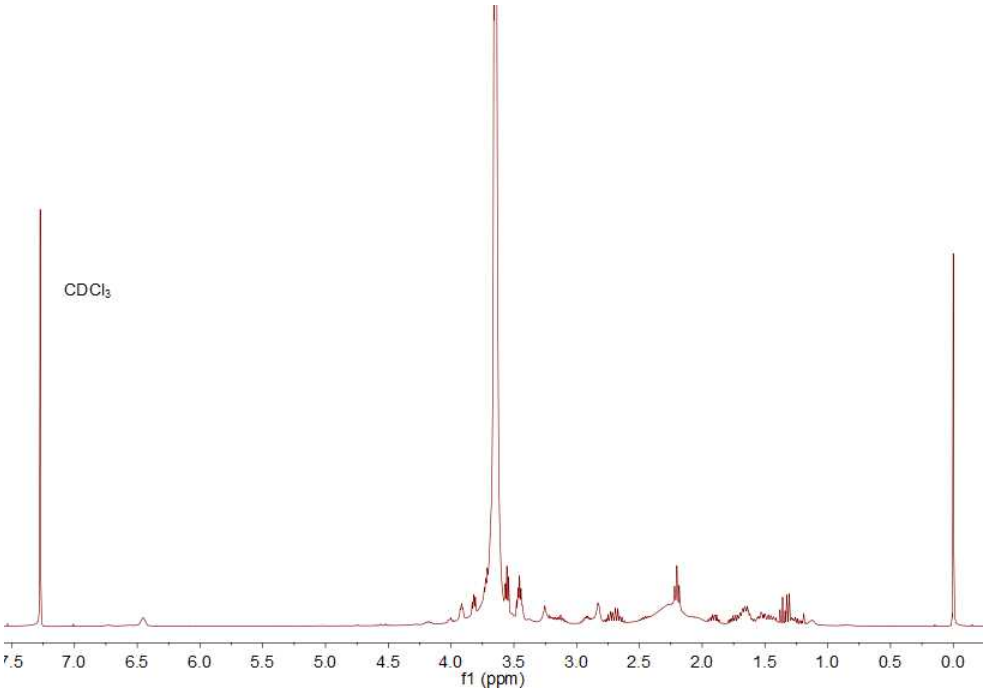


Figure S5. H NMR spectra of SH-PEG$_{2000}$-NOTA.

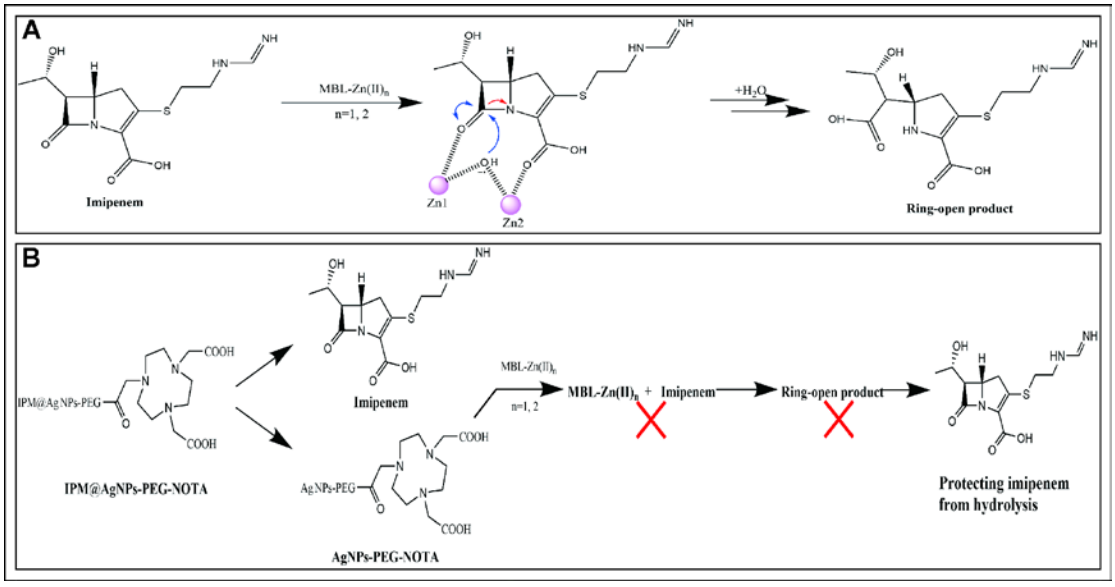


Figure S6. The flow diagram. A. Hydrolysis of imipenem by MBLs. B. The IPM@AgNPs-PEG-NOTA nanocomposite serving as resistance reversal agent.

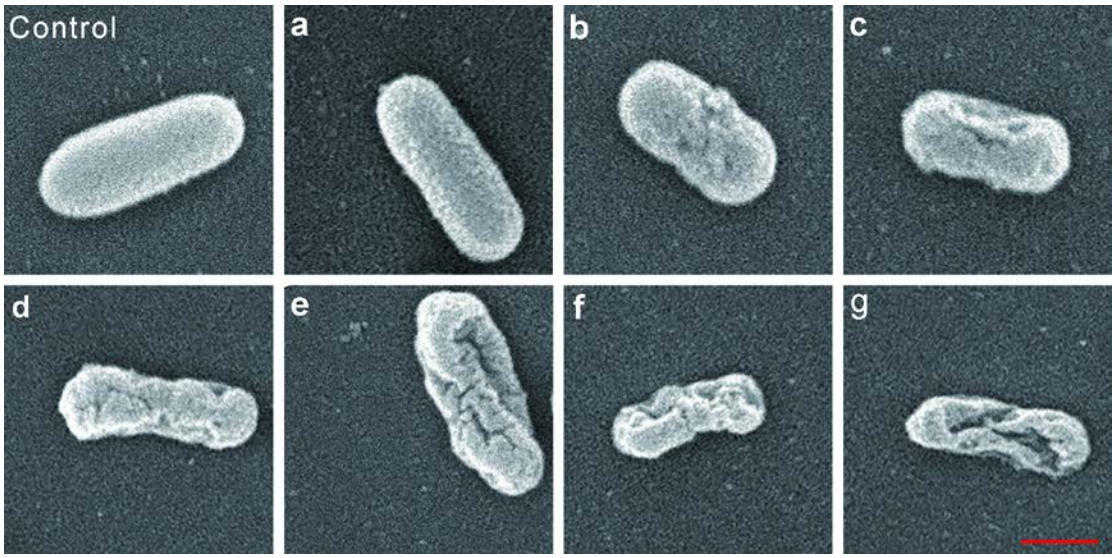


Figure S7. SEM micrographs of carbapenem-resistant *Acinetobacter baumannii* exposed to the respective mateials for 12 h. Control, the normal untreated carbapenem-resistant *Acinetobacter baumannii*; a, PBS; b, imipenem; c,AgNPs; d, AgNPs-PEG-NOTA; e, imipenem+SH-PEG-NOTA; f, AgNPs-IPM; g, IPM@AgNPs-PEG-NOTA. Scale bar: 200 nm.

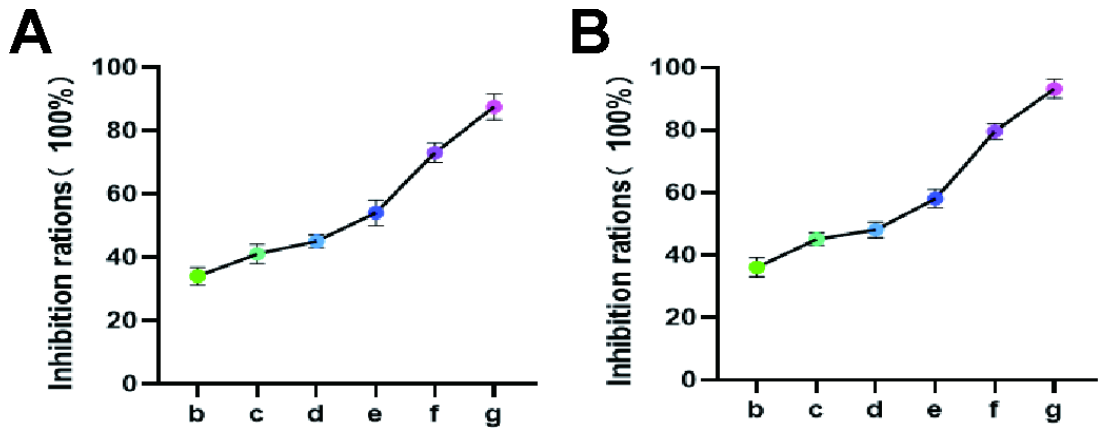


Figure S8. Relative percentage of biofilm inhibition by crystal violet assay for imipenem (b), AgNPs (c), AgNPs-PEG-NOTA (d), imipenem+SH-PEG-NOTA (e), AgNPs-IPM (f), and IPM@AgNPs-PEG-NOTA (g). (A) Treated with 48 h. (B) Treated with 72 h. Data are indicated as mean ± SD (n = 4).

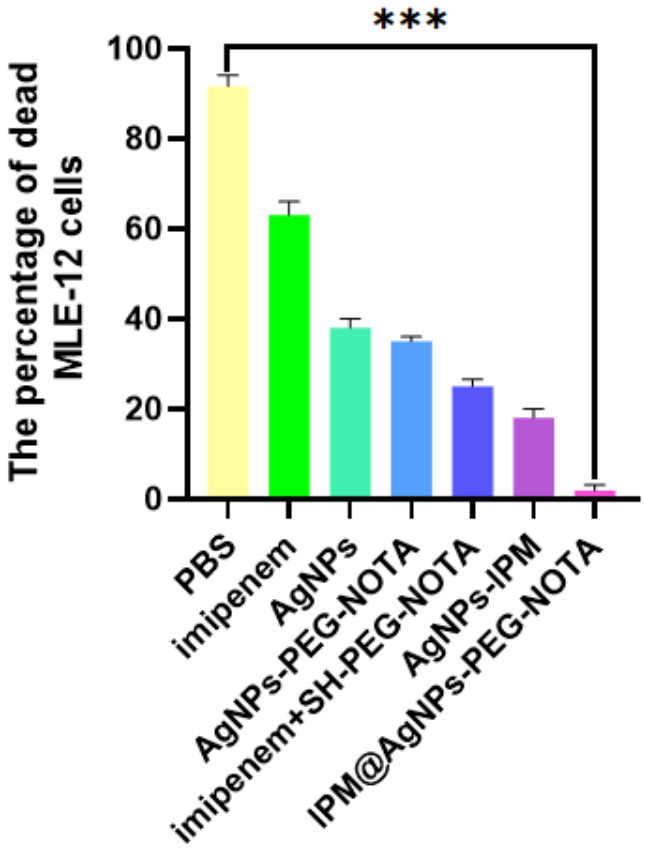


Figure S9. The percentage of dead MLE-12 cells. Data are presented as means ± SD (n = 3). Intergroup comparisons: *** $p < 0.001$.

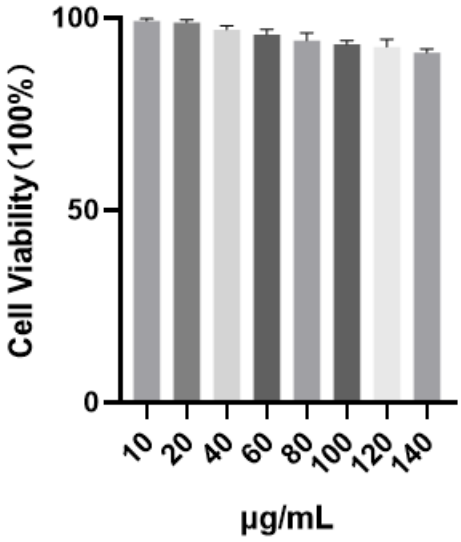


Figure S10. Viability of MLE-12 cells upon the imipenem treatment for 24 h by MTT analysis. Data are presented as means ± SD (n = 3).

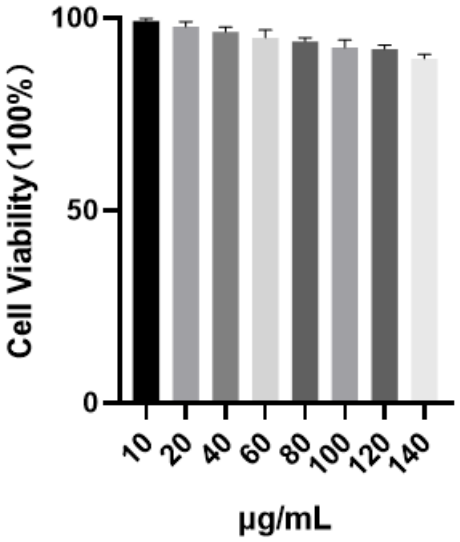


Figure S11. Viability of MLE-12 cells upon the SH-PEG-NOTA treatment for 24 h by MTT analysis. Data are presented as means ± SD (n = 3).

Table S1. Primers for amplification of genes from carbapenem-resistant *Acinetobacter baumannii*.

Target gene	Primer Name	Primer Sequence		Product Length(bp)	Annealing Temp(℃)	Reference
		Forward(5'→3')	Reverse(5'→3')			
bla_{NDM}	NDM	ACTGGATCAAGCAGGAGATCAA	CGGTGATATTGTCACTGGTGTG	279	52	1,2
bla_{IMP}	IMP	GTTTATGTTCATACWTCG	GGTTTAAYAAAACAACCAC	432	45	3,4
bla_{VIM}	VIM	TTTGGTCGCATATCGCAACG	CCATTCAGCCAGATCGGCAT	500	66	3,4

3. References

(1) Bahador, A.; Raoo An, R.; Farshadzadeh, Z.; Beitollahi, L.; Khaledi, A.; Rahimi, S.; Mokhtaran, M.; Mehrabi Tavana, A.; Esmaeili, D. The Prevalence of IS Aba 1 and IS Aba 4 in Acinetobacter baumannii Species of Different International Clone Lineages Among Patients With Burning in Tehran, Iran. *Jundishapur J Microbiol* **2015,** *8* (7), e17167, DOI: 10.5812/jjm.17167v2.

(2) Zendegani, E.; Dolatabadi, S. The Efficacy of Imipenem Conjugated with Synthesized Silver Nanoparticles Against Acinetobacter baumannii Clinical Isolates, Iran. *Biol Trace Elem Res* **2020,** *197* (1), 330-340, DOI: 10.1007/s12011-019-01962-6.

(3) Sarhaddi, N.; Soleimanpour, S.; Farsiani, H.; Mosavat, A.; Dolatabadi, S.; Salimizand, H.; Amel Jamehdar, S. Elevated prevalence of multidrug-resistant Acinetobacter baumannii with extensive genetic diversity in the largest burn centre of northeast Iran. *J Glob Antimicrob Resist* **2017,** *8*, 60-66, DOI: 10.1016/j.jgar.2016.10.009.

(4) Hujer, K. M.; Hujer, A. M.; Hulten, E. A.; Bajaksouzian, S.; Adams, J. M.; Donskey, C. J.; Ecker, D. J.; Massire, C.; Eshoo, M. W.; Sampath, R.; Thomson, J. M.; Rather, P. N.; Craft, D. W.; Fishbain, J. T.; Ewell, A. J.; Jacobs, M. R.; Paterson, D. L.; Bonomo, R. A. Analysis of antibiotic resistance genes in multidrug-resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. *Antimicrob Agents Chemother* **2006,** *50* (12), 4114-23, DOI: 10.1128/AAC.00778-06.