Formulation and Characterization of a Sortase A Inhibitor-Loaded PMMA Bone Cement

Yin-Yu Qi [1, 2†], Lu-Yang Han [1, 2†], Long-Xu Han [1†], Wen-Han Bu[1, 2], Yang Xu [1, 2*] and Jian-Jun Chu [1, 2*]

[†] Yin-Yu Qi, Lu-Yang Han, Long-Xu Han contributed equally to this work

[*]Correspondence: Jian-Jun Chu: chujianj@mail.ustc.edu.cn; Yang Xu: xuyang@hfut.edu.cn

[1] Bengbu Medical University, The Second People's Hospital of Hefei (Affiliated Hospital), Anhui 230011, China.

[2] Department of Pharmaceutical Science and Engineering, Hefei University of Technology, Hefei, Anhui 230009, China.

Table S1: Resistance spectrum of clinically isolated MRSA. The strain is identified as MRSA and shows resistance to multiple antibiotics, including gentamicin (GS). This table is provided by the Department of Laboratory of the Second People's Hospital of Hefei. MIC is the minimum inhibitory concentration that can inhibit the growth of the bacterium determined by the drug susceptibility test. "+" means that the bacteria are resistant to the drug, "-" means that the bacteria are sensitive to the drug.

Bacteria	Methicillin-resistant *Staphylococcus aureus* (MRSA)		
Drug	Measured value	Methodology	Result
Penicillin	⩾0.5	MIC	+
Oxazole penicillin	⩾4	MIC	+
Erythromycin	⩾8	MIC	+
Clindamycin	⩾8	MIC	+
Gentamicin	⩾16	MIC	+
Levofloxacin	1	MIC	-
Moxifloxacin	⩽0.25	MIC	-
Tetracycline	⩽1	MIC	-
Tigecycline	⩽0.12	MIC	-
Linezolid	2	MIC	-
Vancomycin	1	MIC	-
Cotrimoxazole	⩾320	MIC	+
Rifampicin	⩽0.5	MIC	-
Nitrofurantoin	32	MIC	-

Table S2: Change in body weight of mice within 3 days after intraperitoneal injection (n=3) (x±s, g). (*：$p < 0.05$，compared to the same period of antibacterial cement； **：$p < 0.05$；^：$p<0.05$，compared with the other two time points of the same group of bone cement)

	24h	48h	72h
control	0.373±0.039	0.323±0.063*	0.373±0.062
PMMA	0.36±0.033	0.353±0.019*	0.34±0.057
5%AAEK1	0.427±0.082^	0.103±0.179	0.23±0.083
5%GS**	0.387±0.205	-0.123±0.058	0.21±0.088

Table S3: Bone cement formulations and groupings. (The total amount of bone cement is 1g.) This formulation is used to make bone cement used in supplementary experiments (inhibition zone experiment, CCK-8 experiment, hemolysis experiment).

Formulation	Powder(mg)				Liquid(μL)	
	PMMA	BPO	$BaSO_4$	Drug	MMA	DMPT
1.25% AAEK1 cement	517.02	13	100	7.98	379	7.50
2.5%AAEK1 cement	509.05	13	100	15.95	379	7.50

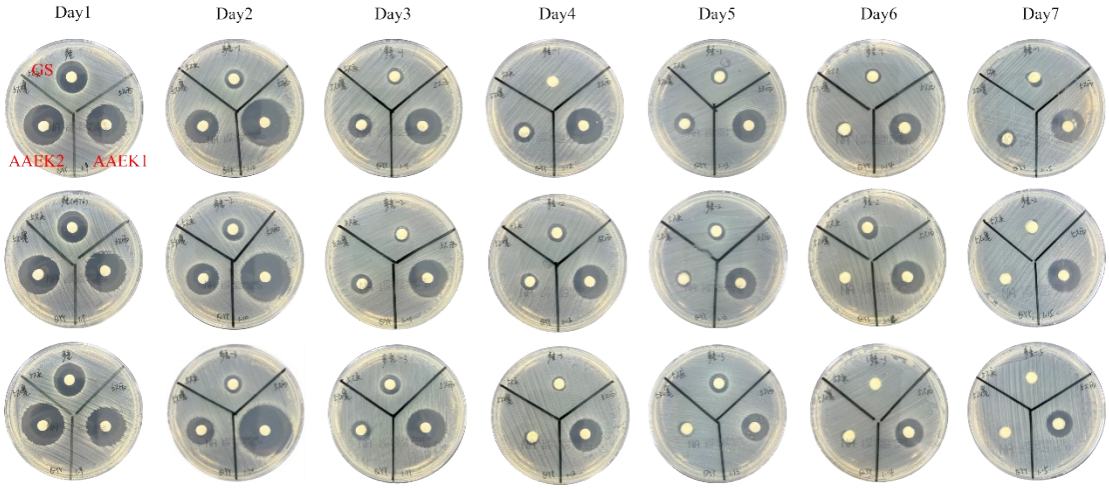

Figure S1: The inhibition zone of 5wt%AAEK1, 5wt%AAEK2 and 5wt%GS bone cement against clinical MRSA for 7 days.

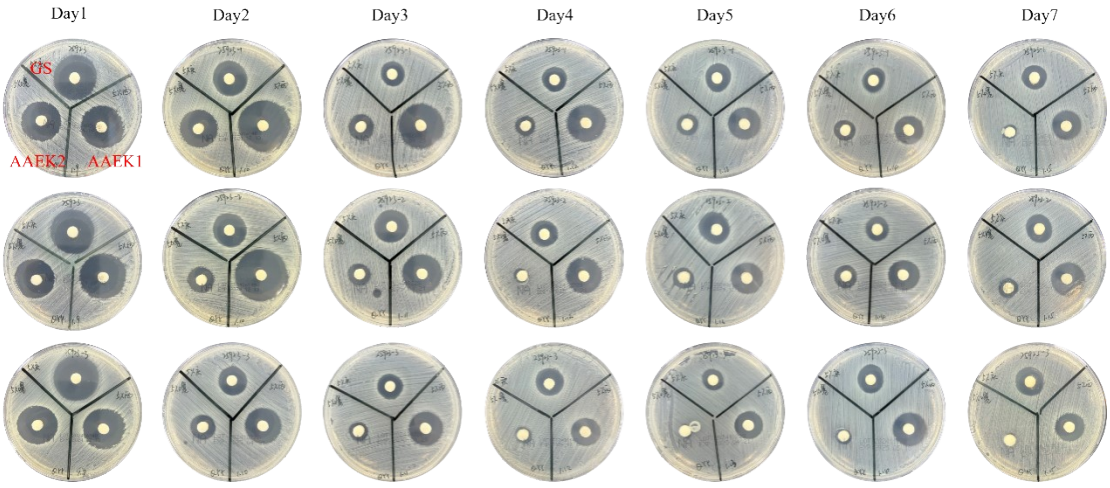

Figure S2: The inhibition zone of 5wt% AAEK1, 5wt%AAEK2 and 5wt%GS bone cement against *S. aureus* for 7 days.

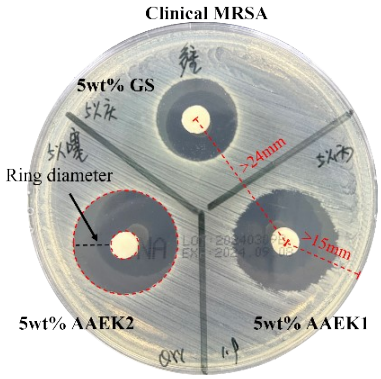

Figure S3: Operation of the inhibition zone of bone cement.

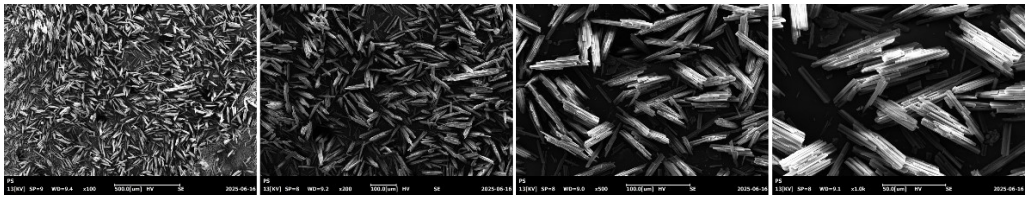

Figure S4: SEM of AAEK1.

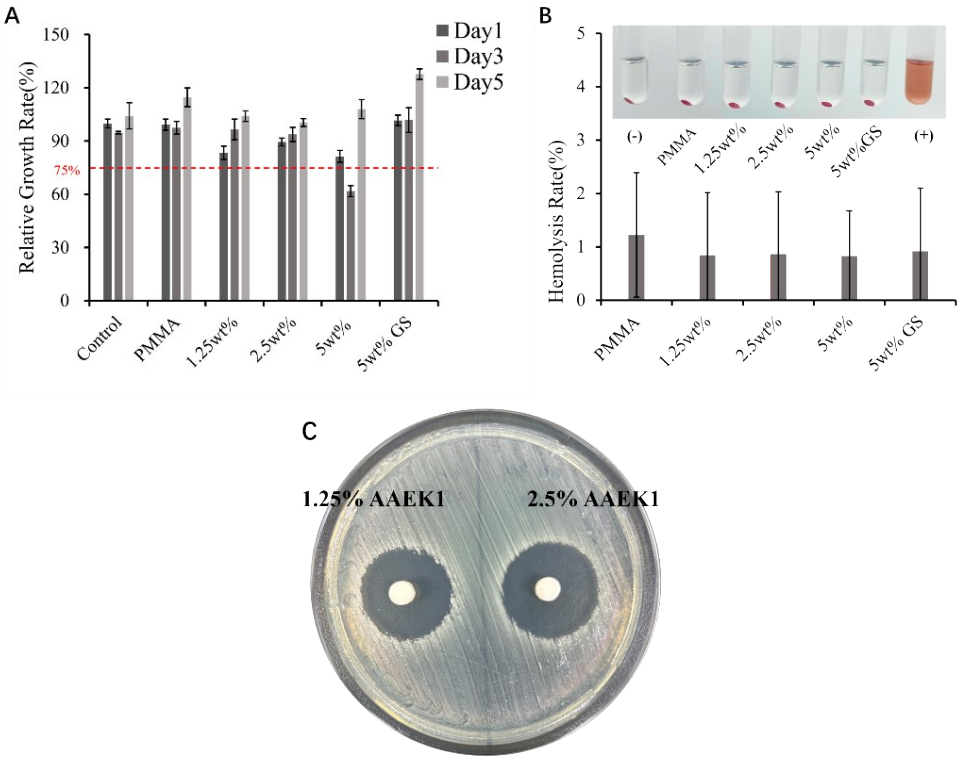

Figure S5: A: CCK-8 with two low-dose AAEK1 bone cements; B: Hemolytic activity with two low-dose AAEK1 bone cements; C: The inhibition zone of two low-dose AAEK1 bone cement.

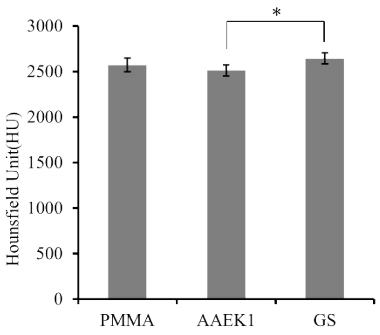

Figure S6: Hounsfield Units of each group of bone cement. (*: $p < 0.05$)

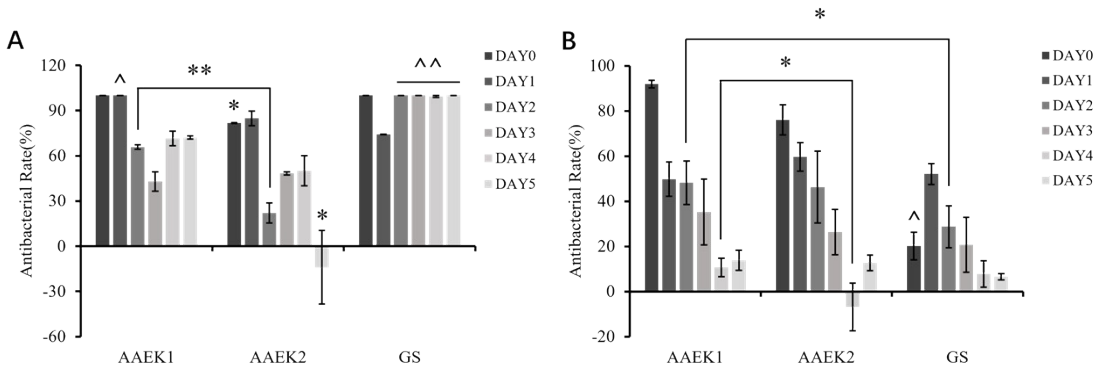

Figure S7: Surface antibacterial experiment of AAEK1, AAEK2 and gentamicin bone cement. A: Anti-*S. aureus* effect of three types of bone cement(*: $p < 0.05$ compared to the other two groups of bone cement at the same time point; **：$p<0.01$; ^: $p < 0.05$, compared to the other two groups of cement; ^^: $p < 0.01$ compared to the other two groups of bone cement); B: Anti-clinical MRSA effect of three types of bone cement(*: $p < 0.05$; ^: $p < 0.01$, there was a significant difference from the other two groups of bone cement). This experiment is based on the fact that Srt A inhibitors achieve antibacterial effect by inhibiting the anchoring and adhesion of bacteria on the surface of bone cement.