

Injectable isoniazid-loaded bone cement based on hydrazone bond achieving long-term release and decent mechanical property

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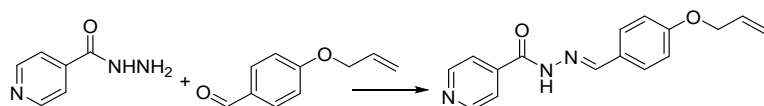
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1. Synthesis of hydrazone based isoniazid allyl monomer



Isoniazid (1.37 g, 10.0 mmol) was mixed with ethanol (20 mL) and the mixture brought to the boil and give a homogeneous solution at reflux. 4-Allyloxybenzaldehyde (1.62 g, 10.0 mmol) was added dropwise over 10 minutes. The reaction mixture was refluxed overnight, then allowed to cool slowly, producing HIA monomer as crystalline solid, which was filtered off and dried. ^1H NMR (400 MHz, CDCl_3) δ 11.34 – 10.73 (m, 1H), 8.71 (dd, $J = 53.6, 4.6$ Hz, 2H), 8.47 – 8.37 (m, 1H), 7.78 (dd, $J = 3.8, 2.2$ Hz, 2H), 7.61 – 7.44 (m, 2H), 6.94 – 6.72 (m, 2H), 6.00 (ddd, $J = 22.2, 10.4, 5.2$ Hz, 1H), 5.35 (dt, $J = 21.8, 12.8$ Hz, 2H), 4.52 (dd, $J = 30.9, 5.0$ Hz, 2H). Yield: 1.65 g (58.7%).

2. SEM images of the surface of PMMA bone cement , HIA bone cement, and INH cement

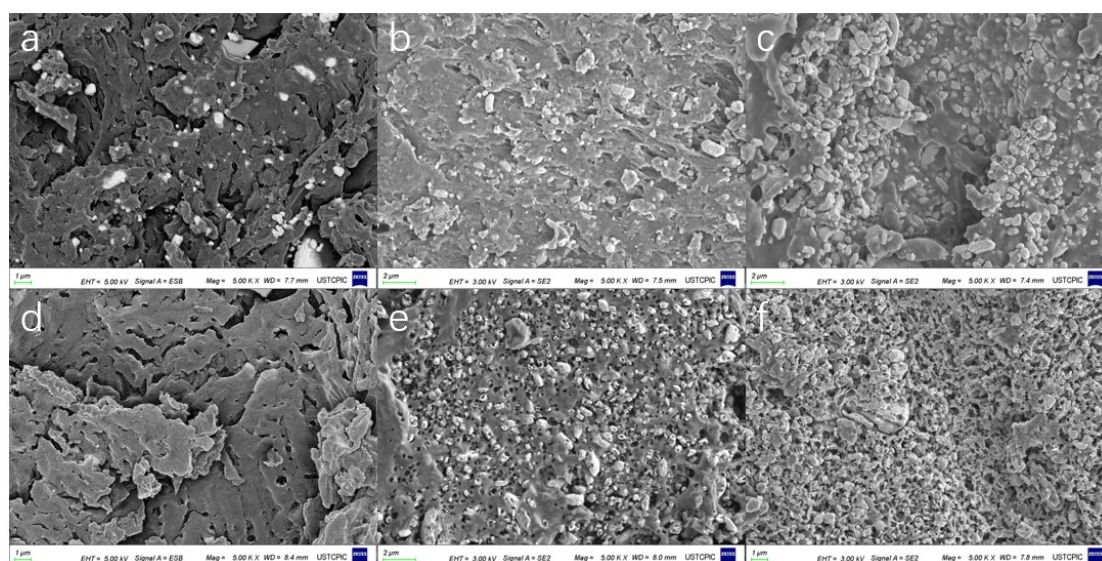


Figure S1 SEM images of the surfaces of PMMA cement, HIA cement, and INH cement. Unsoaked PMMA cement (a), HIA cement (b), and INH cement (c); PMMA cement (d), HIA cement (e), and INH cement (f) after soaking in ddH₂O for 7 days.

3. SEM images of the fracture surfaces of PMMA bone cement , HIA bone cement, and INH cement

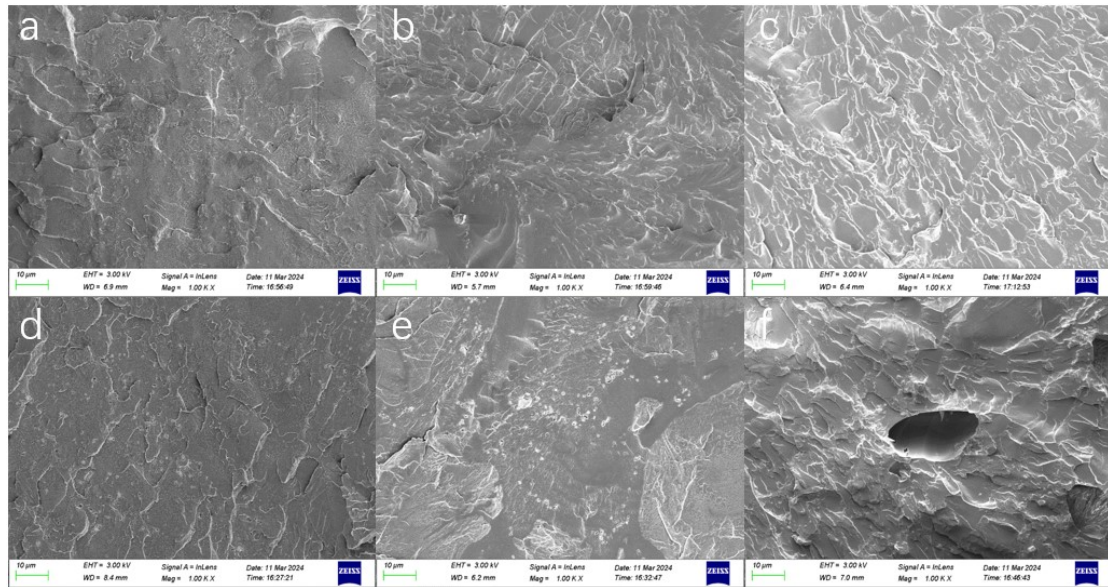


Figure S2 SEM images of the fracture surfaces of PMMA cement, HIA cement, and INH cement. Unsoaked PMMA cement (a), HIA cement (b), and INH cement (c); PMMA cement (d), HIA cement (e), and INH cement (f) after soaking in ddH₂O for 7 days

4. SEM images of the fracture surfaces of INH cement

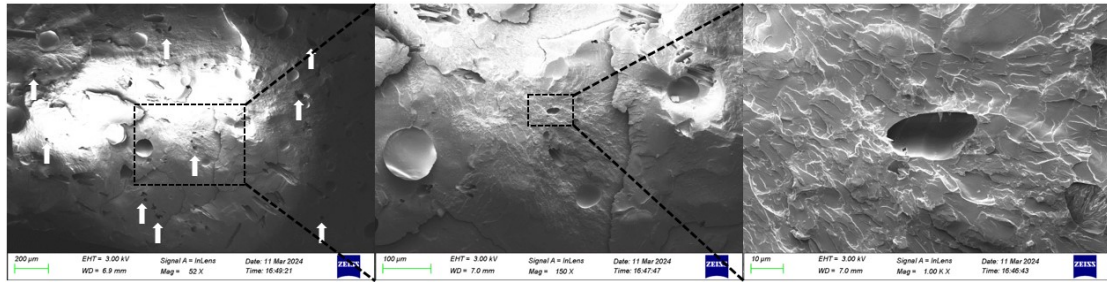


Figure S3 SEM images of the fracture surfaces of INH cement.

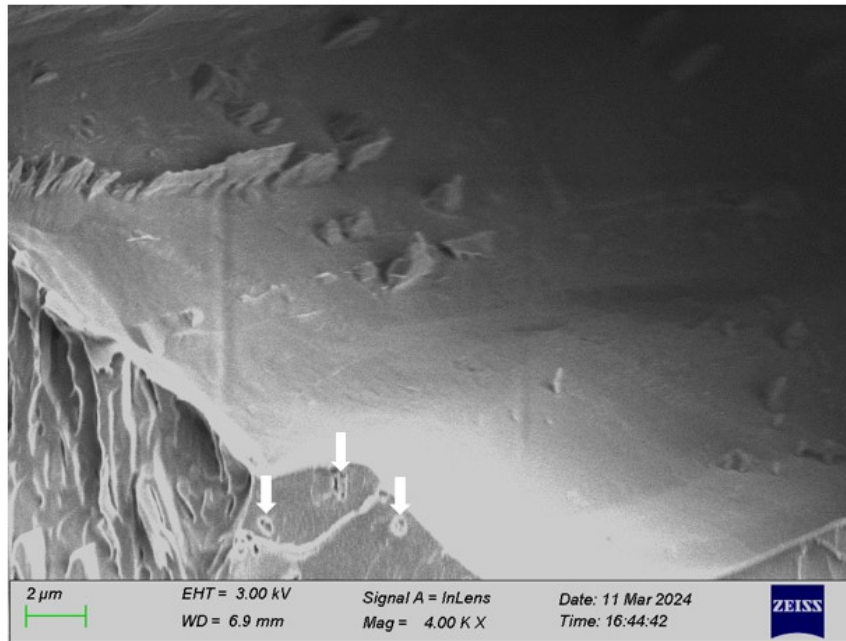


Figure S4 SEM images of the fracture surfaces of INH cement. The white arrow indicates the pores containing incompletely dissolved BaSO₄ cores.

5. SEM images of pores formed by air bubbles on the fracture surfaces of PMMA cement.

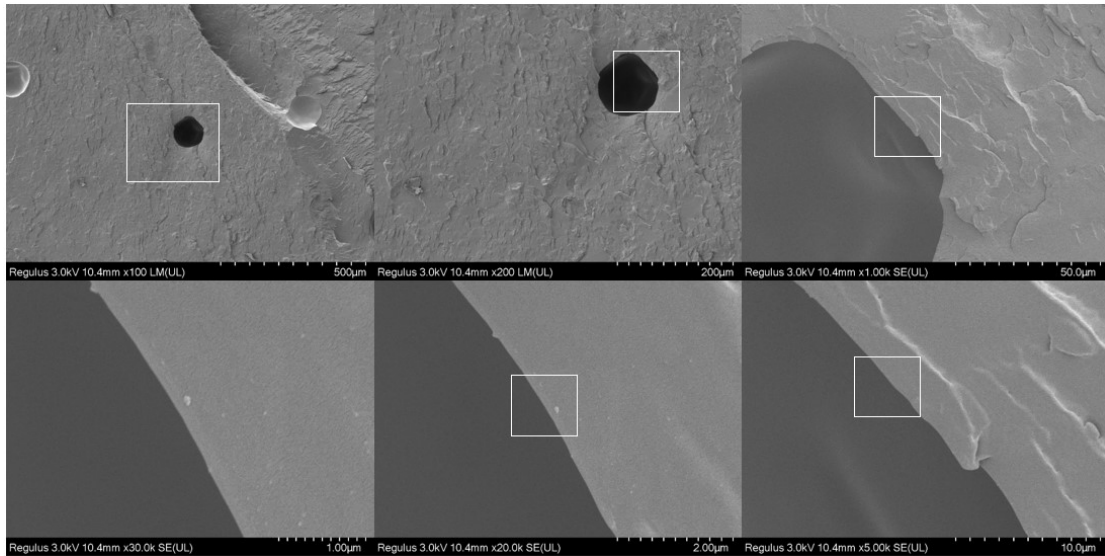


Figure S5 SEM images of pores formed by air bubbles on the fracture surfaces of PMMA cement without BaSO₄. Continue to zoom in on the area enclosed by the white box for observation.

6. SEM images of pores formed by the washout of drugs on the fracture surfaces of INH cement.

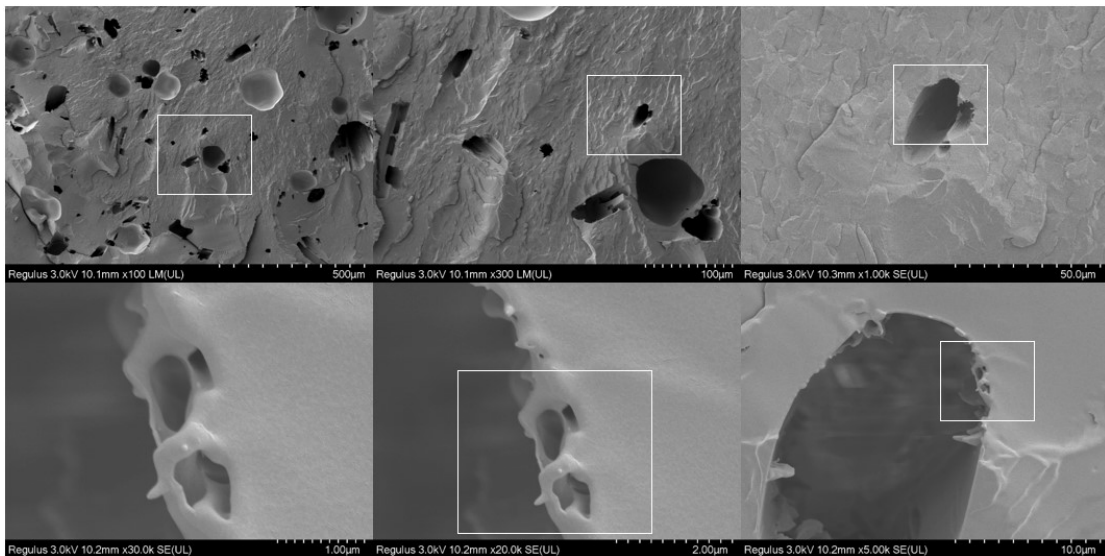
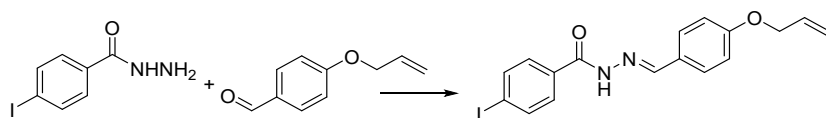


Figure S6 SEM images of pores formed by the washout of drugs on the fracture surfaces of INH cement without BaSO₄. Continue to zoom in on the area enclosed by the white box for observation.

7. Synthesis of hydrazone based isoniazid allyl monomer analog containing iodine and bone cement preparation



4-iodobenzhydrazide (2.62 g, 10.0 mmol) was mixed with ethanol (20 mL) and the mixture brought to the boil and give a homogeneous solution at reflux. 4-Allyloxybenzaldehyde (1.62 g, 10.0 mmol) was added dropwise over 10 minutes. The reaction mixture was refluxed overnight, then allowed to cool slowly, producing iodine-containing analogs of HIA monomer, which was filtered off and dried. ¹H NMR (600 MHz, dmso) δ 11.76 (s, 1H), 8.36 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.66 (dd, *J* = 18.1, 7.9 Hz, 4H), 7.01 (d, *J* = 7.9 Hz, 2H), 6.03 (ddd, *J* = 16.3, 10.1, 4.9 Hz, 1H), 5.38 (t, *J* = 15.5 Hz, 1H), 5.25 (d, *J* = 10.5 Hz, 1H), 4.59 (t, *J* = 10.4 Hz, 2H). Yield: 2.61 g (64.3%).

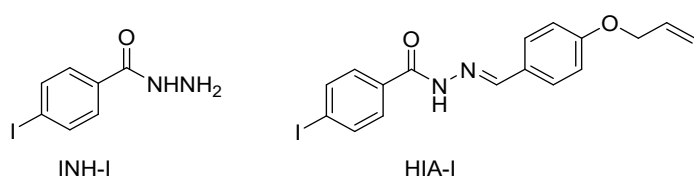


Figure S7 Structure of INH analog containing iodine (INH-I) and HIA analog containing iodine (HIA-I).

We prepared the bone cement according to the formulations outlined in Table 1. Since HIA-I has poor solubility, we reduced the amount added to 5 mg, and accordingly, the amount of INH-I added to the bone cement was adjusted to 16 mg.

8. HAADF-STEM and elemental mapping images of PMMA cement, INH-I cement and HIA-I cement

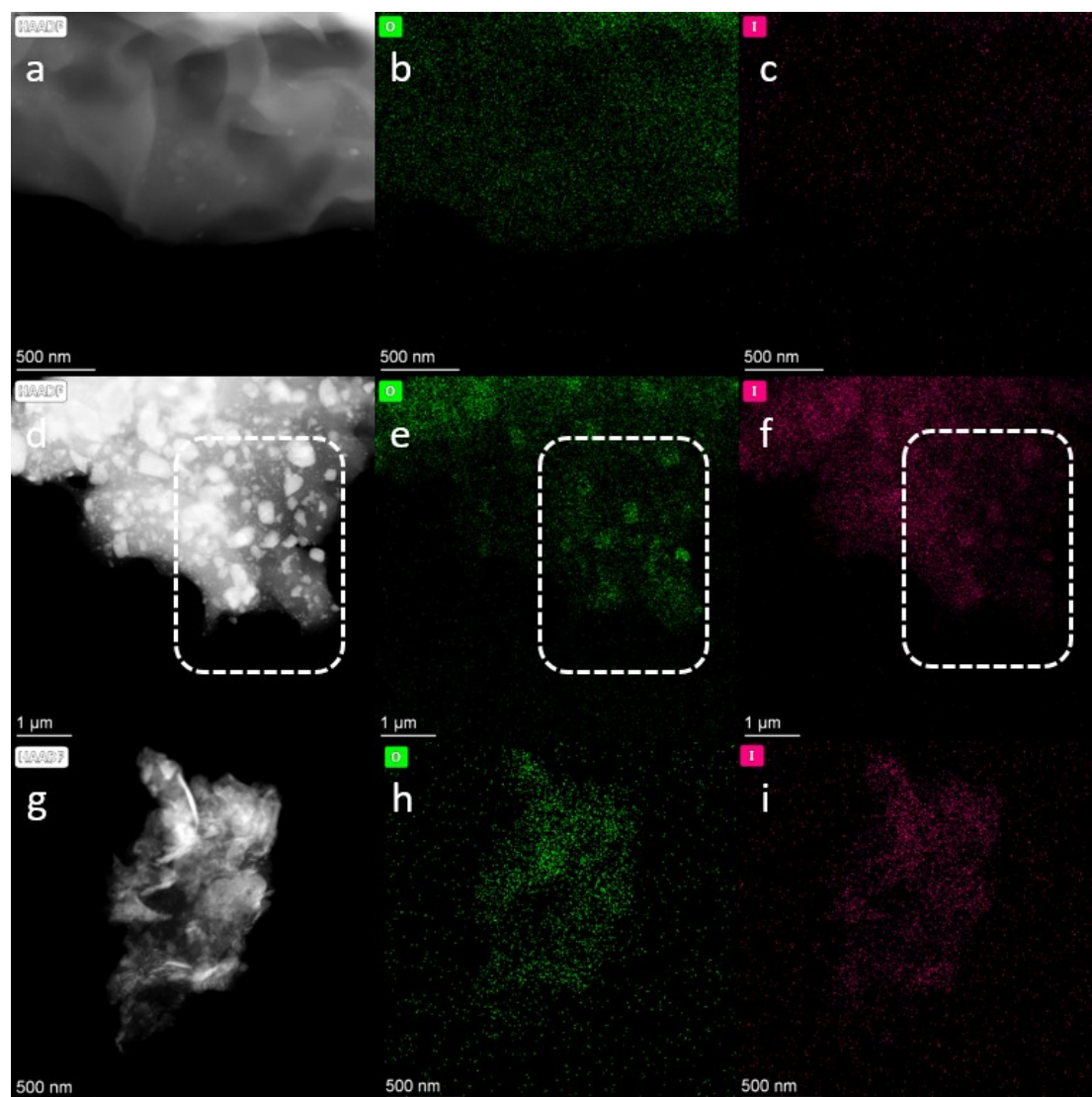


Figure S8 Isoniazid analog containing iodine(INH-I) and HIA analog containing iodine(HIA-I). HAADF-STEM image of PMMA cement (a); EDS mapping images of oxygen and iodine in PMMA cement (b, c); HAADF-STEM image of INH-I cement (d); EDS mapping images of oxygen and iodine in INH-I cement (e, f); HAADF-STEM image of HIA-I cement (g); EDS mapping images of oxygen and iodine in HIA-I cement (h, i).

9. Water contact angle of PMMA cement and HIA cement

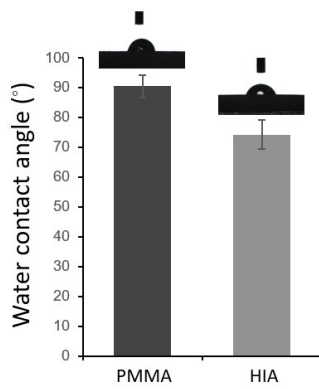


Figure S9 Water contact angle of PMMA cement and HIA cement.

10. Elastic modulus of PMMA cement, HIA cement and INH cement

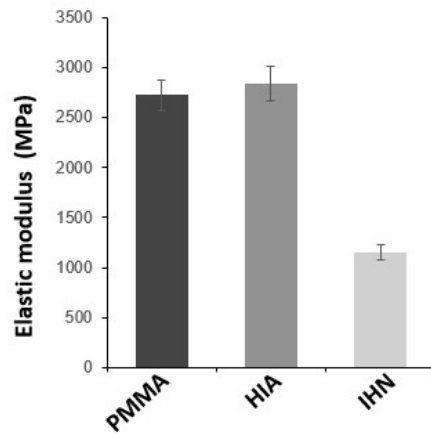


Figure S10 The elastic modulus of PMMA cement, HIA cement and INH cement.

11. Compressive strength and elastic modulus of HIA cement and INH cement after soaking

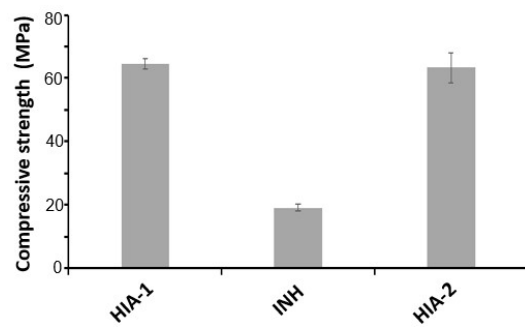


Figure S11 The compressive strength of HIA cement and INH cement. HIA-1 and INH are tested after soaking in ddH₂O for 7 days; HIA-2 is tested after soaking in acidic buffer (pH 6.0) for 7 days.

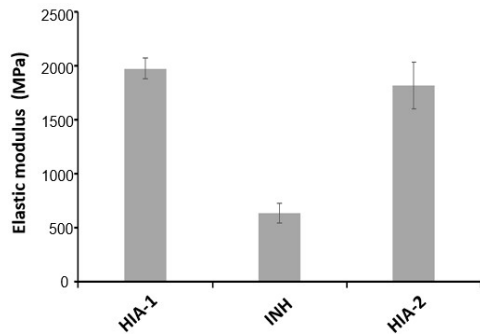


Figure S12 The elastic modulus of HIA cement and INH cement. HIA-1 and INH are tested after soaking in ddH₂O for 7 days; HIA-2 is tested after soaking in a pH 6 buffer for 7 days.

12. Drug release of INH cement at different pH

We used cylindrical samples with a diameter of 6mm and a height of 12mm, and immersed them in 3mL buffer. After soaking for 12 hours, samples were taken, representing the release from 0 to 12 hours. These samples were then tested for drug release amount using HPLC. Subsequently, the buffer was replaced with fresh buffer, and the samples were continued to soak for another 24 hours, representing the release from 12 to 36 hours. The total drug release amount from 0 to 36 hours is the sum of the two sampling measurements.

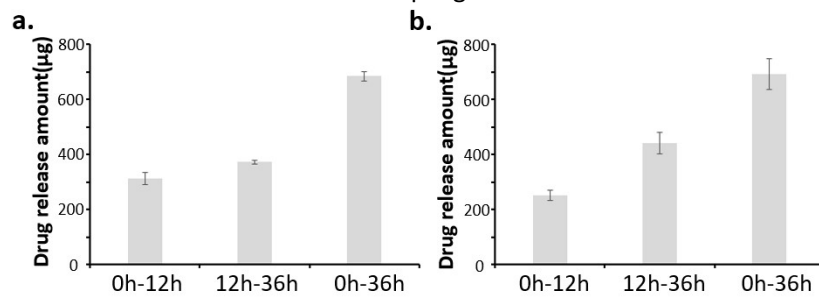


Figure S13 Drug release of INH cement at pH 5.0 (a) and pH 7.4 (b).

13. Hemolytic activity of PMMA cement, INH cement and HIA cement

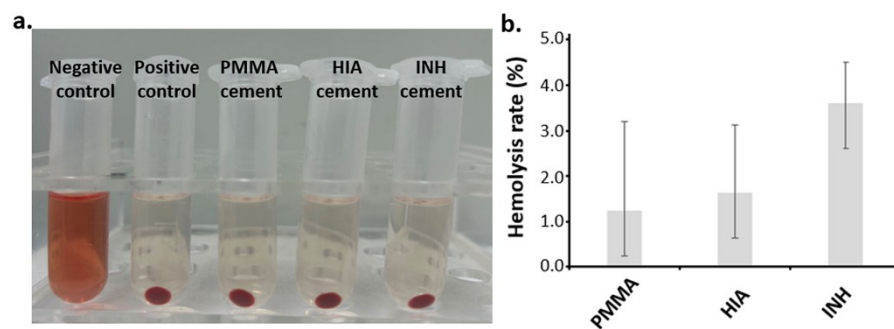


Figure S14 Hemolytic activity of PMMA cement, INH cement and HIA cement. Photograph of hemolysis test (a); hemolysis rate (b).

14. Biocompatibility study in vivo

Table S1 Body weight changes of mice in each group ($\bar{x}\pm s$)

	24h	48h	72h	P(ANOVA)
Control	0.162 ± 0.742	0.162 ± 1.033	0.254 ± 1.009	P=0.984
PMMA	-0.074 ± 0.408	0.17 ± 0.465	0.518 ± 0.373	P=0.120
P(T Test)	P=0.550	P=0.988	P=0.598	
HIA	-0.298 ± 0.302	-0.896 ± 0.453 ^b	-0.952 ± 0.295 ^{a,c}	P=0.024
P(T Test)	P=0.235	P=0.069	P=0.033	
INH	-0.396 ± 0.485	-1.284 ± 0.731 ^a	-2.132 ± 0.964 ^{a,c}	P=0.011
P(T Test)	P=0.197	P=0.034	P=0.005	

^a There is a significant difference between this group and the control group on the same day ($p<0.05$)

^b The difference between this group and the previous day is significant ($p<0.05$)

^c There was a significant difference between the HIA group and the INH group on the same day ($p<0.05$)

15. Imaging study of injectable HIA bone cement in porcine vertebral

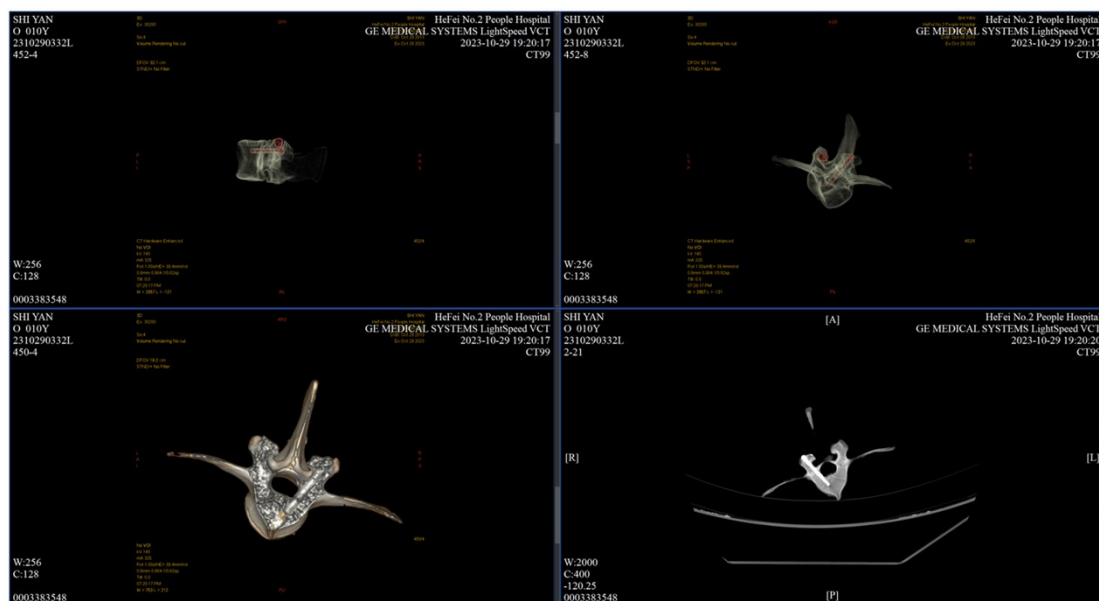


Figure S15 The porcine vertebral was reconstructed by CT after injection of HIA cement.