Supporting informations for

An injectable thermosensitive hydrogel with a self-assembled peptide coupled with an antimicrobial peptide for enhanced wound healing

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1. Materials

Resin was purchased from Tianjin Nankai Synthetic Technology Co., Ltd. 20 kinds of amino acids such as Fmoc Arg (PBF) - Oh (purity at > 99%) and Fmoc ASP (otbu) - Oh (purity at > 99%) were purchased from Chengdu Chengnuo New Technology Co., Ltd.Palmitic acid, Fmoc ACP Oh were purchased from Budweiser Technology Co., Ltd. DMF and DCM were purchased from Korea. MeOH was purchased from Japan. Diea (99%) was purchased from Xinde Chemical Industry Group Co., Ltd. HBTU (99%) was purchased from Haofan Biotechnology Co., Ltd. Piperidine (99%) was purchased from Sinopharm Shanghai Chemical Reagent Company. Phenol reagent (99%), pyridine reagent (99%) and ninhydrin reagent (99%) were purchased from Beijing Chemical Industry Group Co., Ltd. TFA (99%) was purchased from J.T.Baker. Tis (98%) and EDT (98%) were purchased from Shanghai DARUI Fine Chemical Industry Co., Ltd. Anhydrous ether (measured 99.7%) was purchased from Shanghai experiment.

PNIPAM (M_w = 20000−40000 Da) was purchased from Sigma-Aldrich. MGF E peptide (YQPPSTNKKTKSQ(d)R(d)R(d)RKGG, purity at > 96%) was purchased from top-peptide Co., Ltd (Shanghai, China). Escherichia coli (*E. coli*, ATCC25922) and Staphylococcus aureus (*S. aureus*, ATCC6538) were donated by China Agricultural University. Commercially available dressing (Liquid wound dressing, F-SK-I-8, Sinopharm, mainly made by Isopropyl acid, isopropyl palmitate, castor oil and ethyl acetate). The other chemicals were all purchased from Sigma-Aldrich and used as received. Water (18 MΩ cm) was purified from a Milli-Q system and used in all of the experiments.

2. RA-Amps synthesis (From C to N)：

RA-Amps peptide (Ac-RADARADARADARADA-Acp-RRWRVIVKW was prepared by 12-channel semi-automatic peptide synthesizer (China-Peptides Co., Ltd).

2-chlorotrityl Chloride Resin was swelled by DCM, followed by oscillating for 30 min and subsequently removed the solvent. Fmoc-Gly-OH, DMF and DIEA were added and shook for 60 min. The component was sealed with methanol. Then, remove the DMF from the component, 20% piperidine DMF solution (15mL g^{-1}) was added for 5 min, whereupon 20% piperidine DMF solution was added into the mixture for 15 min. Next, the mixture was washed with DMF, DCM and DMF. Adding three times of Protect amino acid and HBTU which were dissolved by little DMF into the reaction tube, DIEA was added into the reaction tube immediately and reacted for 30 min to complete condensation. The mixture was washed again. Whereafter, palmitate Pal is linked by joining the amino acids in the sequence from right to left using the same steps above. The resin was washed and dried. Finally, polypeptide was cut from resin. The crude peptides were isolated and purified by high performance liquid chromatography. The target polypeptide solution was collected and lyophilized into a white powder.

3. Purification and identification of RA-Amps

The peptides were purified by high performance, liquid chromatography (HPLC; on Kromasil 100-5C18, 4.6 mm×250 mm, 5 microns, with a 21-79 % acetonitrile gradient (20 min) in 0.1% trifluoroacetic acid (TFA)-water, at a flow rate of 1 mL/min, with detection at a wavelength of 220 nm) (Fig. S1, Table S1). The identification of the peptide was identified by mass spectrometry (MS; Waters ZQ2000, Positive ion mode (Ion Source: ESI, Capillary (KV): ± (2500~3000), Desolvation (L/hr):800, Desolvation Temp:450℃, Cone(V): 10-30，Run Time: 1 min). Dissolve pure peptide (1.0 mg) in water (1 mL) and shake it with ultrasonic until it is clear and transparent. Move the sample to the injection bottle, put it into the automatic injector, and conduct automatic injection for detection (Fig. S2).

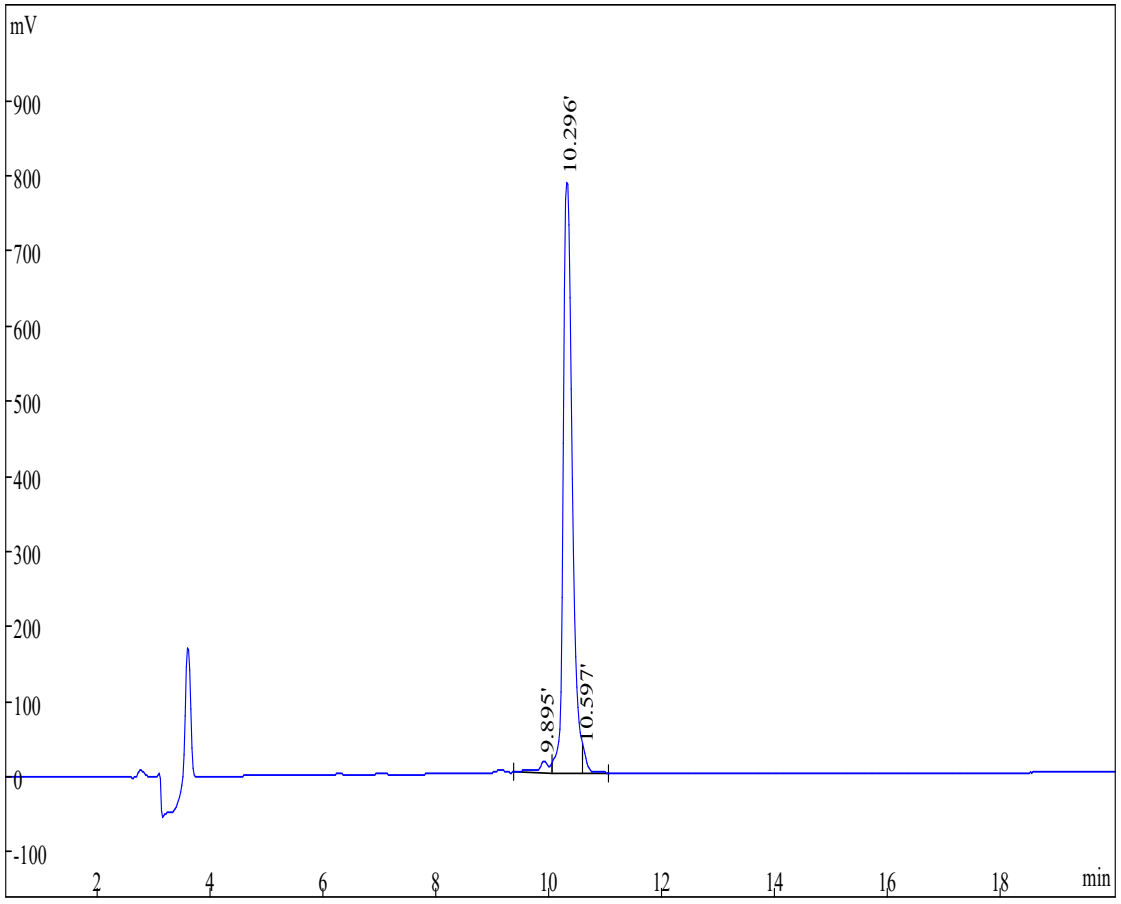

Fig. S1 The purity of RA-Amps determined by high performance liquid chromatography (HPLC).

Table S1 Retention times and signals of main peaks of crude RA-Amps

Rank	Time	Conc.	Area
1	9.895	2.512	206913
2	10.296	96.06	7911570
3	10.597	1.433	118025
Total		100	8236508

Fig. S2 Characterization of molecular weight of RA-Amps in a mass spectrometer (MS).

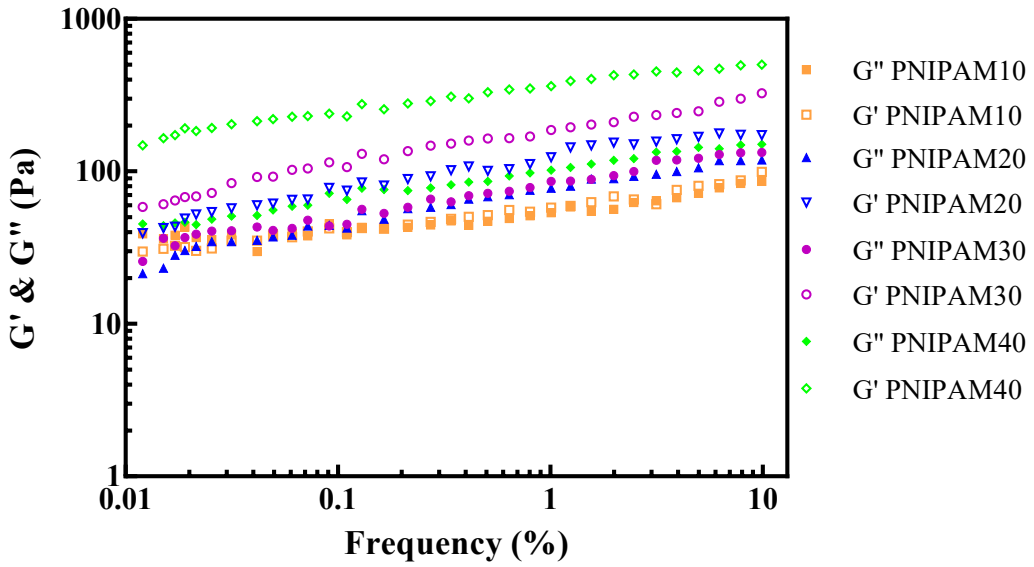

Fig. S3 Linear viscoelasticity experiment measured from rheometer by following the storage modulus (G') and loss modulus (G") as a function of frequency of different PNIPAM systems at 37 °C. PNIPAM10 represents the concentration of PNIPAM (10 mg mL^{-1}), PNIPAM20: 20 mg mL^{-1}, PNIPAM30: 30 mg mL^{-1}, PNIPAM40: 40 mg mL^{-1}.

Table S2 Physical state and phase changed time of PNIPAM at different temperature.

PNIPAM concentration (mg mL^{-1})	Gelation time (37 ^{o}C)	Nature of hydrogel
5	--	White fluid
10	--	White fluid
20	~45 s	White, unstable gel
30	~34 s	White, gel
40	~29 s	White, gel
50	~27 s	White, gel

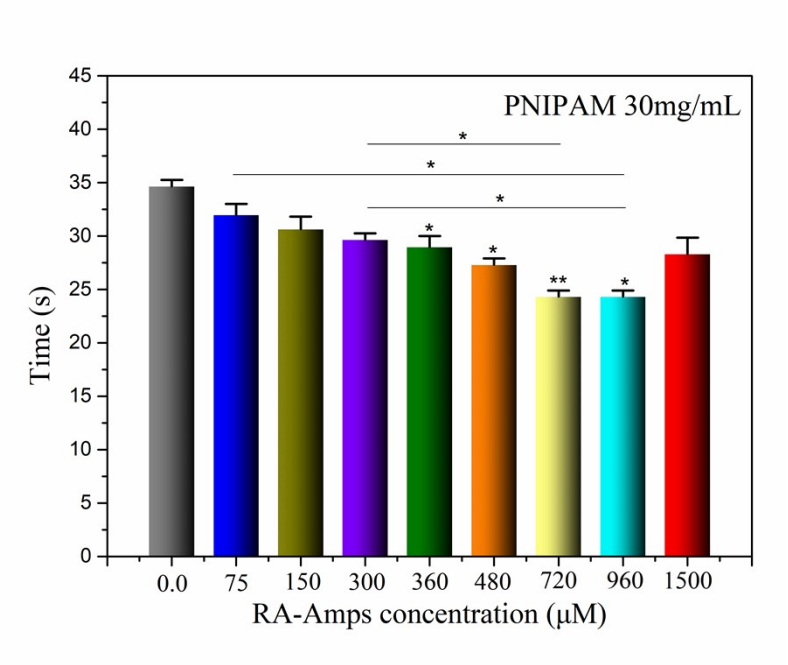

Fig. S4 Gel time of the mixture of PNIPAM (30 mg mL^{-1}) and different concentrations of RA-Amps. (±SD, $n = 3$); $^{*}p < 0.05$, $^{**}p < 0.01$.

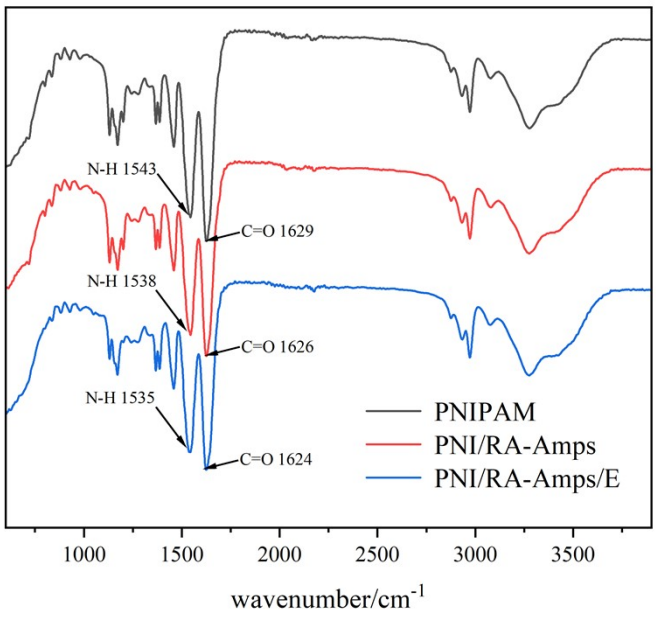

Fig. S5 The FTIR spectra of the PNI/PAM and PNI/RA-Amps/E.

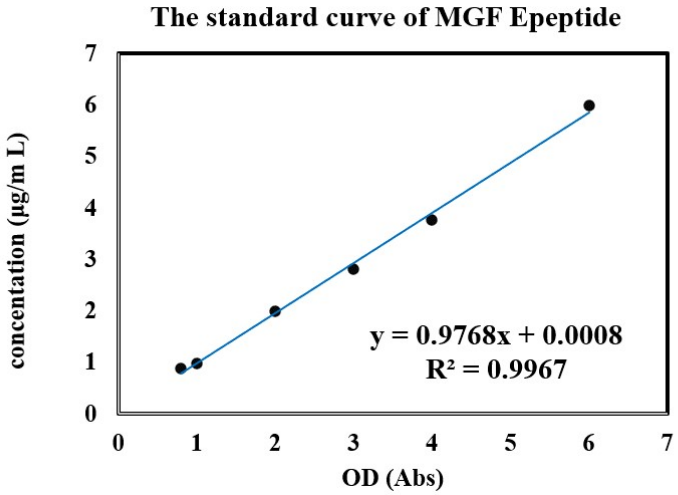

Fig. S6 The standard calculation curve derived by linear fitting of the absorbance at 191 nm of MGF E peptide

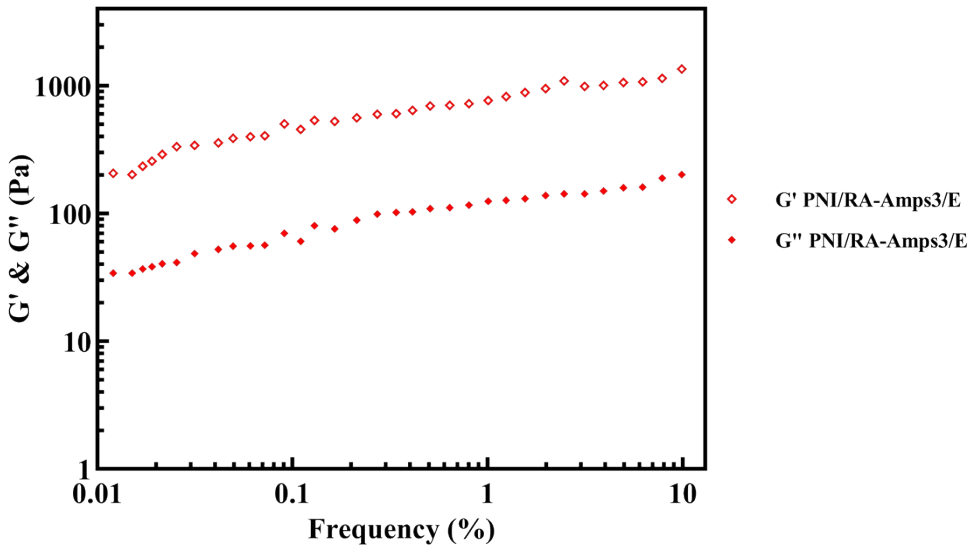

Fig. S7 Storage modulus (G') and loss modulus (G") of PNI/RA-Amps3/E (50 ng mL^{-1}) as a function of frequency at 37 ℃.