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Communication

Supramolecular hydrogels of self-assembled zwitterionic-peptides

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ABSTRACT

Zwitterionic polymer materials have been extensively studied, but zwitterionic peptides supramolecular hydrogel materials are rarely studied. In this study, the preparation of two zwitterionic hydrogels using self-assembled peptides were reported. The hydrogels could be fabricated easily by changing the temperature or enzyme catalysis in a short time. And the differences in structure and function of the zwitterion peptide hydrogels caused by the two preparation methods were also be compared. We found that the hydrogel prepared by enzyme induced self-assembly has better solubility and lower cytotoxicity than that prepared by the heating-cooling process. The result showed the enzyme induced self-assembly way to form zwitterionic peptides supramolecular hydrogel materials could have further biomedical applications.

Zwitterionic materials have emerged as a novel biomaterial with excellent anti-fouling property. This unique property is due to the distribution of both anionic and cationic groups in the same zwitterionic molecule, which leads to various biomedical applications where resistance to non-specific adhesion of proteins or cells are needed. The surface or coating of zwitterionic materials has ultra-low fouling properties and can efficiently resist the adhesion of non-specific molecules, proteins and cells [1–4]. Zwitterionic hydrogel is widely used in the development of antibacterial materials, wound healing dressing and cell cryopreservation technique [5,6]. Meanwhile, zwitterion extends the circulation half-life *in vivo*, which can be used to manufacture create multi-functional nanoparticles for targeted delivery and diagnosis [7–10]. In addition, Jiang and co-workers reported that zwitterionic materials can improve the therapeutic efficacy of proteins more than PEG due to its less immunogenicity and organ toxicity [11]. Despite these achievements in zwitterionic polymers, there is still a need for a simple but effective manufacturing technology of zwitterionic materials, in order to design diverse zwitterionic materials that can adapt to different applications.

Taking the advantage of the spontaneous process of self-assembling, molecular self-assembly has become an attractive method for preparing nanomaterials [12–16]. Supramolecular gels have been proved to be a suitable platform for rearrange of molecules, several works were reported that self-assembly behavior could be adjusted by improving self-assembly methods, such as heating-cooling, enzyme catalysis and glutathione reduction [17–21]. And H-bonding and π-π stacking are the important intermolecular forces to form the gelation process. The formation of ordered nanostructures of self-assembled peptides is the result of the synergistic interaction of various non-covalent bonds between molecules. The formation of ordered nanostructures of self-assembled peptides is the result of the synergistic interaction of various non-covalent bonds between molecules. In self-assembling hydrogel of peptides, there are strong π-π stacking and hydrophobic interactions between the phenyl group of phenylalanine. These non-covalent forces are the driving force of self-assembly to form nanofibers. The hydrophobic groups tend to distribute inside the fibers, and the hydrophilic part distributed on the surface of the fiber [22]. Supramolecular hydrogels from self-assembled peptides are biocompatible and easily to be chemically modified, which have many applications in bioengineering, drug delivery, imaging and cancer treatment [23–25]. Peptides can self-assemble into diverse nanomaterials such as nanofibers, nanospheres, nanosheets and nanotubes [26,27]. Different nanostructures may endow with materials different biological functions [18,28–32]. Until now, there

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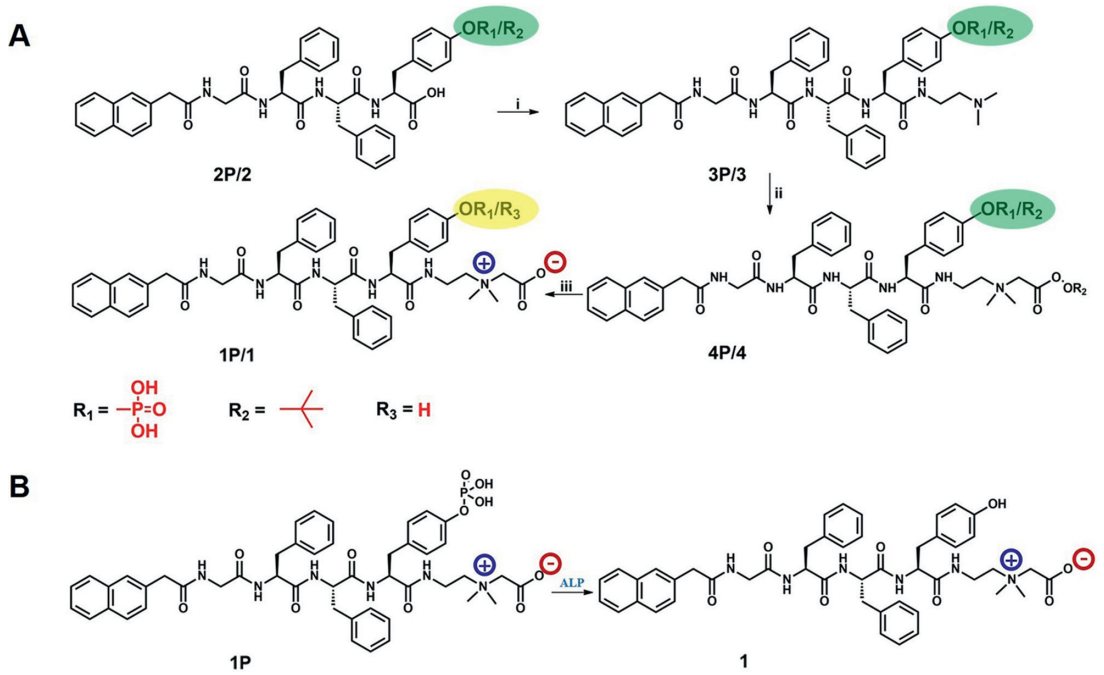

Scheme 1. (A) Synthetic route of Nap-GFFY-CBPA (**1**) and Nap-GFFpY-CBPA (**1P**). (i) *N,N*-Dimethylethylenediamine, DIPEA, HBTU, DMF, r.t.; (ii) *tert*-butyl 2-iodoacetate, DIPEA, THF:DMF = 8:2; (iii) 95% TFA, 2.5% TIS, 2.5% H_2O. (B) The EISA process of **1P** to **1**.

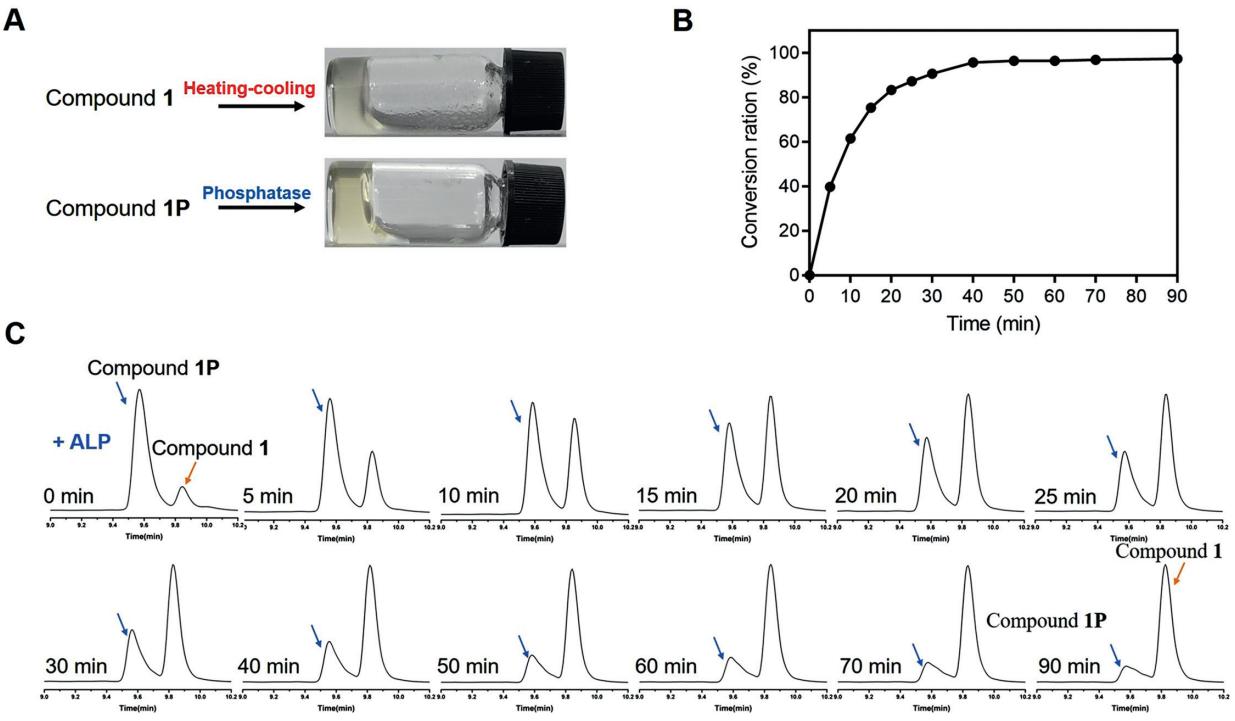

Fig. 1. (A) The optical images of the hydrogels of **1** and **1P** (B) The conversion ratio of **1P** catalyzed by the phosphatase at 37 °C. (C) The detailed enzymatic process at different time points detected by HPLC.

have been many preparation methods of zwitterionic polymer hydrogels by physical or chemical polymerization, but few reports about the synthesis of zwitterionic peptide hydrogels. Therefore, we explored two methods for the manufacture of supramolecular hydrogels of zwitterionic peptide, and compared the difference in structure feature, hoping to have a widely range of biomedical applications.

We synthesized two zwitterionic peptides: **1** (Nap-GFFY-CBPA) and **1P** (Nap-GFFpY-CBPA), which could self-assemble into hydrogel by heating-cooling and enzyme-induced self-assembly (EISA), respectively. They could be obtained by combing standard Fmoc-solid phase peptide synthesis and liquid phase synthesis (Scheme 1). The detailed synthesize route is shown in Scheme S1 (Supporting information). Compounds **1** and **1P** were purified by high performance liquid chromatography. And the HR-MS of all intermediate compounds were shown in Figs. S1-S8 (Supporting information). Compound **1** was difficult to completely dissolve in H_2O or PBS buffer at room temperature (25 °C), while **1P** could

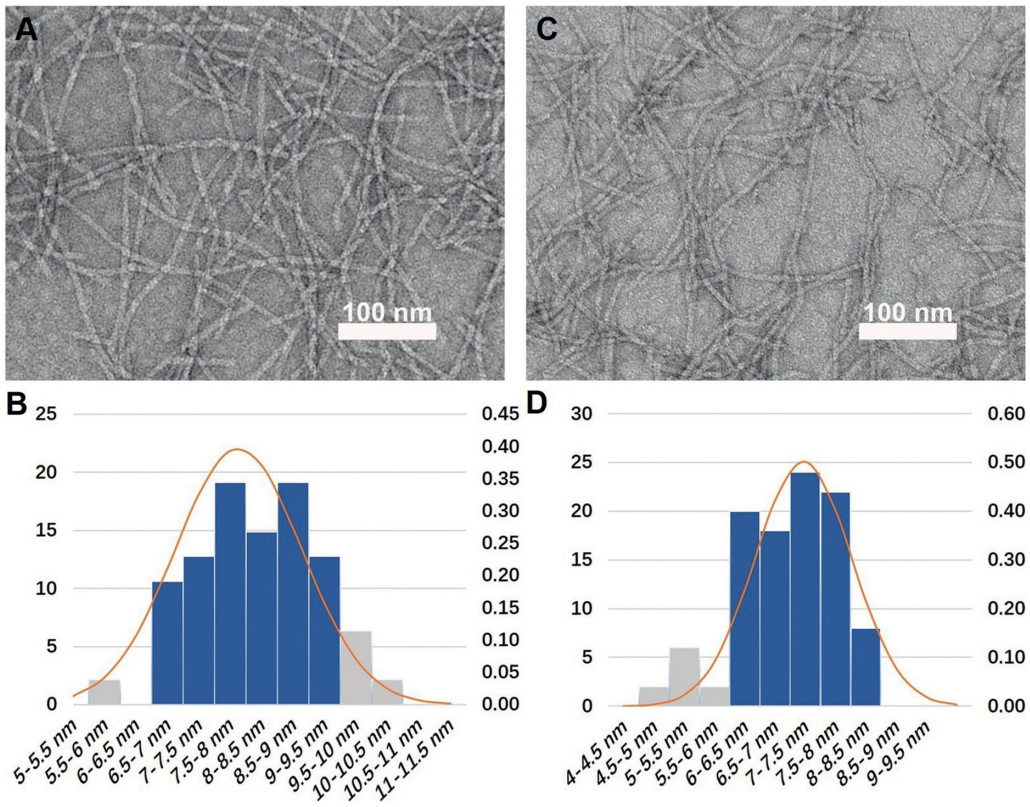

Fig. 2. TEM images of the hydrogels of **1** (A) and **1P** (C). Scale bars represent 100 nm. Frequency distribution histograms of the fiber diameter of compound **1** (B) and compound **1P** hydrogels (D).

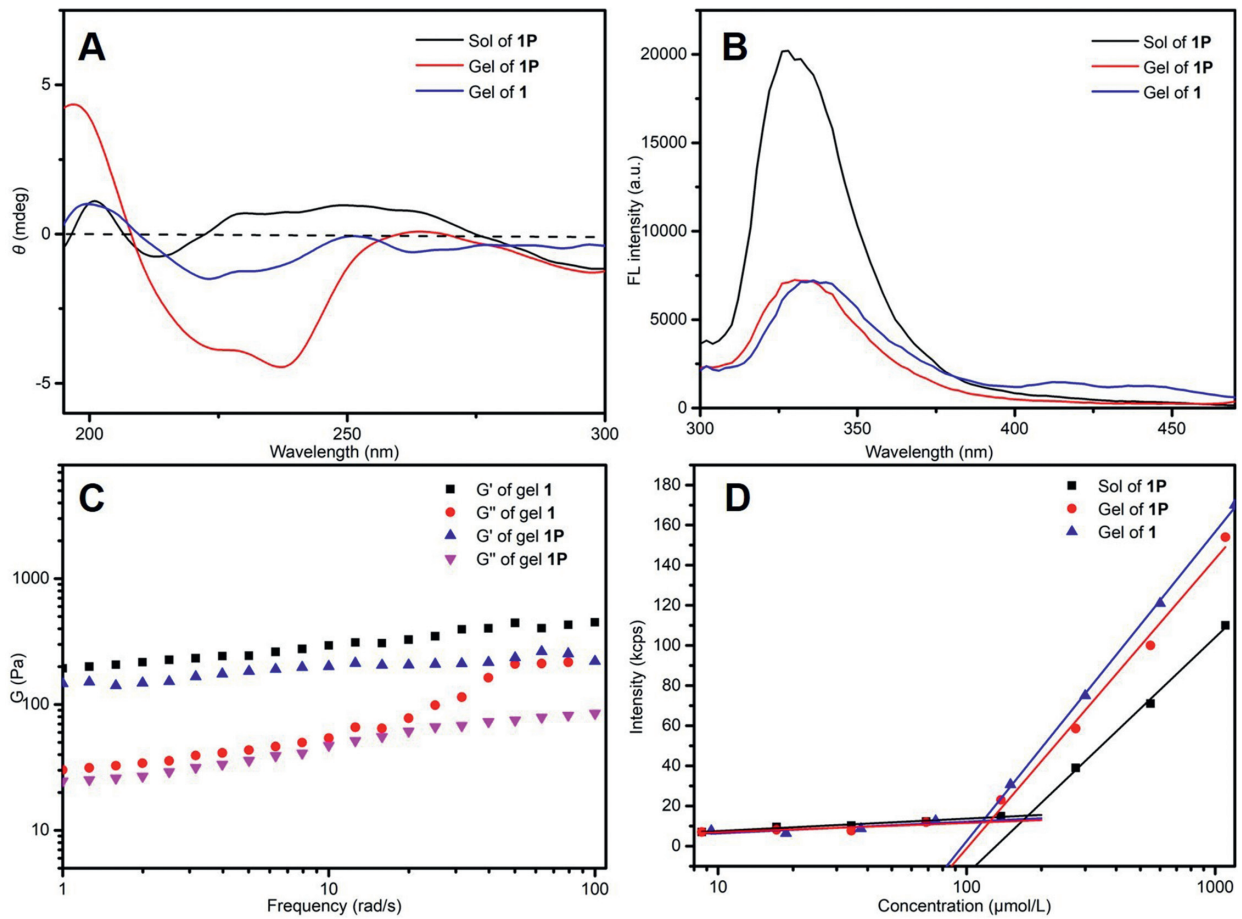

Fig. 3. (A) CD spectra of the solution of **1P**, the hydrogel of **1P** formed by EISA at 37 °C and the hydrogel of **1** formed by heating-cooling process. (B) The florescence spectra of the hydrogels and the solutions (the concentration is 1 mmol/L). (C) Oscillatory rheology dynamic frequency scanning of the hydrogels of **1** and **1P**. (D) The critical aggregation concentration (CAC) of the three compounds.

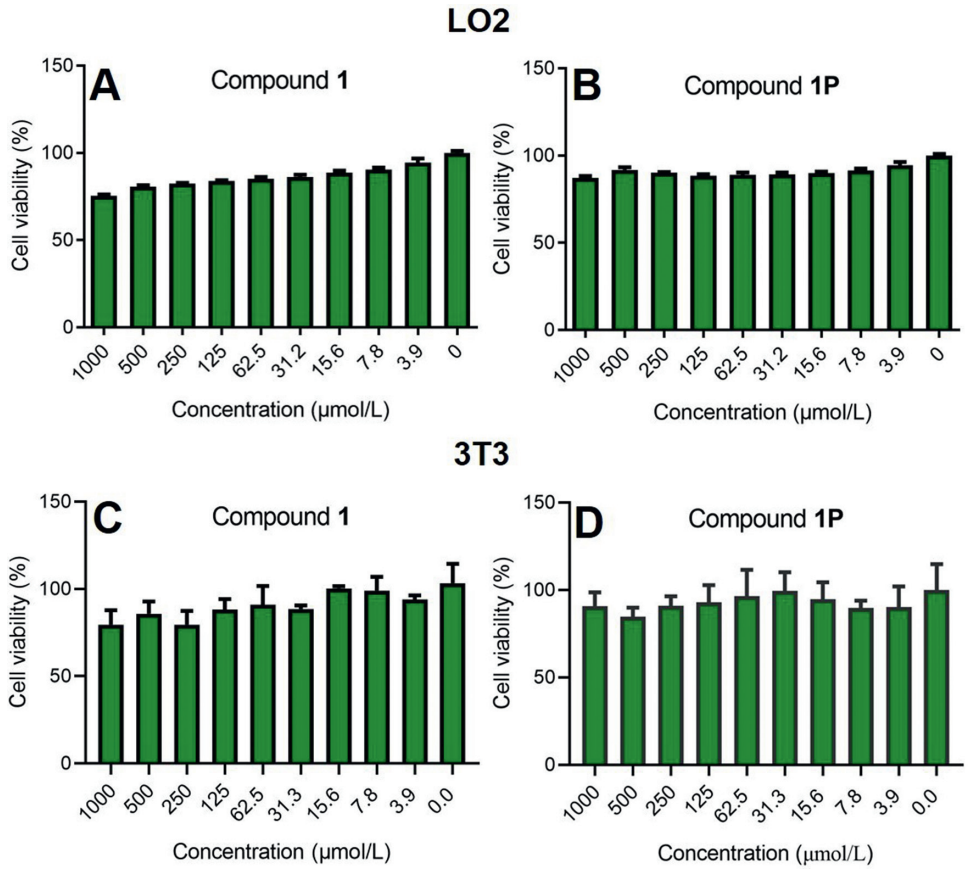

Fig. 4. The cytocompatibility of compound **1** and **1P** with liver cells (A, B) and mouse embryonic fibroblast cells (C, D).

dissolve well in both solvents at physiological pH (Fig. S9 in Supporting information). Nevertheless, both of **1** and **1P** could form hydrogels in H_2O (pH 7.4) at the concentration of 0.2% by the weight under the process of heating-cooling or enzyme catalysis (Fig. 1A). The undissolved compound **1** dissolved in solvents after being heated to 90 °C, and the solution formed a hydrogel after cooling to room temperature. Under the catalysis of alkaline phosphatase (ALP), **1P** could self-assemble and formed a clear hydrogel within 20 min at 37 °C. And the final ingredient of the two gels almost the same except for some extra ALP. The critical gel concentration of compounds **1** and **1P** were confirmed to be 0.2% and 0.1% by weight, respectively. Both hydrogels were stable at room temperature for several months without any morphological variations (Figs. S14 and S15 in Supporting information). We then determined the conversion ratio of **1P** to **1** by ALP (1 U/μL) at 37 °C with LC-MS. We found that **1P** was converted to **1** almost completely within approximately 40 min (Fig. 1B). And the detailed enzymatic process of the compound **1P** to **1** detected by LC-MS at different time points are shown in Fig. 1C.

We used transmission electron microscopy (TEM) to characterize the nanostructures in solutions and hydrogels of the two compounds. From the TEM images, we observed nanofibers both in the solution and hydrogel. As shown in Fig. S13 (Supporting information), the solution of **1** showed nanosheets like morphology with an average diameter of around 12 nm. After the heating-cooling process, the nanostructures turned into dense and thin nanofibers with a diameter of around 6.5–9.5 nm (Figs. 2A and B), which indicating structural remodeling of molecules under temperature changes (the detailed statistical method of fiber diameter is shown in Supporting information). The diameter of nanofibers in the solution of **1P** and hydrogel formed by **1P** were almost the same, which were both around 6–8.5 nm (Figs. 2C and D, Fig. S13). However, the self-assembly increased the degree of cross-linking of the nanofibers, resulting in sol-to-gel phase transition.

Next, we characterized circular dichroism (CD) spectra of the two compounds in solutions and hydrogels. In Fig. 3A, the hydrogel of **1** exhibited a positive peak at 200 nm and two negative peaks at 223 and 235 nm, suggesting that the peptide adopted an α-helix conformation. Besides, there is no signal of the solution **1** (dissolved in H_2O with 5% DMSO, pH 7.4), which means that **1** did not adopt ordered conformation (Fig. S11 in Supporting information). And the hydrogel of **1P** showed the same α-helix conformation with a positive peak at 200 nm and negative peaks at approximately 224 and 237 nm. Meanwhile, the solution of **1P** exhibited a positive peak at 201 nm and a negative peak at 213 nm, which suggested that the solution of **1P** adopted a β-sheet conformation. This result indicated that the zwitterionic peptides compound **1P** has a β-sheet conformation in solution, and after assembly into a hydrogel triggered by the enzyme, the gel of **1P** exhibited an α-helix conformation. The same situation was found in the hydrogel of 1. To understand the molecular arrangement of naphthalene groups in the solution and hydrogel, we characterized them by fluorescent spectroscopy. As shown in Fig. 3B and Fig. S12 (Supporting information), **1P** in the solution exhibited an obvious peak at 328 nm, which was related to the ^1L_b transition of naphthalene. After the gel formation triggered by enzyme, the peaks from the ^1L_b transition of naphthalene were red-shifted to longer wavelength. The peak of the gel **1P** was found at 330 nm, while the corresponding solution **1** showed the peak at 334 nm. The fluorescence intensity of the solution **1P** was stronger than that of the hydrogel **1P** and the solution **1**, which meant that the molecules

in the hydrogel were stacked more tightly so that the fluorescence decreased due to aggregation-caused quenching.

We then studied the mechanical properties of the two hydrogels. As shown in Fig. 3C, the *G*' value for both gels were higher than the corresponding *G*'' value, which demonstrated the formation of hydrogel. The *G*' of the hydrogel **1** was slightly higher than that of hydrogel **1P**, indicating hydrogel **1** had a stronger mechanical strength than hydrogel **1P**, which was consistent with the dense nanofibers observed in TEM for hydrogel **1**. Besides, we measured the dynamic time scanning pattern of aqueous solutions containing 2% by weight of the **1P** and 3 U/mL of the ALP (Fig. S10 in Supporting information). The result showed that the compound **1P** could self-assemble to form hydrogel in a short time. We also measured the critical aggregation concentration (CAC) of the two compounds. As shown in Fig. 3D, the CAC value of **1P** was 176.31 μmol/L, and it decreased to 116.16 μmol/L after enzyme catalysis, which was almost the same as compound **1** (123.40 μmol/L). The result meant compound **1** and the molecule resulted from enzyme catalysis had similar self-assembly property.

Finally, we evaluated the cytotoxicity of both hydrogels. As shown in Fig. 4, the hydrogel **1** had a little toxicity to a normal liver cell line (L02) when the concentration reached 125 μmol/L or higher. In contrast, the hydrogel of **1P** showed negligible cytotoxicity even at a high concentration of 500 μmol/L. Similarly, we chose another cell line named mouse embryonic fibroblast cells (3T3 cell line) to analysis the cytotoxicity and the result was almost the same. These results together indicated that the hydrogel by EISA had better biocompatibility. And we also obtained images of the cells after incubating with certain concentration of **1** and **1P** for 1 day. We found only very few cells died after the incubating (Fig. S16 in Supporting information).

In conclusion, we pioneeringly reported a pair of zwitterionic hydrogels based on peptide-assembly. The hydrogels were prepared using heating-cooling and EISA protocols. We studied their secondary structures and mechanical strength, *etc.* The zwitterionic peptide hydrogel by EISA showed low cytotoxicity for the normal cell line compared to the heating-cooling progress. Our results may be helpful for exploring diverse zwitterionic materials in the future for biomedical applications**.**

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.04.039.

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