This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
The first supramolecular peptidic hydrogelator containing taurine

Yi Kuang, Yuan Gao, Junfeng Shi, Jie Li, Bing Xu* a

The conjugation of taurine with a dipeptide derivative affords a cell compatible, small molecular hydrogelator to form hydrogels that exhibit rich phase transition behaviors in response to sonication and the change of pH or temperature.

This communication describes the design and construction of a new small molecular hydrogelator based on taurine and peptide. Supramolecular hydrogels, consisting of a three-dimensional network formed by molecular self-assembly to encapsulate water, have received increased attentions in recent years because of their potentials as a new class of biomaterials. For example, supramolecular hydrogels have shown promises in applications like protein binding and separation, cell encapsulation and delivery, tissue engineering, and wound healing. Most of the hydrogelators in those works derive from peptides that consist of proteinogenic amino acids. Although there are considerable number of essential, non-proteinogenic amino acids used by living organisms in nature, few of the non-proteinogenic amino acids have served as the building blocks of hydrogelators. To evaluate the feasibility and to expand the use of non-proteinogenic amino acid for making soft biomaterials, we choose to develop hydrogelators based on taurine (1).

Taurine (1), a natural and non-proteinogenic amino acid, exists in large amount in human tissue (0.1% of total human body weight) and carries out important physiological actions. Besides being an essential amino acid in the synthesis of the bile salt (e.g., taurocholate) and the tRNA conjugation in mitochondria, taurine also acts as an inhibitory neurotransmitter, an intracellular osmolyte, a useful membrane stabilizer, and an antioxidant. Many studies have demonstrated that the rise of taurine level has positive effects to the growth and generation of cells and tissues. For example, recent studies showed that taurine can stimulate the generation of rod photoreceptor cells from retinal progenitor cells through the interaction with a glycine receptor. Despite of the biological importance, taurine rarely served as a building block for biomaterials. Though there are scattered reports of polymer gels based on sulfonic acid group containing gellants (e.g., poly(vinyl sulfonic acid)) and a bio-based surfactant containing taurine, taurine, by itself, has yet be incorporated into peptides for making hydrogelators and forming supramolecular hydrogels.

![Illustration of the synthetic route and the self-assembly of a taurine-containing hydrogelator (3). (A) Synthetic route of 3: i: 3 eq. DIPEA, 1 eq. HBTU in DMF; overnight. ii: diethyl ether, precipitation. (B) Representative self-assembly processes of 3 at pH 3 afford a viscous solution or a hydrogel and the corresponding nanostructures.]

Here, we design and synthesize the first taurine containing hydrogelator (3) by attaching taurine (1) at the C-terminal of a well-established self-assembly motif (2). Our study shows that 3, indeed, acts as a hydrogelator to form a supramolecular hydrogel that contains 98% water. Interestingly, the self-assembly of 3 results in nanostructures that are sensitive to sonication. Cooling the solution of 3 at pH 3 affords a viscous liquid containing largely nanotubes. Simultaneous sonication and cooling of the solution of 3 result in a hydrogel that consists of a network of nanotubes and spray nanoribbons. The unwinding of the nanotubes to nanoribbons by sonication increases the physical cross-linking that is crucial for the hydrogelation. The nanostructures of 3 in the hydrogel or the viscous liquid also exhibit pH dependence due to the presence of sulfonic group. More importantly, 3 exhibits excellent cell compatibility despite the cytotoxicity of 2. This work indicates that the incorporation of taurine not only enriches the morphologies of the nanostructure of the self-assembly of the hydrogelators, but also drastically modulates the biological activity of the hydrogelators.
(3 eq.) with 2 (synthesized by SPPS\textsuperscript{18} in DMF, the addition of diisopropylmethyamine (DIPEA, 3 eq.) and o-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU, 1 eq.) activates the carboxyl group on 2 to couple with the amino group on 1. This simple procedure produces 3 in over 80% yield after the purification by high-performance liquid chromatography (HPLC).

The gelation behavior of 3 largely differs with that of 2. While 2 forms a stable hydrogel at 0.4 wt% upon the change of pH or cooling of hot solution,\textsuperscript{19} 3 forms a hydrogel at much higher concentration (2.0 wt%) in PBS buffer. This difference likely originates from the sulfonic acid group that introduces hydrophilicity to 3. Moreover, a unique feature of 3 is that sonication influences the gelation of 3. As shown in Fig. 2, cooling of a hot solution of 3 (70 °C, 2.0 wt%, pH 3) in 20 °C water bath for one minute results in only a viscous solution (denoted as “C-sol”), which behaves like newtonian fluid in rheological test\textsuperscript{20} (Fig. S1). The increase of the concentration of 3 (to 2.5 wt%) fails to afford a hydrogel by the cooling process alone. However, sonication of the hot solution in 20 °C water bath for five seconds following by another fifty-five seconds of cooling in 20 °C water bath affords a stable hydrogel (denoted as “U-gel”) (Fig. 2), which exhibits the rheological behaviour (Fig. S1) of the viscoelastic materials made of weak cross-linking networks.\textsuperscript{20} Interestingly, the formation of C-sol and U-gel is reversible. By heating the U-gel or C-sol to 70 °C (for 1 min), the hydrogel and the viscous solution become clear solutions, and the C-sol and U-gel form again from the solution upon cooling or sonication plus cooling, respectively. Moreover, unlike many charged peptide derivatives that self-assemble by the formation of ionic bond between molecules with opposite charges,\textsuperscript{21} the addition of triethylamine or poly(allylamine) to the solution containing 2% of 3 (molar ratio ranging from 1:10 to 10:1; pH ranging from 1 to 9), results in only precipitation of the mixture.

To reveal the morphology of the nanostructures in C-sol and U-gel, we used transmission electron microscopy (TEM) to visualize the molecular assemblies of 3 in C-sol and U-gel. As shown in Fig. 3A, the negative stained\textsuperscript{22} TEM images of C-sol show the major morphology as nanotubes of which the hollow central cavity partially shows high density (i.e., the darker region due to the permeation of uranyl acetate used in negative staining\textsuperscript{23}). The nanotubes have a diameter of 9±2 nm. The inset of Fig. 3A shows the terminals of the nanotubes have complete and flat ends. Fig. 3B shows the morphology in U-gel is a mixture of nanotubes and nanoribbons. While the nanotubes have a similar width with those in the C-sol (9±2 nm), the helical nanoribbons have a narrower width (6±2 nm), and the helix pitch of the nanoribbons varies from 35 nm to 55 nm. Moreover, the ends of the nanotubes in U-gel, as shown in the inset of Fig. 3B, split into several nanoribbons, indicating that the helical nanoribbons wind together to form the nanotubes observed in C-sol and U-gel. This result implies that the formation of the nanotubes by 3 fits the hierarchical self-assembly model of peptides proposed by Aggeli et al.\textsuperscript{24} They suggested that peptides with β-sheet-forming ability might associate into twisted ribbons and further entwin into hollow fibrils. Since 3 contains the β-sheet-forming motif (e.g., Phe-Phe) and froms twisted ribbons, it should be possible for the ribbons to form nanotubes. The difference in morphology of C-sol and U-gel explains the effect of sonication in gelation of 3 at pH 3. Cooling alone results in complete formation of unbent nanotubes, which provide too few interactions to form a strong 3-D network, thus resulting in only viscous solution. Sonication during cooling likely unwinds the nanotubes into nanoribbons, and, at the same time, the flexible nanoribbons favor physical cross-linking for networking the rigid nanotubes, thus affording the hydrogel of 3.
4±2 nm and length range from ~100 to 200 nm (Fig. 4C). In another case (Fig. 4B), cooling the solution of 3 at 2 wt% and pH 5 from 70 °C results in a solution that contains nanotubes as major morphology (width = 9±2 nm; similar to that shown in Fig. 3A). Cooling plus sonication of the solution of 3 at pH 5 affords a hydrogel with dominate morphology as helical nanoribbons with width = 6±2 nm, and the pitch length range from 100 to 160 nm (Fig. 4D). Based on these results, the hydrogelation of 3 not only clearly depends on pH, but also depends on sonication, thus resulting in the rich phase behaviors.

Besides examining the gelation properties of 3, we also tested the cell compatibility of 3. Fig. 5 shows the 3-day cell viability of HeLa cells incubated with 3. Differing with its self-assembling peptide motif 2 that shows strong cytotoxicity at aggregated state,17 3 induce little loss of cell viability even at the 3rd day of incubation. The biocompatibility of 3 indicates that the addition of taurine to peptide derivative can significantly alter the cytotoxicity of the molecule, which coincides with that the sulfonic group renders hydrophilicity and charge to the molecule. Moreover, the biocompatibility of 3 promises the incorporation of taurine into other hydrogelators for biological applications.

![Cell viability (%)](image)

**Fig. 5** MTT cell viability assay of the HeLa cells incubated with 3 at different concentrations for 3 days.

In conclusion, the conjugation of taurine and a self-assembling peptide derivative affords the first hydrogelator that consisting of both proteinogenic amino acids and a non-proteinogenic amino acid (e.g., taurine). Besides depending on pH, the gelation behavior of 3 also differs according to different gelation processes (i.e., cooling or cooling plus sonication), which is a distinctive property for conventional amino acid based hydrogelators. Moreover, 3 exhibits little cytotoxicity, which may lead to new type of biocompatible hydrogelators. The use of taurine for creating supramolecular hydrogelators not only demonstrates the possibility of utilizing taurine or other non-proteinogenic amino acid in the construction of biocompatible peptide hydrogelator, but also illustrates the uniqueness of sulfonic acid group for generating supramolecular hydrogels. We envision the incorporation of sulfonic groups might result in small molecular hydrogels that have unique property for physical or biological applications.

**Notes and references**

1 Department of Chemistry, Brandeis University, 415 South St., Waltham, MA 02454, USA. Fax: 781-736-2516; Tel: 781-736-5201; E-mail: bxu@brandeis.edu.

2 Electronic Supplementary Information (ESI) available: [materials; characterization of 3; rheology data]. See DOI: 10.1039/c000000x/

† This work was partially supported by R01CA142746 to B.X. We thank the EM facility at Brandeis University.


