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ARTICLE TYPE

Multi-Responsive Supramolecular Hydrogels for Drug Delivery

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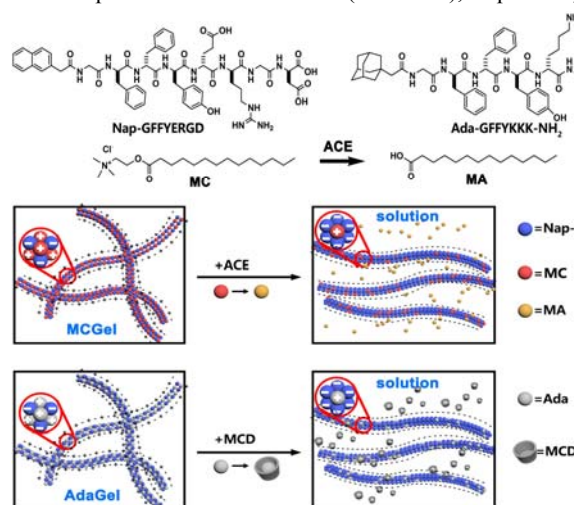
We reported in this study a versatile method to prepare multi-responsive supramolecular hydrogels for drug delivery application.

Peptide-based supramolecular hydrogels have attracted extensive research interests due to the inherent properties of peptides, such as bioactivity, biocompatibility, and degradability.^[1] Among them, short peptide-based hydrogelators hold advantages because of the ease of synthesis of short peptides.^[2] More importantly, functional groups including therapeutic agents,^[3] sugars and nucleic acids,^[4] and fluorescent probes^[5] are easily integrated with short peptides to generate functional hydrogelators. Therefore, short peptide-based hydrogels have been applied for cancer cells inhibition,^[6] cell culture,^[7] drug delivery,^[8] sensing,^[9] and regenerative medicine.^[10] Recent research efforts in the field of short peptide-based hydrogels have moved to the development of novel functional hydrogels and the investigation of their novel applications and practical applications. Among the novel functional hydrogels, responsive hydrogels have big potential in sensing, drug delivery, and regenerative medicine, and multi-component hydrogels possess multi-functions.^[11] Therefore, these two kinds of functional hydrogels are recently extensively investigated. In this study, we report a versatile strategy to construct responsive multi-component hydrogels.

We have reported that positively charged nanospheres of doxorubicin and proteins with multiple binding sites could serve as cross-linkers to cross-link negatively charged supramolecular nanofibers of peptides to form hydrogels.^[12] Such kind of two-component hydrogels were formed by charge interaction or specific protein-peptide interaction. They were formed by simple mixing process and have potential in cell culture and drug delivery. However, they were not responsive hydrogels. Recently, we have also used a responsive selenium-containing polymer to form hydrogels with a self-assembling peptide.^[13] The resulting two component hydrogels could respond to γ -ray irradiation and photo-irradiation, and therefore the gels could be applied for controllable drug delivery. We imaged that, if we used responsive small molecules to interact with opposite charged peptides, we might obtain responsive two-component hydrogels. More importantly, we might use two kinds of responsive components to form hydrogels with peptides. The resulting hydrogels would be multi-responsive ones.

In order to test our hypothesis, we firstly designed and synthesized the short peptide of Nap-GFFYERGD by standard

solid phase peptide synthesis (Scheme 1). We recently found that Nap-GFFYGRGD could only form negatively charged nanofibers, but not hydrogels at the concentrations lower than 0.5 wt%.^[14] We therefore believed that peptide Nap-GFFYERGD would also form negatively charged nanofibers. We selected Myristic Choline (MC) and designed Ada-GFFYKKK-NH₂ (Ada) to interact with the peptide because they were positively charged and could self-assembling into nanoparticles (Scheme 1).^[15] The incorporation of these two components to the peptide solution respectively might lead to hydrogelations due to charge screening (Scheme 1). MC and Ada could respond to the enzyme of Acetyl Cholin Esterase (ACE) and methyl- β -CD (MCD), respectively. Therefore, the resulting hydrogels (MCGel and AdaGel) might show responses to ACE and MCD (Scheme 1), respectively.



Scheme 1. Chemical structures of components used for hydrogelation and schematic illustration for gel-sol phase transitions

As shown in Fig. 1, Nap-GFFYERGD indeed form clear solutions but not hydrogels in phosphate buffer saline (PBS, pH = 7.4) at concentrations lower than 1.0 wt%. The incorporation of 0.25 equiv. of MC or Ada to the peptide solution (0.2 wt%) led to the formation of a slightly opaque MCGel or transparent AdaGel, respectively. In the presence of ACE, the MCGel changed to viscous solution because the positively charged MC was converted by ACE to negatively charged myristic acid (MA). The addition of MCD to the AdaGel also led to a gel-sol phase transition because the hydrophilic MCD would form a complex with the adamantane group of Ada by specific ligand-receptor

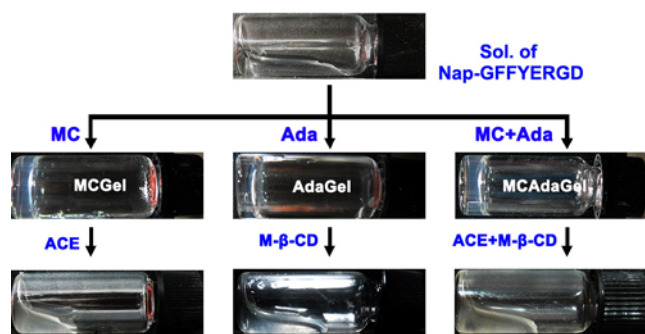


Fig. 1. Optical images to show the formation of hydrogels by mixing negative peptide Nap-GFFYERGD and positive self-assembling components of MC, Ada, or both MC and Ada and the responsive property of resulting gels

interaction. Such interaction made Ada well soluble and therefore could not form hydrogels with the peptide. We also prepared MCAdaGel by incorporation of 0.25 equiv. of MC and 0.25 equiv. of Ada with the peptide. In the presence of single component of ACE or MCD, the MCAdaGel could only be partially destroyed (Fig. S-6), and it could be totally converted to a viscous solution by adding both ACE and MCD (Fig. 1). Adding hydrophilic and positive charged compounds without self-assembling property such as kanamycin and lysine to the peptide solution could not lead to hydrogelations. These observations clearly indicated that responsive multi-component hydrogels could be easily prepared by mixing two responsive self-assembling molecules with opposite charges.

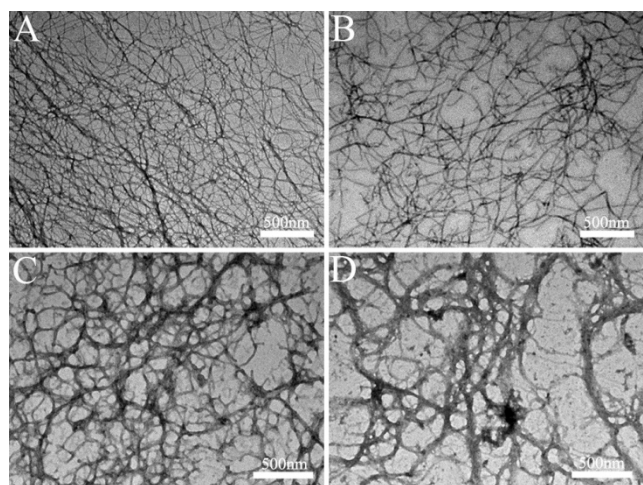


Fig. 2. The transmission electron microscopy (TEM) images of A) PBS solution of peptide, B) MCGel, C) AdaGel, and D) MCAdaGel.

We then used a rheometer to characterize the mechanical properties of the gels. As shown in Fig. S-8, the gels showed weak frequency dependence at the frequency range from 0.1 to 10 rad/s, while showed frequency dependence and the shear-induced thickening property at the high frequency range (>10 rad/s). The G' values of three gels were lower than 100 Pa. These results suggested the gels were mechanically weak ones. As shown in Fig S-12, the mechanical property of hydrogels could be slightly modulated by changing the concentration of positively charged responsive self-assembling components, and gels with more

amounts of positively charged compounds would have bigger G' values. We also used transmission electron microscopy (TEM) to characterize the nanostructures in the solution of the peptide and the gels. As shown in Fig. 2, the peptide itself formed nanofibers with diameters from 16 to 20 nm (Fig. 2A). In the presence of 0.25 equiv. of MC, MCGel exhibited uniform nanofibers with sizes of around 25 nm (Fig. 2B). The AdaGel also showed nanofibers with width of 25-60 nm (Fig. 2C). As shown in Fig. 2D, the sizes of nanofibers in MCAdaGel were the biggest and they were about 50-90 nm, which was consistent with the observation that the G' value of MCAdaGel was bigger than that of MCGel or AdaGel (Fig. S-8). These observations suggested the co-assembly of the negative peptide and positive MC and Ada compounds.

Since our MCAdaGel could respond to the enzyme and the specific ligand-receptor interaction, it might be applied for controlled drug delivery. We therefore incorporated curcumin to the MCAdaGel to test such potential application. As shown in Fig. 3A, the gel partially dissolved in the presence of enzyme ACE or MCD and there was approximately 18.5% and 23.9% of curcumin being released from the gel at 37 °C for 12 hours, respectively. In the presence of both ACE and MCD, most of the gel dissolved and about 33.0% of curcumin got released from the gel during the 12 hours experiment period. The sample with PBS buffer solution slowly and steadily released 13.1% of curcumin, and the hydrogel was basically unchanged for 36 hours (Fig. S-9). The results suggested that our hydrogels could be applied for controllable drug delivery and had multi-responsiveness.

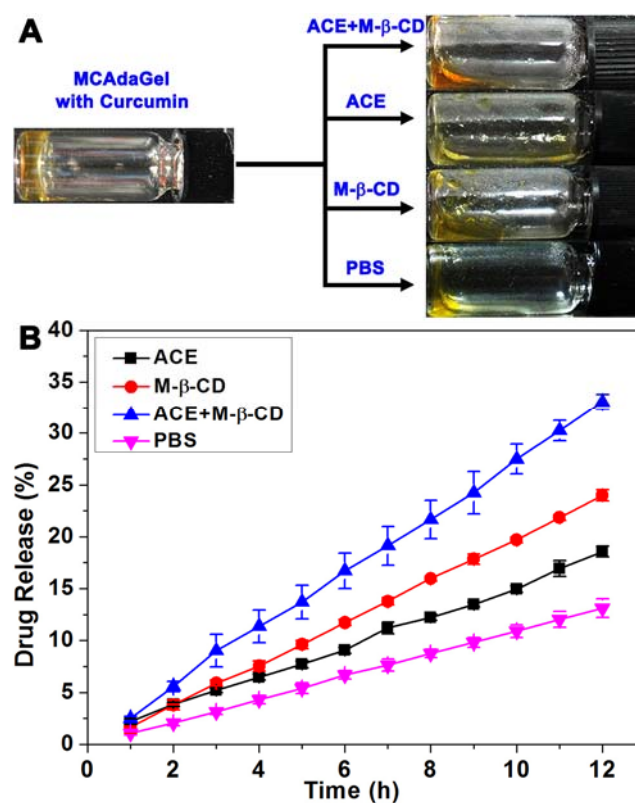


Fig. 3. A) Optical images of curcumin incorporated MCAdaGel and its appearances after releasing experiments for 12h and B) release profile of curcumin from the gel in the presence of PBS, ACE, MCD, and both ACE and MCD

In summary, we have developed a versatile and general method to prepare multi-responsive supramolecular hydrogels. The responsive hydrogels were formed by the co-assembly of self-assembling peptide with negative charge and responsive self-assembling components with positive charge. We could also prepare multi-responsive hydrogels by using multiple responsive components with positive charges. Our responsive hydrogels have potential in controllable drug release application and the release profile of drug molecules could be modulated by the enzymatic reactions or ligand-receptor interaction. Our study provides a novel strategy to prepare multi-responsive supramolecular hydrogels that may be applied for tissue engineering and drug delivery.

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