Self-assembling injectable peptide hydrogel for biomedical applications

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Statement of Purpose: Peptide-based hydrogels have many potential applications in cell culture, tissue engineering, drug delivery, cosmetic product and other areas.[1-5] We designed a self-assembling peptide (h9e) by rationally combining two native sequences from a modified elastic segment of spider silk and a trans-membrane segment of human muscle L-type calcium channel.[6] The h9e peptide can be triggered into high water content hydrogels (greater than 99.5 wt% of water) through different methods. The advanced physiological properties and a special shear-thinning and self-healing character of the h9e hydrogel allow it to be used as an injectable material for many biomedical applications.

Methods: The h9e was synthesized on an automated CEM Liberty microwave peptide synthesizer (CEM Corporation, Matthews, NC) according to the base-labile 9-fluorenylmethoxycarbonyl (Fmoc) strategy with Rink amide resin and Fmoc-protected amino acids. Self-assembly and reassembly in terms of storage and loss moduli (G' and G'', respectively) of h9e hydrogels were determined on a C-VOR 150 rheometer system (Malvern instruments, Malvern, Worcestershire WR141XZ, United Kingdom) with a 20-mm diameter parallel plate geometry and 500 μm gap size. Other methods for hydrogel characterization and applications such as SEM, TEM, CD, FTIR and Confocal Microscopy will be presented in the conference.

Results: The reassembly ability of h9e hydrogel was assessed by a dynamic rheological test. By shear-thinning at 500% strain for 1 min, 1-3 mM hydrogels were converted to a liquid state, showing a storage modules (G') lower than 0.2 Pa (Figure 1a). After the 1-min waiting time for instrument reset, about 80% of hydrogel G' was recovered. The G' of hydrogel at all three concentrations stiffened further with time, and 100% gel strength recovery was observed in minutes during a 1-h recovery test. The hydrogel could maintain this quick reassembly capability even after shear-thinning many times under a multiple circles amplitude sweep test. Figure 1b suggests that although the hydrogel architecture was completely broken into liquid form at the end of each circle, quick reassembly even after shear-thinning multiple times. Based on these rheological properties, we could repeatedly deliver this h9e hydrogel via pipette or syringe without permanently destroying the hydrogel architecture (Figure 1c). In addition, the rheological temperature profile test shows that the G' of hydrogels moves along with temperature and performs 2-3 times higher at 50 ºC than that at 4 ºC (Figure 1d). This thermal response is reversible according to the hydrogel heating and cooling circles. More hydrogel characters and applications will be presented in the conference.

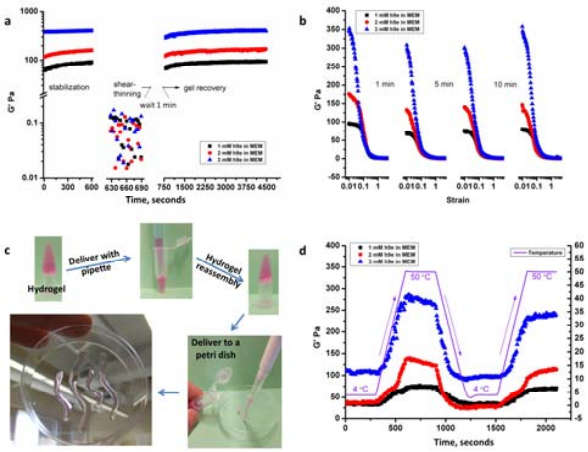

Figure 1. Dynamic rheological study of h9e hydrogel. **a.** Storage modulus G' of shear-thinning and recovery test of 1, 2, and 3 mM peptide hydrogel. **b.** Four times amplitude sweep test with shear strain from 1% to 500% and 1-, 5-, and 10-min breaks. **c.** Peptide hydrogel was delivered via pipette multiple times; hydrogel was shear-thinned but reassembled quickly without permanently destroying the hydrogel architecture. **d.** Temperature profile test of 1-, 2-, and 3-mM peptide hydrogel between 4 °C and 50 °C.

Conclusions: We rationally designed and synthesized novel peptides using two native functional sequences. At physiological pH, the formation of hydrogel with controlled time and gel strength can be modulated by changing the temperature or peptide concentration. The specific shear thinning and rapid recovery rheological properties of hydrogel allow us to inject or deliver this hydrogel material via syringe or pipette multiple times. This peptide hydrogel could be an advanced functional material for 3D cell culture, drug delivery system and many other biomedical applications.

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