

**Supporting information to the article**

**Fenton reaction-initiated formation of biocompatible injectable hydrogels for cell  
encapsulation**

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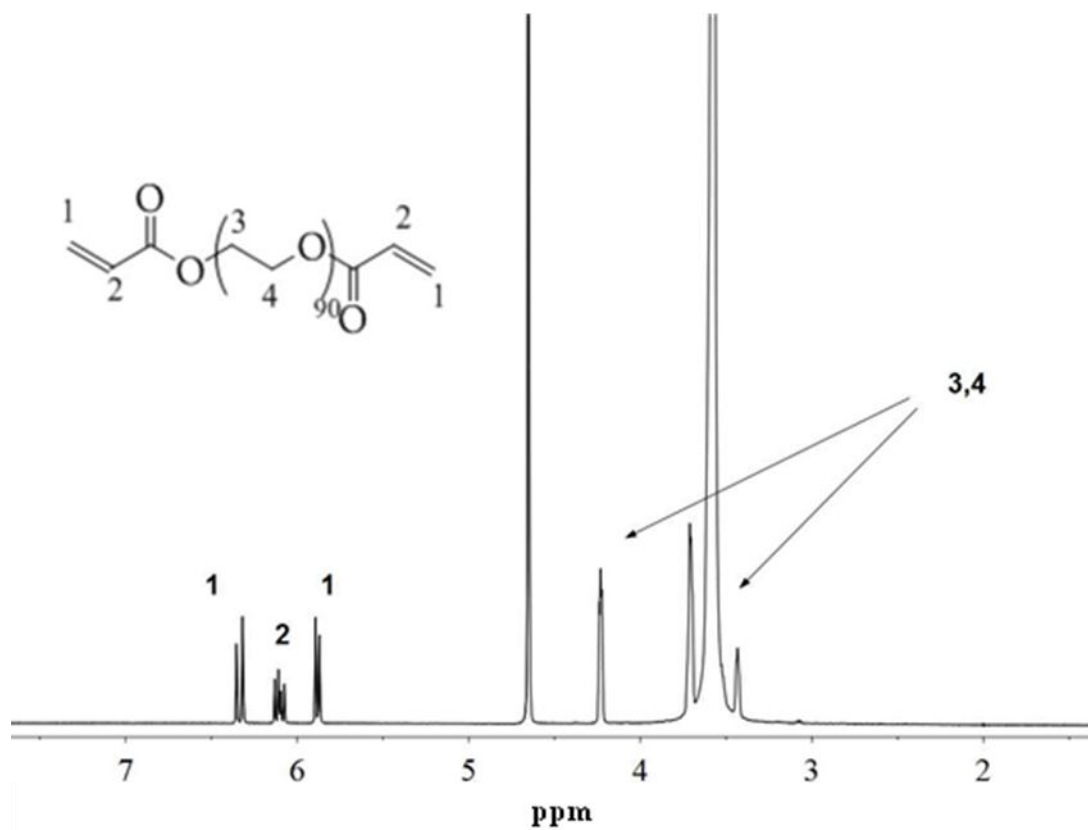


Fig.S1. <sup>1</sup>H NMR spectrum of PEGDA

The degree of substitution of the PEG terminal alcohol for acrylation was determined by <sup>1</sup>H NMR spectrum. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 6.4(2 H, cis CH<sub>2</sub>=CH), 5.83(2 H, trans CH<sub>2</sub>=CH), 4.2(2H, CH<sub>2</sub>=CH-COO-CH<sub>2</sub>), 3.6(4H, CH<sub>2</sub>-CH<sub>2</sub>-O), 4.8(2H, D<sub>2</sub>O). The acrylation substitution, calculated from the integral area, was above 95%.

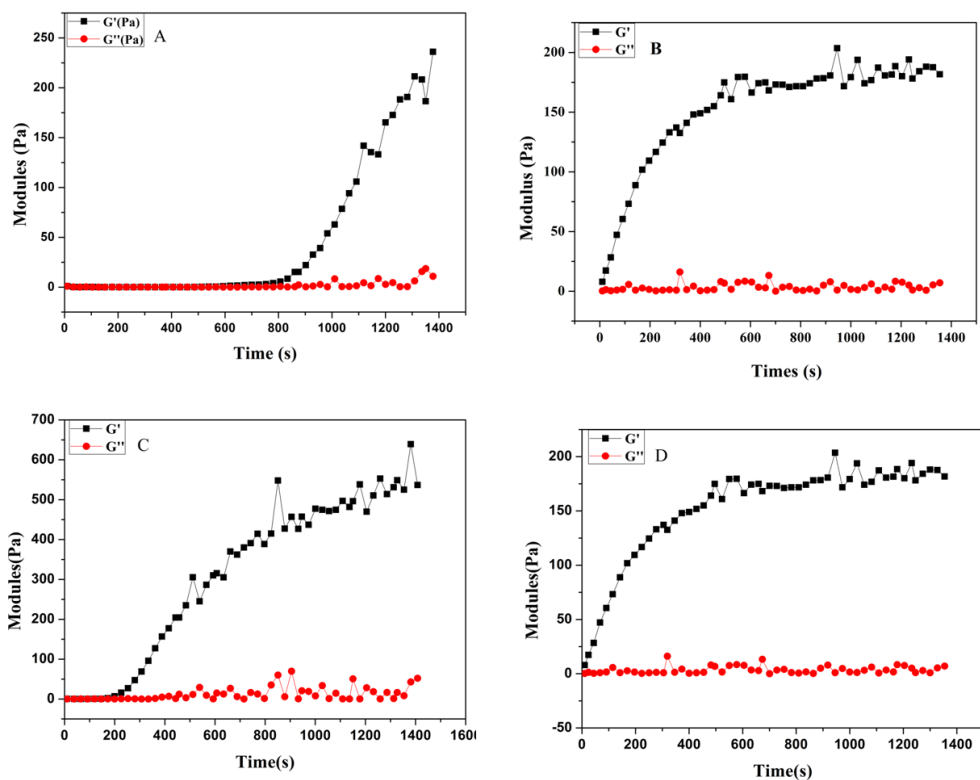


Fig.S2. Storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of HEAA/PEGDA hydrogels (2 wt% HEAA, 1 wt% PEGDA) with different concentrations of Fenton reagents (A: 0.2 mmol/L  $H_2O_2$ ; B: 1 mmol/L  $H_2O_2$ ; C: 1.5 mmol/L  $H_2O_2$ ; D: 2 mmol/L  $H_2O_2$ ).

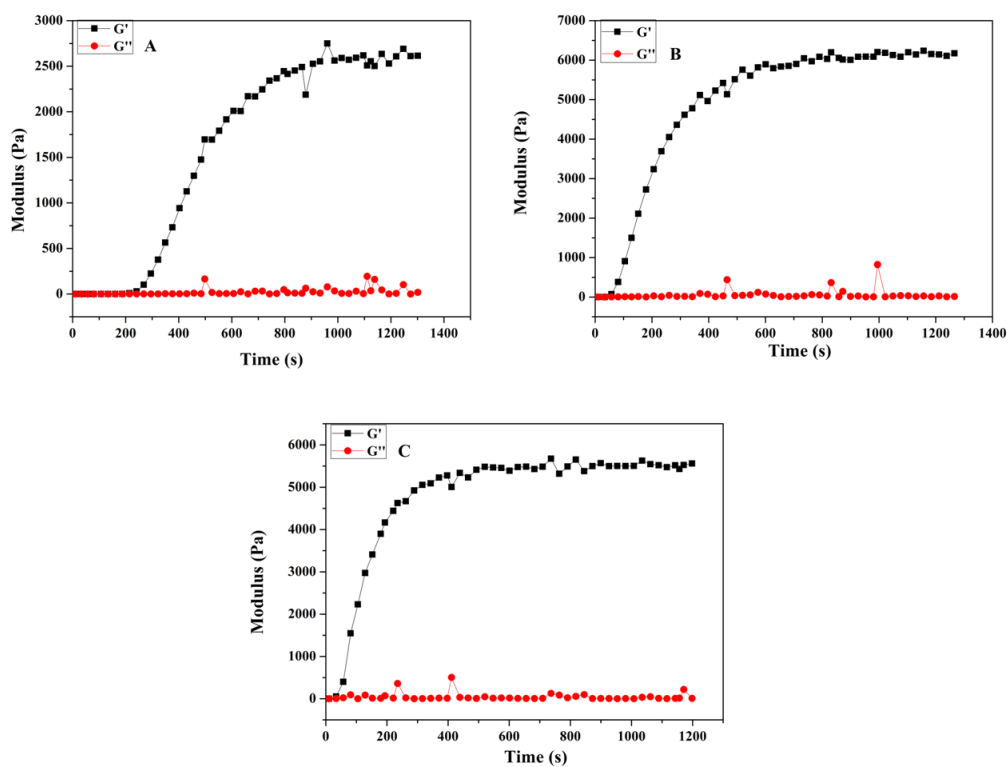


Fig.S3. Storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of HEAA/PEGDA hydrogels (10 wt% HEAA, 1 wt% PEGDA) with different concentrations of Fenton reagents (A: 0.2 mmol/L  $H_2O_2$ ; B: 1 mmol/L  $H_2O_2$ ; C: 1.5 mmol/L  $H_2O_2$ ).

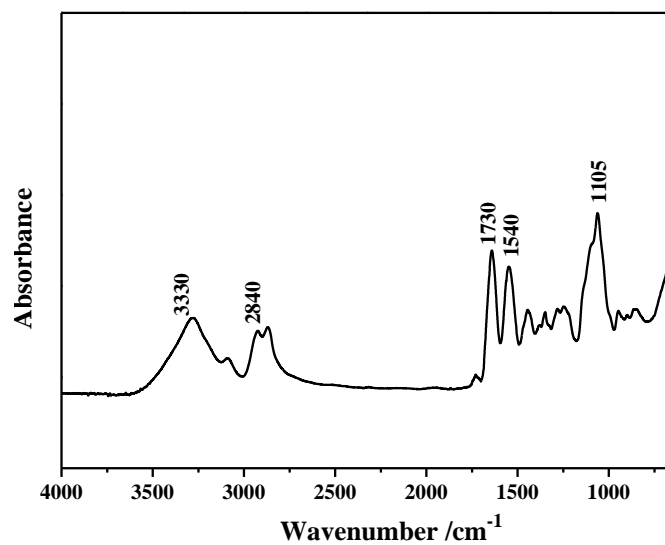


Fig.S4. FTIR spectrum of HEAA/PEGDA hydrogels

Fig.S4 displays the FTIR spectrum of HEAA/PEGDA hydrogel. As shown in the figure, the intense bands at 1105, 1540, 1730 and 3330  $\text{cm}^{-1}$  are separately attributed to stretching vibrations of C-O-C, C-N, C=O and N-H in PEGDA and HEAA. These characteristic peaks confirm the formation of HEAA/PEGDA hydrogel.

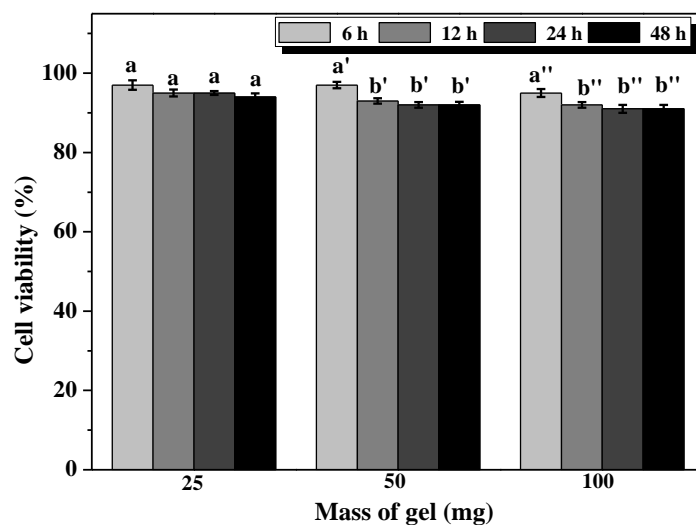


Fig. S5 The cytotoxicity of hydrogel at different time periods. Results were presented as the mean  $\pm$  SD in quadruplicate. The same letters labeled in the figure indicate there was no significant difference and different letters indicated significant difference among the experimental groups.

The HEAA/PEGDA hydrogel was fabricated with the PEGDA (1 wt %) and HEAA (5 wt %) with Fenton reagent ( $\text{H}_2\text{O}_2$  2mmol/L and  $\text{Fe}^{2+}$  0.2mmol/L, which is the same concentration as that used in encapsulation cells.). The different amount of HEAA/PEGDA hydrogel was co-cultured with L929 cells in 48 well plate. At a schedule time, the cytotoxicity was evaluated by MTT assay. The results revealed that the HEAA/PEGDA hydrogel showed low cytotoxicity at all the time period.

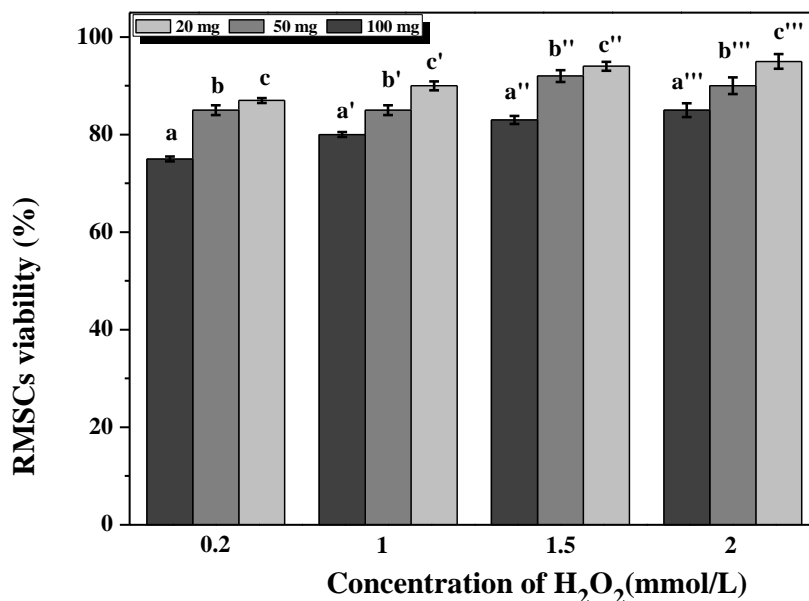


Fig.S6. Cytotoxicity of HEAA/PEGDA hydrogel (HEAA: 5 wt%)

Rabbit bone marrow stromal cells (RMSCs) were harvested from bone marrow aspirates. The cytotoxicity of hydrogel was evaluated by MTT in RMSCs. RMSCs were seeded into a 48-well plate with a density of  $1 \times 10^4$ /well. After 24 h, different mass of hydrogels were added into the well. The viability of cells was determined by MTT assay after 24 h co-culture. Results were presented as the mean  $\pm$  SD in quadruplicate. The same letters labeled in the figure indicate there was no significant difference and different letters indicated significant difference among the experimental groups.

As shown in Fig. S6, when the concentration of H<sub>2</sub>O<sub>2</sub> is 1.5 or 2 mmol/L, the cells have a high viability (>90%) with the different mass of hydrogel (25, 50, 100 mg). The lower concentration of H<sub>2</sub>O<sub>2</sub> leads to relatively higher cytotoxicity, which is attributed to the effect of remaining monomers and initiator. Obviously, the harmful effect of H<sub>2</sub>O<sub>2</sub> is not critical because of the little dose of Fenton reagents, and higher concentration of Fenton reagents accelerated the process of polymerization. The increased mass of gels leads to the higher cytotoxicity. Generally, the viability of cells remains high (>80%) (Table 1). The gelation time increased with the increasing of concentration of Fenton reagents. Water absorption decreased with the increasing of concentration of Fenton reagents.

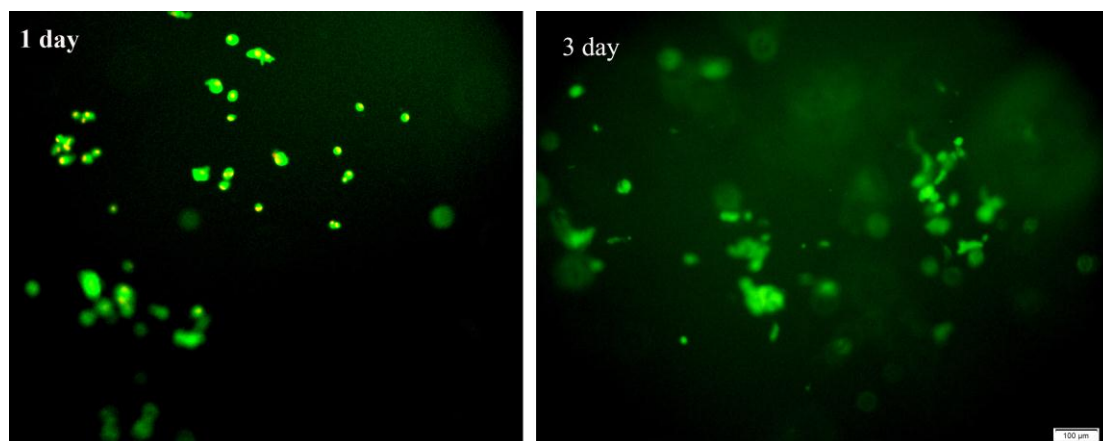


Fig.S7. Fluorescent images RMSCs encapsulated in the hydrogels with the live/dead staining. The live and dead cells were stained green and red, respectively.

RMSCs were encapsulated into the hydrogel in the same method as L929. As shown in the figure, after 1-day culture, only several dead cells were visible. The radical polymerization caused the limited damage to RMSCs. After 3-day culture, the live cells increased obviously and the cell aggregates appeared.



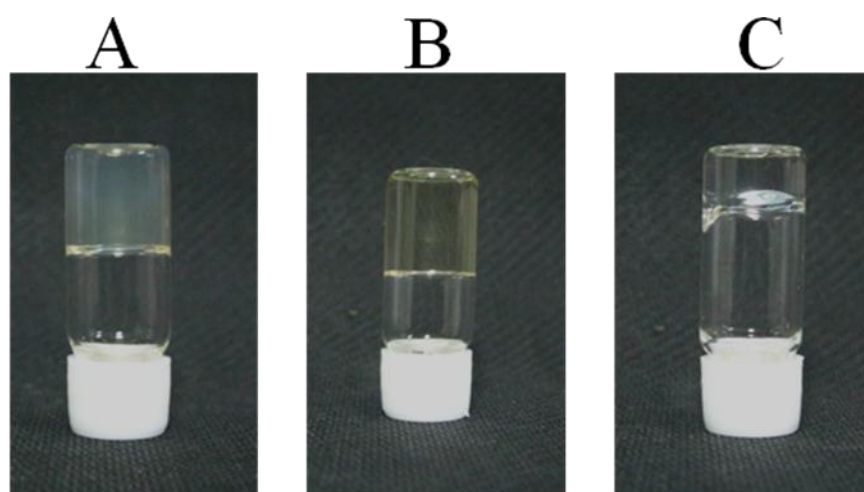


Fig.S8. Appearance of hydrogel ( $\text{H}_2\text{O}_2$  was fixed at 2 mmol/L). A: 5 wt% PEGDA575 only; B: 5 wt% PEGDA575 and 5 wt% acrylic acid; C: 5 wt% PEGDA575 and 5 wt% acrylamide.