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

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A chemical-crosslinked, biocompatible and injectable hydrogel was formed by Fenton reaction initiated polymerization. The gelation time of *N*-(2-hydroxyethyl)acrylamide and PEG-diacrylate, which are two representative monomers, was shown to be tunable from instant to 15 min at an H_2O_2 concentration below several mmol L^{-1} in neutral medium. The strength of the hydrogel could be regulated by the concentration of the monomer and the Fenton's reagent. The hydrogels prepared by H_2O_2/Fe^{2+} initiation showed low cytotoxicity. The bone marrow mesenchymal stem cells and L929 cells encapsulated in the gels exhibited high viability even after 7 days of co-culture. Both the L929 cells encapsulated *in situ* into the hydrogels and those co-cultured with the hydrogel showed negligible cell death and apoptosis. It is anticipated that the familiar Fenton reaction may act as a new initiator system to fabricate biocompatible injectable hydrogels.

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1 Introduction

Hydrogels are formed from hydrophilic polymer chains held together by chemical or physical crosslinks. They are characterized by their ability to uptake large amounts of water while retaining their three dimensional structure. A variety of physical and chemical methods have been used to fabricate biomedical hydrogels. Physical-crosslinking always results in hydrogels with poor mechanical properties. Chemical crosslinked hydrogels which are relatively strong and have stable mechanical properties have been widely used.[1–6] Chemical crosslinking between soluble precursor materials *in situ* can be achieved through a variety of chemical processes including Michael type addition,[7,8] enzyme catalysis,[1,9] photo-intiation,[10,11] and click reactions.[12–14] Although photo-initiation is most frequently employed for the generation of cell encapsulated hydrogels, photo-initiation is limited by cure depth and difficulty of making the injectable gel. An injectable system is a low viscous aqueous solution or suspension before administration, which can be solidified in the body *via* either chemical crosslinking or physical association. Gel formation after injection brings about some advantages: an injectable hydrogel can be implanted in the human body with minimal surgical wounds, and bioactive molecules or cells can be incorporated simply by mixing before injection. Following gelation, these matrices can become drug delivery deposits in pharmaceutics or cell-growing depots for tissue regeneration. In addition, *in situ* cell encapsulation is also beneficial for filling an irregular defect.[5,15] Redox systems generating free radicals under mild conditions have been employed to initiate the crosslinking process, including the redox pair of hydrogen peroxide and ferrous salt (Fenton's reagent).[16] A Fenton system involving hydrogen peroxide and ferrous salts can generate highly reactive hydroxyl radicals (as shown in the equation below):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \dot{O}H$$

Fenton discovered in 1894 that several metals have special oxygen transfer properties which could improve the use of hydrogen peroxide. Nowadays, Fenton's reaction is been used to treat water pollution.[17,18] Also, Fenton's reagent has been used as a radical initiator in vinylic polymerization or grafting for more than 50 years.[19,20] A hydrogel from poly(*N*-vinyl-2-pyrrolidone) aqueous solution was produced by Fenton's reagent, and the hydrogels prepared by H_2O_2/Fe^{2+} did not impose any toxic or irritating effects in a dermal inflammation test in rabbits.[4] In another research work, poly(vinyl alcohol) (PVA) hydrogels were initiated by ferrous salt and hydrogen peroxide, and the gels obtained demonstrated unique and advantageous drug release profiles compared with UV cured PVA hydrogels.[21]

Although toxicity tests for Fenton-initiated hydrogels revealed no toxic effects, there have been no reports on cell encapsulation with injectable hydrogels initiated by Fenton's reagent. There could be several reasons for this. Firstly, the Fenton reaction is generally carried out with H_2O_2 and Fe^{2+} in acidic aqueous solution.[22] It was reported that the optimum conditions in PVP hydrogels have been determined as pH = 2.5.[4] Second, the

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toxicity of the oxidative components is the biggest concern to fabricating a cell-loaded hydrogel, and the optimum concentration of H_2O_2 was found to be 50 mmol L^{-1} to construct the hydrogels in previous research.[4] These harsh requirements are unsuitable for fabricating biocompatible injectable hydrogels for cell encapsulation. Alternatively, many researchers have tried to use glucose oxidase (GOD) or horseradish peroxidase (HRP) to react with H_2O_2.[1,23–25] Nevertheless, the stability and repeatability were of big concern in the enzyme catalysed reaction.[26,27] Third, the Fe^{3+} produced in the Fenton reaction may bring about an opaque yellow appearance.

Previous work demonstrated that both H_2O_2 and Fe^{2+} show no cell toxic effects at initial concentrations below 5 mmol L^{-1} and 0.5 mmol L^{-1}, respectively.[28,29] This cytocompatibility motivated us to fabricate cell-loaded injectable hydrogels initiated by Fenton's reagent. In this study, we reported biocompatible injectable hydrogels (Fig. 1) for encapsulating cells by the Fenton reaction under benign conditions. *N*-(2-Hydroxyethyl) acrylamide (HEAA) and PEG-diacrylate (PEGDA) could polymerize into robust and transparent hydrogels quickly in a neutral medium with an H_2O_2 concentration below several mmol L^{-1}. Several other hydrogels such as PEGDA only, acrylic acid/PEGDA, and acrylamide/PEGDA were used successfully to construct the injectable hydrogels with Fenton's reagent. Additionally, the strength and gelation time of the hydrogels could be controlled by varying the concentration of the monomers and H_2O_2. FDA approved Fe^{2+} and H_2O_2 are much cheaper and easily accessible than enzymes and photo-initiators. All these features of the Fenton reaction will meet the requirements of a broad range of applications in the biomedical field.

2 Materials and methods

2.1 Materials

Hydrogen peroxide (30%), sodium chloride and ferrous chloride were obtained from Tianjin Guangfu Fine Chemical Research Institute. *N*-(2-Hydroxyethyl)acrylamide (HEAA), poly(ethylene glycol) (PEG, M_w 4000 Da) and acryloylchloride were purchased from Sigma-Aldrich. Annexin V-FITC Apoptosis Detection Kit was purchased from Beytime (Nanjing, China). Murine fibrosarcoma L929 cells were obtained from Peking Union Medical College (Beijing, China). Hydrogen peroxide was freshly prepared before use.

2.2 Preparation of PEG-diacrylate (PEGDA)

PEGDA4000 was synthesized as in the method in ref. 30. Briefly, PEG (2.5 mmol) and triethylamine (20 mmol) were dissolved in dichloromethane. Acryloylchloride (13.6 mmol) was added to the flask dropwise at 0 °C under nitrogen. Then, the reaction was allowed to proceed at room temperature for 30 h. After that, an excess amount of diethyl ether was added into the solution to precipitate the product which was dialyzed and lyophilized.[31] The PEGDA was characterized by nuclear magnetic resonance (^{1}H-NMR, Varian INOVA 500 MHz) to confirm that the acrylation substitution was above 95% (Fig. S1†).

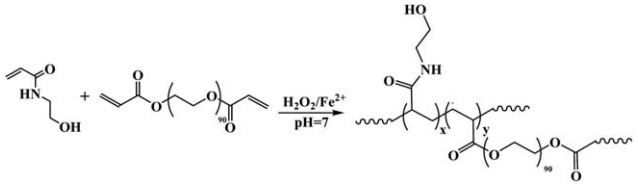

Fig. 1 Schematic diagram of the hydrogel.

2.3 Fabrication of HEAA/PEGDA hydrogel

PEGDA (1 wt%) and different concentrations of HEAA (2 wt%, 5 wt%, 10 wt%) were prepared in NaCl (0.9 wt%) solution. In this work, the molar ratio of $H_2O_2/FeCl_2$ was fixed at 10 : 1, which was suggested as the optimum efficiency.[4] The concentration of PEGDA was fixed at 1 wt%. It is noted that in the following description, only the ultimate concentration of H_2O_2 in the mixed solution is stated. The HEAA/PEGDA hydrogel was fabricated using the following procedures. H_2O_2 (1 μmol) was added into 0.5 mL solution (PEGDA: 1 wt%, HEAA: 2 wt%). Similarly, $FeCl_2$ (0.1 μmol) was also added into an equivalent solution. Then, these two solutions were mixed together and gelled. The gelation time was determined through physical observation, and recorded when a solid mass was formed. The gels were swollen in PBS at 37 °C for 48 h and the sol fraction was determined.

2.4 Characterization of the hydrogel

The oscillatory shear measurements were performed on a stress-controlled rheometer Relogica Instruments AB using a bob-cup geometry. In each measurement 0.6 mL of a polymer solution was loaded into the bob-cup by a micropipette. The scanning interval was 30 seconds. The storage moduli (G') and loss moduli (G'') of the polymer solutions were measured as a function of time, at a constant angular frequency of 0.8 rad s^{-1} and shear strain of 0.2 at 37 °C.[32] Concisely, solutions with varying compositions of HEAA/PEGDA and initiator (H_2O_2/Fe^{2+}) were formulated, vortexed and loaded in the bob-cup by a micropipette. Once the combined solution was loaded, a time sweep test was conducted during the crosslinking reaction of the HEAA and PEGDA, to measure the reaction kinetics and change in modulus of the hydrogel with time. Time sweeps were conducted at pre-determined parameters within the linear viscoelastic region of these materials. Complete gelation was considered to have occurred when G' had increased to a platform.

After polymerization, 1 mL of the hydrogel was immersed in 4 mL of water for 24 h. The residual concentration of H_2O_2 at different times was measured by a Quantofix Pweoxid 25 (MACHEREY-NAGEL Company, LOT: 319302).

Fourier transform infrared (FTIR) spectra of the dry hydrogel were recorded on a PerkinElmer spectra 100 (USA).

The compression tests of the hydrogels were measured on a WDW-05 electromechanical tester (Time Group Inc., China) at room temperature. The crosshead speed was set at 10 mm min^{-1}. The samples were cut into cylinders (9.0 mm in diameter and 9.0 mm in height), and three specimens were tested for each group.

The equilibrium water content was measured according to the previously published protocol.[33] The gel fraction was defined as the ratio of dry mass of the hydrogel to the feed quantity.

2.5 Cytotoxicity

L929 cells were seeded into a 48-well plate with a density of 8×10^4 per well. After 24 h, different masses of the hydrogels were added into the wells. The cell viability was determined by MTT assay after 24 h of co-culture.

2.6 Cell encapsulation *in situ*

The solution (HEAA, PEGDA) was sterilized with a 0.22 μm filter. 0.5 μmol H_2O_2 was added into 0.125 mL HEAA and PEGDA as solution A. 0.05 μmol $FeCl_2$ was added into another identical mixture as solution B. The L929 cells (2.5×10^5) were first suspended in solution B, following by quick addition of solution A. Then, the two component solutions were mixed together. A firm and transparent hydrogel encapsulating the cells was formed within 1 min.

After a scheduled culture time, the cell viability in the hydrogel was imaged by a fluorescence microscope (Olympus, CKX41) after use of a live/dead staining kit (CFSE, Dojindo Laboratorise, Japan).

The L929 cell apoptosis was characterized by an Annexin V-FITC Apoptosis Detection Kit after encapsulation in the HEAA/PEGDA hydrogels for 8 h.

Flow cytometry is always used to characterize the cells in solution, and it is very difficult to separate the cells encapsulated in the hydrogel. In an alternative model, the L929 cells co-cultured with different amounts of HEAA/PEGDA hydrogel (fabricated with the same formulation as that used for the encapsulation of the cells) were stained with Annexin V-FITC/PI and evaluated by flow cytometry.

2.7 Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was performed using the two population Student's *t*-test. The level of significance was set as $p < 0.05$.

3 Results and discussion

3.1 Gelation kinetics

Rheometry was employed to analyze the gelation kinetics. The storage moduli (G') and loss moduli (G'') of the hydrogels are shown in Fig. 2 and 3 and Fig. S2 and 3.† The gelation time (defined as the point at which G' crosses G'') decreases as the amount of H_2O_2 is increased. The gelation time is a significant factor to consider in the design of injectable hydrogels for cell encapsulation. Faster gelation rates could contribute to the efficiency of encapsulation.[34] However, it is impractical for the surgeon if the kinetics of gelation are too fast for operation. As shown in Fig. 2, the time of the sol–gel transition increases with a decreasing H_2O_2, ranging from several seconds to 15 min. The time is also dependent on the monomer concentration. At the same monomer concentration, the gelation time also has a wide range within 15 min. These two factors can achieve the goal of tailoring the time of the sol–gel. Movie 1 (ESI†) exhibits the sol–gel transition of the monomer solution with concentrations of H_2O_2 of 0.2, 1 and 1.5 mmol L^{-1}, and matches well with Fig. 2. The solution can gel instantly while the concentration of H_2O_2 is above 1.5 mmol L^{-1}, but the color of the hydrogel changes from light to deep yellow upon increasing the concentration of Fenton's reagent (Fig. 4).

The above results indicate that both the H_2O_2 and HEAA concentrations affect the final elastic modulus of the gels. The storage modulus of the hydrogels varies from 0.2 to 6 kPa. The time to reach the final modulus can be tuned from 5 to 23 min, which is an appropriate time period for manipulation by researchers or surgeons. The gelation time for these hydrogels needs to be within a clinically feasible time scale so that it is slow enough to be able to deliver the hydrogels without premature polymerization in the delivery device, and meanwhile fast gelation is expected once it is placed in the body. Although injectable hydrogels have great potential in the biomedical field, different bio-applications, such as injectable embolizing agents,[35] vitreous substitutes,[36] and heart-failure post-myocardial infarction models *via* a catheter,[37] have different clinically acceptable times and mechanical properties. In our work, by controlling the narrow-range of concentration of Fenton's reagent, diverse physical properties and a wide range of sol–gel times could be tailored.

The compression strength ranged from 500 Pa to 0.8 MPa and could be controlled by varying the concentration of HEAA and Fenton's reagent (Fig. 5). With a constant concentration of HEAA and PEGDA, the compression strength increased incrementally with initiator concentration (1, 1.5 and 2 mmol L^{-1}). The results suggest that the polymerization process was more completed when the amount of initiator was increased. In the whole range of Fenton's reagent concentration, the highest compression strength was achieved when the concentration of HEAA was 10%. When the concentration of HEAA was over 15%, the hydrogel became brittle due to a high crosslink density. Meanwhile, the HEAA/PEGDA hydrogels could recover from 75% of the deformation for all the formulations. This ability to withstand larger amounts of deformation suggests their potential application in medicine.

As shown in Table 1, the HEAA/PEGDA hydrogel showed a high water content with water absorption more than 95%. The water absorption decreased slightly with an increasing concentration of Fenton's reagent. The efficiency of gelation is another important parameter. The gel fraction increased with the concentration of initiator (Table 1). The gel fraction was less than 90% when the concentration of H_2O_2 was below 1.5 mmol L^{-1}, while it increased to 95% at a concentration of 2 mmol L^{-1}.

3.2 Cytotoxicity of the hydrogels

To evaluate the cytotoxicity of the HEAA/PEGDA hydrogel, different amount of gels (25, 50 and 100 mg) initiated by different amounts of Fenton's reagent were co-cultured with L929 cells. As shown in Fig. 6, the cells had high viability (>90%) while the concentration of H_2O_2 was 1.5 or 2 mmol L^{-1}.

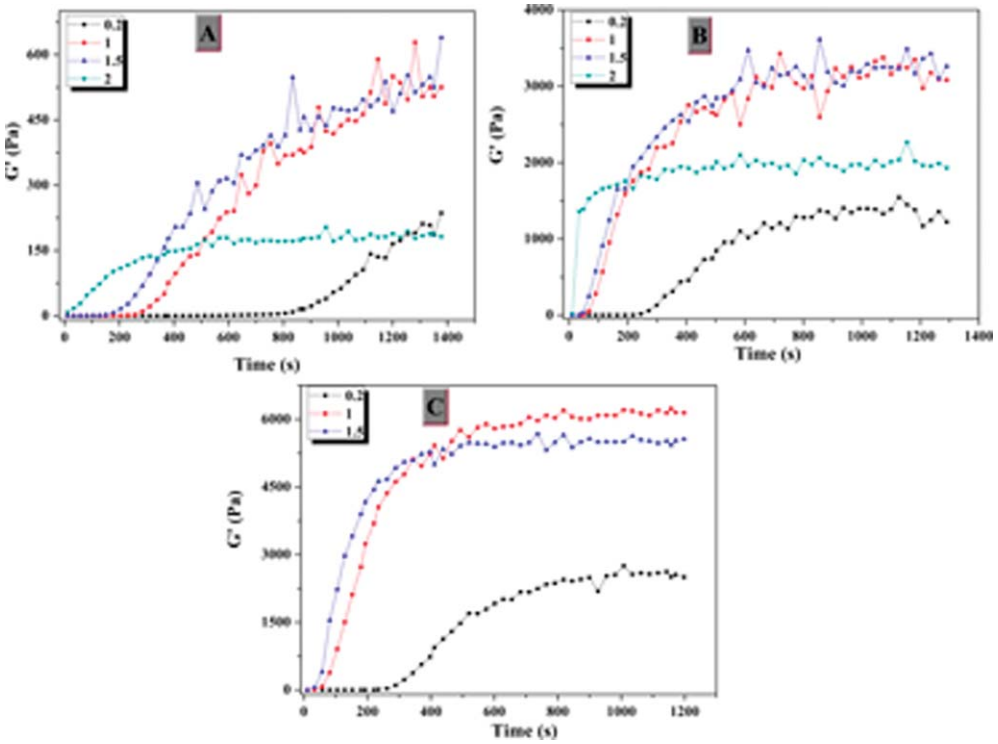

Fig. 2 Rheological records of the hydrogels. Storage modulus of hydrogels of 2% HEAA (A), 5% HEAA (B) and 10% HEAA (C) were measured by rheometer. Different lines represent the different concentrations of H_2O_2. A line could not be record in the (C) graph, because the hydrogel was formed instantaneously at 10% HEAA with 2 mmol L^{-1} H_2O_2.

Interestingly, the cell cytotoxicity of the hydrogels was decreased when the amount of H_2O_2 was increased. The relatively higher cytotoxicity caused by a low concentration of H_2O_2 may be attributed to the effect of the remaining monomers (Table 1 proves the speculation). A higher concentration of Fenton's reagent could accelerate the process of polymerization and result in more complete polymerization. This cytotoxicity result reveals that the harmful effect of H_2O_2 was not critical because of the trace residual amount of H_2O_2 in the hydrogel after polymerization. However, the increased mass of the gels led to a slight increase in the cytotoxicity. Generally, the viability of the cells remained high (>80%).

The cytotoxicity of the HEAA/PEGDA hydrogel was further evaluated with a different time period. The HEAA/PEGDA hydrogel was fabricated with the same formulation as used for the encapsulation of the cells. A different amount of the HEAA/PEGDA hydrogel was co-cultured with L929 cells in a 48 well plate. At a scheduled time, the cytotoxicity was evaluated by MTT assay (Fig. S5†). The results revealed that the HEAA/PEGDA hydrogel showed low cytotoxicity after all the time periods. The residual amount of H_2O_2 in 1 mL HEAA/PEGDA hydrogel was 1×10^{-7} mol. There was only 1×10^{-8} mol H_2O_2 per 100 mg hydrogel, which had little effect on the cell viability as shown in the MTT.

The HEAA/PEGDA hydrogel initiated by Fenton's reagent showed slight cytotoxicity, which was consistent with the reported PVP and PVA hydrogels.[4,21] In those research studies, ferrous gluconate dehydrate (0.4 mg mL^{-1}) and H_2O_2 (1.8 vol%) were used to initiate acrylated PVA to fabricate hydrogel and showed moderate cell growth inhibition, the amounts of which were two orders of magnitude greater than those in this study.[38] The concentration of H_2O_2 (50 mmol L^{-1}) and pH = 2.5 employed to fabricate the PVP hydrogel showed no cytotoxicity either.[4]

3.3 Cell encapsulation in the hydrogel

Free radical polymerization is commonly used to grow polymer chains from monomer units using initiators. However, cell membranes or proteins are also subjected to oxidative damage by high-energy radicals, leading to cell death and apoptosis for *in situ* cell encapsulation. In order to avoid cell damage from the H_2O_2 and residual monomers, 2 mmol L^{-1} H_2O_2 was used.

The L929 cells (1×10^6 per mL) encapsulated in the HEAA/PEGDA hydrogel were stained by live/dead staining after a period of culture. More than 90% of the cells were alive after 1 day (Fig. 7), indicating that the process of polymerization did not cause much damage to the cells. After 7 days of culture, live cell aggregates were observed in the hydrogels (Fig. 7).

To demonstrate the biocompatibility and explore the applications of the injectable hydrogel, rabbit bone marrow stromal stem cells (RMSCs) were encapsulated in the hydrogel. The HEAA/PEGDA hydrogel showed a similar cytotoxicity to that with the L929 cells, shown in Fig. S6.† The viability of the encapsulated cells in the hydrogel was observed by the method mentioned above. Fig. S7† shows the RMSCs encapsulated in

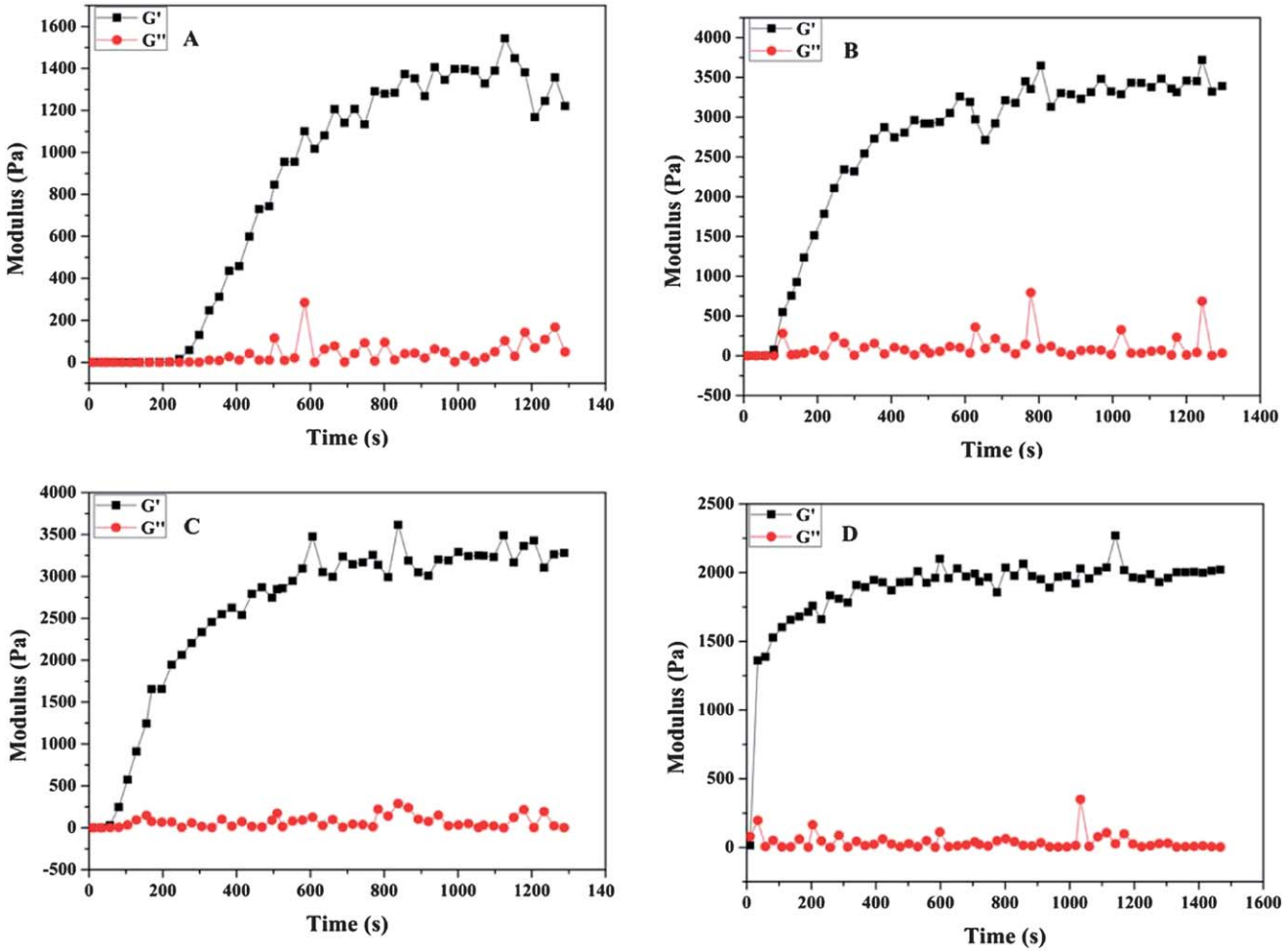

Fig. 3 Storage modulus (*G'*) and loss modulus (*G''*) of HEAA/PEGDA hydrogels (5 wt% HEAA, 1 wt% PEGDA) with different concentrations of Fenton's reagent ((A) 0.2 mmol L^{-1} H_2O_2; (B) 1 mmol L^{-1} H_2O_2; (C) 1.5 mmol L^{-1} H_2O_2; (D) 2 mmol L^{-1} H_2O_2).

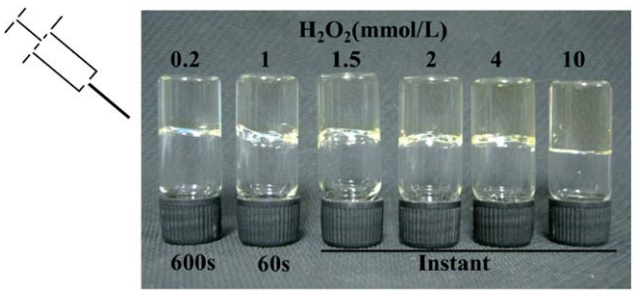

Fig. 4 Appearance of the hydrogels with different amounts of H_2O_2 (mmol L^{-1}).

the HEAA/PEGDA hydrogel after 1 and 3 days. After 3 days, the RMSCs displayed high viability.

H_2O_2 is commonly used to evoke cell apoptosis. It was reported that more than 80% of human lung carcinoma cells were killed after 24 h of exposure to 200 μmol L^{-1} H_2O_2.[39] We found that 27% of cells were apoptosis evoked by 100 μmol L^{-1} H_2O_2 for 1 h. Herein, 2 mmol L^{-1} H_2O_2 was employed to initiate the polymerization, so the cell apoptosis caused by the HEAA/PEGDA hydrogel was a big concern for use of the hydrogel in the

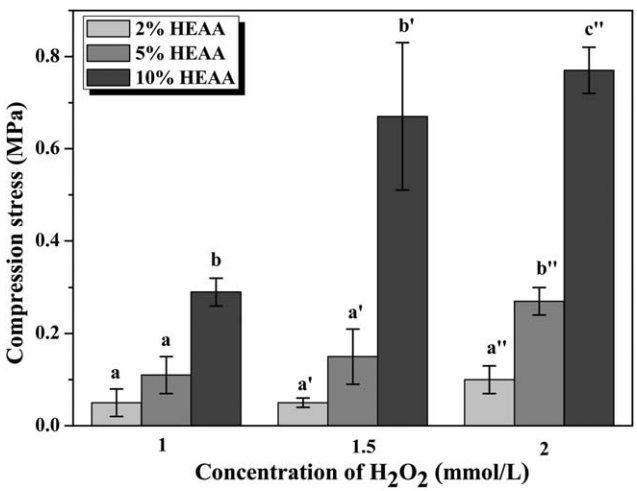

Fig. 5 Compression strength of HEAA/PEGDA hydrogel formed at different concentrations of Fenton's reagent and HEAA. Results are presented as mean ± SD in quadruplicate. Labels of the same letters indicate that there was no significant difference and different letters indicate a significant difference between the experimental groups.

Table 1 Water absorption and gel fraction of the HEAA/PEGDA hydrogel

Concentration of H_2O_2 (mmol L^{-1})	Water absorption (%)	Gel fraction (%)
0.2	98.1 ± 0.3	80.1 ± 0.7
1	97.4 ± 0.2	89.2 ± 0.5
1.5	97.2 ± 0.2	93.4 ± 0.4
2	96.9 ± 0.2	95.1 ± 0.5

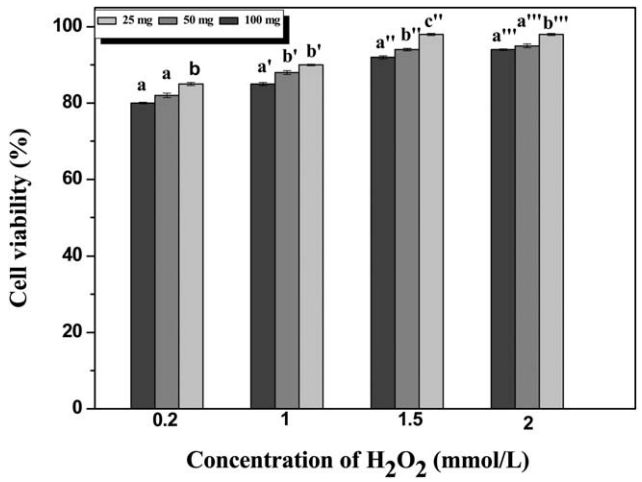

Fig. 6 Cytotoxicity of the hydrogels to L929 cells estimated by MTT assay. Results are presented as mean ± SD in quadruplicate. Labels of the same letters indicate that there was no significant difference and different letters indicate a significant difference between the experimental groups.

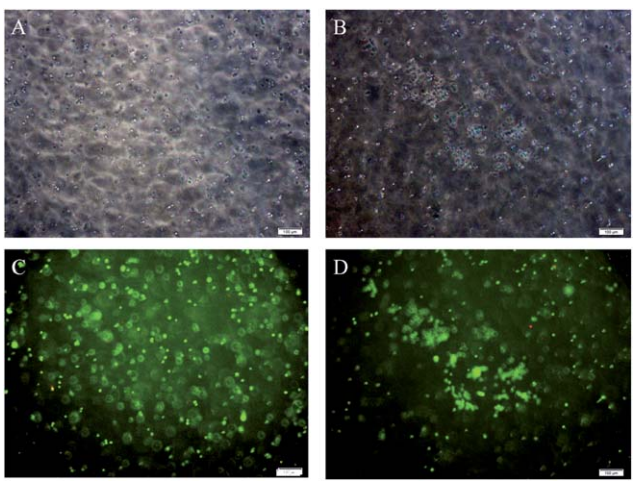

Fig. 7 Microphotograph of L929 cells encapsulated in the HEAA/PEGDA hydrogels after 1 (A and C) and 7 (B and D) days. A and B are the light contrast images, while C and D are the fluorescent images after the live/dead staining by CFSE and PI. The green dots indicate the living cells, while the red dots indicate the dead cells.

biomedical field. The cell apoptosis after encapsulation *in situ* into the hydrogels was characterized by an Annexin V-FITC Apoptosis Detection Kit. Fig. 8 shows that there were negligible apoptotic and dead L929 cells encapsulated in the hydrogels.

Flow cytometry is always used to characterize the cells in solution, and it is very difficult to separate the cells encapsulated in the hydrogel. In an alternative model, the L929 cells cultured with different amounts of the hydrogel were stained with Annexin V-FITC/PI and evaluated by flow cytometry (Fig. 9). The results show that there was only 1–3% apoptotic and dead L929 after 24 h co-culture.

Recently, the hydrogel with cells encapsulated with the enzyme mediated system was investigated. Enzyme-mediated initiation systems involve oxidase enzymes that produce H_2O_2 for rapidly polymerizing hydrogels. Park *et al.* fabricated an injectable hydrogel catalyzed by HRP–H_2O_2. The hydrogel was fabricated at 0.0025 mg mL^{-1} HRP and 0.025% H_2O_2 (about 7 mmol L^{-1}). The hydrogels injected subcutaneously into the backs of mice were shown to be biocompatible.[24] With the HPR, the concentration of H_2O_2 was 3.5 times higher than that used in our study. In the GOD initiator system, H_2O_2 was replaced by GOD and glucose. Ferrous ions (1.25 mmol L^{-1}), were employed according to the quantity of H_2O_2 produced by GOD and glucose to fabricate the cell encapsulation hydrogel, which demonstrated a high cellular viability.[23] The amount of ferrous ions (0.2 mmol L^{-1}) used in this research was much lower than in the GOD system, which may indicate indirectly that the concentration of H_2O_2 was much lower than that of the GOD system. The lower concentration of Fenton's reagent can be applied safely in a wider range of tissue engineering applications.

A cell encapsulation hydrogel requires biocompatible, crosslinked and high molecular weight macromers, typically with a molecular weight greater than 3 kDa.[40] However, small molecule monomer HEAA and macromonomer crosslinker PEGDA4000 were employed to fabricate the cell-loaded injectable hydrogel in this research. The low viscosity of the feed solution could be achieved by mixing the small molecule monomer, which allowed a more uniform cell distribution and rapid controllable injection process. The feed solution may form a firm hydrogel according to the needs of the situation. However, this small molecule monomer HEAA may bring about slight cytotoxicity. In order to explore more applications, several other hydrogels such as PEGDA only, acrylic acid/PEGDA, and acrylamide/PEGDA were successfully used to construct the injectable hydrogel with Fenton's reagent (Fig. S8†), and the gelation time and mechanical properties of hydrogel were all controllable and tailorable.

Overall, these results demonstrate the first use of the Fenton's reagent radical initiation system for the generation of biocompatible hydrogel for encapsulation of L929 cells and RMSCs with high cellular viability and negligible cell apoptosis. In these studies, the Fenton-mediated cellular encapsulation was performed at ambient temperature. Given the rapid and predictable nature of the initiation, the Fenton system may offer benefits for additional biomedical applications.

4 Conclusion

In this study, the familiar Fenton reaction was employed for the first time as an initiator system to fabricate injectable hydrogels

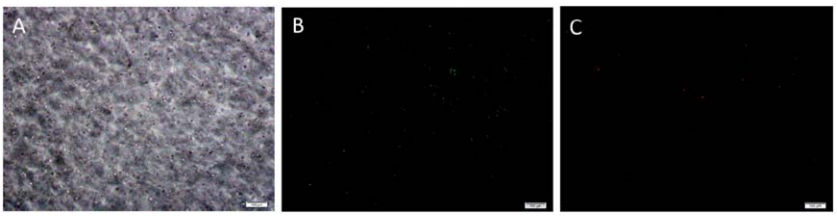

Fig. 8 Fluorescent images of the Annexin V-FITC/PI stained L929 cells encapsulated in the HEAA/PEGDA hydrogels after 8 h. A is the light contrast image, while B and C respectively show the green and red fluorescence after staining with Annexin V-FITC and PI. The green dots indicate the apoptosis cells, while the red dots indicate the dead cells.

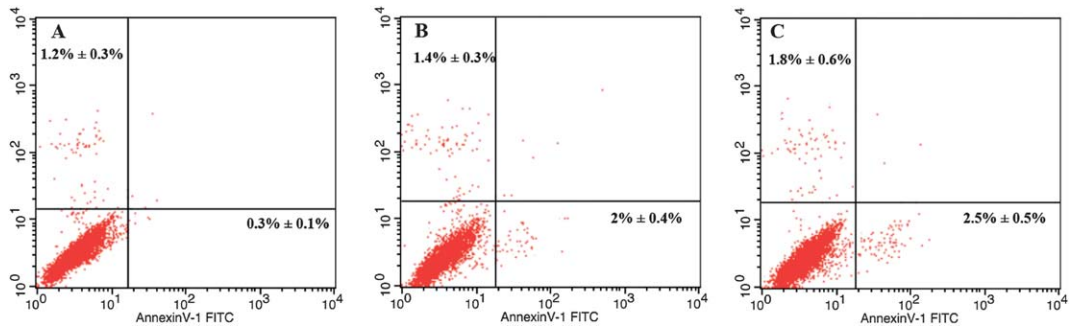

Fig. 9 The L929 co-cultured with the hydrogel after 24 h staining by Annexin V-FITC/PI evaluated by flow cytometry. The cells in the upper left quadrant were positively stained with PI, indicating the dead cells, and those in the right lower quadrant were positively stained with Annexin V-FITC, indicating the apoptotic cells. The data in the different quadrants show the corresponding statistical data.

to encapsulate seed cells. The robust and transparent hydrogels could be formed under benign conditions. The gelation time could be conveniently adjusted by H_2O_2 at several mmol L^{-1} concentrations. The strength of the hydrogel could be regulated by the concentration of the monomer and Fenton's reagent. The hydrogel only imposed slight cytotoxicity and apoptosis on L929 cells. The RMSCs and L929 cells maintained a good activity after encapsulation into the hydrogels. It is our belief that the Fenton reaction will offer a facile method to fabricate a biocompatible injectable hydrogel for cell encapsulation.

Conflict of interest

The authors declare no competing financial interest.

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