

Hydrogels of Chemically Cross-linked and Organ-metallic Complexed Interpenetrating PEG Networks*

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Abstract The aim of the present work was to prepare a well-defined hydrogel of chemically cross-linked and organ-metallic complexed interpenetrating PEG networks. The hydrogel was synthesized *via* the reaction of copper(I)-catalyzed 1,3-dipolar azide-alkyne cycloaddition (CuAAC) with poly(ethylene glycol)-dopamine (PEG-DA) (“Click Chemistry”) followed by complexation with Fe³⁺ ions to crosslink the polymeric network. The chemical composition and morphology of the resulting hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR), ¹H-NMR and scanning electron microscopy (SEM). Swelling ratio, mechanical strength, conductivity, and degradation behaviors of the hydrogels were also studied. The effect of the polymer chain length on properties of hydrogels was explored. The compressive strength of hydrogels could reach as high as 13.1 MPa with a conductivity of $2.2 \times 10^{-5} \text{ S} \cdot \text{cm}^{-1}$. The hydrogels also exhibited excellent thermal stability even at a temperature of 300 °C, whereas degradation of the hydrogel after 7 weeks was observed under a physiological condition. In addition, the hydrogel exhibited a good biocompatibility based on its *in vivo* performance through an *in vivo* subcutaneous implantation model. No inflammation and no obvious abnormality of the surrounding tissue were observed when the hydrogel was subcutaneously implanted for 2 weeks. This work is a step towards creating a new pathway to synthesize hydrogels of interpenetrating networks which could be of important applications in the future research.

Keywords: Hydrogel; Interpenetrating network; Dopamine; Click chemistry; Ions complexation.

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INTRODUCTION

Hydrogel could be regarded as a cross-linked polymeric network, which can absorb and retain a large amount of water without dissolving^[1–3]. Because of their high degree of similarity to biological tissues, high permeability to nutrients and metabolites^[4–6], hydrogels are extensively studied for their potential usages in controlling drug release, as tissue engineering scaffolds, organ transplants, and other medical devices as well^[7–10]. Applications of hydrogels of single network (SN) have been limited for their multiple barriers, such as poor mechanical strength, biocompatibility, and responsiveness *etc.*^[11–13]. As a result, copolymerization^[14, 15], and blending^[16] have been

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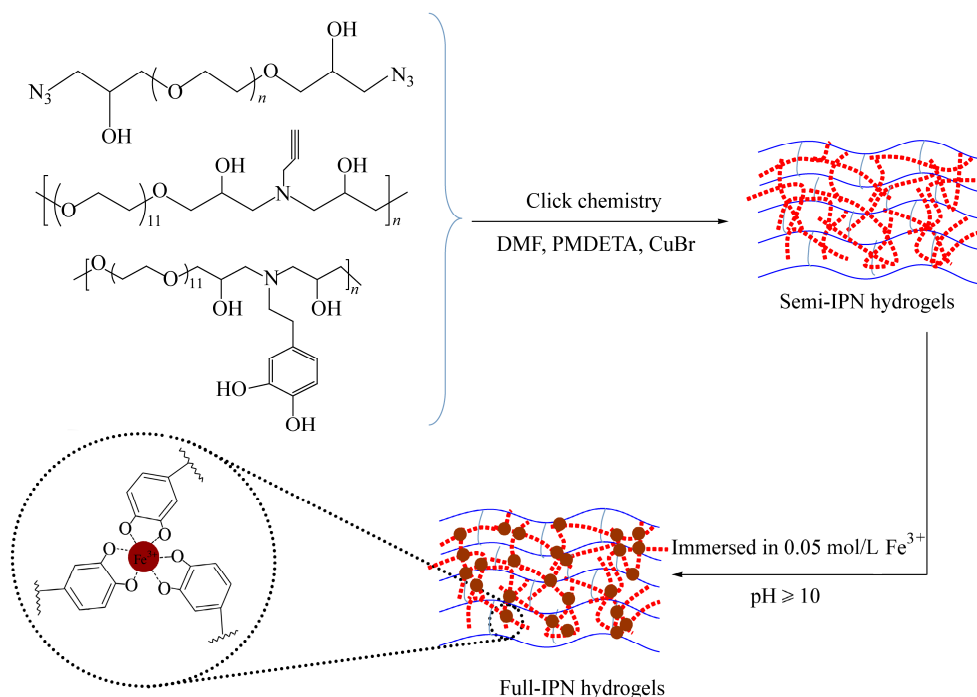
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frequently used to improve the properties of those traditional hydrogels.

Interpenetrating network (IPN) is a unique type of polymer blends. It is an interwoven network consists of two or more crosslinked polymers interpenetrating into each other^[17, 18]. It opens up new ways for the preparation of polymeric materials with special properties. Hydrogels based on IPN were formed by physically or chemically cross-linked bonds including full-interpenetrating network (full-IPN) and semi-interpenetrating network (semi-IPN)^[19]. IPN hydrogels with high swelling ratio and mechanical strength were already described in many literatures. For example, an IPN hydrogel composed of poly(vinyl alcohol) (PVA) and chitosan was prepared by Kim, and the resulting hydrogel exhibited a high swelling ratio^[20]. Myung *et al.* presented a systematic development of mechanically enhanced IPN hydrogels that were formed by synthesis of a crosslinked poly(acrylic acid) (PAA) network within an end-linked poly(ethylene glycol) (PEG) macromonomer network^[21]. IPN hydrogels prepared by ATRP and click chemistry reported by Fu *et al.* gave a new direction for preparing IPN hydrogels^[22]. However, semi-IPN is a way of blending two polymers where only one is cross-linked in the presence of another to produce a mixture. Moreover, the non-cross-linked macromolecules will shift out when being immersed in a solution, resulting in a weaker mechanical strength^[23, 24].

Physical cross-linking is one of the most commonly used methods in the synthesis of hydrogels^[25–27]. Physical hydrogels are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding or hydrophobic forces, which render them unique properties. Especially, physical hydrogels which are formed by ion-cross-linking, may degrade, eventually disintegrate and dissolve. Hence they are used extensively as drug and cell carriers, and as tissue engineering matrices. All of these interactions are reversible, and can be disrupted by changing physical conditions such as ionic strength, pH, and temperature, by the application of stress, or by the addition of specific solutes^[28].

Recently, click chemistry plays a vital role in fabricating various functional and well-defined hydrogels^[29, 30], due to its fast reaction, high efficiency, reliability, no by-products formation, good stability, and superior selectivity. Inspired by the promising structural features of IPN and advantages of click chemistry, a novel method by combining ion-crosslinking with click chemistry to synthesize IPN hydrogels was presented (Scheme 1). In this work, dopamine was introduced into the PEG networks as it can further complex with



Scheme 1 Synthetic routes of PEG-based Semi-IPN and Full-IPN hydrogels

Fe^{3+} ions. Dopamine was of interest here due to the fact that it does not infringe on human cells or trigger the body's immune response with a good record of cell compatibility, biocompatibility, and non-toxicity^[31, 32]. Therefore, it is expected that the prepared full-IPN hydrogel could exhibit high mechanical performance, swelling ratio, and conductivity without compromising its good biocompatibility.

EXPERIMENTAL

Materials

PEG ($M_n = 500, 2000$), poly (ethylene glycol) diglycidyl ether ($M_n = 500$), dopamine hydrochloride, and epichlorohydrin (99%) were purchased from Aldrich Chemical. Sodium azide (NaN_3 , 98%) was purchased from Alfa Aesar. CuBr, NaH (60%), NH_4Cl , THF, methylene chloride (99.9%), and *N,N*-dimethylformamide (99.9%) were purchased from Shanghai Chemical Reagent. Propargylamine (80%) and pentamethyldiethylenetriamine (PMDTA, 99%) were purchased from J&K Scientific and were distilled before use.

Synthesis of DEP_{45} , $\text{PEG}_{11}(\text{N}_3)_2$, $\text{PEG}_{45}(\text{N}_3)_2$, $(\text{PEG}_{11}(\text{C}\equiv\text{CH}))_m$ and DA Groups-pendant Linear PEG_{11} Derivatives $((\text{PEG}_{11}(\text{DA}))_n$

Synthesis of DEP_{45} , $\text{PEG}_{11}(\text{N}_3)_2$, $\text{PEG}_{45}(\text{N}_3)_2$ and $(\text{PEG}_{11}(\text{C}\equiv\text{CH}))_{20}$ have been reported in our previous work^[33, 34]. The molecular weight of $\text{PEG}_{11}(\text{N}_3)_2$, $\text{PEG}_{45}(\text{N}_3)_2$ and $(\text{PEG}_{11}(\text{C}\equiv\text{CH}))_{20}$ are ~ 580 , ~ 2080 , and ~ 11000 g/mol, respectively. Synthesis of $(\text{PEG}_{11}(\text{DA}))_n$ copolymer was carried out directly *via* amine-epoxy click polymerization (AECPP) according to the work of Wilms^[35]. In brief, dopamine hydrochloride (1.9 g) was dissolved in a round-bottom flask containing 5 mL pyridine under N_2 atmosphere with constant stirring at room temperature for 3 h. Equal mole of DEP_{11} (5.0 g) was added into the above solution under N_2 with constant stirring. The reaction mixture was heated to 80 °C and maintained at this temperature for 5 d. The resulting viscous liquid was precipitated in diethyl ether and washed with water quickly. The solid residue obtained after filtration was dried under reduced pressure overnight. The molecular weight of $(\text{PEG}_{11}(\text{DA}))_n$ calculated by Gel permeation chromatography (GPC), was ~ 6800 g/mol, thereby its molecular formula could be confirmed as $(\text{PEG}_{11}(\text{DA}))_{10}$.

Synthesis of Hydrophilic Semi-IPN and Full-IPN Hydrogels via Fe^{3+} Ions Complexation and Click Chemistry
 $(\text{PEG}_{11}(\text{DA}))_{10}$ (0.136 g) and $(\text{PEG}_{11}(\text{C}\equiv\text{CH}))_{20}$ (0.22 g) were dissolved in DMF (0.2 mL) in a vial by sonication in order to dissolve them completely. $\text{PEG}_{11}(\text{N}_3)_2$ (0.042 g) was then added to the above solution. CuBr (2.3 mg) was further added to the above mixture after its dechlorization. The reaction mixture was degassed with N_2 for 10 min. PMDTA (3.46 mg) was added to the above mixture by a syringe. Finally, the vial was ultrasonically agitated at room temperature. The gelation of hydrogel occurred within 15 min and a uniform solid structure was obtained by maintaining it at room temperature for 20 h. After complete gelation, hydrophilic semi-IPN hydrogel was obtained (Gel-semi-IPN- PEG_{11}). Hydrophilic full-IPN hydrogel was then obtained by immersing it into FeCl_3 solution (0.05 mol/L) for 6 h and subsequently transferring it into NaOH solution ($\text{pH} \geq 10$) for further complexation. After that, the gel was immersed into water to remove the copper ions and PMDTA. The obtained hydrogel was named as Gel-full-IPN- PEG_{11} . Synthesis of hydrophilic PEG_{45} -based hydrogels (Gel-semi-IPN- PEG_{45} and Gel-full-IPN- PEG_{45}) was similar to the above method except replacing $\text{PEG}_{11}(\text{N}_3)_2$ by $\text{PEG}_{45}(\text{N}_3)_2$.

Characterization

Fourier transform infrared (FTIR) spectra were recorded on a Fourier Transform Infrared Spectrometer (TENSOR27 PMA 50). Molecular weights (M_n) and their distributions (PDI) were measured through gel permeation chromatography (GPC) (Waters 1515) using THF as an eluent (flow rate of 1.0 mL/min, 40 °C) and through GPC (Waters 515) using DMF as an eluent (flow rate of 1.0 mL/min, 40 °C). Morphology of the hydrophilic hydrogels was observed on a scanning electron microscope (FEI Inspect F50) at an accelerating voltage of 15 kV. An electron universal testing machine (CMT 4503) was used to measure the compression testing of the hydrogel with a cross-head speed = 0.5 mm/min. Viscoelastic properties of hydrogels were

characterized by dynamic shear oscillation measurements. The rheology study was performed on a rheometer (Thermo Scientific HAAKE MARS III) with a 20 mm diameter parallel plate accessory. A series of hydrogels were pre-formed into hydrogel disks of a same size (25 mm diameter, 10 mm thickness) before measurements. In particular, the dynamic storage modulus was determined over a frequency range of 0.1–100 Hz. All of the measurements were carried out at 25 °C. Thermal stability of the hydrophilic semi-IPN and full-IPN hydrogels was determined by a thermogravimetric analyzer (TA, SDT Q-600) with a temperature range of 50–750 °C at a heating rate of 10 K/min under nitrogen atmosphere. DSC measurements were performed under a nitrogen atmosphere on a Perkin Elmer DSC7 calorimeter. All samples were pre-heated to 150 °C to erase the thermal history, then cooled to –50 °C at a rate of 10 K/min, and further heated to 150 °C again at a rate of 10 K/min.

Swelling Behavior of Hydrogels

The swelling degree of the hydrophilic semi-IPN and full-IPN hydrogels was measured according to a gravimetric method. Briefly, the weight of a gel dried to a constant weight was first recorded (m_0). The gel was then immersed into excessive deionized water at room temperature. At predetermined time points, the gel was taken out and weighed until a constant quantity of the gel was obtained (m_n). The following equation was used to calculate the swelling ratio (SR) of the hydrogel:

$$SR = [(m_n/m_0) - 1] \times 100\%$$

Measurement of Hydrogel Conductivity

Conductivity of semi-IPN and full-IPN hydrogels was characterized by a four-point probe technique with a linear probe 90 head (ST2253 Jingge Electronic Co., LTD, China). All samples were of unified specification (4 cm × 4 cm × 0.1 cm). All samples before testing were thoroughly soaked in deionized water to reach their equilibrium swelling. The surface of the test sample shall be dried and kept at the test conditions at room temperature. The bulk resistance of hydrogels (R) was calculated from the applied current (I) and recorded voltage (V), using Ohm's law: $R = V/I$, with four separate measurements made for each sample.

In-vitro Degradation

The *in-vitro* degradation of PEG-based hydrogels was carried out at 37 °C in a phosphate-buffer solution (PBS, pH = 7.4, 0.01 mol/L) containing sodium azide (1.0 mmol/L) as a bacteriostatic agent. The PEG-based hydrogel of 100 mg (m_0) was immersed in a small bottle containing 20 mL of the above buffer solution with gentle shaking. The buffer solution was renewed every 7 days. The hydrogel samples were periodically removed from the buffer and washed with distilled water. Their final mass (m_t) were obtained after complete drying in a freeze-dryer. The following equation was used to calculate the percentage of the mass loss:

$$\text{Mass loss (\%)} = (m_0 - m_t)/m_0 \times 100\%$$

In-vivo Subcutaneous Implantation of Full-IPN Hydrogels in a Porcine Model

The experimental protocol involving animals was approved by the Animal Care and Experiment Committee, School of Medicine, Shanghai Jiao Tong University China. Full-IPN hydrogels were used for *in vivo* subcutaneous implantation in a porcine model for assessing their biocompatibility and their evoked inflammation condition. Full-IPN hydrogels (9 mm in length, 5 mm width and 5 mm thick) were sterilized in 75% ethanol for 30 min and then washed extensively with sterile 0.9% NaCl. Mini pigs of 8 weeks weighing 10–15 kg were anesthetized through intravenous injection of 0.25% pentobarbital sodium. The abdomen of the pig was then shaved and prepared for surgery. Four lateral incisions (1 cm each) in two rows were made subcutaneously at each side of abdomen in one pig. The subcutaneous tissue was separated from the full skin layer to form a cavity. Each incision was inserted with one disc which was around 2 cm long in distance from the exact site of incision. Moreover, a distance of 8 cm was ensured between two incisions. After that, the incisions were sutured. Postoperatively, normal activities with a regular laboratory diet were resumed after the pigs were recovered from anesthesia.

Histological Examination

The implants with surrounding tissue were harvested after 2 weeks postoperatively. The harvested specimens were fixed in 4% paraformaldehyde in PBS for 2 days at 4 °C, embedded in paraffin, and sectioned in 5 mm slices. The slices were stained with hematoxylin and eosin (H&E) and then subjected to optical microscopic observations.

RESULTS AND DISCUSSION

Characterization of Major Compounds

FTIR spectra of DA, (PEG₁₁(DA))₁₀ and (PEG₁₁(C≡CH))₂₀ are shown in Fig. 1, respectively. As for the FTIR spectrum of (PEG₁₁(C≡CH))₂₀, the appearance of a characteristic absorption peak at 2100 cm⁻¹ indicated the presence of alkyne groups^[36]. As for the FTIR spectrum of DA, the bands at around 1190 cm⁻¹ and 1270 cm⁻¹ can be assigned to the C—N and C—O single bond vibration, respectively, whereas the absence of a sharp band at 1490 cm⁻¹ was due to the lower wavenumber ν (C=C) of aromatic C=C moieties. Compared with DA, the absorption peaks of (PEG₁₁(DA))₁₀^[37] at 1100 cm⁻¹, 1451 cm⁻¹, 1289 cm⁻¹, and 866 cm⁻¹ were attributed to the C—O—C, C—N, catechol C—O, and 1,2,4-trisubstituted aromatic ring in its molecular structure, respectively. ¹H-NMR spectrum of (PEG₁₁(DA))₁₀ is provided in Fig. S1. The molecular weight and polydispersity index (PDI) of (PEG₁₁(C≡CH))₂₀ and (PEG₁₁(DA))₁₀ were characterized by using GPC. The number-average molecular weights (M_n) of (PEG₁₁(C≡CH))₂₀ and (PEG₁₁(DA))₁₀ are approximately 11260 and 6680, with a PDI of approximately 1.47 and 1.53, respectively. The characteristic results of the samples obtained were given in Table 1.

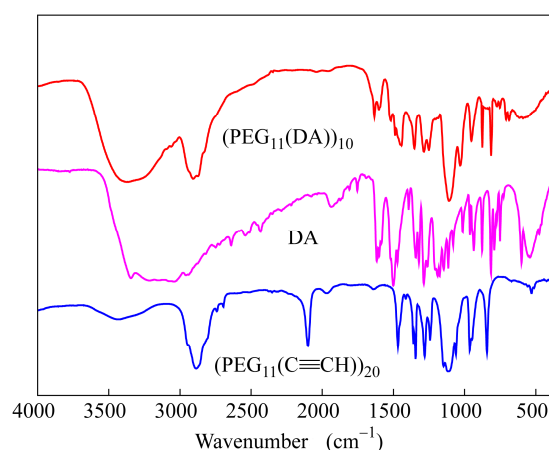


Fig. 1 FTIR spectra of DA, (PEG₁₁(DA))₁₀ and (PEG₁₁(C≡CH))₂₀

Table 1. GPC characterization of PEG₁₁(C≡CH))₂₀ and (PEG₁₁(DA))₁₀

PEG derivative	Numbers of functional groups	M_n (g/mol)	M_w (g/mol)	PDI
(PEG ₁₁ (C≡CH)) ₂₀	21	11260	16550	1.47
(PEG ₁₁ (DA)) ₁₀	11	6680	10510	1.53

Morphological Characterization of Hydrogels

The semi-IPN hydrogel, Gel-semi-IPN-PEG₁₁, was synthesized by click chemistry by mixing PEG₁₁(N₃)₂, (PEG₁₁(C≡CH))₂₀, and (PEG₁₁(DA))₁₀ in DMF with CuBr as a catalyzing agent. The full-IPN hydrogel, Gel-full-IPN-PEG₁₁, was obtained by immersing Gel-semi-IPN-PEG₁₁ in FeCl₃ aqueous solution. The syntheses of PEG₄₅-based hydrogels (Gel-semi-IPN-PEG₄₅ and Gel-full-IPN-PEG₄₅) were similar to the above methods except replacing PEG₁₁(N₃)₂ by PEG₄₅(N₃)₂. The morphology of all the hydrogels was investigated by SEM

observation. SEM images of PEG-based hydrogels are shown in Fig. 2. It can be seen that the surface of hydrophilic Gel-semi-IPN-PEG₁₁ hydrogel was smooth and exhibited a non-porous network structure (Fig. 2a). The surface of hydrophilic Gel-full-IPN-PEG₁₁ is shown in Fig. 2(b). Its surface was uneven with many folds. Probably, this is due to the immersion of Gel-full-IPN-PEG₁₁ in 0.05 mol/L NaOH solution (pH $\geq$ 10), which leads to the swelling of the network. After being freeze-dried, the folds emerged on the surface of Gel-full-IPN-PEG₁₁.

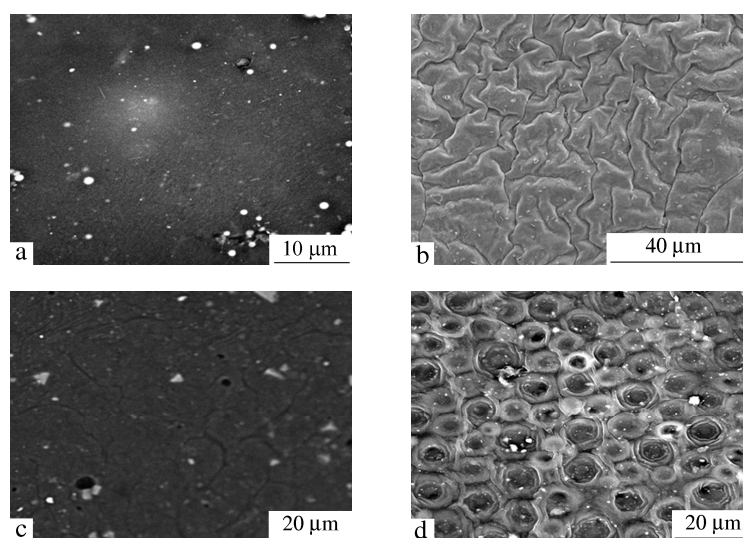


Fig. 2 SEM images of freeze-dried (a) Gel-semi-IPN-PEG₁₁, (b) Gel-full-IPN-PEG₁₁, (c) Gel-semi-IPN-PEG₄₅ and (d) Gel-full-IPN-PEG₄₅

Compared with Gel-semi-IPN-PEG₁₁, the surface of Gel-semi-IPN-PEG₄₅ was rougher (Fig. 2c). The surface of Gel-full-IPN-PEG₄₅ (Fig. 2d) also had many folds and defects. Before SEM observation, all the samples were dried by freeze-drying. The introduction of metal complex and the formation of the second network may result in the phase separation of Gel-full-IPN-PEG_n, which accounts for the formation of lattice structure in Figs. 2(b) and 2(d). Therefore, the surface of hydrophilic Gel-full-IPN-PEG₄₅ hydrogel exhibited a hole-distributed structure and more macroscopic defects (Fig. 2d).

Swelling Behavior of Hydrogels

The swelling degree of all the hydrogels was measured at certain time intervals by a gravimetric method. As shown in Fig. 3, Gel-full-IPN-PEG₁₁ exhibited a swelling ratio of about 380.4%, which was lower than that of Gel-semi-IPN-PEG₁₁ (425.5%). Similar trend was observed in the swelling ratios of PEG₄₅-based hydrogels. The swelling ratios of Gel-semi-IPN-PEG₄₅ and Gel-full-IPN-PEG₄₅ were 758.8% and 687.3%, respectively. Of note, it seems that PEG₄₅-based hydrogels exhibited much higher swelling ratio, when compared with their respective PEG₁₁-based hydrogels of the same network (*e.g.* Gel-semi-IPN-PEG₄₅ versus Gel-semi-IPN-PEG₁₁; Gel-full-IPN-PEG₄₅ versus Gel-full-IPN-PEG₁₁). On the other hand, when the hydrogels were of the same PEG chain length, the full-IPN structure would render the hydrogel having a lower swelling ratio. A few reasons might account for the above phenomena. First, PEG₄₅(N₃)₂ and PEG₁₁(N₃)₂ have different chain lengths of space lattice. Therefore, it is possible that the network of the PEG₄₅-based hydrogels is looser, and it could absorb more water in its structure. Secondly, the addition of a second chain would increase the cross-linking density of the first network^[38, 39].

Mechanical Properties

As a potential candidate for biomedical or tissue engineering application, a hydrogel should have a robust mechanical strength to keep its structural integrity under external force. The stress-strain curves of Gel-semi-

IPN-PEG₁₁, Gel-full-IPN-PEG₁₁, Gel-semi-IPN-PEG₄₅ and Gel-full-IPN-PEG₄₅ were then evaluated, and the results are shown in Fig. 4. The water content of all the hydrogels is 70%.

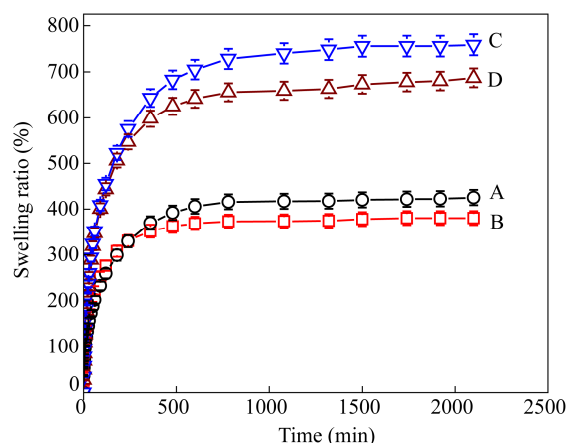


Fig. 3 Swelling curves for (A) Gel-semi-IPN-PEG₁₁, (B) Gel-full-IPN-PEG₁₁, (C) Gel-semi-IPN-PEG₄₅ and (D) Gel-full-IPN-PEG₄₅

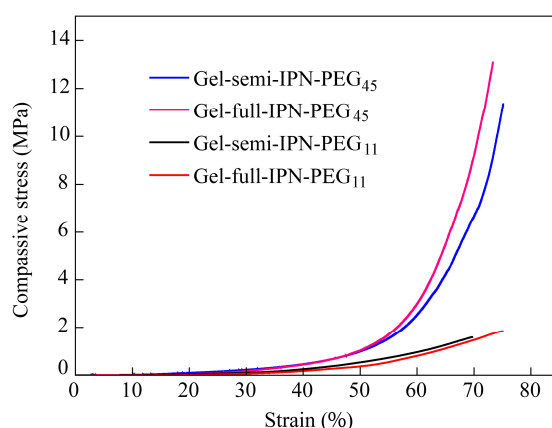


Fig. 4 Stress-strain curves of Gel-semi-IPN-PEG₁₁, Gel-full-IPN-PEG₁₁, Gel-semi-IPN-PEG₄₅ and Gel-full-IPN-PEG₄₅ under uniaxial compression

The fracture stress of Gel-semi-IPN-PEG₁₁, 1.57 MPa, was reached at 69.7% of its strain, and then the hydrogel was crushed. As to Gel-full-IPN-PEG₁₁, the gel still maintained its structure with only a little fracture even at 75.0% of its strain with a pressure of 1.84 MPa. The maximal compressive strength of Gel-semi-IPN-PEG₄₅ was 11.3 MPa and it was crushed at a strain of 75%. As for Gel-full-IPN-PEG₄₅, it started to fracture when the compressive strength was 13.1 MPa (at the strain of 73.3%). It could be seen that PEG₄₅-based hydrogels have a higher toughness than those of PEG₁₁-based hydrogels. Compared to the semi-IPN hydrogel, the full-IPN hydrogel would not completely break even at a great pressure. The above results can be attributed to the presence of a second layer network constituted by poly-dopamine. It had a certain adhesion which resulted in the higher mechanical strength. The two networks were gelled together and formed a relatively complete hydrogel^[40].

The rheological properties of all the hydrogels were studied by small-deformation oscillatory tests. As shown in Fig. 5(a), both Gel-semi-IPN-PEG₁₁ and Gel-full-IPN-PEG₁₁ exhibited typical gel rheological behaviors. The storage modulus (G') of the hydrogel was greater than the loss modulus (G'') over the entire tested range of angular frequencies (0.1–100 rad/s) for each sample. This phenomenon implied that the elastic

response was predominant and the hydrogels possessed a network structure. The G' and G'' of the hydrogels were sensitive to angular frequency and did not cross over each other in the tested frequency range, which is one of the characteristics of a gel with a high degree of crosslinking^[41]. However, the Gel-full-IPN-PEG₁₁ hydrogel had a higher G' , greater than 100 kPa, than the Gel-semi-IPN-PEG₁₁. This was mainly because the strong interpenetration in the hydrogel could provide high cross-linking strength. The same results were also obtained from PEG₄₅-based hydrogels, as shown in Fig. 5(b). However, their G' and G'' values were lower than those of PEG₁₁-based hydrogels, respectively. The above phenomenon was mainly due to the cross-linking density of the hydrogels. PEG₁₁-based hydrogels were denser than the PEG₄₅-based hydrogels.

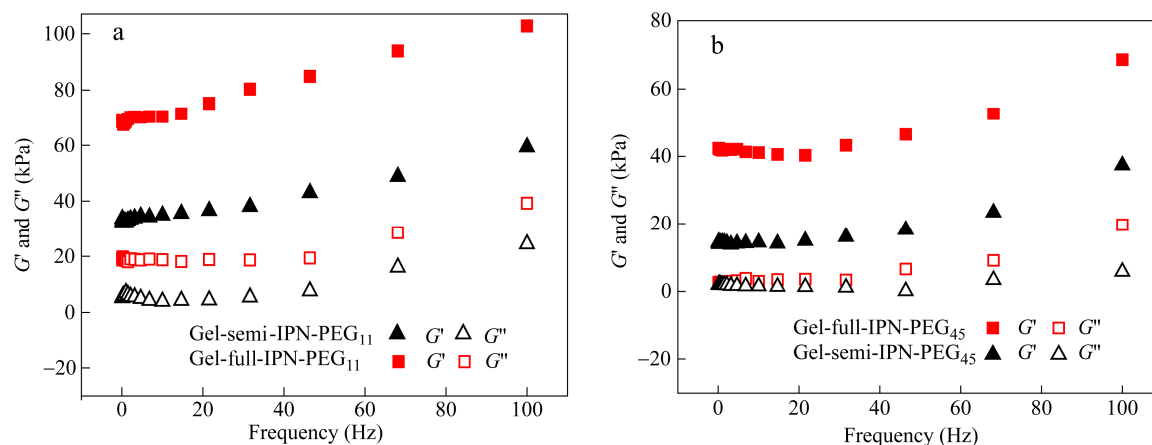


Fig. 5 Dynamic rheological behaviors of (a) PEG₁₁-based and (b) PEG₄₅-based hydrogels

Thermal Analysis

Thermal degradation behavior of dry hydrogels was measured by TGA measurements. It is shown in Fig. 6 that Gel-semi-IPN-PEG₁₁ (curve i) and Gel-full-IPN-PEG₁₁ (curve ii) hydrogels had a same decomposition temperature (260 °C), indicating that the chemical bonds needed to break in the heating process were almost the same. After the completion of thermal degradation, the relative percentage of residuals in full-IPN hydrogel was almost the same as that of the semi-IPN hydrogel. As for the Gel-semi-IPN-PEG₄₅ (curve iii) and Gel-full-IPN-PEG₄₅ (curve iv) hydrogels, their decomposition temperatures were both shifted to 267 °C. After the completion of thermal degradation, the relative percentage of residuals in semi-IPN hydrogel was lower than that of the full-IPN hydrogel.

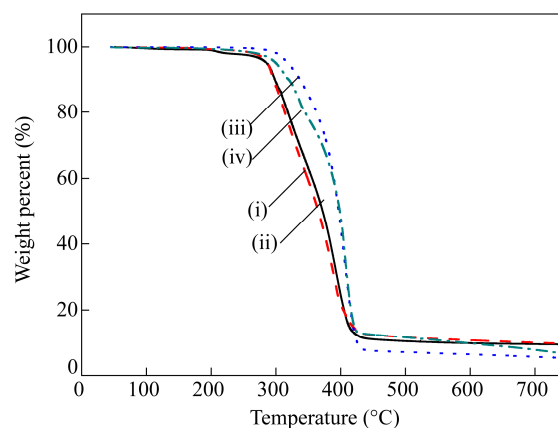


Fig. 6 TGA curves of (i) Gel-semi-IPN-PEG₁₁, (ii) Gel-full-IPN-PEG₁₁, (iii) Gel-semi-IPN-PEG₄₅ and (iv) Gel-full-IPN-PEG₄₅

The DSC heating curves of Gel-semi-IPN-PEG₄₅ and Gel-full-IPN-PEG₄₅ are shown in Fig. 7. Gel-semi-IPN-PEG₄₅ exhibited an endothermic peak at 18.67 °C, which is the T_m of PEG segments. The T_m of Gel-full-IPN-PEG₄₅ was 14.30 °C, lower than that of Gel-semi-IPN-PEG₄₅. This result was consistent with the fact that the higher the cross-linking, the less the defects in Gel-full-IPN-PEG₄₅.

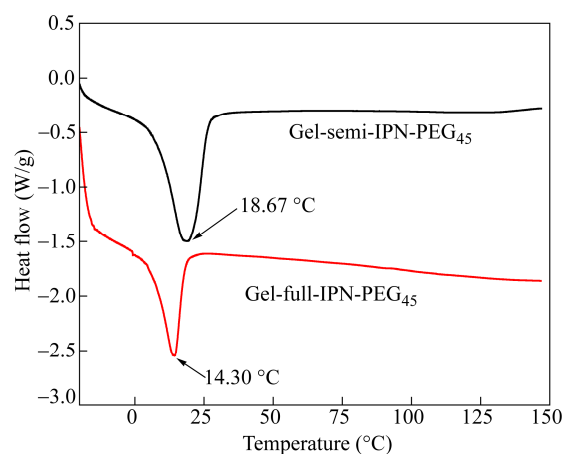


Fig. 7 Heating curves of DSC measurements of Gel-semi-IPN-PEG₄₅ and Gel-full-IPN-PEG₄₅

Conductivity of the Hydrogels

Table 2 shows the conductivity of the semi-IPN and full-IPN hydrogels. The conductivity of Gel-semi-IPN-PEG₁₁, Gel-semi-IPN-PEG₄₅ hydrogels were 8.3×10^{-6} and $7.7 \times 10^{-6} \text{ S} \cdot \text{cm}^{-1}$, respectively. The conductivity of Gel-full-IPN-PEG₁₁ and Gel-full-IPN-PEG₄₅ hydrogels were improved to 3.6×10^{-5} and $2.2 \times 10^{-5} \text{ S} \cdot \text{cm}^{-1}$, respectively. The electrical activity of polydopamine is attributable to the catechol functional groups in dopamine. Electro-active substance is used to deliver electron, since the active center of the active substance is far away from the electrode surface and it needs electroactive species as mediator to transfer electrons. PEG-based IPN hydrogels were attained by chelating Fe^{3+} ions on the basis of semi-IPN hydrogels. The increase in conductivity is assigned to the iron complexed with six phenolic hydroxyl groups which make them connected. Therefore, the PEG-based IPN hydrogels containing polydopamine have good conductive properties and biocompatibility.

Table 2. Conductivity of PEG₁₁-based hydrogels

Samples	Gel-semi-IPN-PEG ₁₁	Gel-full-IPN-PEG ₁₁	Gel-semi-IPN-PEG ₄₅	Gel-full-IPN-PEG ₄₅
Conductivity ($\text{S} \cdot \text{cm}^{-1}$)	8.3×10^{-6}	3.6×10^{-5}	7.7×10^{-6}	2.2×10^{-5}

In vitro Degradation

The degradation study of all hydrogels was carried out in $0.01 \text{ mol} \cdot \text{L}^{-1}$ PBS at 37 °C. The weights of hydrogels prior to and after being degraded for predetermined time periods, were measured and recorded as shown in Fig. 8. Interestingly, all hydrogels had experienced obvious degradation after 7 weeks, indicating that they were degradable under a physiological condition. It could be seen that the degradation rate of Gel-semi-IPN-PEG₁₁ was faster than that of Gel-full-IPN-PEG₁₁. As for the degradation rate of PEG₄₅-based hydrogels, the same trend was obtained. The difference in degradation rates of semi-IPN and full-IPN might be due to the Fe^{3+} complexation in full-IPN hydrogels. The full-IPN hydrogels had stronger binding ability, thereby the degradation rate was slower. The difference in relative ratios of C—N and OH bonds over C—O and C—C bonds would also affect the degradation rate. The lower the cross-linking density and higher the contents of C—N bonds, the faster will be the degradation rate^[34].

Compared to Gel-semi-IPN-PEG₁₁, Gel-semi-IPN-PEG₄₅ had a faster degradation rate. Gel-full-IPN-PEG₁₁ also had a lower degradation rate compared with Gel-full-IPN-PEG₄₅. At the end of 7 weeks, it was found that Gel-full-IPN-PEG₁₁ had about 57.7% of its initial weight left, whereas Gel-full-IPN-PEG₄₅ had only about 51.6% of its initial weight left. Most probably, the degradation was mainly due to the breakdown of C—N bonds with time. The longer the PEG chains, the lower the densities of C—N bonds and crosslinking in polymer networks. Thus, it is plausible to tune the degradation rate of the hydrogels by using the PEG with different molecular weights. Therefore, the PEG molecular weights could also affect the degradation property of hydrogels, and it seems that the higher the PEG molecular weight, the faster the degradation rate if the hydrogels are of the same type.

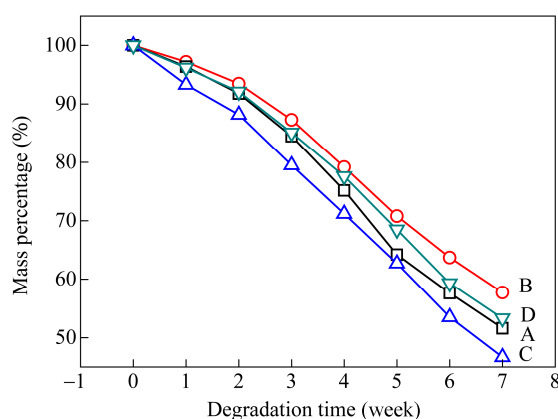


Fig. 8 Mass loss profiles of (A) Gel-semi-IPN-PEG₁₁, (B) Gel-full-IPN-PEG₁₁, (C) Gel-semi-IPN-PEG₄₅ and (D) Gel-full-IPN-PEG₄₅

Histological Morphology of Subcutaneously Implanted Full-IPN Hydrogel

To assess the inflammation reaction of the hydrogels and also to infer their biocompatibility, the *in-vivo* implantation of Gel-full-IPN-PEG₁₁ hydrogel was carried out in the subcutaneous area of pigs. The representative gross observation of the pig with Gel-full-IPN-PEG₁₁ implanted immediately and 2 weeks postoperatively are shown in Figs. 9(a) and 9(b), respectively. It can be seen that the wound areas created during implantation were healed after 2 weeks without any signs of tissue swelling or ichorous (Fig. 9b). No obvious abnormality of the surrounding tissue was observed after cutting off the subcutaneous pocket (Fig. 9c).

Histological observation of the Gel-full-IPN-PEG₁₁ after being implanted for 2 weeks is shown in Fig. 10. Two localized histological images indicating the interface between the implanted Gel-full-IPN-PEG₁₁ and tissue, were representatively shown in Figs. 10(a) and 10(b). No obvious inflammation reaction was observed in the surrounding tissue in consistence with the above gross observation. It seems that tissue grew quite homogeneously with the Gel-full-IPN-PEG₁₁. Moreover, a well integration between the hydrogel and adjacent tissue was achieved, indicating its excellent biocompatibility.

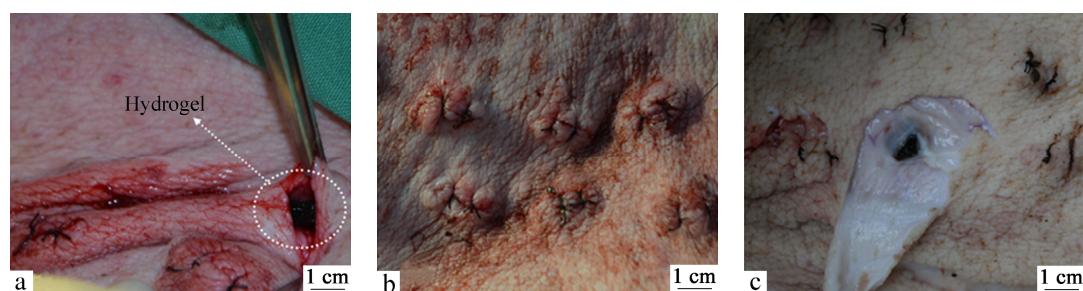


Fig. 9 (a) Gross observation of the pig with Gel-full-IPN-PEG₁₁ implanted immediately and (b) 2 weeks postoperatively; (c) Surface image of the implanted area after cutting off the subcutaneous pocket

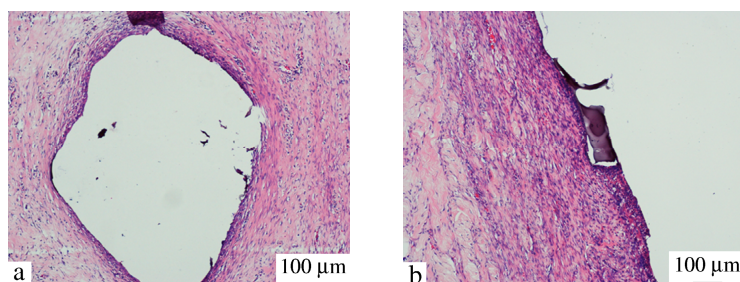


Fig. 10 Histological observation of the Gel-IPN-PEG₁₁-(PEG₁₁(DA))₁₀ hydrogels after being implanted for 2 weeks: Overall (a) and localized (b) histological morphology of implanted Gel-full-IPN-PEG₁₁ with surrounding tissues

CONCLUSIONS

A novel type of well defined IPN hydrogels prepared *via* Fe³⁺ ions complexation and copper-catalyzed 1,3-dipolar azide-alkyne cycloaddition (CuAAC) was designed and synthesized. The corresponding interpenetrated cross-linking structure between PEG-based hydrogel and (PEG₁₁(DA))₁₀ renders the respective hydrogels excellent mechanically strength, thermal stability, biodegradable and conductive properties. The excellent biocompatibility of such full-IPN hydrogel makes it promising as a biomaterial in tissue engineering.

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