Polymer Degradation and Stability 241 (2025) 111514

Contents lists available at ScienceDirect

Polymer Degradation and Stability

journal homepage: www.journals.elsevier.com/polymer-degradation-and-stability

Self-healing Poly(γ-glutamic acid)/hyaluronic acid hydrogels with *in situ* Hydroxyapatite formation

Rina Kugimiya , Yu-I Hsu *, Hiroshi Uyama *

Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Keywords:
Poly(γ-glutamic acid)
Hydrogels
Self-healing
SCHIFF-base reaction
Bioadhesion

ABSTRACT

Biocompatible hydrogels have potential applications as scaffold materials for repairing bone defects. However, they lack strength suitable for bone tissue and are easily damaged. Therefore, the development of hydrogels with high mechanical strength and self-healing properties is strongly desired. Poly(γ-glutamic acid) (PGA), a polysaccharide derived from natto, and hyaluronic acid (HA), a major component of the extracellular matrix (ECM), are both biocompatible and biodegradable, and thus expected to be used as potential biopolymers. In this study, self-healing hydrogels using PGA modified with adipic acid dihydrazide (PADH) and oxidized HA (OHA) with *in situ* hydroxyapatite (HAp) formation were developed *via* the Schiff base reaction. The hydrogel was easily obtained at room temperature by blending the two polymer precursor solutions and was injectable using a syringe with a short gelation time. The resulting hydrogels showed a significant increase in the mechanical strength as the amount of HAp in the hydrogels increased. The hydrogels demonstrated self-healing properties through reversible acylhydrazone bonds, although the self-healing efficiency was reduced in hydrogels with a high HAp content. Moreover, the hydrogels exhibited a low swelling ratio, 3D porous structures, and adhesion to chicken bone, suggesting that they are suitable for treating bone defects. In summary, our results indicate the potential of PGA/HA/HAp hydrogels given their injectability, superior mechanical strength relative to typical hydrogels, self-healing properties, and adhesion to bone.

1. Introduction

Bone grafting is often used to treat bone defects and fractures caused by disease or injury. However, these methods involve a heavy surgical burden and high risk of inflammation [1,2]. Various alternative materials such as metals, bioceramics, and biodegradable polymers have been considered, but challenges remain in developing materials with sufficient strength, morphology, osteoinductivity, and compatibility [3–7]. Therefore, bone regeneration *via* tissue engineering using hydrogels has attracted significant attention in recent years [8]. Hydrogels are polymeric materials with three-dimensional structure formed of hydrophilic polymer chains that can absorb water. Its porous structure and high biocompatibility promote bone regeneration by facilitating cell growth. In addition, injectable hydrogels can conform to various bone-defect shapes owing to their flexibility [9–11]. However, conventional hydrogels often lack mechanical properties and are easily damaged, limiting their applications as hard bone repair materials [12]. Therefore, a method for implanting a combination of hydroxyapatite (HAp) and hydrogel into bone has been investigated. HAp, which is composed of $Ca_5(PO_4)_3OH$, is the main component of bone and has attracted attention as a biomaterial owing to its stability against temperature and pH, and high biocompatibility. HAp exhibits excellent osteoconductive properties, thereby supporting bone regeneration [3,13,14]. Injection of HAp-containing hydrogels into the bone may improve mechanical strength and promote the repair of bone defects. Kuang et al. reported an injectable nanocomposite hydrogel for bone regeneration by *in situ* growth of calcium phosphate nanoparticles using dimethylaminoethyl methacrylate (DMAEMA) and 2-hydroxyethyl methacrylate (HEMA) for bone regeneration [15].

Furthermore, to enable long-term use of the material in the body, this study focused on self-healing hydrogels. Self-healing hydrogels that can spontaneously repair damage have attracted considerable interest [16,17]. Self-healing mechanisms can be divided into physical and chemical. Physical self-healing occurs through non-covalent interactions, such as hydrophobic interactions, host-guest interactions, and hydrogen bonding. In contrast, chemical self-healing occurs through the formation of covalent bonds such as disulfide bonds, acylhydrazone bonds, and Diels-Alder reactions [18,19]. These self-healing hydrogels have

* Corresponding authors.
E-mail addresses: yuihsu@chem.eng.osaka-u.ac.jp (Y.-I. Hsu), uyama@chem.eng.osaka-u.ac.jp (H. Uyama).

https://doi.org/10.1016/j.polymdegradstab.2025.111514
Received 3 March 2025; Received in revised form 20 June 2025; Accepted 23 June 2025
Available online 25 June 2025

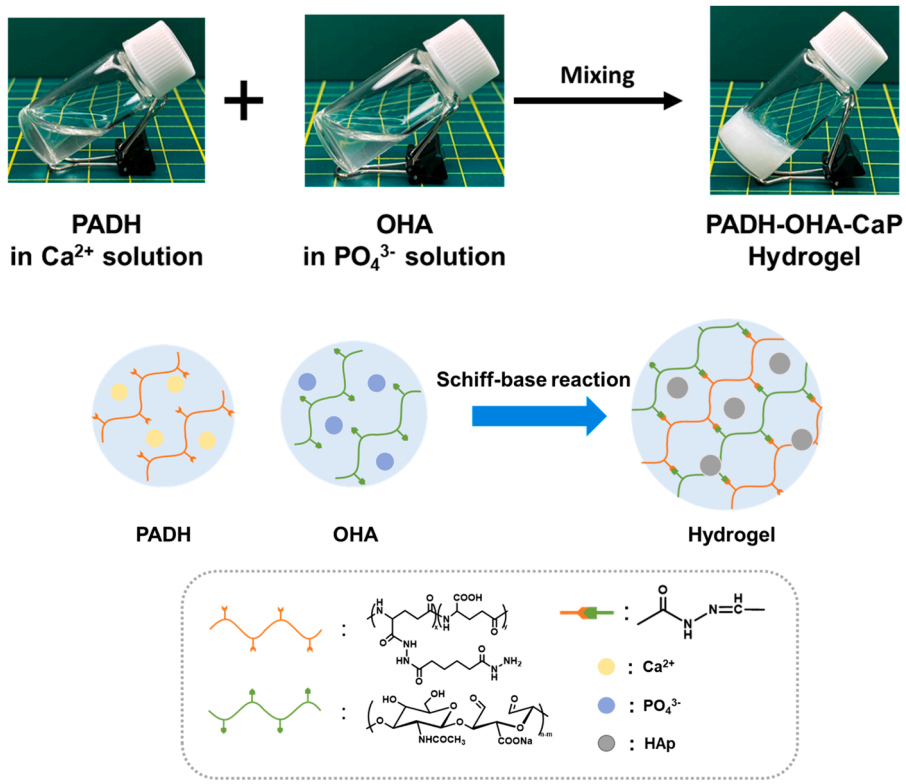

Fig. 1. Schematic representation of hydrogel formation.

applications in wearable electronics, biosensors, and drug delivery because they do not lose their functionality once damaged, thereby improving the safety and lifetime of these materials [20–22]. We focused on acylhydrazone bonds, reversible covalent bonds formed by the Schiff base reaction, and attempted to fabricate self-healing hydrogels using naturally derived biopolymers Hyaluronic acid (HA) and poly (γ-glutamic acid) (PGA).

HA is a major component of the extracellular matrix (ECM), and possesses biocompatibility, biodegradability, and low immunogenic activity [23]. These features render it a promising material for use in tissue engineering, drug delivery, and wound repair [24,25]. Li et al. reported that hydrogels consisting of hydrazide-modified HA, aldehyde-modified HA, aldehyde-modified cellulose nanocrystals, and platelet-rich plasma exhibited excellent self-healing properties and promoted wound healing [26]. However, many conventional hyaluronic acid hydrogels have weak strength and insufficient stability in the body, so introducing cross-linking or combining them with other materials has been considered [27,28].

PGA is a highly biodegradable, biocompatible, and nontoxic biopolymer derived from natto [8]. PGA is synthesized in nature by Bacillus subtilis and formed by amide bonding of α-amino and γ-carboxyl groups of two glutamic acids [29,30]. PGA has carboxyl groups on its side chain, which allow for hydrogen bonding and electrostatic interactions with other polymers, as well as the easy introduction of various functional groups, making it suitable for further applications like drug delivery [31,32]. In addition, PGA is degraded into glutamic acid, a component of collagen that promotes the nucleation of hydroxyapatite [8]. Several injectable hydrogels using PGA have been developed and are expected to be used *in vivo* as scaffold materials for tissue engineering and drug delivery [33]. Clarke et al. reported that β-sheet peptides grafted poly(γ-glutamic acid) hydrogel exhibits self-healing, tailor-made mechanical properties and can be a promising platform for future tissue engineering scaffolds and biomedical applications [34]. However, previous study has reported that it takes approximately 14 days for HAp to form in PGA-only hydrogels [35]. In addition, hydrogels containing dynamic covalent crosslinking formed by a thiol-aldehyde addition reaction between thiol-modified PGA and oxidized HA self-repaired in 8 hours but were prone to degradation in phosphate-buffered saline (PBS) [36]. Therefore, we focused on introducing acylhydrazone bonds to create more stable and stronger PGA-based hydrogels at low cost.

In this study, we successfully fabricated poly(γ-glutamic acid)/ hyaluronic acid/ hydroxyapatite hydrogels through rapid, simple, and mild gelation method. The hydrogels were prepared by mixing hydrazide-modified poly(γ-glutamic acid) (PADH) and oxidized hyaluronic acid (OHA) in the presence of calcium and phosphate ions. Within a few minutes, gelation occurred *via* Schiff base reaction and hydroxyapatite (HAp) formation *in situ* (Fig. 1), requiring no harsh reaction conditions, such as high temperature or extreme pH, nor expensive reagents. This method allows for easy fabrication of mechanically robust hydrogels, thanks to both covalent acylhydrazone cross-linking and the uniform dispersion of HAp crystals formed within the hydrogel matrix. The introduction of HAp not only improved the osteoconductivity but also led to a marked enhancement in compressive strength, making the hydrogels suitable for bone repair applications. Moreover, the flexibility and injectability of the hydrogels allowed it to fit into the irregular shape of damaged bone areas, and short gelation time reduce the risk of leakage of the precursor solution. Furthermore, the introduction of the covalent acylhydrazone bond not only increased the strength of the hydrogel, but also gave the hydrogel self-healing properties due to its reversibility. This study achieved a rapid and simple method of gelation PGA/HA hydrogels including HAp, and evaluated the mechanical properties and self-healing capacity.

2. Materials and methods

2.1. Materials

Poly(γ-glutamic acid) (PGA) (Mw = 1.6×10^5 g mol^{-1}) was provided by Kookmin University (Seoul, Korea). Hyaluronic acid sodium salt,

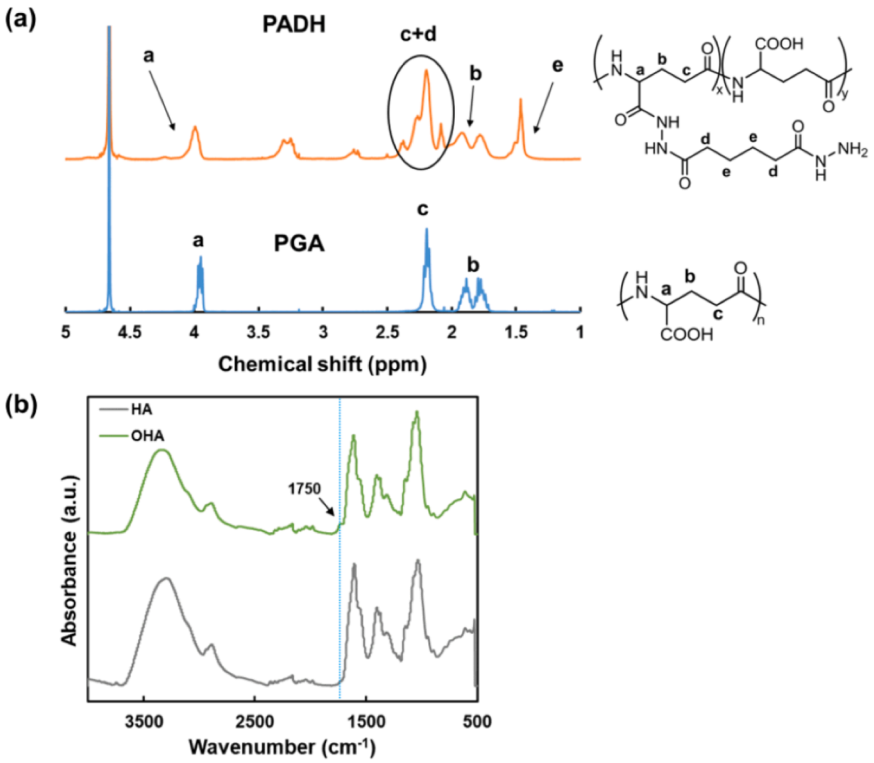

Fig. 2. (a) ^{1}H NMR spectra of PGA and PADH (b) FT-IR spectra of HA and OHA.

sodium periodate ($NaIO_4$), 2-morpholinoethanesulfonic acid (MES), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), calcium nitrate tetrahydrate ($Ca(NO_3)_2·4H_2O$) were purchased from Wako Chemicals (Osaka, Japan). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), and adipic dihydrazide (ADH) were purchased from Tokyo Chemical Industry (Tokyo, Japan). All the reagents were used without further purification.

2.2. Synthesis of PADH

Poly(γ-glutamic acid) (1.3 g, 10 mmol) was dissolved in 100 mL of MES buffer solution prepared to 0.01 mol/L (pH 5.5). Following which, EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (2.3 g, 12 mmol) and NHS (*N*-hydroxysuccinimide) (1.2 g, 12 mmol) were added, stirring at 25 °C for 30 min to activate the carboxylic groups. Then, ADH (adipic dihydrazide) (2.6 g, 15 mmol) was dissolved in 10 mL of deionized water, which was added to the flask and reacted at 25 °C for 24 h. The solution was precipitated with ethanol and acetone and dialyzed with deionized water using a 10 kD membrane for 3 days, followed by lyophilization to obtain PADH as a white solid.

2.3. Synthesis of OHA

Sodium hyaluronate (0.4 g, 1 mmol) was added to a round-bottom flask with 100 mL of deionized water and stirred overnight at 25 °C. Following which, $NaIO_4$ (0.3 g, 1.5 mmol) was dissolved in 6 mL of deionized water and added to a flask and reacted for 6 h in dark condition at 25 °C. Subsequently, ethylene glycol (0.093 g, 1.5 mmol) was added and stirred for 30 min to stop the reaction. The reaction solution was dialyzed with deionized water using a 10 kD membrane for 3 days. A white solid was obtained after lyophilization.

2.4. Preparation of hydrogels

Phosphate and calcium ion solutions were prepared by $(NH_4)_2HPO_4$, $Ca(NO_3)_2·4H_2O$ using 0.1 M Tris buffer (pH 8). The synthesized PADH and OHA were dissolved in a phosphate ion solution (0.5 mL) and calcium ion solution. Hydrogels were obtained by mixing the solutions at 37 °C. In this study, hydrogels were prepared by varying the concentration of the ion solutions. The prepared hydrogel was named PADH-OHA-CaPx, where x is the concentration of the calcium ion solution. The vial inversion method was used to determine the gelation times of the hydrogels.

2.5. Characterization

The structure of PADH was characterized by ^{1}HNMR (JMN-ECS400) using D_2O as the solvent. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Nicolet iS5 spectrometer, Thermo Scientific) was measured to determine the introduction of carbonyl groups of OHA in the range 500–4000 cm^{-1}. The degree of OHA oxidation was examined by titration with hydroxylamine hydrochloride using Eq. (1).

$$\text{Amount of aldehyde groups of OHA (mmol / g)} = \frac{\text{the concentration of NaOH (mmol/L)} \times \text{volume of NaOH (L)}}{\text{weight of OHA (g)}} \quad (1)$$

The XRD patterns of the obtained hydrogels were analyzed using a

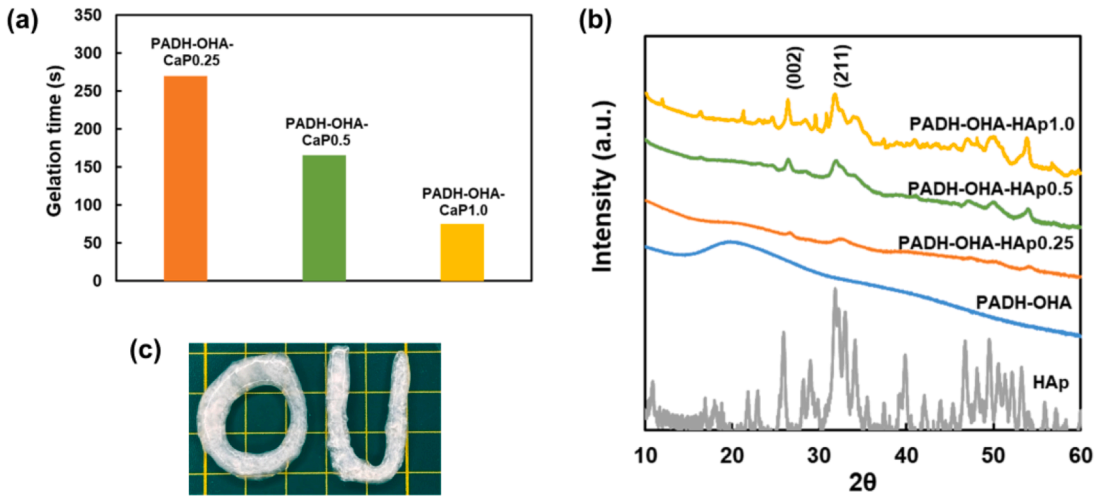

Fig. 3. (a) Gelation time of hydrogels with different Ca^{2+}, PO_4^{3-} concentration (b) XRD results of hydrogels with different Ca^{2+}, PO_4^{3-} concentration (c) Photograph of the injectability of PADH-OHA-CaP0.5.

SmartLab (Rigaku) instrument with a Cu K-beta X-ray source. Measurements were performed in the range of 10°–60° at a scanning rate of 5°/min. PDXL2 (from the ICDD database PDF-2 2011) was used for identification.

Rheological tests were performed using a Hakke Rheostress 6000 (Thermo Scientific) equipped with a 20 mm diameter parallel plate. Hydrogels were placed on the plates, and oscillation frequency sweep was carried out using at 25 °C in the range of 0.1 Hz–10 Hz. Compression tests were performed using a Hakke Rheostress 6000 (Thermo Scientific) equipped with a 20 mm diameter parallel plate. The measurements were performed at 25 °C at a rate of 10.0 mm/s.

The morphologies of the hydrogels were characterized by scanning electron microscopy (SEM, Hitachi, SU3500). The hydrogel samples were freeze-dried 24 hours after gelation and sputtered with an Au-Cd coating using an MSP-1S magnetron sputterer (Vacuum Device Inc.).

A TG/DTA 7200 (Hitachi High-Tech Science Corporation) was used for thermogravimetric analysis to determine the amount of HAp present in the hydrogels. Measurements were performed under a nitrogen atmosphere at a heating rate of 10 °C/min from 40 °C to 800 °C.

2.6. Swelling test

The hydrogels were immersed in 30 mL of phosphate buffer solution (pH 7.4) at room temperature. After a certain amount of time, the hydrogels were removed from the solution, the surface moisture was wiped off with a tissue paper, and their weights were measured. The swelling ratio was calculated using Eq. (2):

$$\text{Swelling ratio } (\%) = \frac{w_t - w_0}{w_0} \times 100 \quad (2)$$

Here, W_0 denotes the original weight of the sample, and W_1 represents the weight at a given time (t).

2.7. Self-healing abilities

The prepared hydrogels were cut into two pieces, the cutting faces placed together and left overnight at 37 °C. The cut faces of the hydrogels were visually examined and pulled using tweezers. In addition, compression tests were performed using a Hakke Rheostress 6000 (Thermo Scientific) equipped with a 20 mm diameter parallel plate at 25 °C at a rate of 10.0 mm/s. The maximum stresses were compared for the hydrogels before cutting and after repair, and the self-healing efficiency was calculated using the following Eq. (3).

$$\text{Healing efficiency } (\%) = \frac{F_H}{F_0} \times 100 \quad (3)$$

Here, F_0 denotes the maximum stress of the original hydrogel (Pa), while F_H represents the maximum stress of the healed hydrogel (Pa).

2.8. Bioadhesion of hydrogels

The precursor solutions of hydrogels were injected into the damaged chicken bone using a syringe and left overnight at 37 °C. The boundary between the hydrogel and the bone was observed using a high-magnification microscope (SE-2000WR, Selmic).

3. Results and discussion

3.1. Characterization of PADH and OHA

PADH was synthesized by modifying the carboxylic acid of PGA with ADH and was characterized by ^{1}HNMR. As shown in Fig. 2(a), a new peak derived from the methylene protons (4H, $-CH_2CH_2-$) of ADH was identified in PADH. The degree of substitution was calculated by comparing the integral areas of the peaks attributed to the protons a and e and it was found to be 27 %. The OHA was prepared by oxidizing HA with sodium periodate. The FT-IR spectra of HA and OHA were measured, and a peak attributed to aldehyde groups was observed at approximately 1730 cm^{-1} in the spectrum of OHA (Fig. 2(b)). The intensity of the broad peak at 3000–3500 cm^{-1} originating from the hydroxyl groups was smaller in OHA than in HA. Therefore, the hydroxyl group of HA was successfully oxidized to obtain OHA aldehyde groups. The amount of aldehyde groups of OHA was determined to be 0.43 mmol/g by titration using hydroxylamine hydrochloride. In addition, the toxicity of OHA and PADH was investigated by examining cell viability after 48 hours (**Figure S1**.). While PADH showed high survival rates of 0.8 or higher at all concentrations, OHA showed a decrease in survival rates in high-concentration solutions. This is thought to be due to the effects of residual oxidising agent used in the synthesis of OHA, and a review of the production method and further investigation of biocompatibility will be required in the future.

3.2. Preparation of hydrogels

Hydrogels were prepared by mixing PADH and OHA in the presence of Ca^{2+} and PO_4^{3-} *via* acylhydrazone bonds. In this study, PADH-OHA-CaP0.25, PADH-OHA-CaP0.5, and PADH-OHA-CaP1.0 hydrogels were

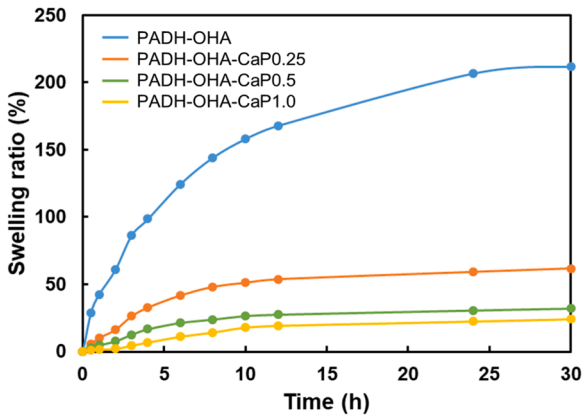

Fig. 4. Swelling ratio of hydrogels with different Ca^{2+}, PO_4^{3-} concentration.

prepared by changing the concentration of calcium ion solution to 0.25 mol/L, 0.5 mol/L, and 1.0 mol/L, respectively, while the molar ratio of calcium and phosphate ions was kept 5:3. PADH-OHA hydrogels without calcium and phosphate ions were also prepared using 0.1 M Tris buffer for comparison. The gelation times were all within 5 min for the PADH-OHA-CaP hydrogels, compared to approximately 1 h for the PADH-OHA hydrogels (Fig. 3(a)). The formation of acylhydrazone bonds by the Schiff base reaction is favored by mildly acidic conditions around pH 6 [37], and the higher concentration of calcium and phosphate ion solutions may have resulted in faster formation of HAp and shorter gelation time because the hydroxyl groups are consumed faster. The short gelation time allowed the solution to fit into the damaged area of the bone without diffusion.

XRD measurements were performed to confirm the formation of HAp (Fig. 3(b)). The main HAp peaks are observed at approximately 26° and 32° [12] for the PADH-OHA-CaP hydrogel, confirming the formation of HAp. Furthermore, the peak intensity increased as the concentrations of the calcium and phosphate ion solutions increased, suggesting that the amount of HAp generated increased.

The injectability of PADH-OHA-CaP0.5 was also confirmed. As shown in the Fig. 3(c), it was possible to inject the letters "O" and "U" using a double syringe. These letters can be injected without clumping for about 3 min after starting the injection; after about 5 min, the solution begins to solidify, so the operation should be carried out quickly. Therefore, the prepared hydrogels could be applied to various bone fracture shapes.

The hydrogels were then immersed in PBS, and the swelling ratio was measured (Fig. 4). The swelling ratio reached 212 % after 30 h for PADH-OHA hydrogels without HAp. In contrast, it decreased significantly in hydrogels containing HAp, with 62 % for PADH-OHA-CaP0.25, 32 % for PADH-OHA-CaP0.5 and 24 % for PADH-OHA-CaP1.0. As the concentrations of the calcium and phosphate ion solutions increased, the hydrogel absorbed less water. Because hydrogels break easily when swollen, a lower swelling ratio is more suitable for application as a bone repair material.

3.3. Mechanical properties

The storage modulus, G', of the hydrogels was measured (Fig. 5(a)). The storage modulus of the hydrogel containing HAp increased significantly compared to that of the hydrogel without HAp, confirming that

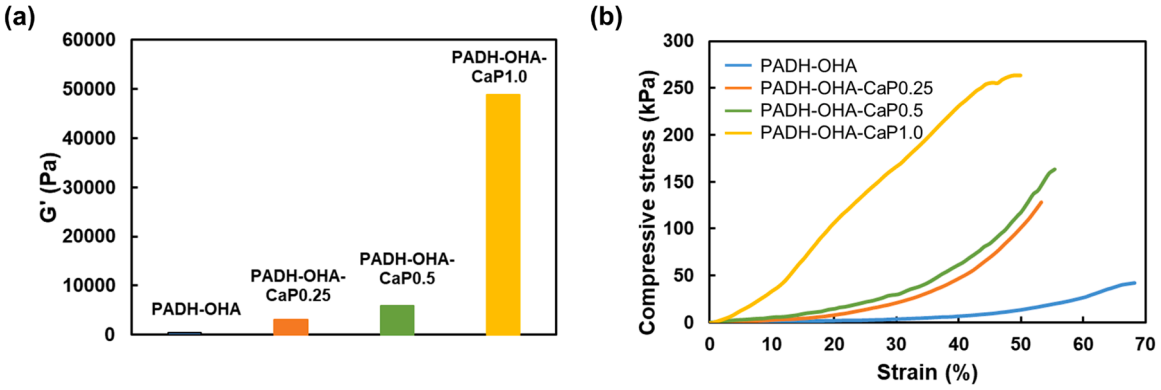

Fig. 5. (a) Rheology (b) Compressive stress of hydrogels with different Ca^{2+}, PO_4^{3-} concentration.

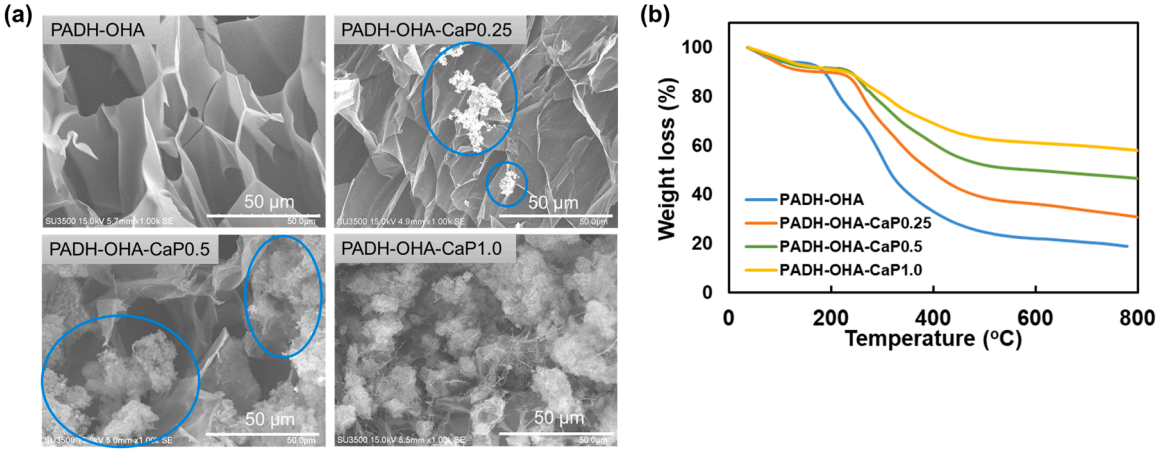

Fig. 6. (a) SEM images (b) TGA results of hydrogels with different Ca^{2+}, PO_4^{3-} concentration.

Table 1
TGA results of hydrogels with different Ca^{2+}, PO_4^{3-} concentration (T=780 °C).

Hydrogel	Weight loss (%)	Content of HAp (%)
PADH-OHA	18.80	–
PADH-OHA-CaP0.25	31.33	12.53
PADH-OHA-CaP0.5	46.89	28.09
PADH-OHA-CaP1.0	58.35	39.55

higher strength hydrogels were obtained. In addition, the G' values increased from 3011 Pa for PADH-OHA-CaP0.25, to 48,908 Pa for PADH-OHA-CaP1.0, indicating that the strength increased when the concentration of the calcium and phosphate ion solutions increased. This could be attributed to the increased amount of HAp formed in the hydrogels.

In addition, the mechanical properties were evaluated using a compression test (Fig. 5(b)). The PADH-OHA-CaP hydrogels showed better mechanical properties than hydrogels without HAp. The maximum stress increased with increasing concentrations of calcium and phosphate ion solutions, exceeding 250 kPa for PADH-OHA-CaP1.0. Similar to the results of the rheological measurements, this trend may be related to the amount of HAp contained in the hydrogel. The resulting PADH-OHA-CaP hydrogels are stronger than existing hydrogels being considered as bone replacement materials [38] and is expected to be used as a fixation material until the damaged bone heals.

3.4. Amount of HAp

The morphology of the hydrogel was observed using SEM (Fig. 6(a)). HAp particles were formed in the PADH-OHA-CaP hydrogel, and the amount of HAp formed increased as the concentration of the calcium and phosphate ion solutions increased. This is believed to be the reason for the improved mechanical properties of the hydrogels.

TGA was performed to determine the HAp content of the hydrogels (Fig. 6(b), Table 1).

Fig. 6(b) depicts the framework decomposition in PADH-OHA-CaP hydrogels started around 230 °C, demonstrating enhanced thermal stability compared to PADH-OHA hydrogel. The remaining weight above 780 °C in the PADH-OHA hydrogel was assumed to be a high-molecular-weight polymer, and the HAp content in the PADH-OHA-CaP hydrogel should be considered, excluding this amount. Table 1 shows that the HAp content of the PADH-OHA-CaP hydrogels increased with increasing concentrations of calcium and phosphate ions. The TGA data showed an increase in HAp, just as the aforementioned SEM results indicate.

3.5. Self-healing properties

To evaluate the self-healing properties of the hydrogels, they were cut into two pieces and left overnight at 37 °C with the cut surfaces touched. As shown in Fig. 7(a), all prepared hydrogels healed to the extent that they did not break when pulled with tweezers. Therefore, the hydrogels with HAp exhibited self-healing properties through reversible acylhydrazone bonds, as illustrated in Fig. 7(c). The self-healing efficiency of each hydrogel was examined from the maximum stress of the compression test (Fig. 7(b)). The values for self-healing efficiency were 100 % for PADH-OHA, 94 % for PADH-OHA-CaP0.25, 52 % for PADH-

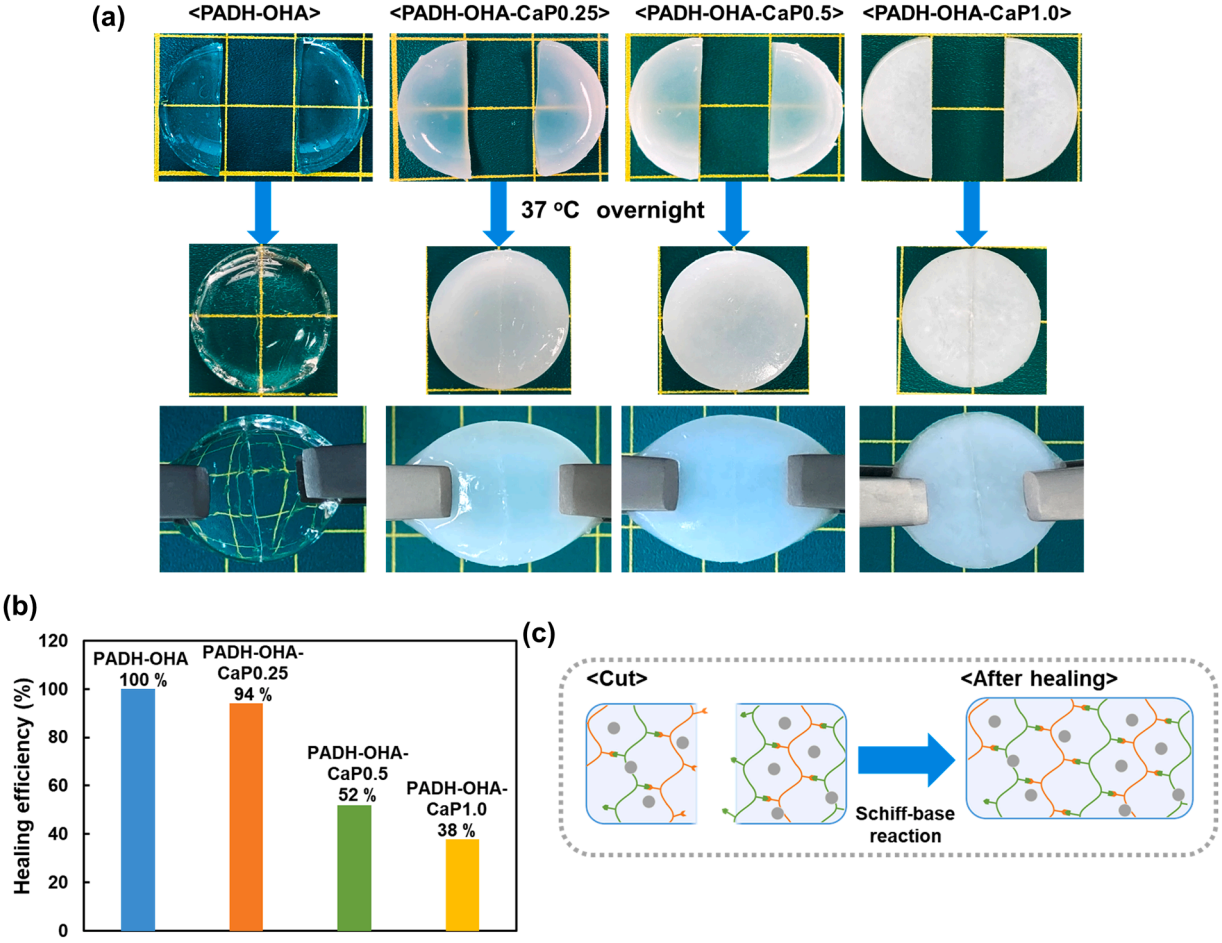

Fig. 7. (a) Photographs of the self-healing ability (b) Self-healing efficiency of hydrogels with different Ca^{2+}, PO_4^{3-} concentration (c) Healing mechanism of PADH-OHA-CaP hydrogels.

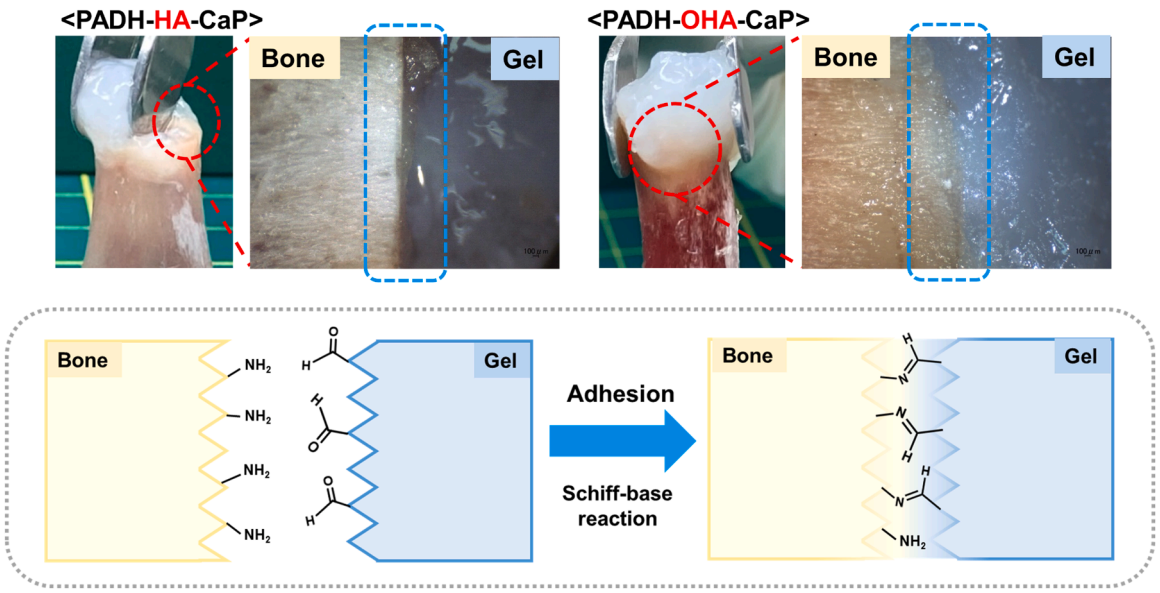

Fig. 8. Bioadhesion of PADH-HA-CaP hydrogel and PADH-OHA-CaP hydrogel to chicken bone.

OHA-CaP0.5 and 38 % for PADH-OHA-CaP1.0. PADH-OHA and PADH-OHA-CaP0.25 showed self-healing efficiency close to 100 %. Such high self-healing properties are expected to contribute to a longer lifetime of the hydrogels. However, the self-healing efficiency was reduced for hydrogels containing large amounts of HAp like PADH-OHA-CaP0.5 and PADH-OHA-CaP1.0, due to the inhibition of the Schiff base reaction between PADH and OHA.

3.6. Adhesion to bone

Hydrogels were directly injected into chicken bones, and the adhesion ability of HA- and OHA-based hydrogels to the bone was compared (Fig. 8). For the hydrogel containing HA, the bone and hydrogel separated when the hydrogel was pulled, and the boundary was observed with a high-magnification scope, showing clear separation of the hydrogel and bone. However, the hydrogel and the bone did not separate when OHA was used, as shown in the image on the right. The boundary between the PADH-OHA-CaP hydrogel and bone was indistinct, with no gap between the two. This was thought to be due to the Schiff base reaction between the aldehyde groups in OHA and the amino groups in the bone. Therefore, the prepared PADH-OHA-CaP hydrogel possessed bone-adhesive properties.

4. Conclusion

Hydrogels with hydroxyapatite (HAp) were prepared using a simple and quick method of mixing hydrazide-modified poly(γ-glutamic acid) (PADH) and oxidized hyaluronic acid (OHA) with calcium ion solution and phosphate ion solution. The prepared PADH/OHA/HAp hydrogels had a gelation time of less than 5 min and could be injected using a dual syringe. Their low swelling ratio also makes them suitable for application in the presence of water. Hydrogels containing HAp showed a significant improvement in mechanical properties compared to PADH/OHA hydrogels. The amount of HAp in the hydrogels increased with increasing calcium and phosphate ion concentrations. Furthermore, it was confirmed that the PADH/OHA/HAp hydrogels also exhibited self-healing properties and adhesion to the bone owing to the Schiff base reaction. These results suggest that injectable PADH/OHA/HAp hydrogels with excellent mechanical properties and self-healing abilities have potential applications in bone repair.

CRediT authorship contribution statement

Rina Kugimiya: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Yu-I Hsu:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Hiroshi Uyama:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by Japan Science and Technology Agency (JST) PRESTO Grant Number JPMJPR23N4, the Environment Research and Technology Development Fund JPMEERF21S11900 of the Environmental Restoration and Conservation Agency of Japan, and Japan Society for the Promotion of Science (JSPS) KAKENHI Grants (22K21348 and 23K26717). The authors would like to express their sincere gratitude to Dr. Yuichiro Ukon, Dr. Hirokazu Mae, and Dr. Akitomo Inoue for their invaluable support and cooperation in conducting the toxicity experiments.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.polymdegradstab.2025.111514.

Data availability

No data was used for the research described in the article.

References

[1] A.A. El-Rashidy, J.A. Roether, L. Harhaus, U. Kneser, Aldo R. Boccaccini, Regenerating bone with bioactive glass scaffolds: a review of *in vivo* studies in bone defect models, Acta Biomater. 62 (2017) 1–28.
[2] E. Jimi, S. Hirata, K. Osawa, M. Terashita, C. Kitamura, H. Fukushima, The current and future therapies of bone regeneration to repair bone defects, Int. J. Dent. 2012 (2012) 148261.

[3] S. Aleksandra, H. Lucyna, C. Emil, Synthesis of hydroxyapatite for biomedical applications, Adv. Colloid. Interface Sci. 249 (2017) 321–330.
[4] N.S. Moghaddam, M.T. Andani, A. Amerinatanzi, C. Haberland, S. Huff, M. Miller, M. Elahinia, D. Dean, Metals for bone implants: safety, design, and efficacy, Biomanuf. Rev. 1 (2016) 1.
[5] S.N. Khan, E. Tomin, JM. Lane, Clinical applications of bone graft substitutes, Orthop. Clin. North Am. 31 (3) (2000) 389–398.
[6] L. Tan, X. Yu, P. Wan, K. Yang, Biodegradable materials for bone repairs: a review, J. Mater. Sci. Technol. 29 (6) (2013) 503–513.
[7] D. Puppi, F. Chiellini, A.M. Piras, E. Chiellini, Polymeric materials for bone and cartilage repair, Prog. Polym. Sci. 35 (4) (2010) 403–440.
[8] M. Wei, Y.I. Hsu, T. Asoh, M.H. Sung, H. Uyama, Design of injectable poly (γ-glutamic acid)/chondroitin sulfate hydrogels with mineralization ability, ACS. Appl. Bio Mater. 5 (2022) 1508–1518.
[9] Y. Xiong, Y. Xiong, Applications of bone regeneration hydrogels in the treatment of bone defects: a review, J. Mater. Sci. 57 (2022) 887–913.
[10] J. Kopeček, Hydrogel biomaterials: a smart future? Biomaterials 28 (2007) 5185–5192.
[11] X. Bai, M. Gao, S. Syed, J. Zhuang, X. Xu, XQ. Zhang, Bioactive hydrogels for bone regeneration, Bioactive Mater. 3 (2018) 401–417.
[12] B. Xu, P. Zheng, F. Gao, W. Wang, H. Zhang, X. Zhang, X.Q. Feng, WG. Liu, A mineralized high strength and tough hydrogel for skull bone regeneration, Adv. Funct. Mater. 27 (2017) 1604327.
[13] N.A. Abdul Halim, M.Z. Hussein, M.K. Kandar, Nanomaterials-upconverted hydroxyapatite for bone tissue engineering and a platform for drug delivery, Int. J. Nanomedicine 16 (2021) 6477–6496.
[14] S. Wei, J.X. Ma, L. Xu, X.S. Gu, XL. Ma, Biodegradable materials for bone defect repair, Mil. Med. Res. 7 (2020) 54.
[15] L. Kuang, X.Y. Ma, Y. Ma, Y. Yao, M. Tariq, Y. Yuan, CS. Liu, Self-assembled injectable nanocomposite hydrogels coordinated by *in situ* generated CaP nanoparticles for bone regeneration, ACS. Appl. Mater. Interfaces. 11 (2019) 17234–17246.
[16] Y.S. Zhang, A. Khademhosseini, Advances in engineering hydrogels, Science 356 (2017) eaaf3627.
[17] Z. Deng, H. Wang, P.X. Ma, B. Guo, Self-healing conductive hydrogels: preparation, properties, and applications, Nanoscale 12 (2020) 1224–1246.
[18] W. Zhao, J.H. Yang, J. Zhou, F. Xu, M. Zrínyi, P.H. Dussault, Y. Osada, YM. Chen, Self-healing gels based on constitutional dynamic chemistry and their potential applications, Chem. Soc. Rev. 43 (2014) 8114–8131.
[19] Q. Lia, C. Liu, J. Wen, Y. Wu, Y. Shan, J. Liao, The design, mechanism and biomedical application of self-healing hydrogels, Chin. Chem. Lett. 28 (2017) 1857–1874.
[20] G. Xiao, Y. Wang, H. Zhang, L. Chen, S. Fu, Facile strategy to construct a self-healing and biocompatible cellulose nanocomposite hydrogel *via* reversible acylhydrazone, Carbohydr. Polym. 218 (2019) 68–77.
[21] D.L. Taylor, MI. Panhuis, Self-healing hydrogels, Adv. Mater. 28 (2016) 9060–9093.
[22] V K A Devi, R. Shyam, A. Palaniappan, A.K. Jaiswal, T.H. Oh, AJ. Nathanael, Self-healing hydrogels: preparation, mechanism and advancement in biomedical applications, Polymers. (Basel) 13 (2021) 3782.
[23] Y. Wang, Y. Chen, J. Zheng, L. Liu, Q. Zhang, Three-dimensional printing self-healing dynamic/photocrosslinking gelatin-hyaluronic acid double-network hydrogel for tissue engineering, ACS. Omega 7 (2022) 12076–12088.
[24] X. Ma, X. Liu, P. Wang, X. Wang, R. Yang, S. Liu, Z. Ye, B. Chi, Covalently adaptable hydrogel based on hyaluronic acid and poly(γ-glutamic acid) for potential load-bearing tissue engineering, ACS. Appl. Bio Mater. 3 (2020) 4036–4043.
[25] S. Trombino, C. Servidio, F. Curcio, R. Cassano, Strategies for hyaluronic acid-based hydrogel design in drug delivery, Pharmaceutics 11 (2019) 407.
[26] S. Li, Q. Dong, X. Peng, Y. Chen, H. Yang, W. Xu, Y. Zhao, P. Xiao, Y. Zhou, Self-healing hyaluronic acid nanocomposite hydrogels with platelet-rich plasma impregnated for skin regeneration, ACS. Nano 16 (2022) 11346–11359.
[27] Y. Luo, J. Tan, Y. Zhou, Y. Guo, X. Liao, L. He, D. Li, X. Li, Y. Liu, From crosslinking strategies to biomedical applications of hyaluronic acid-based hydrogels: a review, Int. J. Biol. Macromol. 231 (2023) 123308.
[28] K. Yang, J. Yang, R. Chen, Q. Dong, Y. Zhou, Fast self-healing hyaluronic acid hydrogel with a double-dynamic network for skin wound repair, ACS. Appl. Mater. Interfaces. 16 (2024) 37569–37580.
[29] M. Wei, T. Inoue, Y.I. Hsu, M.H. Sung, T. Fukuoka, S. Kobayashi, H. Uyama, Preparation of pH-responsive poly(γ-glutamic acid) hydrogels by enzymatic cross-linking, ACS. Biomater. Sci. Eng. 8 (2022) 551–559.
[30] A. Ogunleye, A. Bhat, V.U. Irorere, D. Hill, C. Williams, I. Radecka, Poly-γ-glutamic acid: production, properties and applications, Microbiology 161 (2015) 1–17.
[31] S.B. Park, M.H. Sung, H. Uyama, DK. Han, Poly(glutamic acid): production, composites, and medical applications of the next-generation biopolymer, Prog. Polym. Sci. 113 (2021) 101341.
[32] B. Manocha, A. Margaritis, A novel method for the selective recovery and purification of γ-polyglutamic acid from Bacillus licheniformis fermentation broth, Biotechnol. Prog. 26 (3) (2010) 1–17.
[33] M. Wei, Y.I. Hsu, T. Asoh, M.H. Sung, H. Uyama, Injectable poly(γ-glutamic acid)-based biodegradable hydrogels with tunable gelation rate and mechanical strength, J. Mater. Chem. B 9 (2021) 3584–3594.
[34] D.E. Clarke, E.T. Pashuck, S. Bertazzo, J.V.M. Weaver, MM. Stevens, Self-healing, self-assembled β-sheet peptide-poly(γ-glutamic acid) hybrid hydrogels, J. Am. Chem. Soc. 139 (2017) 7250–7255.
[35] E.H. Lee, Y. Kamigaito, Seki S Tsujimoto, H. Uyama, S. Tagawa, M.H. Sung, Preparation of poly(γ-glutamic acid) hydrogel/apatite composites and their application for scaffold of cell proliferation, SEN'I Gakkaishi. 66 (2010) 4.
[36] R. Yang, X. Liu, Y. Ren, W. Xue, S. Liu, P. Wang, M. Zhao, H. Hong Xu, B. Chi, Injectable adaptive self-healing hyaluronic acid/poly (γ-glutamic acid) hydrogel for cutaneous wound healing, Acta Biomater. 127 (2021) 102–115.
[37] G. Deng, F. Li, H. Yu, F. Liu, C. Liu, W. Sun, H. Jiang, Y. Chen, Dynamic hydrogels with an environmental adaptive self-healing ability and dual responsive sol-gel transitions, ACS. Macro Lett. 1 (2012) 275–279.
[38] Y. Qiao, X. Liu, X. Zhou, H. Zhang, W. Zhang, W. Xiao, G. Pan, W. Cui, A. Hélder, H. A. Santos, Q. Shi, Gelatin templated polypeptide Co-cross-linked hydrogel for bone regeneration, Adv. Healthcare Mater. 9 (2020) 1901239.