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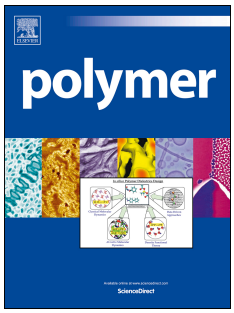

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Yusheng Liu: Conceptualization, Methodology, Data Curation, Formal analysis, Writing - Original Draft

Yigang Liu: Funding acquisition, Resources, Project administration

Qiuxia Wang: Visualization, Funding acquisition

Yugui Han: Validation Data, Curation

Yebang Tan: Conceptualization, Supervision, Project administration Writing - Review & Editing

Graphical abstract

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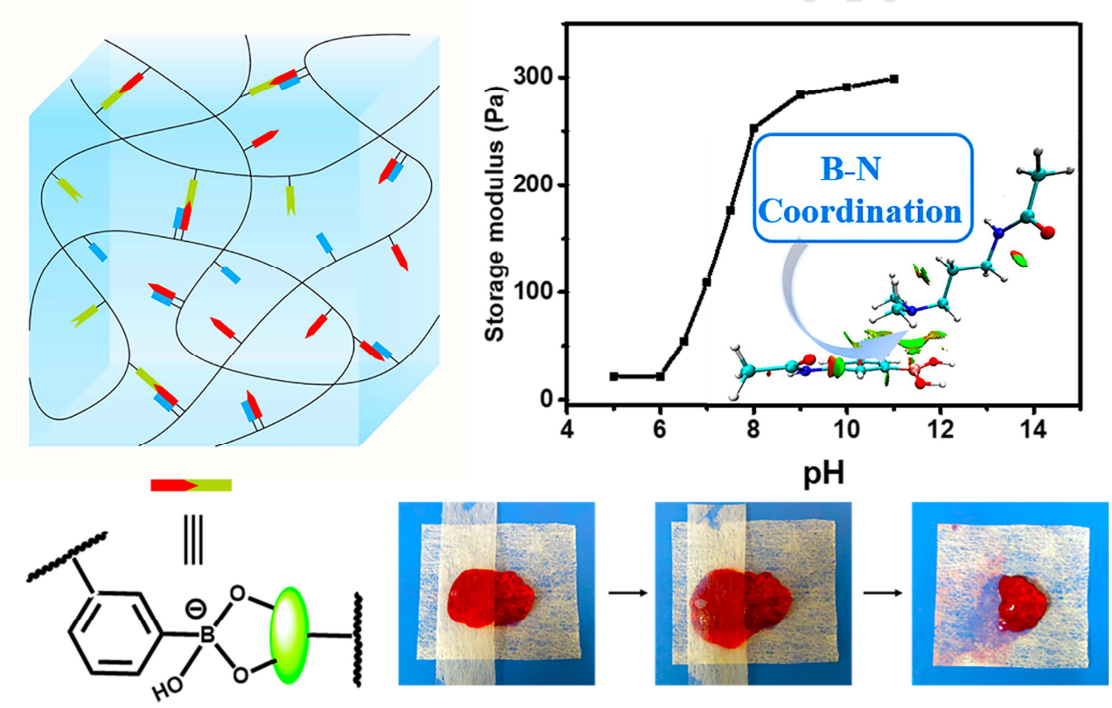


Boronic Ester-Based Self-Healing Hydrogels Formed by Using Intermolecular B-N Coordination

Yusheng Liu [a,b], Yigang Liu [b], Qiuxia Wang [b], Yugui Han [b], Yebang Tan [a,b]*

[a] School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, People's Republic of China ; The Key Laboratory of Special Functional Aggregated Materials, Ministry of Education, Shandong University, Jinan 250100, People's Republic of China

[b] CNOOC, Ltd, Tianjin Branch, Bohai Oilfield Research Institute, Tanggu Tianjin 300452, China

* Corresponding author. Tel.: +86-531-88363502; Fax: +86-531-88564464

E-mail address: ybtan@sdu.edu.cn

Abstract

The copolymer poly(acrylamide-*co*-2-acrylamido glucose) (PA) and poly(acrylamide-*co*-*N*-acryloyl-3-aminophenylboronic acid-*co*-*N*-(3-dimethylaminopropyl) acrylamide) (PB) were separately prepared by radical polymerization, and the hydrogel was readily constructed by simple mixing the two kinds of copolymers. The amine-containing monomer, *N*-(3-dimethylaminopropyl) acrylamide was incorporated into the polymer chain to enhance the stability of the complex between boronic acid and glucose units under physiological condition. The mechanical properties of the hydrogel can be readily engineered by adjusting the pH, solid content, and [phenylboronic acid]/[glucose] ratios. Moreover, the hydrogel possesses excellent intrinsic self-healing ability. Benefitting from the reversible property of the boronic ester, the hydrogel can be dissociated by adding competitive sugar molecules, which makes it easy to remove from medical gauze or skin. Additionally, cell cytotoxicity studies revealed that the hydrogel has good biocompatibility. With its superior properties, this dynamic phenylboronic ester-based hydrogel has great potential to be a component of new generation of wound dressings.

Keywords: hydrogel, water soluble polymer; self-healing, rheological behaviour, biocompatibility

1. Introduction

Recently, hydrogels have been considered as ideal materials for wound dressing owing to their biocompatibility and high water content.[1-4] The high water content provides a moist environment, which has been shown to be beneficial for wound healing.[5] However, traditional hydrogel dressings adhere to or entangle with the wound to some degree, which can cause secondary injuries to newly formed tissues and thus additional pain to the patient during the removal of the dressing material.[6] It is therefore necessary to develop easily removable dressing materials, which can be degraded in a rapid and controllable manner to prevent secondary injuries to patients.[7] Use of dynamic covalent bonds (DCBs), which can selectively undergo reversible breakage and reformation without irreversible side reactions,[8, 9] represents a new strategy for the design and preparation of wound dressings. The dynamic nature of DCBs endows the resulting hydrogels with self-healing, controllable degradation, and stimuli-response properties.

As a representative DCB, the boronic ester bond, which is formed through the condensation of boronic acid and 1,2- or 1,3-diols, has been successfully used to prepare hydrogels with self-healing and stimuli-response properties.[10-16] However, the equilibrium between the breakage and reformation of the boronic ester bond is controlled by the pH of the environment.[17-19] Typically, a dynamic bond forms

only at a pH near or above the pK_a of the boronic acid derivatives, which is typically 8–10.[20] In aqueous media, phenylboronic acid (PBA) exists in equilibrium between a hydrophilic triangular form and hydrophilic tetrahedral form, depending on the pH.[20,21] This pH-dependent dynamic bond formation severely inhibits the practical application of these materials.

Significant effort has been devoted to reducing the apparent pK_a of PBA-based materials in order to facilitate the formation of dynamic boronic esters under physiological conditions.[22,23] A typical method is to synthesize boronic acid derivatives with a lower pK_a by modifying the structure of PBA with electron-withdrawing groups.[10,24,25] However, the modification of the molecular structure or synthesis of these special PBA derivatives often involves complicated synthetic chemistry, thus limiting its applicability. Recent reports have shown that the pH dependence and viscoelasticity of boronic ester-based hydrogels can be engineered by designing the chemical structure of the polymer chain.[11,26] Accordingly, a suitable amino group was introduced into the polymer backbone to decrease the apparent pK_a of the PBA moiety.[27,28] The amino groups present in PBA-containing polymers enhanced the stability of the boronic ester owing to the coordination interaction between PBA and the amide groups.[27,29-31] Okano *et al*. used this approach to prepare microgels to control the release of insulin under physiological conditions.[31] However, to the best of our knowledge, there has been no report on boronic ester-based self-healing bulk hydrogels with excellent physiological usability formed via B-N intermolecular coordination.

Herein, we report a boronic ester-based hydrogel formed under physiological conditions as an ideal wound dressing material. The hydrogel was readily constructed by simple mixing the boronic acid-containing poly(acrylamide-*co*-2-acrylamido glucose) and diol-containing poly(acrylamide-*co*-*N*-acryloyl-3-aminophenylboronic acid-*co*-*N*-(3-dimethylaminopropyl) acrylamide). To fabricate the hydrogel under physiological pH, amine-containing *N*-(3-dimethylaminopropyl) acrylamide (DMAPAA), was introduced into the polymer chain. The composition-property relationship of the hydrogels in terms of the pH, [PBA]/[glucose] ratios, solid content was investigated by the rheological study. The self-healing property and degradability of the hydrogel were investigated comprehensively. Moreover, the effective biocompatibility of the hydrogel renders it an ideal platform for developing easily removable wound dressing materials.

2. EXPERIMENTAL SECTION

2.1 Materials

D-glucosamine hydrochloride, aminophenylboronic acid, acryloyl chloride, 2,2′-azobis(isobutylronitrile) (AIBN), and *N*-(3-dimethylaminopropyl) acrylamide (DMAPAA) were obtained from J&K Scientific and used as received. Acrylamide (AM), sodium carbonate, sodium hydrogen carbonate, hydrochloric acid, dimethyl sulfoxide (DMSO), and acetone were obtained from Sinopharm Chemical Reagent Co. and used as received.

2.2 Characterisation

The ^{1}H NMR spectra were recorded on Bruker Avance 400 or 300 NMR

spectrometer. The feed ratios of the copolymers were determined by elemental analyse using an Elementar Vario E1 III elemental analyser (Germany) at 1150 °C. Static light scattering (SLS) was performed on a Dawn Heleos light scattering instrument (Wyatt Technology) equipped 18-angle light scattering detectors. Morphological observations were performed using a Hitachi S-4800 scanning electron microscope (SEM). Samples of the hydrogel were freeze-dried and sputter-coated with platinum. The rheological behaviour of the samples was characterised using a Thermo Scientific Haake MARS III rheometer equipped with a 20 mm parallel plate at 25 °C. All hydrogels were aged for 24 h before testing.

2.3 Synthesis of a 2-acrylamido glucose (AAG)

AAG was synthesized according the literature via the reaction of D-glucosamine hydrochloride and acryloyl chloride.[32] D-glucosamine hydrochloride (8.6 g) and $NaNO_2$ (0.14 g) were dissolved in 40 mL of 2 M K_2CO_3 aqueous and cooled in a low-temperature reactor at -5 °C with magnetic stirring. Acryloyl chloride (4.0 g) was added dropwise to the solution. The mixture was reacted at -5 °C for 120 min, and then warmed to room temperature for 24 h. Subsequently, absolute ethyl alcohols (200 mL) were poured into the reactant and then the white solid was removed by filtration. The solution was concentrated by rotary evaporation and the crude product was obtained. The crude product was purified on silica gel column chromatography and recrystallization with a methanol/ethyl acetate mixture. The product was obtained and the yield of the product is 41.5%. ^{1}H NMR (400 MHz, D_2O) δ 6.34 – 6.08 (m, 4H), 5.72 (dd, *J* = 10.0, 1.7 Hz, 2H), 5.15 (d, *J* = 3.6 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H),

3.96 – 3.33 (m, 10H).

2.4 Synthesis of *N*-acryloyl-3-aminophenylboronic acid (AAPBA)

3-aminophenylboronic acid (5.478 g) was dissolved in 80 mL of 2 M NaOH and cooled in a low-temperature reactor at -5 °C with magnetic stirring for 10 min. Subsequently, acryloyl chloride (5.20 mL) was added dropwise. The reaction mixture was reacted at -5 °C for 30 min and then was carried out at room temperature for 120 min, and the pH of the reaction mixture was adjusted to 2 with a 2 M HCl solution. The precipitate was filtered off and washed 3 times with 25 mL of cold deionized water. The precipitate was dissolved in hot water (70 °C) and filtered off the insoluble impurities. The hot filtrate was left to a refrigerator overnight and the product was obtained by filtered off. The yield of AAPBA was 69.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.08 (d, J = 16.0 Hz, 1H), 8.01 (s, 2H), 7.94 – 7.77 (m, 2H), 7.50 (dt, J = 7.3, 1.2 Hz, 1H), 7.30 (q, J = 8.5, 7.7 Hz, 1H), 6.45 (dd, J = 17.0, 10.0 Hz, 1H), 6.24 (dd, J = 17.0, 2.2 Hz, 1H), 5.73 (dd, J = 10.0, 2.2 Hz, 1H).

2.5 Preparation of copolymers

The copolymer, poly(AM-*co*-AAG) with 5.0 mol% AAG, referred to as PA-5, was synthesized by radical polymerization. First, AM (6.752 g) and AAG (1.168 g,) were dissolved in 52.8 mL of DMSO. The mixture was degassed under a nitrogen atmosphere with magnetic stirring for 1 h, and then an initiator solution (1.0 mL) containing 0.025 g of AIBN was injected into the reaction mixture. The mixture was allowed to react at 70 °C for 24 h. After polymerization, the solution was transferred to a semi-permeable membrane (MWCO: 3500) and purified against deionized water

for 7 d to remove impurities. The product was obtained after lyophilisation. Another polymer referred to as PA-10 containing 10 mol% AAG was prepared in the same way.

The copolymer, poly(AM-*co*-AAPBA-*co*-DMAPAA) with 5.0 mol% AAPBA, referred to as PB-5, was also synthesized by radical copolymerization. First, AM (6.04 g), AAPBA (0.96 g), and DMAPAA (1.56 g) were dissolved in 57.1 mL of DMSO. The mixture was degassed under a nitrogen atmosphere with magnetic stirring for 1 h, and then an initiator solution (1.0 mL) consisting of 0.025 g of AIBN in DMSO was injected into the reaction mixture. The mixture was allowed to react at 70 °C for 24 h. After polymerisation, the solution was transferred to a semi-permeable membrane (MWCO: 3500) and purified against deionized water for 7 d to remove impurities. The product was obtained after lyophilisation. Another polymer referred to as PB-10 containing 10 mol% AAPBA was prepared in the same way.

2.6 Fabrication of hydrogels

Hydrogels were prepared by mixing PB and PA solutions with mild stirring at room temperature. To investigate the effect of pH, the polymers were dissolved in phosphate-buffered saline (PBS) solutions with different pH values (ranging from 5 to 11). The pH value in experiment was repeatedly adjusted by adding 1 M HCl aqueous solution or 1 M NaOH aqueous solution to the PBS solutions. The solid contents were kept constant at 5.0 wt%. The [PBA]/[glucose] ratio was adjusted by varying the volumes of the PA and PB polymer solutions used for hydrogel fabrication.

2.7 Cell cytotoxicity

The cytotoxicity of the dynamic hydrogels was evaluated by Cell Counting Kit-8 (CCK-8) assay using both human embryonic kidney (HEK293) cells and HeLa cells. Typically, hydrogels with different compositions and amounts were incubated in 4 mL of the Dulbecco's modified eagle medium (DMEM) (Corning, USA) for 24 h. Cells were seeded in 96-well plates at a density of 7×10^3 cells/well and incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. Then, the cells were incubated with either fresh DMEM medium or DMEM medium containing gel extracts for another 24 h. Each well was washed twice with PBS after removing the medium. Then, a mixture of CCK-8 (Shanghai Yeasen Biological Engineering Technology, China) and DMEM medium (1:10; 100 μL/well) was added to the wells and the cells were cultured for 2 h. The cell numbers were counted using a microplate reader (Model 680 Bio-RAD) at 450 nm; DMEM medium without cells but with added CCK-8 was used to record the background signal.

2.8 Decomposition of hydrogels

The hydrogel formed from PB-5 and PA-5 in PBS (polymer content of 10 wt%, [PBA]/[glucose] = 1) was used to investigate their responsiveness to sugar. To test the sugar responsiveness, a hydrogel plate coloured by carmine was cut into two equal pieces, and the halves were incubated in a PBS (pH 7.3) solution with or without 2 M fructose. The dissociation of the hydrogels was monitored visually at different time intervals. Moreover, a piece of hydrogel was attached to a medical gauze, and a fructose-soaked gauze was applied to half the hydrogel to test its controllable degradability.

3. Results and discussion

3.1 Characterization of copolymers and fabrication of hydrogels at various pH

The boronic acid-containing copolymer PB and diol-containing copolymer PA were separately prepared by conventional radical polymerisation. The structures of the polymers were characterized by ^{1}H NMR spectra as shown in Figure 1, and the molecular weight of the copolymers was determined by SLS (Table 1). The actual molar ratios of monomers in copolymers were calculated from the results of elemental analyse (Table S1). Rheological properties of the PB solutions with varying concentrations were also investigated, as shown in Figure S3. For the PB-5 and PB-10 solutions, the loss modulus is always prevailing on the storage modulus over entire investigated range of the oscillatory strain. Moreover, loss modulus dominates the whole frequency range with increasing tendency of both storage and loss modulus. The PB itself cannot form gels using B-N as cross-linking at the polymer concentration use in experiments.

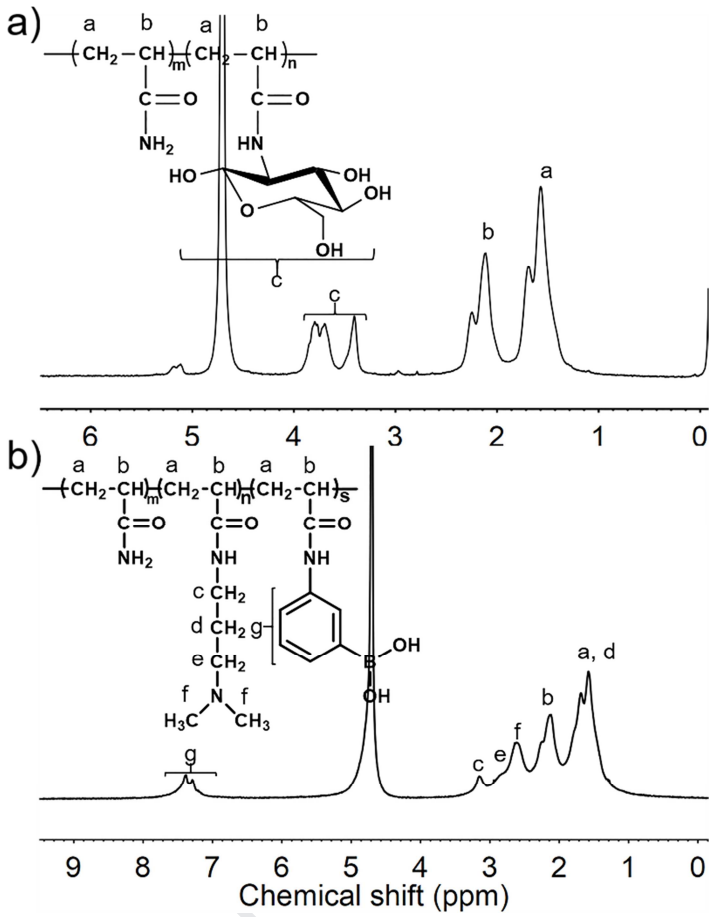


Figure 1. 400 M ^{1}H NMR spectra of a) PA-5 and b) PB-5. The spectra were recorded at 298 K in D2O.

Hydrogels were prepared by mixing boronic acid-containing PB copolymer and diol-containing PA copolymer solutions (Scheme 1a). The amino groups present in the boronic-containing polymer enhanced the stability of the boronic ester, which is likely related to the electrostatic attraction between electron-deficient boron in the PBA group and electron-rich nitrogen in the amino group at an intermediate pH. Scheme 1b and Figure S4 qualitatively illustrates the intermolecular B-N interactions in the polymer mixture. [33,34] It is clear that the middle system (pH = 7) has stronger attractive interactions between boron in the PBA moiety and nitrogen in the amino moiety. It is worth noting that other weak non-covalent interactions occur in parts

other than those involving B-N interactions, when the pH is greater than or less than 7.

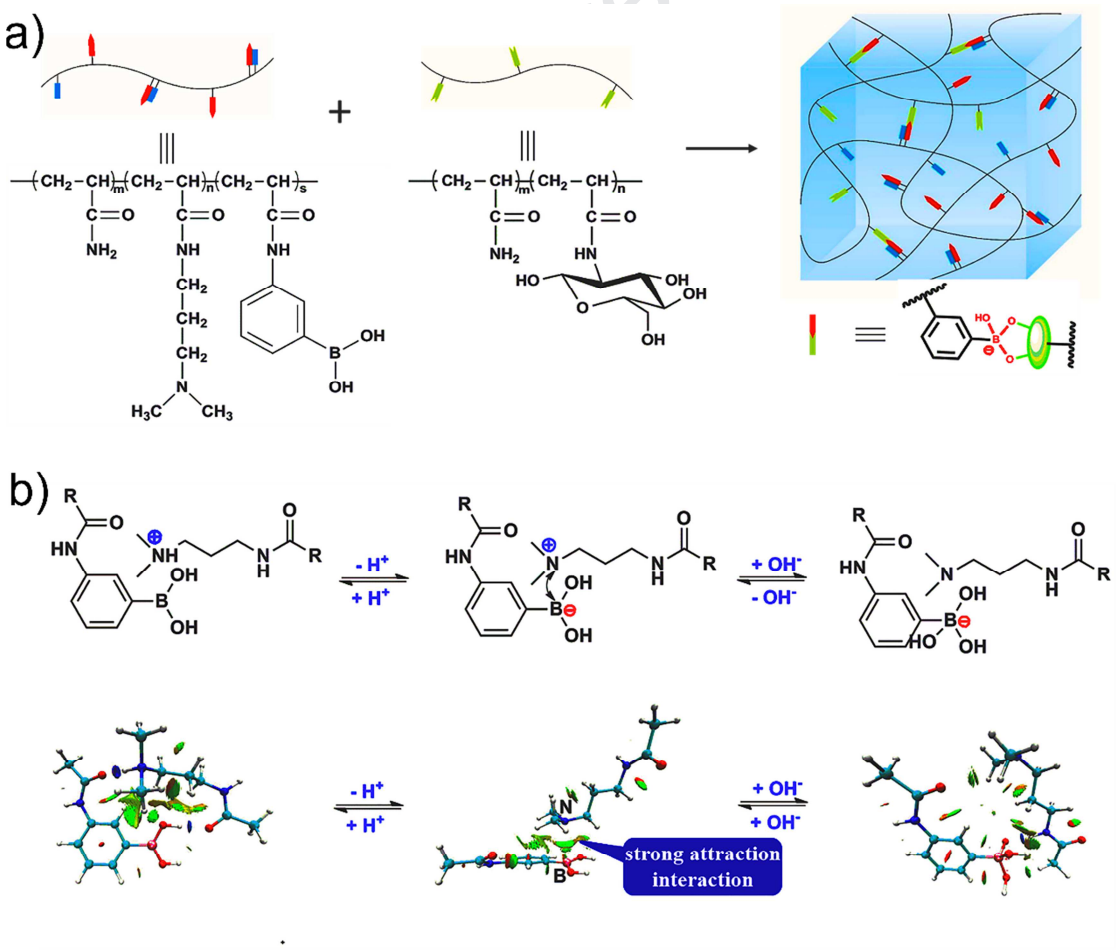
Table 1 Characterization of copolymers.

sample	Feed ratio (%)	Determined ratio[a] (%)	$M_{w,\,app}$[b] (10^4 g/mol)	A_2[b] (10^{-3} mol mL/g^2)	Yield (%)
PB-5	85.0: 10.0: 5.0[c]	80.53: 14.11: 5.36[c]	7.29 ± 0.63	0.112	92.4
PB-10	80.0: 10.0: 10.0[c]	73.23: 17.56: 9.21[c]	8.10 ± 1.42	-0.098	93.8
PA-5	95.0: 5.0[d]	92.12: 7.88[d]	11.2 ± 1.58	0.692	87.5
PA-10	90.0: 10.0[d]	83.37: 16.63[d]	9.63 ± 1.88	0.743	86.4

[a] Calculated from elemental analyse ;[b] Obtained by SLS measurements ; [c] Molar ratios of AM: DMAPAA: AAPBA in PB ; [d] Molar ratios of AM: AAG in PA.

Hydrogel formation at varying pH values was qualitatively confirmed through a vial inversion test, as shown in Figure 2a. The mixture was converted from a viscous liquid to an elastic hydrogel with a change in pH from acidic to alkaline one. At pH 6.5, the system exhibited a semisolid state and flowed under gravity on a long-time scale, indicating the formation of a hydrogel. Moreover, gelation occurred immediately upon mixing and gently stirring the two polymer solutions. To obtain further insight into the gel-formation kinetics, a dynamic time sweep experiment was conducted to determine the hydrogelation process, as shown in Figure S5. The hydrogel could form within 60 s, as indicated by the crossover point of G' and G'' curves. These results indicated that the hydrogel can formed with the pH > 6.5. To

demonstrate the influence of the intermolecular B-N coordination on this system, the control experiments were carried out. The copolymer, poly(AM-*co*-AAPBA) with 5.0 mol% AAPBA and without the DMAPAA, referred to as PN-5, was synthesized. Rheological properties of hydrogels (formed from PN-5 and PA-5, 5.0 wt%, [PBA]/[glucose] = 1) at different pH were also investigated as shown in Figure S6 and Figure S7. It was clearly that the hydrogel can formed when the pH > 8.2. The existence of the DMAPAA in the polymer backbone can provide a weak alkaline microenvironment due to the coordination interaction between PBA and the amide groups, which can decrease the apparent pK_a of the AAPBA.



Scheme 1. (a) Schematic illustration of the formation of the dynamic hydrogels. (b)

Non-covalent interactions of the polymer mixture system. Regions of red, green, and blue indicate strongly repulsive, weakly attractive, and strongly attractive interactions, respectively.

To quantitatively investigate the effects of pH on the mechanical properties, hydrogels prepared at various pH were subjected to oscillatory rheological measurements as a function of angular frequency. As shown in Figure 2b, frequency-dependent modulus curves were observed for all hydrogels. At high frequencies, the hydrogels behaved like an elastomer with $G' > G''$, whereas at low frequencies, a liquid-like state was observed with $G' < G''$. Typically, unlike gels cross-linked by permanent covalent bonds, gels cross-linked by dynamic covalent bonds usually display frequency-dependent moduli. This reversible association-dissociation mechanism often contributes to a characteristic relaxation time τ_c, which characterises the dynamics of the system.

The τ_c value was obtained from the inverse of the crossover frequency f_c, where G' equals G''.[35,36] At low angular frequencies, there is sufficient time for the bonds to restructure when perturbed because the time scale probed in the test is longer than the lifetime of the reversible cross-linking, whereas the system does not have enough time to dissociate at high angular frequencies. The hydrogel formed at pH 6.5 has the highest f_c of 0.16 Hz corresponding to a relaxation time τ_c of 6.67 s. With increasing pH, τ_c increased as f_c shifted to the low-frequency region. For example, τ_c increased to 7.38 s and 20.11 s for the hydrogels formed at pH 7.0 (f_c = 0.135 Hz) and 10.0 (f_c = 0.043 Hz), respectively. Moreover, the storage modulus in the frequency-independent

region increased with increasing pH. The plateau *G′* values are summarised in Figure 2c. The improved mechanical properties and lifetime of the network can be reasonably attributed to the increased binding affinity between the PBA group and glucose moiety at an alkaline pH. The relative binding constants of PBA and sugar are pH-dependent, and a higher pH favours the formation of the boronic ester bond under specific conditions.[37,38]

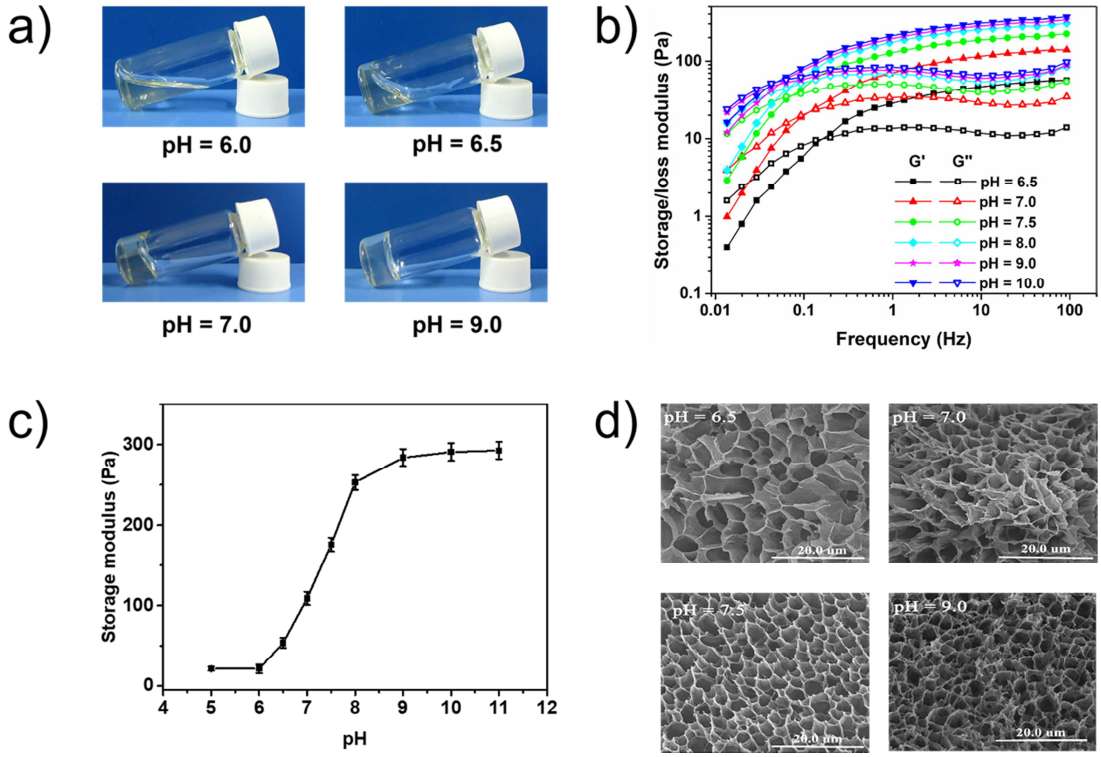


Figure 2. (a) Photographs, (b) dynamic oscillatory angular frequency sweep, (c) storage modulus of the hydrogels formed at different pH. (d) SEM images of the dynamic polymer dry gels formed at different pH. The hydrogels were formed from PB-5 and PA-5 (5.0 wt%, [PBA]/[glucose] = 1).

The structural features of the hydrogels were also characterised according to the network parameters (Table S3) calculated from Equations 1–3.[39,40]

$$M_e = \frac{\mathrm{CRT}}{G'_p} \quad (1)$$

$$\nu_e = \frac{G'_p \mathrm{N_A}}{\mathrm{RT}} \quad (2)$$

$$\xi = \left(\sqrt[3]{\nu_e}\right)^{-1} \quad (3)$$

where c (g/L) is the polymer concentration, R is the universal gas constant, N_A is the Avogadro constant and T is the temperature, the G'_p was determined from the frequency independent regime of the storage modulus.

The cross-linking density υ_e increased with increasing pH, and therefore, the cross-linking distance ξ between effective cross-links decreased. The calculated molecular weight of the polymer segment between effective cross-links M_e was used to evaluate the number of effective cross-links per polymer chain. To investigate the interior morphology of the hydrogels formed at different pH values, SEM images were obtained, and are shown in Figure 2d. All the hydrogels possessed an interconnected porous network structure. The pore size decreased at an alkaline pH, indicating an increase in the cross-linking density.

Table 2. Network parameters for polymer gels formed with varying pH.

pH	τ_c (s)	G'_p (Pa)	M_e (kg/mol)	v_e (10^{21} m^{-3})	ξ (nm)
6.5	5.03	21.5	5756.4	5.2	57.6
7.0	7.38	52.0	2381.4	5.2	57.6
7.5	10.84	126.2	981.6	12.6	42.9
8.0	15.91	209.1	592.7	30.7	31.9
9.0	18.06	253.2	489.6	50.8	27.0
10.0	20.11	284.3	436.2	61.5	25.3

The rheological properties of the hydrogel formed from PB-10 and PA-10 were also investigated (Figure S9). The moduli of the polymer gels formed by complexing the PB-10 and PA-10 copolymers were much greater than those of the hydrogels consisting of PB-5 and PA-5 at the same pH. Moreover, the crossover frequency shifted to lower angular frequencies as compared with those of the hydrogels formed from PB-5 and PA-5, indicating a slowing of the hydrogel dynamics.[36] Increasing the number of cross-linking moieties in the polymer chain increased the number of effective cross-links per polymer chain, which is correlated with τ_c.

3.2 Effect of [PBA]/[glucose] on hydrogels

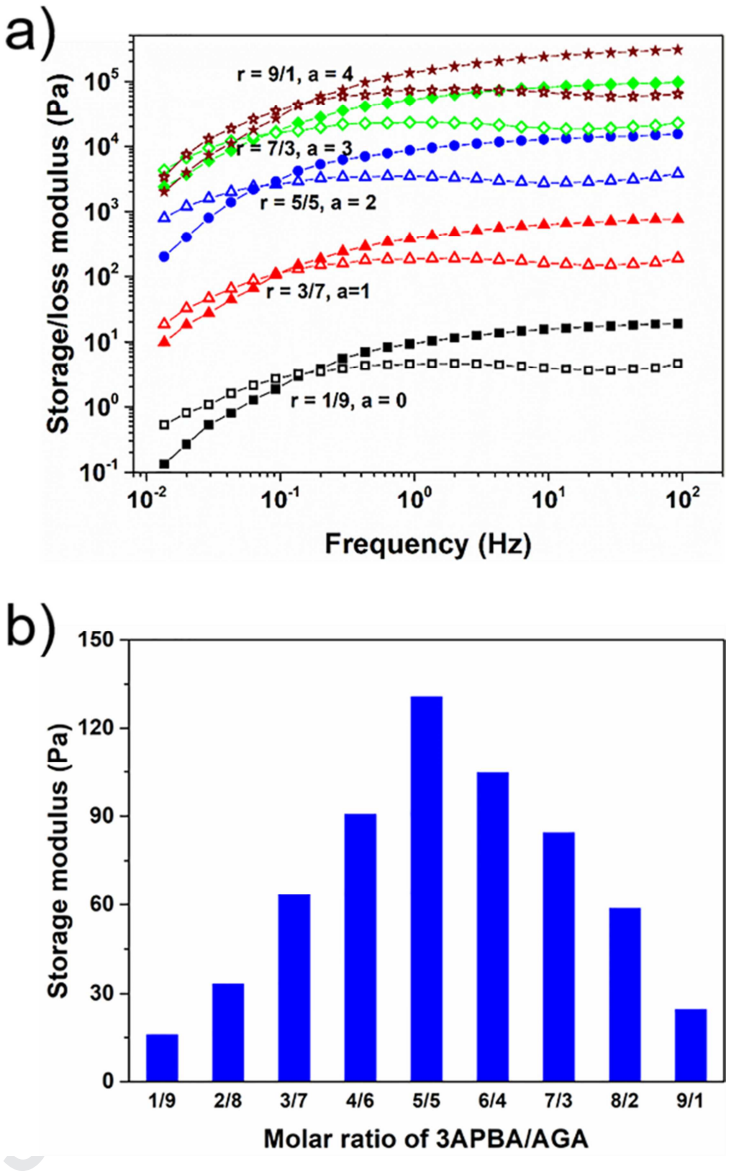


Figure 3. (a) Dynamic oscillatory angular frequency sweep of the hydrogels formed from PB-5 and PA-5 (pH = 7.0, 5.0 wt%) with different [PBA]/[glucose] ratios. The data have been vertically shifted by 10^{a} to avoid overlapping. (b) summarization of plateau storage modulus with various [PBA]/[glucose] ratios.

The mechanical properties of the polymer gels could also be manipulated by altering the ratio of [PBA]/[glucose]. Figure 3a and Figure S10 show the G' and G'' as a function of f for the gels obtained from PB-5 and PA-5 with different

[PBA]/[glucose] ratios at a constant 5.0 wt% solid content. The frequency-independent plateau G' are summarised in Figure 3b. As expected, the largest plateau G' occurred at the stoichiometric ratio (1: 1) of [PBA]/[glucose] due to the increased cross linkages. The network parameters were calculated, and are presented in Table S2. The hydrogel with a [PBA]/[glucose] ratio of 5:5 has the highest cross-linking density υ_e and thus the lowest cross-link distance ξ between two points.

3.3 Effect of solid content on hydrogels

The mechanical properties of the dynamic boronic ester-based hydrogels can be further engineered by adjusting the solid content. Polymer gels formed from PB-5 and PA-5 (pH = 7.0, [PBA]/[glucose] = 1) with different polymer concentrations were chosen for testing. The G' and G'' as functions of strain obtained from oscillatory amplitude sweep are shown in Figure S11. As expected, both storage and loss moduli increased with increasing polymer content due to the increase in the cross-linking density. The hydrogels with various concentrations demonstrated similar linear viscoelastic regions up to 100% strain. In this region, G' remained above G'', indicating that the hydrogel networks were rigid and that the networks remained intact under relatively large deformations. With increasing strain amplitude, gel-liquid transition points, where the G'' curve crosses over the G' curve occurred at 500% strain, indicating the beginning of the destruction of the hydrogel network. However, both G' and G'' show little strain-hardening behaviours under oscillatory strain before the destruction of the network structure, implying that both the creation and

destruction of dynamic bonds occurred during this process and that their creation occurred more quickly than their destruction.[41,42]

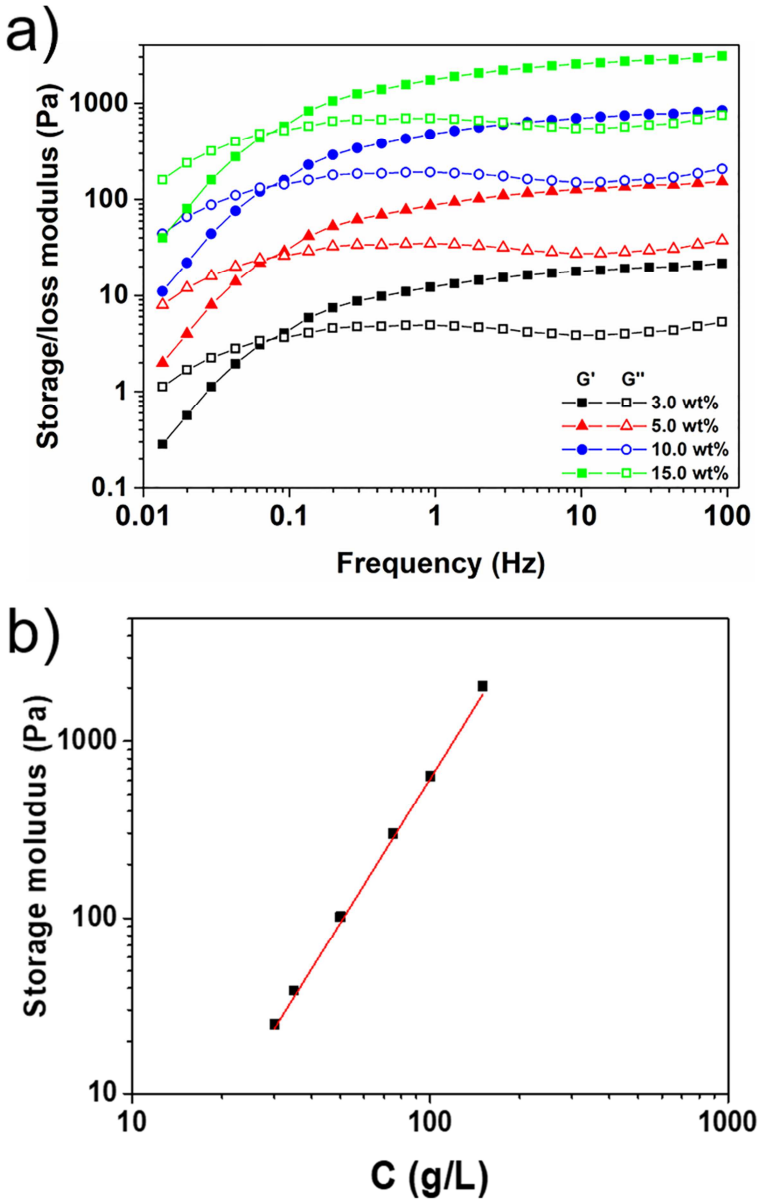


Figure 4. (a) Dynamic oscillatory angular frequency sweep of the hydrogels formed from PB-5 and PA-5 (pH = 7.0, [PBA]/[glucose] = 1) with different polymer concentrations. (b) storage modulus as functions of the polymer concentrations.

Figure 4a depicts the storage and loss moduli versus angular frequency at a constant strain (10%) for hydrogels with various polymer concentrations. The crossover frequency of the *G′* and *G″* curves was independent of the concentration at

approximately 0.083 Hz in the concentration range of 30–150 g L^{-1}. There have been reports that both the *G′* and *G″* curves of dynamic hydrogels with different solid contents can be superimposed by only vertical shifting.[36] This indicated that the polymer concentration did not alter the lifetime of the cross-links arising from the reversible nature of the DCBs. The plateau storage modulus versus polymer concentration plots are presented in Figure 4b, showing a curve approximating a straight line on a double logarithmic coordinate system. The correlation between the mechanical strength and polymer concentration offers an effective approach to design hydrogels with tailored mechanical properties.

The rheological properties of the hydrogel formed from PB-10 and PA-10 were also investigated (Figure S12). The modulus of the polymer gels formed by complexing the PB-10 and PA-10 copolymers were much higher than those of the PB-5 and PA-5 gels. The plateau storage modulus of the hydrogel with a polymer concentration of 15 wt% was 8.6 KPa, indicating that the mechanical strength could be tuned over a large range.

3.4 Hydrogel self-healing behaviour

Hydrogel wound dressings with self-healing abilities can improve the reliability of dressings and provide better wound protection.[43-45] To qualitatively evaluate the self-healing behaviour of the dynamic polymer gels at intermediate pH, three parts of a cylindrical hydrogel (formed from PB-5 and PA-5, 10 wt%, [PBA]/[glucose] = 1) prepared under physiological conditions were placed together and contacted without external treatment. As expected, the gels merged autonomously into a whole

cylindrical hydrogel after contact for 40 min. Furthermore, the cracks in the attachment region disappeared completely (Figure 5a). The hydrogel after healing was strong enough to be stretched to a length several times its initial length without breakage. This self-healing behaviour can be explained by the dynamic equilibrium of the formation and breakage of the phenylboronic ester bond.[46] In aqueous media, free boronic acid and diol groups still exist in the hydrogels due to the reversibility of the boronic ester bonds. Thus, the boronic ester formation and rearrangement across the adjacent interfaces contributed to the self-healing of the hydrogels.

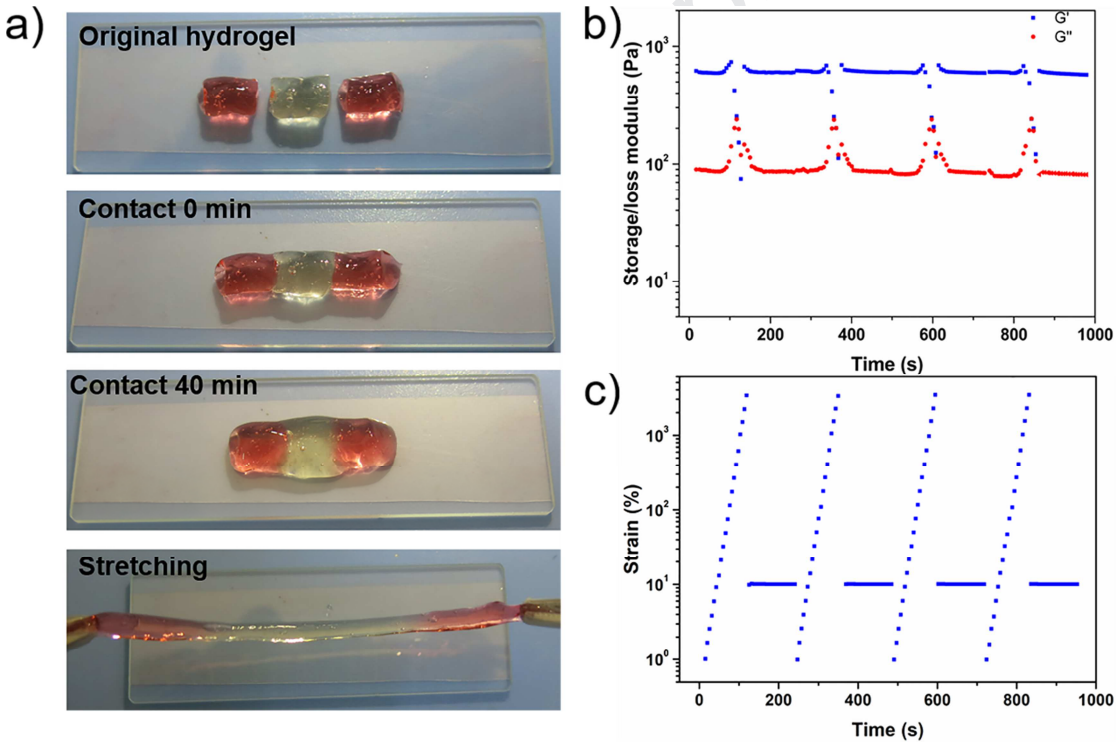


Figure 5. a) Optical images showing the self-healing behaviour of dynamic hydrogels, the red hydrogels were dyed by carmine, and self-healing of hydrogels after fracture, the plot b) shows the change in modulus during the strain ramp described by the bottom plot c).

The self-healing properties of the dynamic polymer gels were also investigated by

dynamic rheological experiments. The hydrogel was subjected to increasing strain to induce a large-amplitude deformation (1300% oscillatory strain) far beyond the linear viscoelastic region, followed by a recovery period under small-amplitude (10% oscillatory strain) deformation conditions. As shown in Figure 5b, the gel recovered its original mechanical properties in 100 s at 10% strain after being destroyed, indicating its excellent self-healing performance. Furthermore, the modulus value of the hydrogel did not decrease significantly with each repeated cycle of recovery. This repeatable and efficient self-healing performance of the hydrogel is beneficial for bioengineering functions. To study the injectable properties, hydrogel was loaded into a syringe, then injected on glass substrate. The self-healing hydrogel could pass through a 26-gauge needle without clogging (Figure S13), indicating its excellent injectability. This injectable self-healing hydrogel dressing could be easily applied to irregular wound sites and adhered to wounds or medical gauze *in situ*. To illustrate the adhesion properties, the hydrogel was adhered to human skin, and the image was shown in Figure S14.

3.5 Cell cytotoxicity

Biocompatibility is an essential requirement for well-designed hydrogels for wound dressing.[4,5] Therefore, a relative cell viability assay based on the standard CCK-8 was performed for both HEK293 cells and HeLa cells, and the results are shown in Figure 6. Considering the appropriate mechanical strength and good self-healing ability,[5,7,47] the hydrogel formed from PB-5 and PA-5 (10 wt%, [PBA]/[glucose] = 1) was chosen for evaluation. Mechanical tensile test showed that

the hydrogel possessed good stretchability (21 kPa, 210% of the elongation-at-break), which could assure their integrity during the application (Figure S15). The hydrogel exhibited low cytotoxicity, with over 95% cell viability remaining for both HEK293 and HeLa cells at a concentration of 400 mg gel in 4 mL of DMEM. When the hydrogel content was increased to 800 mg, slightly lower yet still good viabilities of 88.7% and 87.5%, respectively, were obtained for HEK293 and HeLa cells. Typically, boronic derivatives are considered to have low cytotoxicity for most cell lines.[48] The good biocompatibility of the hydrogel can be attributed to the good biocompatibility of the glycopolymer PA and the low content of PBA groups in PB-5. Therefore, this boronic ester-based hydrogel formed under physiological conditions has great potential as a wound dressing material.

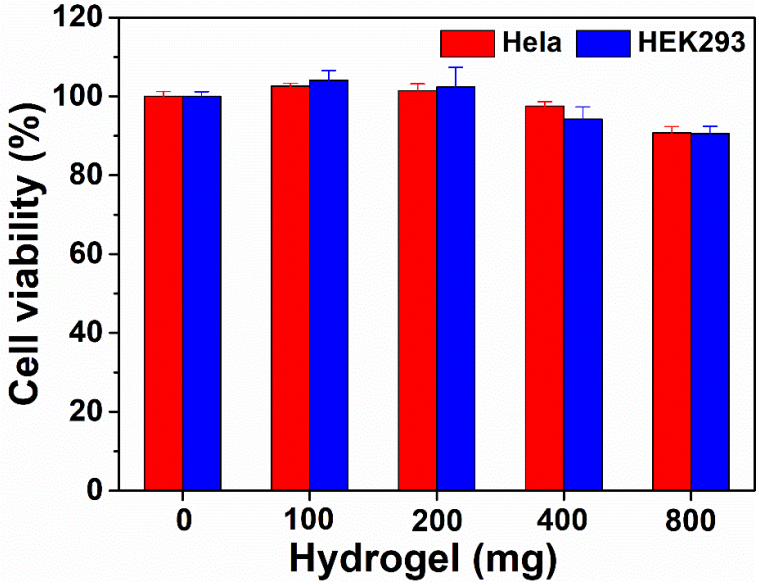


Figure 6. Cell viability of HeLa cancer cells and HEK293 cells after 24 incubation with gel extracts, the hydrogels were formed from PB-5 and PA-5 (10 wt%, [PBA]/[glucose] = 1).

3.6 Decomposition of hydrogels

Wound dressing materials are usually mechanically removed from the wound, which may cause secondary injury and additional pain to the patient. The dynamic boronic acid ester bond can be dissociated in the presence of another competitive molecule due to a shift in the equilibrium,[49,50] which can be effectively used to prevent any new injuries arising from the removal of the material. Fructose was selected for experiments owing to its high sensitivity to complexing with PBA.[16,51] The hydrogel could completely degrade into a viscous liquid in 3 min after the addition of excess fructose (Figure 7a), indicating the efficient dissociation of phenylboronic-glucosamine interactions. The dissociation of carmine-dyed hydrogels in 2 M fructose is depicted in Figure 7b. The hydrogel incubated in the fructose solution completely disappeared within 30 min because of the cleavage of the cross-links due to competition from fructose for binding with the PBA groups in the system. Moreover, there was no obvious volume change of the hydrogel in the PBS solution without fructose, indicating that the hydrogel was stable under physiological conditions. Figure 7c shows the dissolution process on medical gauze. One half of the carmine-dyed hydrogel coated on fructose-soaked gauze disappeared completely within 5 min; that is, only half of the hydrogel remained. These results confirmed that the demonstrated dynamic hydrogel with its controllable degradability can effectively improve the performance of wound dressings, as it can be easily removed to avoid damage to newly formed tissues.

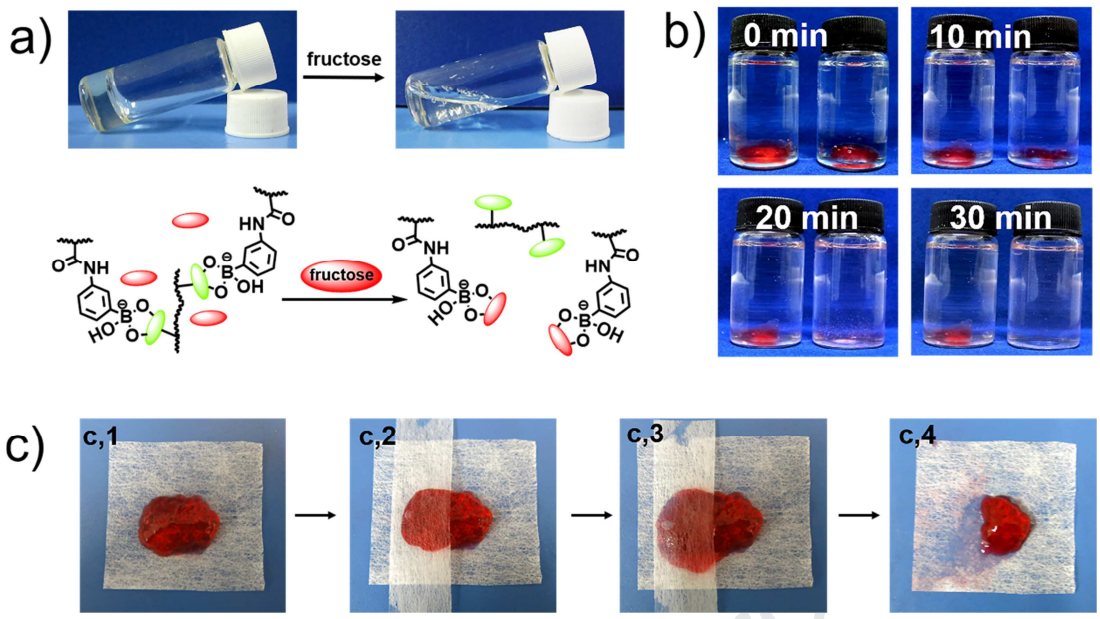


Figure 7. (a) Degradation of the hydrogel by adding excess of the fructose, (b) Hydrogel stability in PBS with (right vial) and without (left vial) 2.0 M of fructose, (c) photographs of gel degradation process. (c, 1) original hydrogel, (c, 2) fructose-soaked gauze was applied to half of the hydrogel, (c, 3) after 5.0 min and (c, 4) gauze was removed and only half of the hydrogel remained. Hydrogels were dyed by carmine for better observation and the gauze was immersed in a 10 M fructose solution for 10 min before used.

4. CONCLUSIONS

In summary, we fabricated bulk boronic ester-based hydrogels with excellent physiological usability using the B-N coordination interaction for wound dressings. The hydrogels were readily prepared by mixing the copolymer poly(AM-*co*-AAG) and poly(AM-*co*-APBA-*co*-DMAPAA) in aqueous solution. Amine-containing DMAPAA was incorporated into the polymer backbone to facilitate the formation and increase the stability of the cross-links. The cross-linking density and inner structure of the polymer hydrogels could be readily engineered by controlling the pH,

[PBA]/[glucose] ratio, solid content of the polymer, thus offering great versatility for preparing functional hydrogels with tuneable mechanical properties. Moreover, the hydrogels exhibited excellent biocompatibility for both HEK293 cells and HeLa cells. Benefitting from the dynamic nature of the boronic ester, the hydrogels can be dissociated by adding competitive sugar molecules, making them easy to remove from medical gauze or skin. This approach of designing a polymer architecture to regulate the pH dependence and mechanical properties of a gel is a promising strategy for preparing functional hydrogels. The amine-containing dynamic hydrogel is a practical platform for designing multifunctional, smart soft materials, and has great potential for biomedical applications, especially for wound dressings.

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Declaration of interest: There are no conflicts to declare.

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The captions of Scheme and Figure

Scheme 1. (a) Schematic illustration of the formation of the dynamic hydrogels. (b) Non-covalent interactions of the polymer mixture system. Regions of red, green, and blue indicate strongly repulsive, weakly attractive, and strongly attractive interactions, respectively.

Figure 1. 400 M ^{1}H NMR spectra of a) PA-5 and b) PB-5. The spectra were recorded at 298 K in D2O.

Figure 2. (a) Photographs, (b) dynamic oscillatory angular frequency sweep, (c) storage modulus of the hydrogels formed at different pH. (d) SEM images of the dynamic polymer dry gels formed at different pH. The hydrogels were formed from PB-5 and PA-5 (5.0 wt%, [PBA]/[glucose] = 1).

Figure 3. (a) Dynamic oscillatory angular frequency sweep of the hydrogels formed from PB-5 and PA-5 (pH = 7.0, 5.0 wt%) with different [PBA]/[glucose] ratios. The data have been vertically shifted by 10^{a} to avoid overlapping. (b) summarization of plateau storage modulus with various [PBA]/[glucose] ratios.

Figure 4. (a) Dynamic oscillatory angular frequency sweep of the hydrogels formed from PB-5 and PA-5 (pH = 7.0, [PBA]/[glucose] = 1) with different polymer concentrations. (b) storage modulus as functions of the polymer concentrations.

Figure 5. a) Optical images showing the self-healing behaviour of dynamic hydrogels,

the red hydrogels were dyed by carmine, and self-healing of hydrogels after fracture, the plot b) shows the change in modulus during the strain ramp described by the bottom plot c).

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Figure 7. (a) Degradation of the hydrogel by adding excess of the fructose, (b) Hydrogel stability in PBS with (right vial) and without (left vial) 2.0 M of fructose, (c) photographs of gel degradation process. (c, 1) original hydrogel, (c, 2) fructose-soaked gauze was applied to half of the hydrogel, (c, 3) after 5.0 min and (c, 4) gauze was removed and only half of the hydrogel remained. Hydrogels were dyed by carmine for better observation and the gauze was immersed in a 10 M fructose solution for 10 min before used.

Highlights

- New polymer architecture is designed to regulate pH dependence of gel formation
- Hydrogels based on dynamic covalent bonds show significant self-healing properties
- pH and polymer content/ratio are varied to control their mechanical properties
- They show low cytotoxicity to both cancer and normal cells
- They are readily dissolved by fructose, making it easy to removeable for wound dressings

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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.