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Facile Preparation of Mussel-Inspired Polyurethane Hydrogel and Its Rapid Curing Behavior

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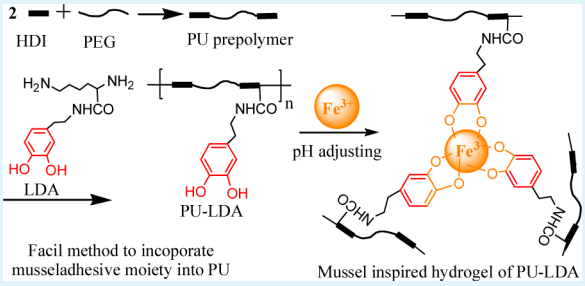
Supporting Information

ABSTRACT: A facile method was found to incorporate a mussel-inspired adhesive moiety into synthetic polymers, and mussel mimetic polyurethanes were developed as adhesive hydrogels. In these polymers, a urethane backbone was substituted for the polyamide chain of mussel adhesive proteins, and dopamine was appended to mimic the adhesive moiety of adhesive proteins. A series of mussel mimetic polyurethanes were created through a step-growth polymerization based on hexamethylene diisocyanate as a hard segment, PEG having different molecular weights as a soft segment, and lysine-dopamine as a chain extender. Upon a treatment with Fe^{3+}, the aqueous mussel mimetic polyurethane solutions can be triggered by pH adjustment to form adhesive hydrogels instantaneously; these materials can be used as injectable adhesive hydrogels. Upon a treatment with $NaIO_4$, the mussel mimetic polyurethane solutions can be cured in a controllable period of time. The successful combination of the unique mussel-inspired adhesive moiety with a tunable polyurethane structure can result in a new kind of mussel-inspired adhesive polymers.



KEYWORDS: *mussel mimetic, polyurethane, adhesive hydrogel, dopamine, gelation time*

1. INTRODUCTION

Mussels can adhere tightly to all types of surfaces, even to adhesion-resistant PTFE surfaces, and in a wet and turbulent environment by secreting adhesive proteins as liquids that rapidly harden to form adhesive plaques. Several research groups (the Deming,[1,2] Messersmith,[3] Wilker,[4] Waite,[5] and Yamamoto[6,7] groups) demonstrated that the universal and water-resistant adhesive ability of mussel adhesive proteins (MAPs) is due to the presence of the unusual amino acid DOPA and its catechol group, central to its unique adhesion property.

To engineering this adhesive performance, other than by the extraction of MAPs, several approaches to mimic this adhesive performance are being pursued, including by expressing MAPs via recombinant DNA technology,[8,9] chemical synthesis of polypeptides containing DOPA,[2,7,10] synthesis of mussel-inspired adhesive moiety (DOPA and catechol derivates such as dopamine) functionalized natural polymers (such as plant proteins, chitosa, hyaluronic acid, and gelatin),[11−13] and the generation of synthetic polymers containing the mussel-inspired adhesive moiety.[14−18] In the 1980s, the Biopolymer Co. extracted adhesive proteins from mussels and developed a super biomedical adhesive "Cell-Tak".[19,20] However, "Cell-Tak" is very expensive because 10 000 mussels provide only 1 g of the mussel adhesive. Hwang et al.[8] successfully expressed mussel adhesive proteins via recombinant DNA technology.

In general, the extraction and cloning of MAPs proved to be time-consuming, complicated, and expensive. Obtaining large quantities of these biological adhesive materials for application development has proven to be problematic. The difficulties of working with adhesive proteins at large scale have fueled the design of polymer mimics. Deming[2] reported the synthesis and adhesion of mussel mimetic polypeptides with high molecular weight through copolymerization of lysine and DOPA *N*-carboxyanhydride monomers. Li et al.[11] prepared plant proteins containing the mussel-inspired adhesive moiety by grafting soybean protein with dopamine based on an amidation reaction. Because of the low-cost and wide-use of synthetic polymers, the design of synthetic polymers containing the mussel-inspired adhesive moiety has emerged as a promising strategy for the development of new biomimetic adhesives. The approach of combining synthetic polymer with DOPA/dopamine and its catechol derivatives to form mussel mimetic adhesive polymers may have numerous applications in clinical and industrial arenas.

Recently, different polymers containing mussel-inspired adhesive moiety and different synthetic approaches have been reviewed[21,22] for this hot research field. Messersmith et al.[23,24] have end-capped poly(ethylene glycol) with DOPA by

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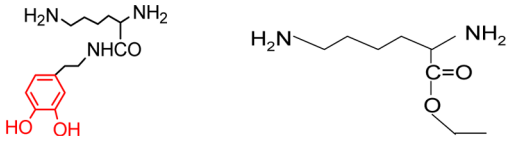
amidation to prepare MAP mimetic hydrogels. Our group[26] has modified carboxyl-containing polymer with dopamine by a grafting procedure. Mussel-inspired adhesive moiety can be also incorporated into acrylate[27] or styrene[15,28] monomers for incorporation via free radical or anionic polymerization. However, due to the limitations of the grafting ratio for grafting modification of polymers, the free radical scavenging effect of catechol for free radical polymerization, and the complex and strict conditions needed for anionic polymerization, new approaches need to be developed for the incorporation of mussel-inspired adhesive moiety into novel adhesive polymers needed for biomedical applications.

Polyurethanes (PU) are a unique class of synthetic materials with alternating "soft" and "hard" segments, and are widely used in many applications such as adhesives, coatings, and thermoplastic elastomers. Because of their excellent biocompatibility and mechanical properties, many types of PU have gained U.S. Food and Drug administration (FDA) approval and been extensively investigated as biomaterials. PU can be easily synthesized from diisocyanate, polyol, and chain extender in a step growth polymerization process. By suitable choice of diisocyanate, polyol, and chain extender, PU can be easily tailored for desired performance with tunable structure, segment distribution, and morphology. It is hypothesized that a mussel-inspired adhesive moiety could be introduced as a chain extender, and that the water solubility of the PU could be tailored by use of different polyols such as PEG. Furthermore, the content of mussel-inspired adhesive moiety can be adjusted as needed.

The goal of our work is to combine the unique chemistry of marine adhesive proteins with the flexibility, facile synthesis, and low cost of PU. We began our studies with a novel functionalized molecule lysine-dopamine (LDA), synthesized in our lab (Scheme 1), which was employed as a chain extender in

Scheme 1. Chemical Structures of LDA and LOE



Lysine-dopamine (LDA) Lysine ethylester (LOE)

PU polymerization. PEG having different molecular weights was adopted as polyols to contribute water solubility. The content of adhesive moiety dopamine (DA) can be finely tuned; the structure of PU can be easily designed, and the gelation time can be tuned according to the needs of the application.

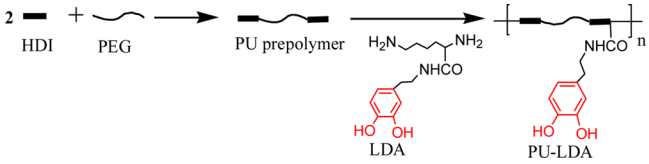
In this Article, we incorporate mussel-inspired adhesive moiety into polymer chains through a new approach (step growth polymerization) based on lysine-dopamine (LDA) and create a new type of mussel mimetic polyurethanes (PU-LDA). To our knowledge, this is the first report of the new incorporation method and the type of mussel mimetic polyurethanes. The synthesized PU-LDA can be used as an adhesive hydrogel. Upon injection of an aqueous solution of PU-LDA/$FeCl_3$ under alkaline conditions, or mixing an aqueous solution of PU-LDA with aqueous $NaIO_4$, adhesive hydrogels can be formed instantaneously because of rapid pH-sensitive gelation of PU-LDA/$FeCl_3$ based on the conversion between different Fe^{3+}-catechol complexes, or pH-accelerated gelation of PU-LDA/$NaIO_4$ through oxidative cross-linking. The pH-sensitive or pH-accelerated sol−gel transition and detailed mechanisms of cross-linking were examined.

2. EXPERIMENTAL SECTION

2.1. Materials. 1,6-Hexamethylene diisocyanate (HDI) (BASF), lysine ethylester dihydrochloride (LysOEt·2HCl) and stannous octoate ($Sn(Oct)_2$) (Aldrich), and triethylamine (TEA) and diethyl ether anhydrous (Sinopharm Chemical Reagent Co., Ltd.) were used without further purification. PEG (M_w = 1000, 2000, 3000, Aldrich) was dried under vacuum at 100 °C for 2 h before use. Lysine-dopamine (LDA) was prepared in our laboratory.[29] *N*,*N*-Dimethylformamide (DMF) (Sinopharm Chemical Reagent Co., Ltd.) was refluxed with calcium hydride (CaH_2) for 4 h, distilled under vacuum at 60 °C, and stored in the presence of 4 Å molecule sieves before use.

2.2. Synthesis of Mussel Mimetic Polyurethane. The mussel mimetic polyurethanes based on HDI, PEG, and chain extender LDA were synthesized using a multistep solution polymerization in DMF (shown in Scheme 2). Briefly, PEG was dissolved in DMF, and then

Scheme 2. Synthesis of Mussel Mimetic PU



HDI was added to the solution under a dry nitrogen atmosphere at 70 °C. Two drops of $Sn(Oct)_2$ (catalyst) were added. The prepolymerization was carried out for 2 h under mechanical stirring. The detailed feed ratios are shown in Table 1. After prepolymerization, the solution was transferred to an ice−water bath, and the prepolymer solution was cooled to near 0 °C. Chain extender LDA in DMF was added, and TEA was injected dropwise into the solution to neutralize the hydrochloric acid. The chain extension was carried out for another 16 h. After purification by separation and precipitation in anhydrous diethyl ether, the final product was dried under vacuum at 40 °C for 24 h. Polyurethane prepared with lysine ethylester (LOE) as chain extender served as a control.

2.3. Formation of PU-LDA Adhesive Hydrogel. *Fe^{3+} Cross-Linked Hydrogels.* PU-LDA was dissolved in Milli-Q water at a concentration of 140 mg/mL. The polymer aqueous solution was then mixed with $FeCl_3$ water solution at Fe^{3+}/dopamine molar ratio of 3. The corresponding concentration of dopamine is 60 mM for PU-LDA2, while it is 45 mM for PU-LDA3. As to PU-LDA2, 200 μL of polymer solution was mixed with 50 μL of 80 mM $FeCl_3$. As to PU-LDA3, 200 μL of polymer solution was mixed with 50 μL of 60 mM $FeCl_3$. A green color developed immediately upon mixing. NaOH was then added to adjust to the desired final pH. This resulted in instant gelation and color development at gel pH. The above green solution can also be injected onto a substrate in an alkaline environment to form an injectable adhesive hydrogel.

$NaIO_4$ Cross-Linked Hydrogels. PU-LDA was dissolved in Milli-Q water at a concentration of 140 mg/mL. The polymer aqueous solution was then mixed with $NaIO_4$ water solution at IO_4^-/dopamine molar ratio of 0.2, 0.3, or 0.5. The corresponding concentration of dopamine is 60 mM for PU-LDA2, while it is 45 mM for PU-LDA3. As to PU-LDA2, 200 μL of polymer solution was mixed with 30 uL of 80, 120, or 200 mM $NaIO_4$. As to PU-LDA3, 200 μL of polymer solution was mixed with 30 uL of 60, 90, or 150 mM $NaIO_4$. Upon mixing, it resulted in immediate orange color development and gelation. The above protocols were also carried out by substituting Milli-Q water with buffer solutions of different pH values (6.2, 7.4, 8.2, and 10.0) for the gelation rate test.

Gelation Time by the Stir Bar Method. Gelation time was measured by the stir bar method (also can be measured by the vial tilt

Table 1. Theoretical Composition and Molecular Weight of Mimetic PU and Its Control

	molar ratio						
			chain extender			molecular weight	
sample	HDI	soft segment	LOE	LDA	DA content (wt)	M_n (g/mol)	M_w/M_n
PU-LDA1	20	10 (PEG1000)		11	10.2%	24 785	2.68
PU-LDA2	20	10 (PEG2000)		11	6.4%	27 304	2.82
PU-LDA3	20	10 (PEG3000)		11	4.6%	26 133	2.91
PU-LOE1	20	10 (PEG1000)	11		0	19 554	2.87
PU-LOE2	20	10 (PEG2000)	11		0	25 888	3.27
PU-LOE3	20	10 (PEG3000)	11		0	28 316	3.17

method). Briefly, polymer aqueous solution was added to a small glass vial with a diameter of 10 mm. An 8 mm × 4 mm Teflon-coated stir bar was placed in the vial, and was rotated at 300 rpm. $NaIO_4$ or $FeCl_3$ aqueous solution then was added to the vial. Gelation was defined as the time when the stir bar stopped spinning. Molar ratios and pH were varied to determine the optimal gelation time.

Rheological Analysis of Hydrogels. Oscillatory rheometry was used to monitor the progress of gelation. Cross-linking reagent ($NaIO_4$) was added to a solution of PU-LDA, and the well-mixed solution was immediately loaded into a Bohlin VOR rheometer (a 25 mm diameter cone and plate fixture with a cone angle of 2.5°). The time sweep analysis for gelation time was performed at a frequency of 0.1 Hz at a strain of 1%.

The mechanical and self-healing properties of PU-LDA/$FeCl_3$ hydrogel were tested at 25 °C using a TA ARGII rheometer with parallel plate geometry (50 mm diameter rotating top plate). The strain sweep (1%−1000%) was performed at a frequency of 1 Hz to monitor the storage modulus (G'), and the recovery test was performed by straining the hydrogel to failure (1000% strain, 1 Hz) immediately followed by linear condition (5% strain, 1 Hz) while monitoring the recovery of storage modulus (G_r').

2.4. Characterization. *FTIR Spectroscopy.* Fourier transform infrared (FTIR) spectra of the PU samples were obtained on a PerkinElmer Paragon 1000 spectrophotometer between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

NMR Spectroscopy. ^1H NMR and ^{13}C NMR spectra were obtained with a Varian (Mercury plus-400) NMR spectrometer at room temperature in DMSO-d_6, and chemical shifts were reported in ppm relative to tetramethylsilane (TMS).

UV−Vis Spectroscopy. The UV−vis spectra were recorded on a PerkinElmer (Lambda 750S) UV−vis spectrometer using DMF or H_2O as solvent. All measurements were performed in quartz cuvettes. The scan range was 200−800 nm, and the scan rate was 960 nm/min.

Gel Permeation Chromatography (GPC). GPC was performed by a PerkinElmer Series 200 using *N,N*-dimethylformamide (DMF)/LiBr as eluent against polystyrene (PS) standards. The sample concentration was 1.0 mg/mL, and the flow rate was 1.0 mL/min.

Thermogravimetry Analysis (TGA). Thermal decomposition of all samples was carried out with a TA Instruments Q800 under a nitrogen flow (100 mL/min) from 40 to 600 °C at a heating rate of 20 °C/min.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Mussel Mimetic Polyurethane. The dopamine containing chain extender LDA was successfully synthesized as illustrated in Supporting Information Scheme S1. It is known that the primary amine group has a particularly high reactivity (several hundred times of phenol group) with isocyanate group. Hence, a series of polyurethanes with an appended dopamine group were prepared using LDA as a new chain extender. The synthesis and the structure of polyurethanes are shown in Scheme 2. The chain extender lysine ethylester (LOE) was used to prepare control polyurethanes, the PU-LOE series, for comparison.

The structure of the mussel mimetic polyurethane (PU-LDA) was confirmed by FTIR, ^1HNMR, and UV−vis spectroscopy. In the FTIR spectrum (Figure 1) for PU-LDA,

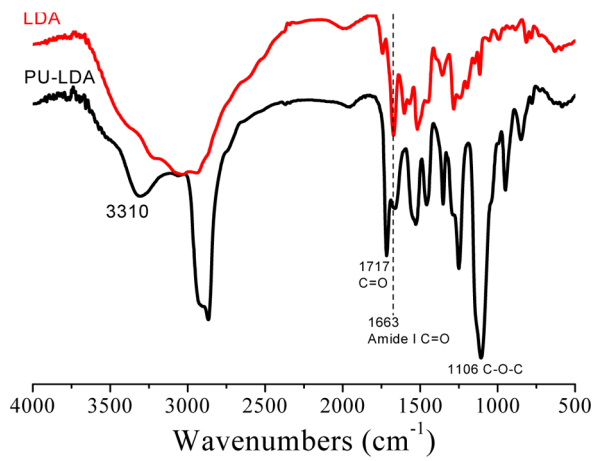
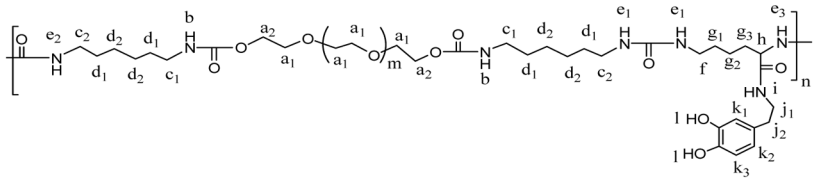


Figure 1. FTIR spectra of PU-LDA and LDA.

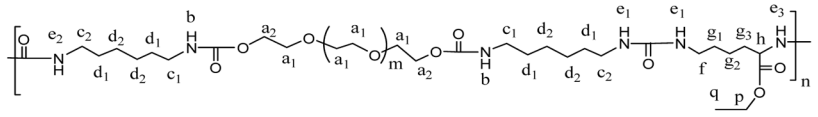
the 1717 cm^{-1} band is associated with the stretching vibration of the carbonyl C═O in −NH−COO−, which indicated the successful prepolymerization between the −OH of PEG with the −NCO of HDI. The 1663 cm^{-1} band is associated with the stretching vibration of the carbonyl C═O in −NH−CO−NH−, which indicated the successful chain extension of the prepolymer with LDA.

The ^1H NMR spectrum (Figure 2) shows that LDA with two primary amine groups is copolymerized into the chain of the polyurethane. For PU-LOE, the chemical shifts between 5.5 and 6.5 ppm corresponds to protons on −NH−CO−NH−, resulting from the successful chain extension reaction. As compared to PU-LOE, new chemical shifts for PU-LDA at 8.6−8.9, 7.6−7.9, and 6.7−6.3 ppm corresponding to protons on $-Ar(-O\underline{H})_2$, $-CO-N\underline{H}-CH_2-$, and the Ar of dopamine reveal that the dopamine containing chain extender was successfully introduced into the polyurethane backbone. In the ^{13}C NMR spectrum (Supporting Information Figure S1) of PU-LDA, chemical shifts at 158.6 and 158.1 ppm correspond to carbons in the two kinds of urea groups (−NH−$\underline{C}$O−NH−), indicating $-NH_2$ of LDA reacted with −NCO of PU prepolymer. Chemical shifts attributed to carbons in $-Ar-(-OH)_2$ of PU-LDA are the same as that in LDA, which confirms there is no side reaction between $-Ar(-OH)_2$ of LDA and −NCO of PU prepolymer.

In the UV−vis spectrum (Figure 3), there is an absorbance peak for the catechol group at 280 nm in PU-LDA, which confirms the successful introduction of LDA into the polyurethane. All in all, the FTIR spectra, ^1H NMR spectra,



PU-LDA



PU-LOE

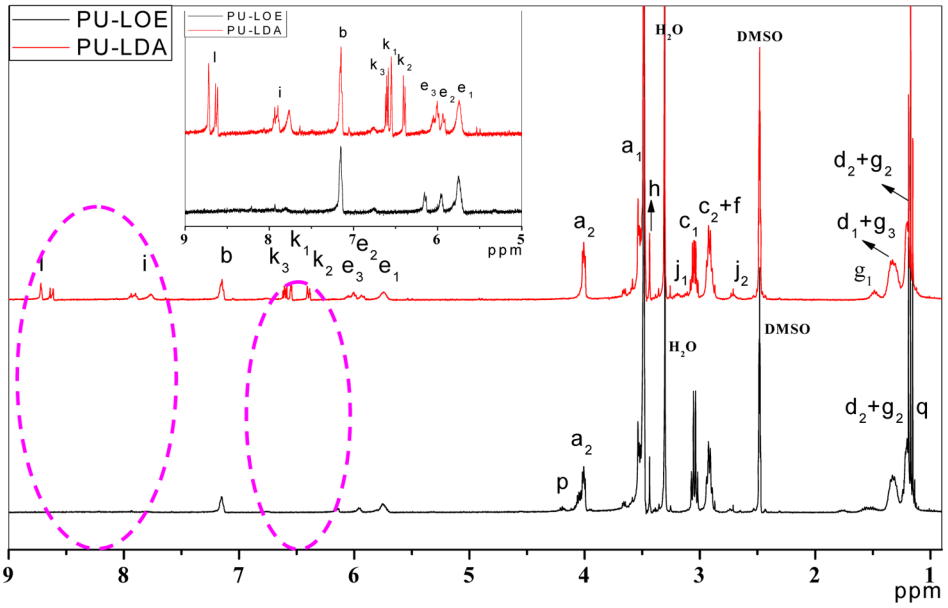


Figure 2. Structures and ^{1}H NMR spectra of PU-LDA and PU-LOE.

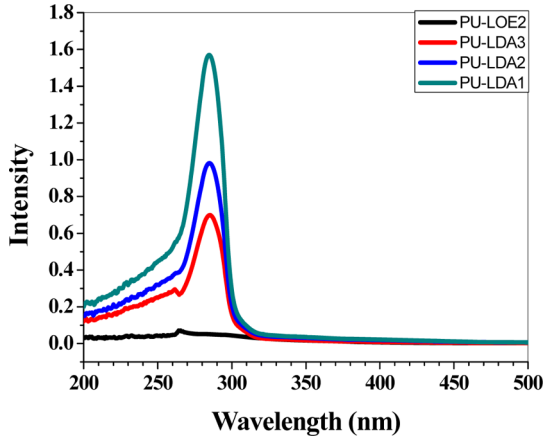


Figure 3. UV−vis spectra: different synthesized PU samples at 1 mg/mL in DMF.

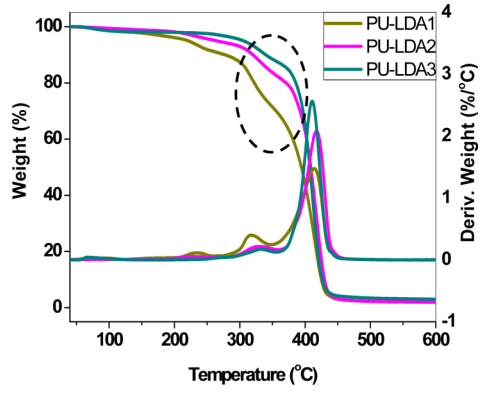


Figure 4. TGA of PU-LDA.

and UV−vis spectrum confirm the successful preparation of mussel mimetic PU using LDA as a novel chain extender.

By changing the PEG chain length, the content of dopamine (DA) attached to the hard block could be controlled. It is hypothesized that the DA content will increase with a decrease in PEG chain length. TGA analysis confirms the content of mussel-inspired adhesive moiety DA can be controlled and the structure of the polyurethane can be easily modified (Figure 4). The first two stages (weight loss from 200 to 270 °C and from 270 to 360 °C) of the thermal decomposition of PU are due to cleavage and degradation of the urethane and urea linkage,[30] attributed to the hard segment including HDI and the chain extender LDA, while the last stage (weight loss from 360 to 460 °C) is attributed to the degradation of the soft segment PEG. As compared to PU-LDA1, less thermal decomposition in the second stage (270−360 °C) for PU-LDA2 and PU-LDA3

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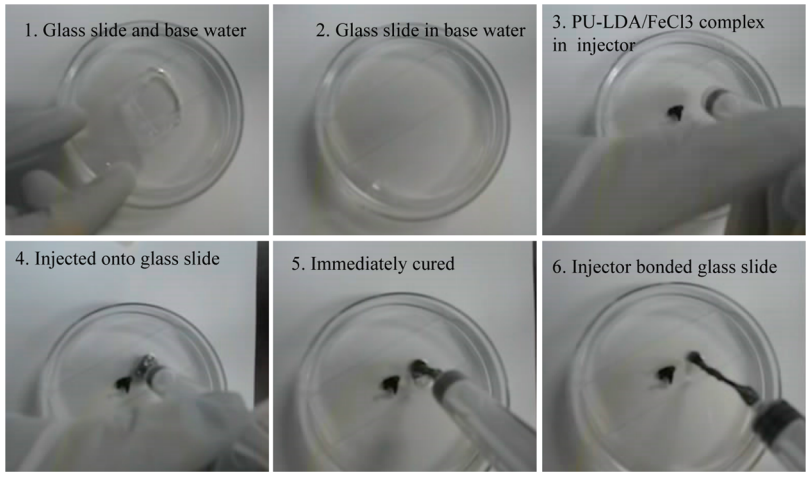


Figure 5. Selection from the movie of mimetic PU gel formation.

indicates there is less LDA in the polymer chains. Calculated from the second stage of decomposition, the DA contents in PU-LDA1, PU-LDA2, and PU-LDA3 are 10.1%, 6.5%, and 5.1%, respectively. The results are consistent with DA content from LDA addition as shown in Table 1.

For different PUs, at the same concentration of 1 mg/mL, the UV absorbance intensity increases when PEG chain length decreases (Figure 3). Therefore, the UV−vis spectra indicated that PU samples with differing dopamine contents can be successfully prepared. As listed in Table 1, the theoretical contents of dopamine for the synthesized PUs are in the range of 0−10.2 wt %. GPC was used to determine the polymer number-average molecular weight (M_n) and the sample polydispersity (PDI). The M_n of all of the samples was between 19 000 and 29 000. It should be noted that by changing the ratio of prepolymer and chain extender, the molecular weight of the mussel mimetic PU could be further controlled.

All in all, mussel mimetic polyurethanes (PU-LDA) with tunable content of adhesive moiety were successfully synthesized through a facile method based on LDA as PU chain extender. The urethane backbone similar to the amide backbone of MAPs and the appended catechol groups from adhesive moiety promise that the new waterborne PU can be developed as a novel mussel mimetic hydrogel.

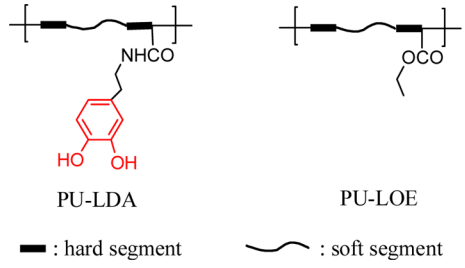
3.2. Hydrogel from Mussel Mimetic Polyurethane. In mussel mimetic polyurethane PU-LDA, the soft segment with different molecular weight PEGs contributes to the water solubility. Under consideration of the large water solubility (controlled by the ratio soft segment and the hard segment), PU-LDA2 and PU-LDA3 with different content of mussel-inspired adhesive moiety dopamine are focused to study the hydrogel behavior.

3.2.1. Gel Formation by Fe^{3+}−Catechol Ligand Coordination. Adhesive byssus formation[25,31] has been reported to be due to the mussels' secretion of liquid adhesive proteins, which cures upon release into seawater. The liquid MAPs stockpiled in the foot (acidic environment, pH = 5−6) were secreted and injected on the substrate under seawater environment (pH = 8) to form byssus including byssal thread and byssal plaque. The seawater exposure drives the liquid mussel adhesive proteins to undergo immediate cross-linking.

The synthesized polyurethane PU-LDA can fully mimic the behavior of mussel. Inspired by the pH jump induced curing of mussel adhesive proteins, we premixed a concentrated solution of mussel mimetic PU with Fe^{3+} under acidic conditions to form monodopamine−Fe^{3+} complexes (green color) in a buret or injector, then injected it onto a substrate in an alkaline environment. A spontaneous cross-linking was induced for the formation of adhesive hydrogel via the formation of bis- and/or tris-dopamine−Fe^{3+} complexes (red color). The whole process of PU-LDA/$FeCl_3$ being injected onto a glass slide under alkali water had behavior similar to that of MAPs secreted onto the surface of glass tank under seawater. Some selections of short movie illustrating gel formation of PU-LDA//$FeCl_3$ were shown in Figure 5. From the last selection, the injector was bonded to the glass slide (substrate) by the cured hydrogel. Therefore, this material may be developed as an injectable adhesive hydrogel.

Mussel mimetic hydrogel can be formed immediately through pH jump for the PU-LDA/$FeCl_3$ system, while gel could not be formed at any pH value for the PU-LOE/$FeCl_3$ system. The difference was due to the presence of catechol group (Scheme 3).

Scheme 3. Schematic Drawings of PU-LDA and PU-LOE



For PU-LOE, there was no absorbance peak in the UV−vis spectrum as shown in Figure 6a. When PU-LOE was mixed with $FeCl_3$, there was no change of absorbance, and $FeCl_3$ shows a peak at 295 nm. For PU-LDA, the absorbance peak appears at 280 nm in the UV−vis spectrum as shown in Figure 6b. When PU-LDA was mixed with $FeCl_3$, new peaks at 395 and 760 nm appeared, and the peak at 295 nm for $FeCl_3$ disappeared, indicating an interaction between $FeCl_3$ and PU-LDA. The catechol group in LDA molecule incorporated into mussel mimetic polyurethane (PU-LDA) plays the key role for

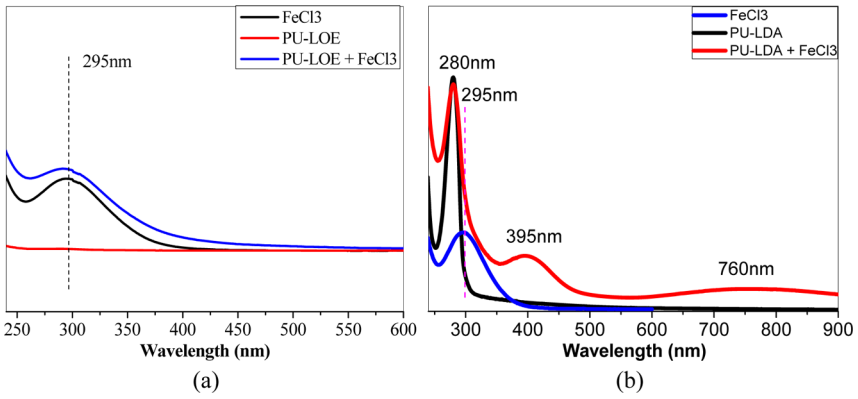


Figure 6. UV−vis spectra of PU-LOE (a) or PU-LDA (b) mixed with $FeCl_3$ (Fe^{3+}/dopamine = 1/3).

Table 2. Gelation Time of Mimetic PU Based on Different pH Values

	PU-LDA2 (140 mg/mL, DA 60 mM)				PU-LDA3 (140 mg/mL, DA 45 mM)			
pH ($FeCl_3$/DA = 1/3)	6.2	7.4	8.2	10	6.2	7.4	8.2	10
gelation time (s)	∞	1−2	1−2	1−2	∞	1−2	1−2	1−2

the gel formation due to the stoichiometry of catechol−Fe^{3+} complexes.

The instantaneous formation of adhesive hydrogels was due to the rapid pH-sensitive formation of catechol/$FeCl_3$ complex in the PU-LDA/$FeCl_3$ system. As shown in Table 2, the gel cannot be formed under acidic conditions, while the gel can be instantaneously formed under alkaline conditions. All of the hydrogels based on different PU-LDA samples exhibited similar characteristics.

During the observation of gel formation, the change of color and state was apparent. PU-LDA water solution was a colorless to light-colored liquid (Figure 7a). When mixing PU-LDA solution with $FeCl_3$ solution, a green color developed immediately, and the liquid state was kept for the acidic environment (Figure 7b). Through pH adjustment by adding NaOH, the gel was established for instant gelation, and green color turned to red (Figure 7c). The change of color was due to the change of catechol−Fe^{3+} stoichiometry. The detailed mechanism will be more fully described elsewhere (section 3.2.3).

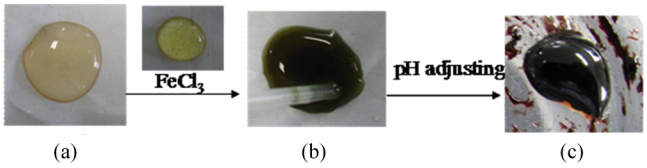


Figure 7. PU hydrogel formed by Fe^{3+}−catechol ligand: (a) PU-LDA in H_2O, (b) PU-LDA solution mixed with $FeCl_3$, and (c) cured mimetic hydrogel by pH adjusting.

The PU hydrogel formed by Fe^{3+}−catechol ligand shows a self-healing property. As shown in Figure 8, the PU hydrogel formed from PU-LDA3 (10 wt %) and $FeCl_3$ at pH = 10 was fractured into two parts (Figure 8a), and after simply contacting the fractured surfaces, the two parts can be fused together rapidly (Figure 8b). The hydrogel recovered cohesiveness in minutes.

Rheological analysis was also used to monitor the self-healing and mechanical property of the PU hydrogel. As shown in Figure 9, the storage modulus (G') of the gel was first tested by the strain sweep (0.1%−1000% strain) as a function of time at a constant frequency of 1 Hz. The storage modulus (G') was found to be strain independent in the linear regime at about 3.2 kPa, much higher than that of the early example of hydrogel formed from 4-arm-PEG-dopa (the same concentration in the final gel at 10 wt %) and Fe^{3+} reported by Messersmith group (G' at about 1 kPa).[25] It may contribute to the unique structure of PU containing PEG as a "soft" segment and diisocyanate as a "hard" segment. When the strain further increased, the gel failed, and then the recovery test could be performed. After the gel failure under 1000% strain, it was immediately followed by

Figure 8. Self-healing of PU hydrogel formed from PU-LDA and $FeCl_3$: (a) cut into two pieces, and (b) fused together in minutes.

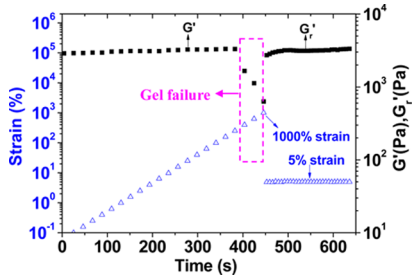


Figure 9. Rheological analysis of PU hydrogel formed from PU-LDA3 (10 wt %) and $FeCl_3$ at pH = 10: the self-healing was monitored by the storage modulus (G') and recovery storage modulus (G_r') at 1 Hz.

linear condition (5% strain, 1 Hz), and the recovery of storage modulus (G_r') was monitored. The PU hydrogel formed from PU-LDA3 and $FeCl_3$ can recover its initial storage modulus. The recovery process is quick, and it can be finished in less than 1 min at 5% strain.

3.2.2. Gel Formation by $NaIO_4$ Oxidation. Treated with Fe^{3+}, the aqueous mussel mimetic polyurethane solution PU-LDA can be triggered by pH adjustment to form an adhesive hydrogel instantaneously, just like mussel adhesive hydrogel formed from the mussel adhesive proteins. The gelation time was just 1−2 s. Treated with $NaIO_4$, concentrated aqueous solutions of the mussel mimetic polyurethane PU-LDA can be cured in a controllable period of time. The process of hydrogel formation was recorded in Figure 10.

Figure 10. Formation of PU hydrogel by $NaIO_4$ oxidation: (a) PU-LDA solution in a vial, (b) PU-LDA solution mixed with $NaIO_4$, (c) gelation as time progresses, and (d) inversion of the vial.

As shown in Figure 10, when $NaIO_4$ was added to PU-LDA solution (IO_4^-/dopamine = 0.5), an orange color immediately developed (Figure 10b), and gelation took place shortly thereafter (Figure 10c and d). The gelation time measured by the stir bar method is listed in Tables 3 and 4.

As shown in Table 3, the gelation time of mimetic PU samples can be adjusted by the ratio of $NaIO_4$/LDA. PU-LDA3 exhibited a gelation time of less than 29 min at the ratio of $NaIO_4$:LDA = 1:2, while more than 2 h at the ratio of $NaIO_4$:LDA = 1:1. In the periodate-initiated gelation, mimetic PU samples exhibited a minimum gelation time at approximately $NaIO_4$:LDA = 1:2. The same trend is also proved in PU-LDA2. At this ratio, the semiquinone (described in section 3.2.3) was formed and dominated, which can be further transferred to biscatechol. The reaction from semiquinone (free radical) may contribute to rapid gel formation.

The gelation time of mimetic PU samples can be also adjusted by the content of DA. As described previously, the DA content of PU-LDA2 is about 6.4%, while the DA content of PU-LDA3 is about 4.6%. Under the same condition, the gelation time of PU-LDA2 is much shorter than that of PU-LDA3 for its relative higher DA content. As compared to the gelation time of about half of an hour for PU-LDA3 at the ratio of $NaIO_4$:LDA = 1:2, the gelation time for PU-LDA2 at the ratio of $NaIO_4$:LDA = 1:2 can be shortened to about 7 min.

Also, the gelation time of mimetic PU can be shortened by increasing the pH value. As shown in Table 4, mimetic PU can gel in as fast as a few seconds under alkaline conditions, as compared to minutes under acidic conditions. Therefore, mimetic PU gelation by $NaIO_4$ exhibited base accelerated oxidative cross-linking.

Oscillatory rheology was used to monitor the process of gel formation. For example, PU-LDA3 and $NaIO_4$ were mixed and immediately loaded onto the testing platform in a fluid state. The dynamic storage modulus (G') of PU-LDA fluid was recorded during the periodate-induced curing as shown in Figure 11. G' began to increase approximately 30 min after addition of periodate at pH = 6.2, while about 10 min after addition of periodate at pH = 7.4. The results for gelation time by rheological analysis were close to that by the stir bar method. Rheological analysis of other hydrogels revealed similar characteristics, although the gelation time showed a strong dependence on the $NaIO_4$ to dopamine ratio.

All in all, gelation time can be controlled by molar ratio of $NaIO_4$/DA, DA content, and pH value. We can adjust the gelation time from several seconds to tens of minutes, which can satisfy different requirements, particularly suitable for clinical use as injectable therapies.

As compared to the gel formation of PU-LDA or PU-LOE mixed with $FeCl_3$, there is the same tendency for the gel formation of PU-LDA or PU-LOE mixed with $NaIO_4$. Addition of oxidizing reagent ($NaIO_4$) to PU-LOE solution led to no hydrogel formation, while addition of oxidizing reagent ($NaIO_4$) to PU-LDA solution led to hydrogel formation. The hydrogel formation of PU-LDA is a result of cross-linking of the catechol groups appended to the polymer chains. The catechol group in PU-LDA is oxidized to quinone and then further reacts to give rise to cross-linking. The detailed mechanism will be more fully described elsewhere.

3.2.3. Mechanism of Cross-Linking. To understand the mechanisms behind the cross-linking of PU-LDA, reaction mixtures were monitored over time using UV−vis spectroscopy.

For $NaIO_4$ cross-linked hydrogels at pH = 6.2, as shown in Figure 12a, a peak at 395 nm (*o*-quinone) was observed immediately after the addition of $NaIO_4$, while the peak at 280 nm (catechol) decreased as compared to PU-LDA. Over time, the peak at 395 nm decreased in intensity, while at the same time the absorbances at 300−350, 280, and 500 nm increased in intensity. The formation of a shoulder at about 315 nm (between 300 and 350 nm) suggested the formation of dehydro-dopamine[23] (Figure 13), although no well-defined peak was observed. The decrease in absorbance at 395 nm with time indicated consumption of the quinone,[1,23,32] while the increase in absorbances at 280 and 510 nm indicated the formation of a new product[23,32] (Figure 13). The major product compared favorably to a biscatechol formed via a phenol coupling of 4-methylcatechol.[32] For $NaIO_4$ cross-linked hydrogel at pH = 8.2, as shown in Figure 12b, a peak due to quinone at 410 nm (a small shift under basic conditions) was observed immediately after the addition of $NaIO_4$, and the intensity remained essentially unchanged for 1 min after periodate addition as compared to the $NaIO_4$ cross-linked hydrogel at pH = 6.2.

Table 3. Gelation Time of Neutral Mimetic PU Solutions Based on Different Molar Ratios of $NaIO_4$/DA

	PU-LDA2 (140 mg/mL, DA 60 mM)				PU-LDA3 (140 mg/mL, DA 45 mM)			
$NaIO_4$/DA	0.2	0.3	0.5	1	0.2	0.3	0.5	1
gelation time	10′54″	7′40″	7′28″	19′18″	33′38″	30′46″	28′50″	>120′

Table 4. Gelation Time of Mimetic PU Based on Different pH Values

	PU-LDA2 (140 mg/mL, DA 60 mM)				PU-LDA3 (140 mg/mL, DA 45 mM)			
pH ($NaIO_4$/DA = 0.5/1)	6.2	7.4	8.2	10	6.2	7.4	8.2	10
gelation time	7′28″	0′10″	0′7″	0′4″	28′50″	9′15″	0′11″	0′7″

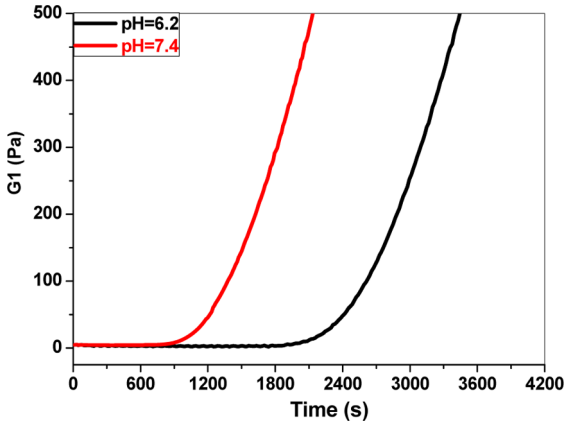


Figure 11. Time sweep of PU-LDA3 mixed with $NaIO_4$ at pH = 6.2 and 7.4.

As to the Fe^{3+}–catechol cross-linked PU-LDA hydrogel, the stoichiometry of catechol–Fe^{3+} complexes is controlled by pH (Figure 14). For the cross-linking, bis- or/and tris-complexes are required to be established, and the required pH is typically reported to be above pH 7.[25] Also, it has been reported that in the mussel body, the adhesive proteins can be found in the adhesive liquid in an acidic environment (pH = 5–6), and that they cure upon release into seawater (pH = 8). For mussel mimetic polyurethane PU-LDA, a green color developed immediately when mixing with $FeCl_3$ solution. Through pH adjustment by adding NaOH, the initial green color turned blue and finally red as the catechol–Fe^{3+} stoichiometry changed from mono- to bis- to tris-, respectively (Figure 14).

UV–vis spectroscopy was carried out to monitor the catechol–Fe^{3+} stoichiometry. The dilute PU-LDA solution showed a sharp peak at 280 nm, attributed to the catechol group in the polymer chain. We then premixed the dilute PU-LDA with $FeCl_3$ at a LDA/Fe ratio of 3:1, and a green color developed immediately for the acidic environment. As shown in Figure 15, the UV–vis spectrum shows the formation of the mono-LDA–Fe^{3+} complexes[25,33] with an absorbance peak at 760 nm (also shown in Figure 6) at about pH = 5. All green curves are pH < 6.5, where the monocomplex dominates.

With increasing pH by adding NaOH, the green color turned blue. The UV–vis spectrum shows the formation of the bis-LDA–Fe^{3+} complexes[25,33,34] with an absorbance peak around 564 nm at pH = 7.4. All blue curves are 6.8 < pH < 8, where the bis-complex dominates. Through increasing the pH by adding NaOH, the solution finally turned red. The UV–vis spectrum shows the formation of the tris-LDA–Fe^{3+} complexes[25,33,34] with an absorbance peak around 495 nm at pH = 8.5. All red curves are pH > 8, where the tris-complex dominates.

All in all, monocomplexes at acidic environment allow fluidity for the PU-LDA/$FeCl_3$ system, and bis- or tris-complexes ensure cross-linking and curing of PU-LDA when injected into a high pH environment, just like natural mussel adhesive proteins.

4. CONCLUSIONS

The mussel adhesive moiety was successfully incorporated into synthetic polymers through a new facile approach using step growth polymerization based on a novel chain extender LDA, and a new kind of waterborne polyurethane (PU-LDA) was created. In this polymer, the urethane backbone is analogous to the mussel adhesive protein polyamide main chain, and dopamine mimics the strong adhesive moiety of the mussel adhesive proteins. The content of mussel adhesive moiety dopamine can be finely controlled; the structure of the polyurethane can be easily varied.

The novel PU-LDA polymers were successfully developed as a novel mussel mimetic adhesive hydrogel. Mixed with $NaIO_4$, PU-LDA aqueous solution can be cured in a controllable time

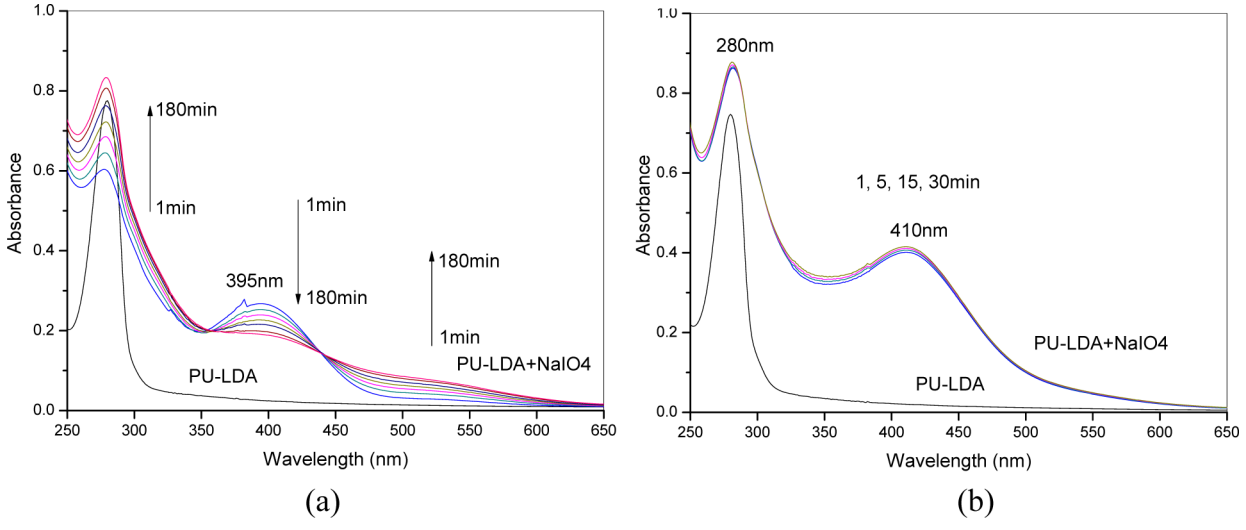


Figure 12. UV–vis spectra of PU-LDA mixed with $NaIO_4$ for different time ($NaIO_4$:LDA = 1:2): (a) pH = 6.2 for 1, 5, 15, 30, 60, 120, 180 min; (b) pH = 8.2 for 1, 5, 15, 30 min.

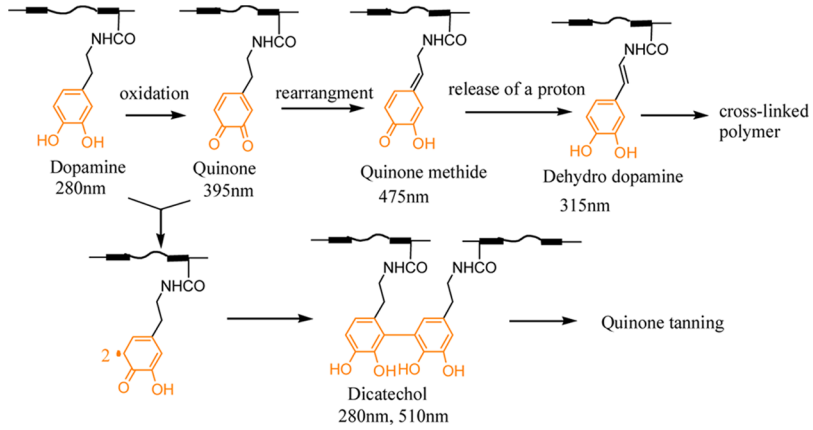


Figure 13. Oxidative reaction and cross-linking pathways of PU-LDA mixed with $NaIO_4$.

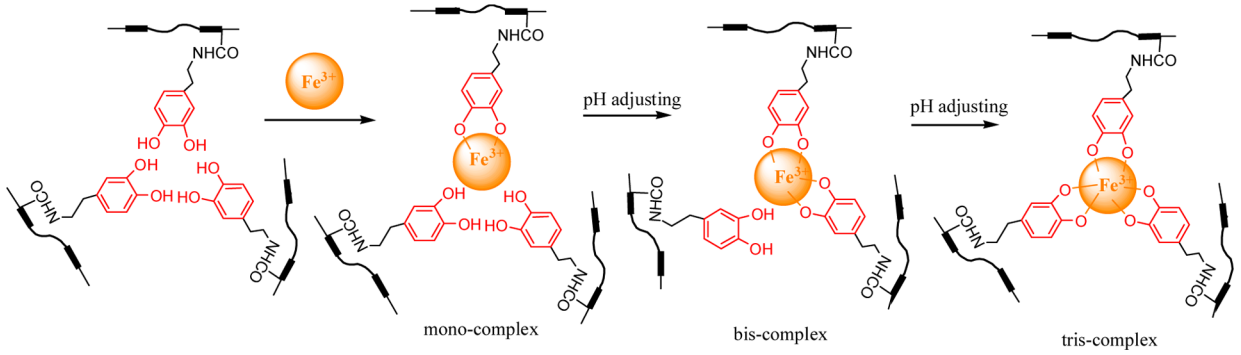


Figure 14. Schematic illustration of pH-sensitive gelation based on PU-LDA and $FeCl_3$.

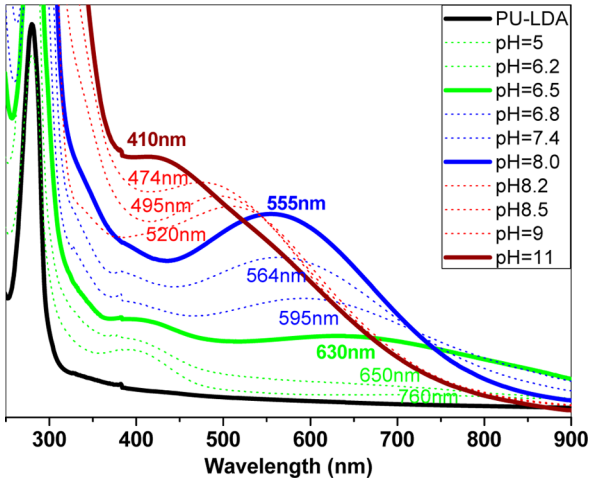


Figure 15. UV−vis spectra of PU-LDA mixed with $FeCl_3$ at different pH values (Fe^{3+}/dopamine = 1/3).

from several seconds to tens of minutes, and it shows a pH-accelerated oxidative cross-linking to a hydrogel. Mixed with $FeCl_3$, PU-LDA aqueous solution can be cured rapidly upon pH adjustment to an adhesive hydrogel and can be used as an injectable adhesive hydrogel under alkali condition, even under physiological condition. The spontaneous curing of PU-LDA/$FeCl_3$ through a pH jump is achieved via conversion of monodopamine−Fe^{3+} complexes to cross-linked bis- and/or tri-dopamine−Fe^{3+} complexes.

The mussel mimetic polyurethane (PU-LDA) successfully combines the unique chemistry of marine adhesive proteins with the flexibility, facile synthesis, and low cost of polyurethanes. Thus, it shows great potential in biomedical material, tissue engineering, and many other applications.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of lysine-dopamine (LDA); ^{13}C NMR spectra of PU-LDA and LDA (Figure S1); a description of the adhesive liquid secreted by a mussel to the surface of tank and the curing of mussel adhesive proteins; and a description of PU-LDA/$FeCl_3$ solution injected to the glass surface by an injector and the curing of mussel mimetic polyurethane. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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