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The DOPA-Functionalized Bioadhesive with Properties of Photocrosslinked and Thermoresponsive

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ABSTRACT: The unusual amino acid L-3,4-dihydroxyphenylalanine (DOPA), which was found in mussel adhesive protein, was recognized as crucial element for the adhesive of mussel. In this work, the synthesis of thermoresponsive and photocrosslinkable bioadhesive with ternary networks prepared by incorporating dopamine acrylamide (DAM) and *N*-isopropylacrylamide (NIPAAm) into a crosslinked network based on poly(ethylene glycol)-triacrylate (PEG-TA) was reported. The effects of DAM and NIPAAm on polymerization kinetics, swelling kinetics, adhesion strength, thermomechanical properties, and cytotoxicity assays were systematically evaluated. The results showed that DAM could affect photopolymerization kinetics of terpolymer due to inhibitory effects of the catechol. The terpolymer has not only strong adhesion strength which was better than that of the commercial fibrin adhesives (0.05 MPa), but also the good humid-resistant property. The thermoresponsive properties of the system were investigated by the measurement of swelling kinetics. The equilibrium swelling ratio of gels was obviously higher at 25°C than at 37°C. The thermomechanical properties of terpolymer indicated that the presence of the catechol moiety increased significantly the glass transition temperature (T_g) and crosslink density of ternary network. The results of cytotoxicity of gels indicated that terpolymer were biocompatible and less cytotoxicity towards the growth of mouse fibroblast cells (L929 cells). The obtained products could have the potential to serve as the bioadhesive in the future. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41102.

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INTRODUCTION

Bioadhesives have attracted great attention as novel biomaterials because of their obvious advantages including rapid bonding, less pain, no suture, and utilized for surgical adhesives, hemostatic agents, wound closing, dental bonding, and so on. However, most artificial adhesives are difficult to form strong adhesion strength with matrix due to the presence of body fluid or humidity, or cannot be as an ideal biomaterials because of biotoxicity.[1,2] Therefore, it is highly desirable and urgent to synthesize a new kind of bioadhesive with biocompatibility and water resistance adhesion. Mussels are well known for their superior adhesion strength and good adhesion diversity under wet conditions,[3–6] even including low-fouling materials, such as paraffin and polytetrafluoroethene (PTFE).[7,8] Mussel adhesive proteins (MAPs) secreted by mussels contain a high content of the unusual amino acid 3,4-dihydroxyphenylalanine (DOPA),[6,7,9–12] which is believed to be mainly responsible for the astounding adhesive of MAPs.[13–15] DOPA was identified by Waite group[16] that it played two essential roles in adhesion process: one was as a catecholic anchor which chemisorbed to various surfaces using diverse mechanisms, including metal bidentate coordination[17–20] and hydrogen bonding,[7,21,22] the other was that it easily oxidized by enzymatic or chemical methods to form DOPA-quinone which acted as a covalent crosslinking unit, reacting with nucleophilic groups via Michael addition, or by means of direct free-radical coupling.[13,23–25] Therefore, DOPA or its derivatives functionalized bioadhesive materials had many potential applications in medicine as surgical adhesive and tissue cement.

DOPA derivatives had been the subject of extensive scientific research over the past decades,[22,26–30] particularly synthesized polymer that contained DOPA and analogous catecholic moieties to mimic the adhesion function of mussels.[12,31–35] Wilker and co-workers[36] synthesized a kind of terpolymer,

including 3,4-dihydroxystyrene, *p*-vinyltolyltriethylammonium chloride and styrene. Adhesion values of terpolymer were lower than those of some commercial popular adhesives in dry conditions, higher in wet conditions. Especially, the terpolymer with ca. 7% cation cantent yielded the highest value of dry adhesion 2.8 ± 0.6 MPa, and underwater adhesion 0.4 ± 0.2 MPa which was better than three commercial products. Yamada et al.[37] investigated the modification of chitosan with dopamine via tyrosinase-catalyzed reactions. The adhesive shear strengths of these modified chitosan samples were over 400 KPa. The Messersmith group prepared a range of polymeric systems contained DOPA derivatives, including polyacrylates, polyethylene glycol.[38–40] The results showed that DOPA derivatives obviously enhanced the adhesive strength of materials.

Photopolymerization is an advanced method that provides lots of technical and economic advantages than that of thermal polymerization. However, relatively few researches reported the utilization of photopolymerization to prepare polymer containing DOPA derivatives.[41,42] In our group, a series of DOPA- or dopamine-containing polymers were synthesized and utilized as photo-crosslinkable bioadhesive.[43–45] These photocurable bioadhesives showed strong adhesion strength, rapid photocurable activity and good biocompatibility.

Thermosensitive materials can change their chemical and/or physical properties depending on environmental conditions, which undergo a coil–globule transition in aqueous solution at lower critical solution temperature (LCST).[46] Poly(*N*-isopropylacrylamide) (PNIPAAm) is a typical thermoresponsive polymer, which has attracted great attention for biomedical applications due to its LCST around 32°C in an aqueous solution.[47–49] Many groups investigated the thermoresponsive properties of PNIPAAm copolymerized with other acrylates or conjugated with various substrates.[50–52] Hydrogels, which prepared from PNIPAAm or the monomer NIPAAm and crosslinkers, exhibited volume phase transition at the LCST. When the temperature increased above the critical point, the phase-transition behavior of hydrogels began with the entropic gain that was caused from water molecules associated with the amide groups of NIPAAm. These hydrogels could act as biomolecular separation, microfluidics, actuators, and so on.[53–55]

In this work, the synthesis of dopamine acrylamide (DAM), three-armed poly(ethylene glycol)-triacrylate (PEG-TA), and further prepared terpolymer that composed of DAM, PEG-TA and the monomer NIPAAm using UV irradiation were reported. The PEG was chosen because of its well water solubility, superior biocompatibility, nonimmunogenicity, and no toxicity. The structures of DAM, PEG-TA were characterized by Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). In addition, the properties of terpolymer containing different proportions of ingredients were investigated, and the effect of DAM via real-time infrared reflection measurement, adhesion strength test, dynamic mechanical analysis, swelling experiments, fluorescence microscopic photos, and the toxicity test were systematically evaluated.

MATERIALS AND METHODS

Materials

Dopamine hydrochloride (Dopamine HCl) was purchased from Beijing Brilliance Biochemical (Beijing, China). Acryloyl chloride was gained from Sigma-Aldrich. Photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure 2959) was supplied by BASF Corporation (Ludwigshafen, Germany). Three-arm PEG (MW: 1500) was gained from Liming Research Institute of Chemical Industry. NiPAAm was purchased from Tokyo Chemical Industry. Mouse fibroblast (L929) was obtained from the Department of Microbiology, Peking University Health Science Center. Other reagents were all purchased from Beijing Chemical Agent Co.

Preparation of DAM

20 g of sodium borate and 8 g sodium bicarbonate were added to 200 mL distilled water to protect catechol group. Both sodium borate and sodium bicarbonate were saturated in distilled water. Then, the aqueous solution was bubbled with N_2 for 30 min and cooled in the ice bath condition. After 10 g dopamine HCl was added to this solution, 25 mL tetrahydrofuran (THF) including 6.0 g acryloyl chloride and 1*M* NaOH solution were alternately added dropwise to it to maintain pH values of the reaction mixture over 9.0. PH indication paper was utilized to check the pH of the solution. After all the reagents were added, the mixture was stirred at room temperature for 1 h with N_2 bubbling. The white suspension was formed and then washed twice with 50 mL ethyl acetate. The resulting solid was filtered and the obtained solution was acidified to pH 2 with 6*M* HCl solution. The acidified solution was extracted three times with 50 mL ethyl acetate. The extracted brown organic layer was dried over $MgSO_4$, filtrated using filter paper, and concentrated by rotary evaporation. The formed solution was added to 300 mL of hexane with effective stirring to obtain brown precipitate, then the resulting suspension was refrigerated to maximize crystal formation size. After filtration, the obtained light brown solid was dissolved in 25 mL ethyl acetate and precipitated in 300 mL of hexane again. After dried in a vacuum, the final powder product with 60% yield was obtained. ^{1}H-NMR (400 MHz, $CDCl_3$, δppm): 3.25–3.27 ppm, (4.0H, S, –$\underline{CH_2}$–$\underline{CH_2}$–NH–), 5.54–5.67 ppm (1.0H, d, CH_2=$\underline{CH}$COO—), and 6.04–6.19 ppm (2.0H, m, $\underline{CH_2}$=CHCOO—), 6.43–6.67 ppm (3.0H, m, —H of benzene ring).[56]

Preparation of PEG–TA

The three-arm PEG (15 g, 10 mmol) was added to toluene and dried by azeotropic distillation using a Dean–Stark trap. After being cooled to room temperature, the mixture was degassed with bubbling nitrogen for 30 min then cooled in the ice bath condition. Dichloromethane (DCM) and triethylamine (TEA, 6.26 mL, 1.5 eq.) were added to above solution with stirring, respectively. Then acryloyl chloride (3.66 mL, 1.5 eq.) was added. The reaction mixture was stirred for 24 h in the dark with N_2 bubbling. The obtained pale yellow suspension was filtered using neutral alumina column, then neutral alumina column was washed by abundant DCM. Sodium bicarbonate was added to the toluene–DCM mixture and stirred for 1 h. After sodium bicarbonate was filtrated, the solution was reduced by rotary evaporation. The product was precipitated by adding

diethyl ether to solution in an ice bath and gained through filtration. The resulting pale yellow powder was washed by diethyl ether again, then refrigerated, finally obtained after filtration with yield more than 70%. ^{1}H-NMR (400 MHz, $CDCl_3$, δppm): 5.94–6.36 ppm (3.0H, m, CH_2═CH─COO─), 3.65 ppm (45.83H,S, -H of PEG main chain). The degree of esterification (DE) of PEG-TA, which was calculated from the proton peaks areas of acrylate groups when compared with those of PEG main chain, was more than 95%.

Preparation of Photocrosslinkable Gels

Gels were fabricated by photocrosslinking of precursor solutions. The precursor solutions were prepared with 33 wt % (feed ratio of NiPAAm: DAM = 10 : 0, 8 : 2, 6 : 4, 5 : 5, 4 : 6, 2 : 8, and 0 : 10) of NiPAAm and DAM blend system, 1.0 wt % photoinitiator 2959 (relative to NiPAAm and DAM blend system), and 33 wt % PEG-TA as crosslinking agent in methanol to produce terpolymer gels, respectively. Details of the photocrosslinkable procedure were as follows: after homogenizing, the precursor solution was injected into a flat round mold consisting of two glass slides separated by a rubber spacer (thick: 2.0 mm). Then, the system was irradiated by UV light source (320–480 nm, EXFOlite, EFOS Corporation) at light intensity 15 mW cm^{-2} for 15 min to form the photopolymerized gels. The obtained gels for swelling test were dried in vacuum.

FTIR Spectra Measurement

FTIR spectra of DAM, PEG-TA products were recorded on a Nicolet 5700 instrument (Nicolet 5700, Thermo Fisher, USA, equipped with an extended range KBr beam splitter and an MCT/A detector). The wavenumber ranging was from 4000 to 650 cm^{-1} with resolution of 4.0 cm^{-1}.

The photocrossing behavior of gels was monitored via series real-time infrared spectroscopy with the resolution of 4.0 cm^{-1}. A horizontal transmission accessory (HTA) was designed to enable mounting of samples in a horizontal orientation for FTIR measurements. The double bond conversion (DC) of the samples was obtained by monitoring the change of C─H absorbance peak area due to C═C double bond at 6110–6250 cm^{-1} in the near-IR range. The UV light intensity was 30 mW cm^{-2}.

Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR)

^{1}H NMR spectra of samples were obtained with a Bruker AV spectrometer at 400 MHz at room temperature. $CDCl_3$ and deuterated dimethyl sulfoxide (DMSO) were as solvent, respectively.

Measurement of Swelling Kinetics

The swelling tests of terpolymer were performed at phosphate buffered solution (PBS, pH 7.4) to investigate the swelling behavior of the gels. The dry gels (weight W_0) were recorded and then immersed in 30 mL PBS at 25°C at certain time intervals. The same experiments were done at 37°C again. After removal of the redundant superficial water, the weight of the swollen gels (W_t) was measured and recorded until no further weight change was detected. The swelling ratio of gels was determined by the following equation:

$$\text{Swelling ratio } (\%) = (W_t - W_0)/W_0 \times 100\% \quad (1)$$

Measurement of Adhesion Strength

The 20 wt % gelatin solution spread uniformly on the surface of glass was used to simulate the living tissue. The dimension of each glass slide was 50 mm × 20 mm × 5 mm. After gelatin solution formed homogenous coating, the blend solution of NiPAAm, DAM, and PEG-TA was dropped and spread on the gelatin. Then, the glass were overlapped in 15 mm × 20 mm, clamped tightly, irradiated by UV light source under light intensity 15 mW cm^{-2} for 15 min. The adhesion strength of glass samples were measured by lap shear test using a testing machine (Model 1185, Instron, USA) with a crosshead speed of 5.0 mm min^{-1} at room temperature. Five samples were measured in each experiment.

The humid-resistant adhesion strength of terpolymer was also investigated. In this test, the terpolymer solution was coated directly on the surface of glass slide. Then, the overlapped glass slides were clamped and exposed under the UV light of light intensity 15 mW cm^{-2}. The resulting samples were stored in a container with humid atmosphere of humidity about 80% for different time (0, 60, 120 min). After that the adhesion strength was measured using above method.

Dynamic Mechanical Analysis of Terpolymer

Dynamic mechanical analysis (DMA) of terpolymer was performed using a mechanical analyzer in tension film mode, which was equipped with a liquid nitrogen cooling system. The dimension of each specimen was 20 mm × 5 mm × 1.5 mm. The temperature range was from −150 to 120°C at a ramp rate of 5 K min^{-1}. For the purpose of investigation of crosslink network structure, the crosslink density (ρ_x) of terpolymer networks was evaluated by the usage of the rubbery plateau storage modulus at T_g +40°C according to the theory of rubber elasticity[57]:

$$\rho_x = E'/2\ (1 + \gamma)\ \mathrm{RT} \quad (2)$$

where E' is the rubbery storage modulus at temperature T, γ is Poisson's ratio (is assumed to be 0.5 for incompressible networks), and R is the gas constant.

Cytotoxicity Assays

The cytotoxicity of the photopolymerization terpolymer gels was evaluated based on a procedure adapted from the ISO10993-5 standard test method. Mouse fibroblasts (L929 cells) were cultured in extraction media Dulbecco's Minimum Essential Medium (DMEM) which contained 10% fetal bovine serum, 1.0% penicillin–streptomycin, and 1.2% glutamine. Culture was maintained at 37°C in a wet atmosphere containing 5% CO_2. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1 × 10^3 M ethylene diaminetetraacetic acid (EDTA).

The viabilities of cells were evaluated by the MTT (3-[4-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The prepared gel samples were soaked in PBS (pH 7.4) for 24 h. Then they were sterilized with highly compressed steam for 15 min and placed in wells of 24-well culture plate which filled with 1 mL DMEM at 37°C for 24 h. The extraction ratio was 100 mg mL^{-1}. At the end of this period, the extracts were

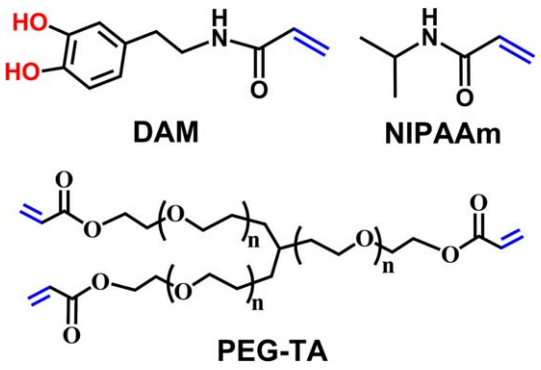

Figure 1. Chemical structures of the monomers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

obtained after removing the photopolymerization gels, and L929 cells were seeded in wells of 96-well plates (density: 8×10^3 cells per well). After 24 h incubation, the culture medium solution was removed and replaced with the as-prepared extraction medium and incubated for another 24 h, then 100 μL MTT stock solution was added to each well. After the cells incubated at 37°C for 4 h, 200 μL of (DMSO) was added to ensure formazan crystals dissolution. The dissolved solution was swirled homogeneously about 10 min by the shaker. The optical density (OD) of the formazan solution was detected by an ELISA reader (Multiscan MK3, Labsystem) at 570 nm. For the reference purpose, L929 cells were seeded to a fresh culture medium (negative control) and a medium containing 0.64% phenol (positive control) at the same culture condition, respectively. Each assay was performed six times. The cell relative prolifertation was obtained by the following equation:

$$\text{Cell relative prolifertation}\,(\%) = \text{OD}\ (t)/\text{OD}\ (0) \times 100\% \quad (3)$$

where OD (t) is the optical density of samples, OD (0) is the optical density of negative control.

The prepared disk terpolymer gels (10 mm in diameter, 2.0 mm in thickness) were incubated in 50 mL phosphate buffered saline (PBS, pH 7.4) for 24 h, then sterilized with highly compressed steam for 20 min. They were rinsed three times using sterile PBS and subsequently placed to a 24-well plate. One milliliter of L-929 cells suspension (1.5×10^4 cell mL^{-1}) were seeded on the samples. After cultured for 48 h, terpolymer gels were washed twice through PBS to remove nonadherent cells, then fixed by 75% alcohol solution, dyed by Hoechst solution. The resulting samples were observed using the fluorescence microscope (HZA9610K, Beijing, China).

RESULTS AND DISCUSSION

The Structures of DAM and PEG-TA

The successful synthesis of DAM and PEG-TA were confirmed by FTIR and ^{1}H NMR, respectively. The structures of monomer were shown in Figure 1.

Compared with the spectrum of Dopamine·HCl [Figure 2(1)], the spectrum of DAM [Figure 2(2)] showed the new peaks at 3296, 1655, 1526, 1443, and 806 cm^{-1}. The broad band of 3296 cm^{-1} was assigned to the N-H stretching vibration of amide group and O-H stretching vibration. Typical absorption

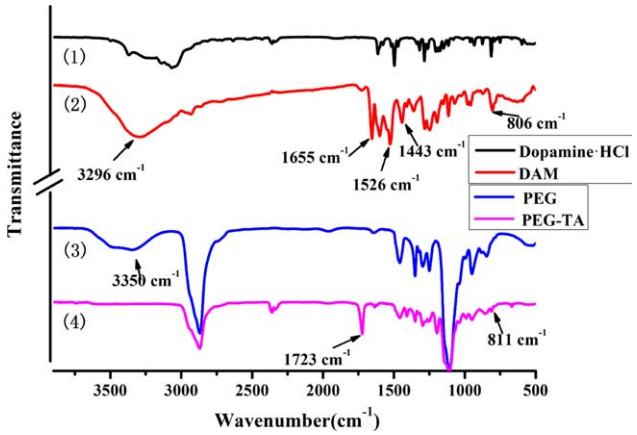

Figure 2. FT-IR spectra: dopamine·HCl (1), DAM (2), PEG raw material (3), and PEG-TA (4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

peak of C=O stretching vibration (amide I) was observed at 1655 cm^{-1}, N–H bending vibration (amide II) at 1526 cm^{-1}, C–H stretching vibration at 1443 cm^{-1}. Peak at 806 cm^{-1} were characteristics of C=C double bonds. The principal spectral features of PEG-TA could be seen in the previous work.[43]

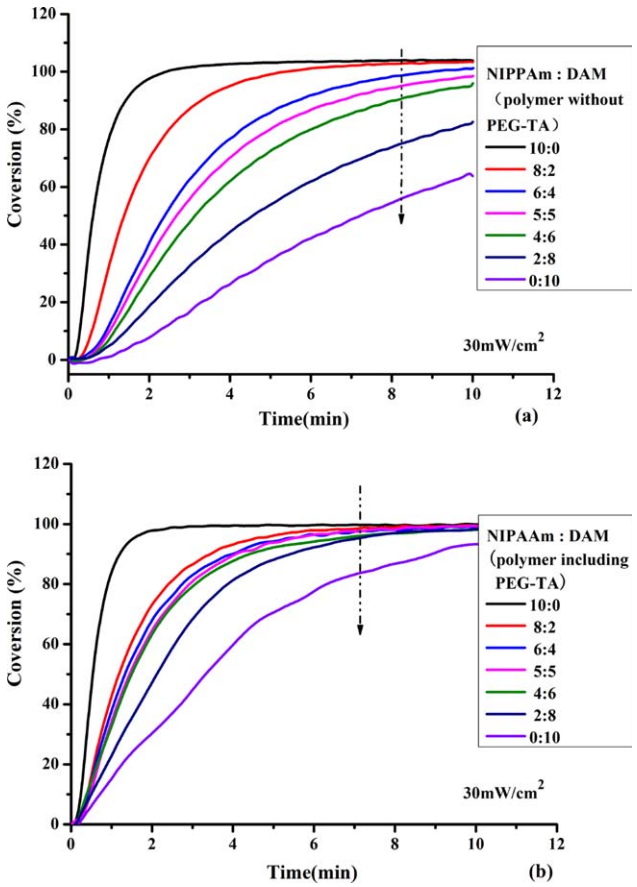

Figure 3. Photocrosslinking kinetics of (a) copolymer of NIPAAm and DAM (b) terpolymer of NIPAAm, DAM, and PEG-TA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

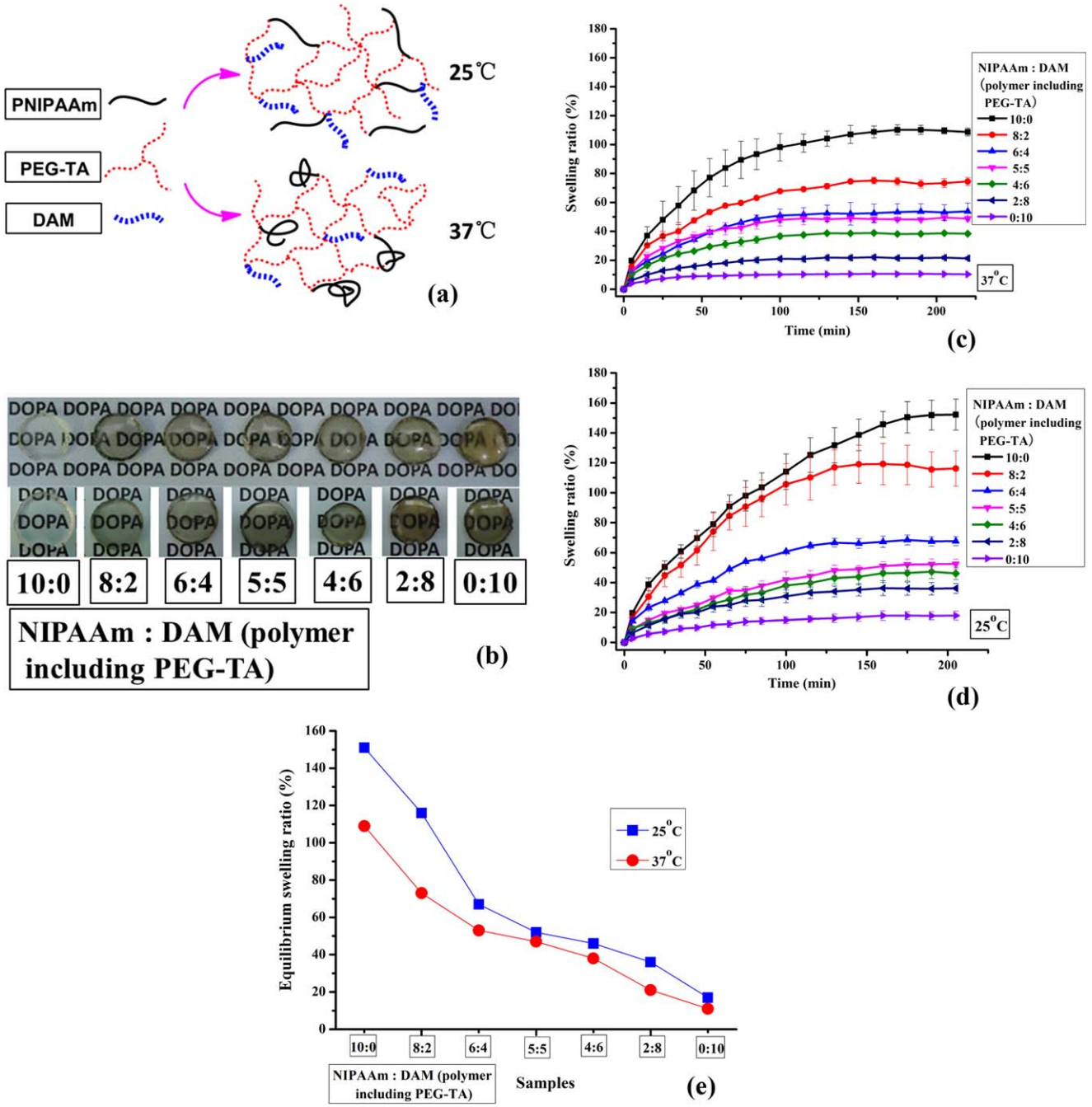

Figure 4. (a) simplified illustration of molecular structure of the terpolymer at 25 and 37°C; (b) morphology of gels: dried samples [upper row, swollen samples (under row); swelling behavior of gels: (c) at 37°C, (d) at 25°C, equilibrium swelling ratio of terpolymer with different molar ratio of NIPAAm:-DAm (e)]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Photocrosslinking Kinetics of Terpolymer

When the prepared solution was irradiated by UV light, the unsaturated C═C double bonds of samples started polymerization and the area of absorption peak attributed to C═C double bond at 6120–6250 cm^{-1} decreased accordingly. In the copolymer system of NIPAAm and DAM (without PEG-TA), the influence of the weight ratio of NIPAAm to DAM on photocrosslinking process was shown in Figure 3(a). It could be found that the max conversion of NIPAAm gels without DAM was about 100% after irradiated 3 min; however, it was about 60% after 10 min for DAM without NIPAAm. In addition, the conversions of the gels were obviously decreased at higher DAM concentrations. The reason was that catechol group of DAM provided a decelerating or inhibitory effect on radical photopolymerization. The result was consistent with the study of Lee and co-workers.[58] However, PEG-TA was added into system, the conversions of almost all of the gels samples increased to 100% at 10 min, as shown in Figure 3(b). Therefore, the problem

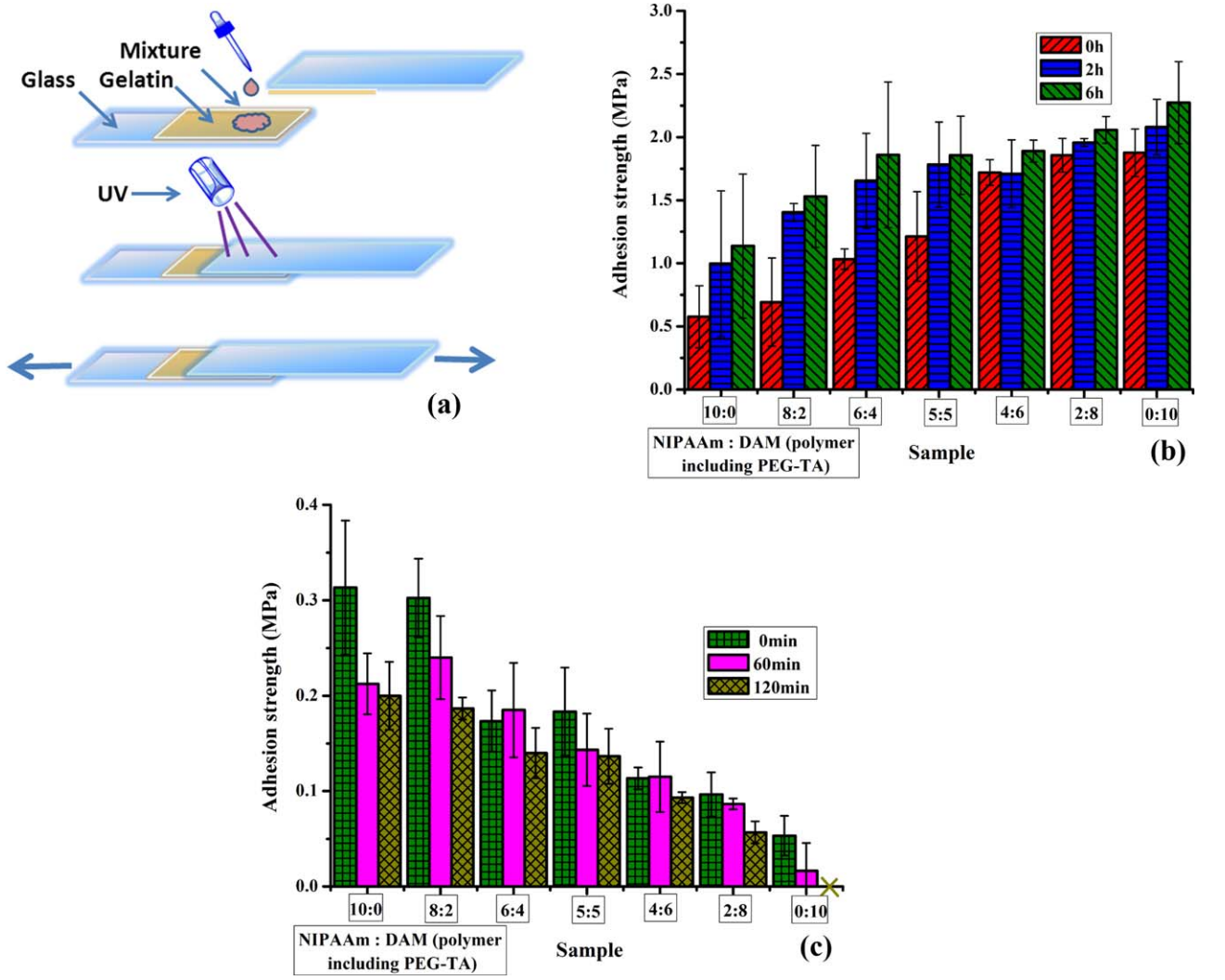

Figure 5. (a) the schematic of preparation of lap-shear test samples, (b) adhesion strength of terpolymers of different time, (c) the humid-resistant adhesion strength of terpolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with extractable monomer did not exist in terpolymer system. High molecular weight PEG-TA which added into the system led to the decrease of density of double bond in the solution. Therefore, the conversion of C═C double bond increased within the same amount of time.

Swelling Kinetics and Equilibrium Swelling Ratio

When polymer was developed as biomaterials for applications in the medical field, swelling ratio was one of important indicators to evaluate the adhesive performance and thermoresponsive properties. The swelling behavior of hydrogels depended on the external temperature. Hydrogels, which prepared from the monomer NIPAAm and suitable crosslinkers, also displayed volume phase transition at the LCST of PNIPAAm and shrunk above the LCST with releasing swelled water. Figure 4(a) showed the simplified illustration of molecular structure of the terpolymer at 25 and 37 °C. Morphology of gels were represented in Figure 4(b). The color of the hydrogels transformed from colorless to brown with the amount of the DAM increased. The reason was that a number of catechol groups of DAM oxidated to form *o*-quinone structure. The *o*-quinone groups not only deepen the color, but also increased intermolecular crosslinking which confined the swelling of samples. Dried samples were on the upper row, swollen samples on the under row. As shown in Figure 4(c) and 4(d), the swelling ratio of terpolymer were increased with the increase in the NIPAAm-to-DAM molar ratio. Simultaneously, the equilibrium swelling ratio of samples at 25°C were larger than that at 37°C [see Figure 4(e)], and the swelling equilibrium time of samples at 25°C were longer than that at 37°C. The reason was that water as a good solvent at solution temperatures of 25°C (below the LCST of PNIPAAm), PNIPAAm chains formed hydrogen bonds with the water and exhibited expanded structures. Nevertheless, when heated to 37°C (above its LCST), terpolymer became hydrophobic to form a compact gel structure by dehydrating.

Analysis of Adhesion Strength

Lap-shear test was utilized to evaluate the adhesive performance of terpolymers. The samples for lap shear test were prepared by placing terpolymers gels between gelatin layers which simulated

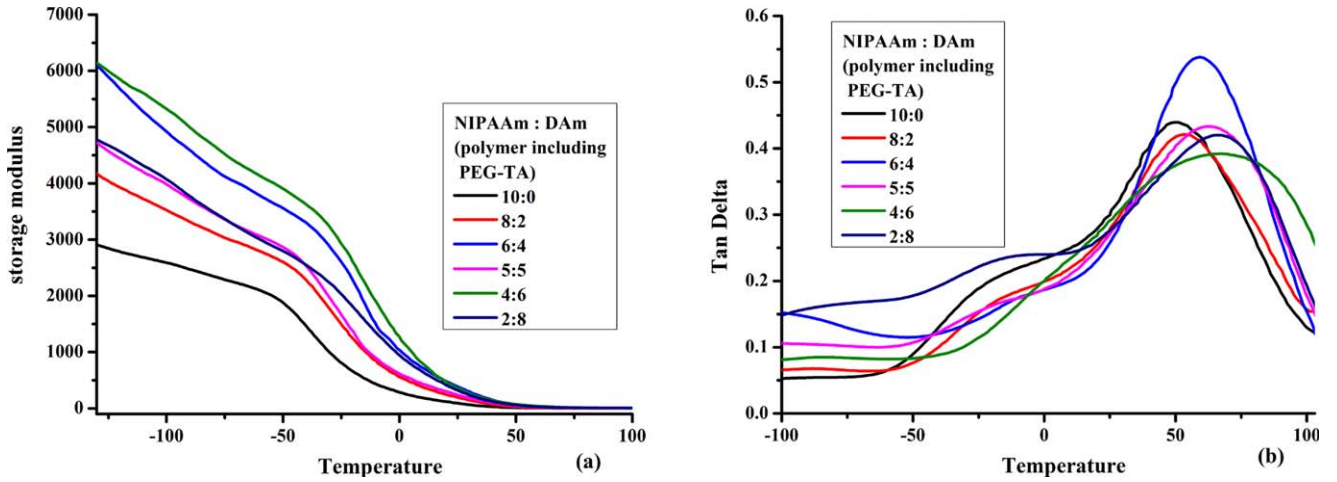

Figure 6. Thermomechanical properties of terpolymer networks with different NIPAAm-to-DAM molar ratio. (a) Storage modulus, (b) tanδ. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. The Crosslink Density (ρ_x) of the Ternary Network

NIPAAm : DAM	10:0	8:2	6:4	5:5	4:6	2:8
ρ_x ($\times 10^3$ mol/cm^3)	0.25	0.26	0.44	0.43	0.47	0.48

the living tissues, as shown in Figure 5(a). The methods were adapted from ASTM F2255-03 "Standard Test Method for Strength Properties of Tissue Adhesive in Lap-Shear by Tension Loading".

Adhesion strength of different composition of samples was shown in Figure 5(b). The adhesion strength was improved from 0.57 ± 0.25 to 1.88 ± 0.19 Mpa with the molar ratio of NIPAAm to DAM increased from 10 : 0 to 0 : 10 at 0 h of the storage time. The solvent evaporated with the increase in the storage time, so adhesive coating gradually formed solidified cutan which produced cohesive force with gelatin layers. Therefore, interfacial adhesion force was from 0.99 ± 0.58 to 2.08 ± 0.22 Mpa at 2 h, from 1.14 ± 0.57 to 2.27 ± 0.33 Mpa at 6 h. The adhesive strength of the gels were higher than that of commercial available bioadhesives Tisseel (0.05 MPa).[59]

Figure 5(c) showed the humid-resistant adhesion strength of samples. The humid-resistant adhesion strength decreased with the increase the weight ratio of NIPAAm to DAM from 0.31 ± 0.07 to 0.05 ± 0.02 Mpa at 0 min storage time, from 0.21 ± 0.03 to 0.02 ± 0.02 Mpa at 60 min, from 0.2 ± 0.04 Mpa to the value which could not be measured at 120 min. It could be seen that the bonding strength of samples without DAM decreased obviously. With the increase of DAM content, more catechols could form strong H-bonding, which enabled samples to acquire more H-bonding site competing with water and benefited for adhesion.

Evaluation of Thermomechanical Properties

DMA was used to investigate the thermomechanical properties of terpolymer.[60] The thermal transitions were determined in tension film mode at a rate of 2°C min^{-1}. The T_g was obtained from the maximum peak value of the tanδ curve. Tanδ, which was defined as the ratio of the loss moduli (E'') to storage (E') moduli, was a damping term that related the dissipation of energy on periodic deformation. As shown in Figure 6(a), the sub-T_g storage modulus increased with the increase of proportion of DAM in ternary network. According to the eq. (2), the crosslink density (ρ_x) was given in Table I. As shown in Table I, ρ_x values increased indeed with the increase of DAM concentration of terpolymer. Figure 6(b) displayed the tanδ curves for the ternary networks. With increasing proportion of DAM content,

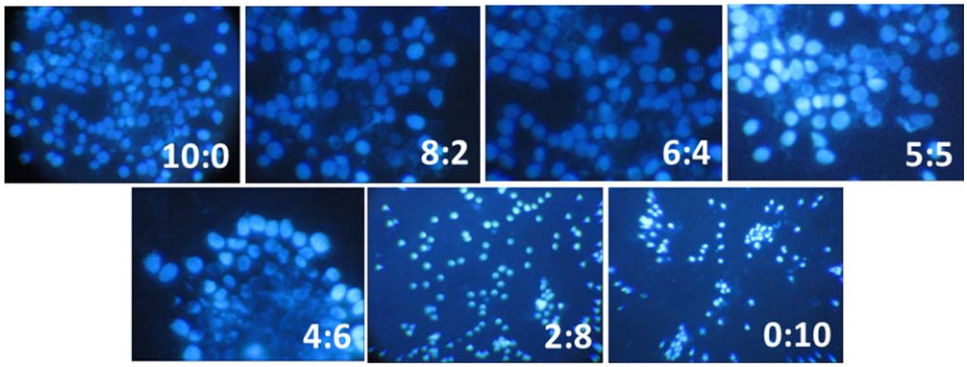

Figure 7. Fluorescence microscope images (200 magnifications) of L929 cells seeded on gels with the NIPAAm-to-DAM molar ratio from 10 : 0 to 0 : 10. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

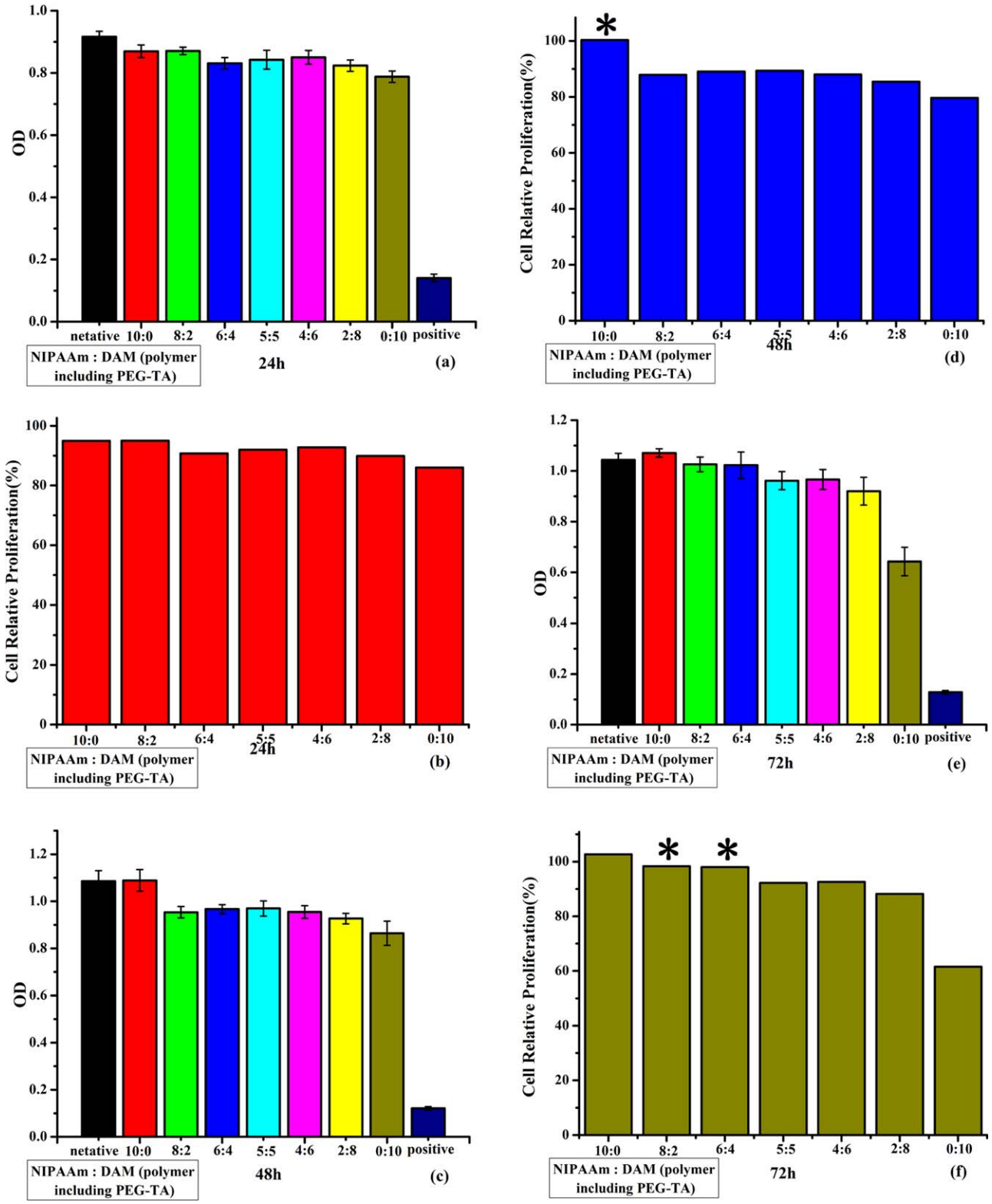

Figure 8. Cytotoxicity test of gels. (a), (c), and (e): MTT test of gels with positive and negative controls at 24, 48, and 72 h, respectively. The data represented mean and standard deviations of six samples; (b), (d), and (f): cell relative proliferation of 24, 48, and 72 h, respectively [*: no statistically significant differences ($P > 0.05$)]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the peak maximum of the T_g increased systematically. The increase of T_g of the ternary networks could be attributed to hydrogen bonding formed between the catechol moieties of DAM and various proton acceptors, such as amides, esters, and ethers. The *o*-quinone structure, which obtained easily from the oxidation of catechol, also could form more crosslinked system.

Cytotoxicity Assays

In this work, cell attachment measurement was used to evaluate the biocompatibility of terpolymer gels. Figure 7 showed fluorescence microscopy images of L929 cells seeded on the surface of the terpolymer gels. The L929 cells spread quite well on the surface of all gels and cell nucleuses were round, which indicated good cell-surface interaction and good cell viability on the gels surface.

Toxicity test was important to evaluate the potential toxicity of biomaterials. An ideal bioadhesive should not release toxic substances or generate adverse reactions. Mouse fibroblast cells (L929 cells) were cultured in the extraction media from specimens, and the absorbance which illustrated the viability of L929 cells, were shown in Figure 8. There was decreasing trend of OD values with increasing DAM content in the material. Maybe this was that a small quantity of quinone groups oxidated from catechol influenced slightly proliferation L929 cells. As Figure 8(d) and 8(f) shown, it could be seen that no statistically significant differences ($P > 0.05$) were observed in the cell activity of some gel extracts at 48, 72 h, compared with negative control. Although statistically significant differences ($P < 0.05$) were obtained in other gels except one which the NIPAAm-to-DAM molar ratio was 0 : 10, the cell viability still reached about 85% of the negative control. The result indicated that terpolymer of NIPAAm-PEG-DAM were less toxic to L929 cells.

CONCLUSION

In this study, DAM and PEG-TA were synthesized, and these compounds were characterized using FT-IR and ^{1}H NMR. In addition, bioinspired terpolymer was successfully prepared via photopolymerization of DAM, NIPAAm, and PEG-TA. The ternary network incorporated varying concentrations of DAM and NIPAAm was investigated, and the effect of DAM and NIPAAm on polymerization kinetics, adhesion strength, swelling kinetics, thermomechanical properties and cytotoxicity assays were elucidated. Increasing the concentration of DAM in the ternary network resulted in a decrease in C═C double bond conversion of photopolymerization likely due to inhibitory effects of the catechol. The results of lap shear tests showed that the adhesion strength of terpolymer not only was significantly superior to the commercial fibrin adhesives, but also had a good humid-resistant property. The thermoresponsive properties of terpolymer were evaluated using measurement of swelling kinetics. The equilibrium swelling ratio of gels at 25°C was obviously higher than that at 37°C. Moreover, the decrease in the swelling kinetics of terpolymer with the increase of DAM concentration was observed due to the decrease in thermoresponsive group. The presence of the catechol moiety increased significantly the glass transition temperature and crosslink density of the ternary networks. The results of MTT assays and cell attachment showed that terpolymer was biocompatible and less cytotoxicity towards the growth of L929 cells. The terpolymer system could be as a bioadhesive and apply to the biomedical field.

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