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An Injectable Thermosensitive Hydrogel Potentially Used for Wound Dressing with Anti-bacterial Properties

Yunxiu Qiu
Washington University in St. Louis

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Student ID # 475212

1 Introduction

A hydrogel is a network of crosslinked polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. Compared to biomaterials fabricated from metals or ceramics, hydrogels can be easily designed to have similarities to soft tissues, and thus they are commonly used in biomedical applications. For example, drug delivery, tissue engineering, would dressing and bio-adhesion. Injectable hydrogels could remain liquid before being injected in vivo while forming scaffold after stimulated in animal or human bodies. Multiple stimuli could be used, including pH, temperature, light, ions in body fluids, etc.[1, 2]

In this project, an injectable hydrogel was prepared through polymer synthesis, and several properties were characterized. It showed thermosensitive property because of the existence of poly (N-Isopropylacrylamide) (PNIPAM)[3], which made it injectable as PNIPAM-based hydrogels would gel at body temperature. Also, the introduction of dopamine and coated silver nanoparticles could provide anti-bacterial property which could potentially used for would dressing.

2 Materials and methods

2.1 Materials

All chemicals included in this project were purchased from Sigma-Aldrich.

Dry chemicals included in this project: N-Isopropylacrylamide (NIPAM), poly(ethylene glycol) methacrylate (MAPEG), acrylate-oligolactide (AOLA), N-acryloxysuccinimide (NAS), benzoyl peroxide (BPO), dopamine hydrochloride (DOPA), silver nitrate ($AgNO_3$).

Solvent included in this project: dioxane, tetrahydrofuran, hexane, diethyl ether, dimethylformamide, triethylamine.

2.2 Synthesis of Polymer I

Polymer I (poly (NIPAM-MAPEG-AOLA-NAS)) was synthesized using 1.60g NIPAM, 1.5g MAPEG, 0.283g AOLA and 0.338g NAS. The monomer ratio of synthesizing Polymer I is listed in Table 1.120ml dioxane was added to dissolve these monomers. After nitrogen protection for 20 minutes, 0.2 wt% (22.3mg) BPO was added into the system as initiator of the reaction dropwise. Then nitrogen was ventilated for another 10 minutes. The whole reaction took 24 hours under 70 ℃, 350 rpm.

The polymer was then purified after reaction. Polymer solution was dropped into 600ml cold hexane with stirring. Precipitation is formed and separated from solution using vacuum filtration. Then the filtered powder is dissolved into 20ml tetrahydrofuran (THF) and dropped into 500ml cold diethyl ether. Again, the newly formed precipitation is vacuum filtered to get the purified Polymer I. The hydrogel was collected by 24 hours-lyophilization thereafter.

2.3 Conjugation of Polymer II

Polymer II was obtained from conjugation of Polymer I and dopamine hydrochloride (DOPA). After calculating the weight ratio of NAS in Polymer I, 50% mole ratio of DOPA (70mg) was added with 1g Polymer I into 10ml dimethylformamide (DMF). After ventilating nitrogen for 30 minutes, 68mg triethylamine (TEA) was also added into the system. The conjugation took 20 hours under 60 ℃.

For purification, the solvent of the polymer solution was evaporated to dryness. Then, the residue was dissolved in 6ml THF, then dripped into 150ml cold diethyl ether. The precipitation was vacuum filtered and then dissolved into 8ml THF. Then the solution was dripped into 160ml cold diethyl ether and the precipitation was filtered again. The Polymer II was collected after 24 hour vacuum dry under 60℃.

2.4 Characterization of synthesized hydrogel

The chemical structure of the Polymer I and Polymer II was confirmed with proton nuclear magnetic resonance (^{1}H NMR).

2.5 Solubility and thermosensitive gelation of synthesized hydrogel

To test the solubility of synthesized hydrogel, 6 wt% and 8 wt% polymer solution of Polymer I and Polymer II was dissolved in PBS under 4℃. Then the solutions were put under 37℃ to test their gelation properties.

2.6 In vitro degradation of synthesized hydrogel

6mL 6 wt% hydrogel solution with DPBS was prepared and sterilized under UV for half an hour. Each micro centrifuge tubes were labeled and weighed w_1. Use 1mL syringe inject exactly 0.2mL hydrogel solution into each tube; Place the tubes in 37℃ water baths for half an hour to allow gelation; add 0.2mL sterilized DPBS as degradation medium into each tube to replace the remaining medium after gelation. Incubate the tubes in 37℃ water baths. When collecting samples at each time point, transfer the medium into labelled separate tubes and store in -80℃ freezer for degradation product toxicity test. The remaining solid gels and tubes are stored in -80℃ freezer. After all samples are collected, freeze dry the tubes with solid gel and weigh the tubes again w_2. Dry weight of hydrogel at each time point are calculated (w_2-w_1). Average dry weight of day0 samples are regarded as original dry weight of the hydrogel (used for normalize the data). Remaining weights (w2-w1) data were recorded at day0, 1, 3, 7, 14, 18, 21, and 28. Data were normalized by day0 data and the percent weight remaining curve was plotted.

2.7 Water content determination

Incubate the day0 samples for at least 5 hours to allow water equilibrium in the hydrogel. Discard the liquid and weight the wet weight of the gel w_3.

Water content (%) = $\frac{w3-w2}{w3-w1}$ *100%

2.8 Coating of Ag nanoparticles

Different concentrations of $AgNO_3$ solution were added into Polymer II solution to form Ag particles coating on the polymer surface. Incubate the 6% Polymer II solution for at least 5 hours, then add 400 μL 0.1M, 0.2M, 0.3M AgNO3 solution into 500μL polymer solution and incubate for another 40 minutes under 60℃.

3 Results and discussion

3.1 Synthesis and characterization of synthesized hydrogel

Polymer I was synthesized through the polymerization of NIPAM, MAPEG, AOLA, NAS, as shown in **Fig. 1(a)**. The representative ^{1}H NMR spectra undertaken with Polymer I and II are shown in **Fig. 1(c)**. The characteristic peak at δ 4.0 ppm (d) in the spectrum represents the hydrogen atom connected to the tertiary carbon in NIPAM. The multi-peaks centered at δ 3.6 and 3.8 ppm (e, f) represent the two methylene groups brought by MAPEG. The peaks centered at δ 5.2 ppm (g) and 2.9 (h) represent the tertiary carbon in AOLA and methylene groups in NAS separately. Polymer II was synthesized through the conjugation of Polymer I and DOPA, under the condition of 60℃, N2 protection, and TEA triggering, as shown in **Fig. 1(b)**. The multi-peaks in 1H NMR spectrum centered at δ 6.6 ppm (j, k, l) represent the methylene groups from DOPA, meaning that dopamine hydrochloride was successfully grafted onto Polymer I.

The feed ratio and real ratio of synthesized Polymer I are listed in **Table 1**. The real ratio was calculated from the integration of ^{1}H NMR peaks. The critical components MAPEG and NAS has the similar ratio compared with feed ratio, as MAPEG enhances the solubility of the hydrogel and NAS provides possibility of conjugating DOPA. The real conjugation ratio of DOPA is also listed in **Table 1**. The conjugated DOPA could provide further crosslinking and anti-bacterial applications.

3.2 Solubility and thermosensitive-gelation properties of synthesized hydrogel

Solubility tests were done under 4℃, with a concentration of 6 weight percentage and 8 weight percentage. 2ml of polymer solution was placed under 4℃ overnight to completely dissolve the polymer. The solubility results were shown in **Fig. 2**. Both 6% and 8% Polymer I and II solution could dissolve well in PBS at 4℃, and gel in different times at 37℃.

At lower temperatures, PNIPAM will fix itself in the solution to hydrogen bond with the water molecules that have been arranged. The water molecules must be redirected around the non-polar region of PNIPAM, which causes a decrease in entropy. At lower temperatures (such as room temperature), the negative enthalpy term produced by the hydrogen bonding effect dominates the Gibbs free energy, causing the PNIPA to absorb water and dissolve in solution. At higher temperatures, the entropy term dominates, causing the PNIPA to release water and phase separate which can be seen in the following demonstration[4].

3.3 In vitro degradation and water content of synthesized hydrogel

The data of day0, 1, 3, 7, 14 was retrieved and remaining hydrogel weights were calculated and plotted against day number. **Fig. 3** shows that this hydrogel could degrade comparably fast, meaning that it will not stay for a long time at would surface or in human tissues. It could be an advantage as some tissues require quick degradation, but could also limits would healing.

3.4 Ag nanoparticles coated on polymer surface

After adding $AgNO_3$ solution and incubate under 60℃ for 40 minutes, the solution turned brown and some precipitation was formed. The Ag+ ion could be reduced to Ag by the hydroxyl groups on dopamine, and the Ag particles could improve the anti-bacterial properties of the hydrogel. UV-Vis tests will be done to test the coating level of silver particles.

4 Conclusion and outlook

In this project, an injectable thermosensitive hydrogel was synthesized and characterized through different ways. It showed good thermal-sensitivity and gelation properties, while its mechanical properties could be improved by adjusting its chemical components. With the addition of dopamine and Ag particles coated on the polymer surface, the anti-bacterial properties of the hydrogel could be significantly improved, which would be done in further research. Therefore, this hydrogel could be applied for would dressing. More tests of the hydrogel need to be done, including biocompatibility and anti-bacterial behaviors.

Figures and Tables

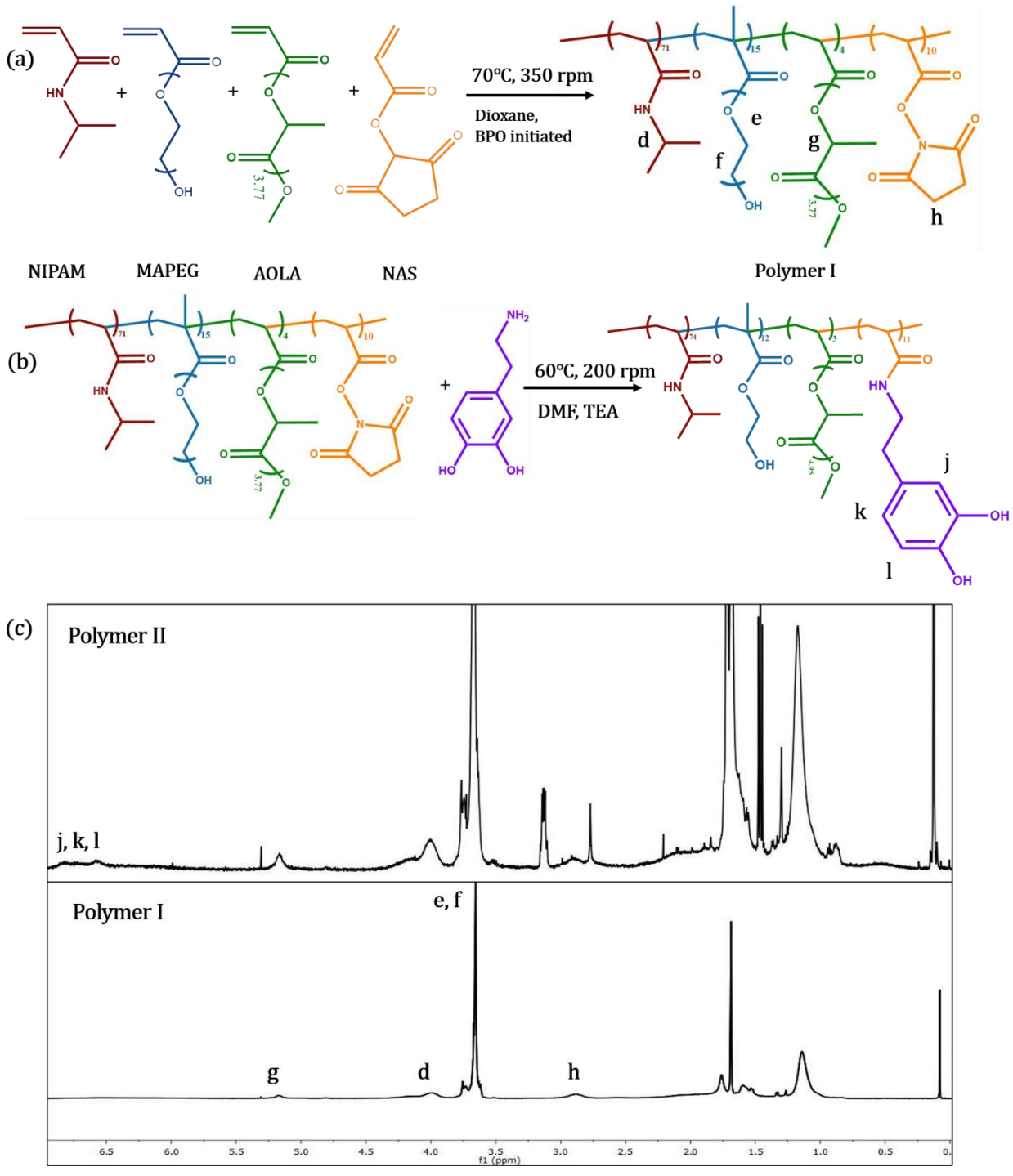

Fig. 1 (a) Synthesis of Polymer I; (b) Synthesis ^{1}H NMR spectrum of Polymer I, solvent: $CDCl_3$.

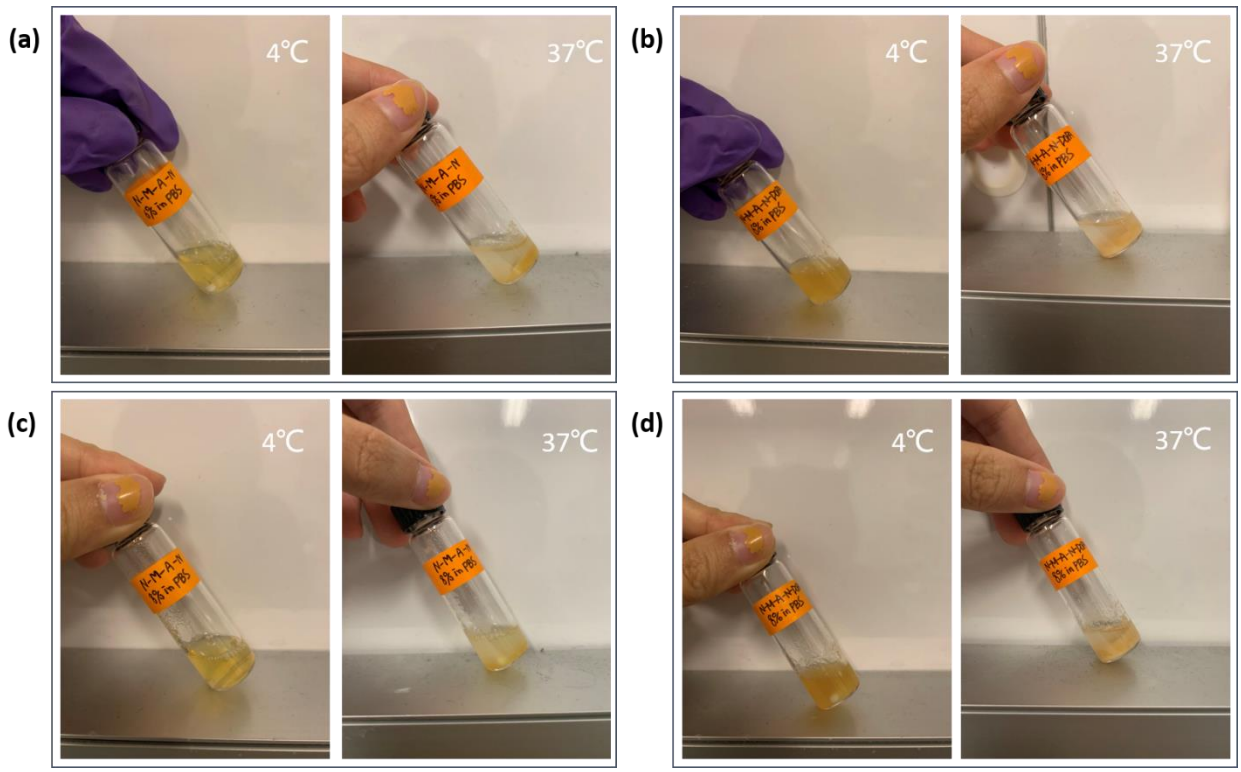

Fig. 2 Solubility at 4℃ and thermosensitive gelation at 37℃. (a) Polymer I, 6% in PBS; (b) Polymer II, 6% in PBS; (c) Polymer I, 8% in PBS; (d) Polymer II, 8% in PBS.

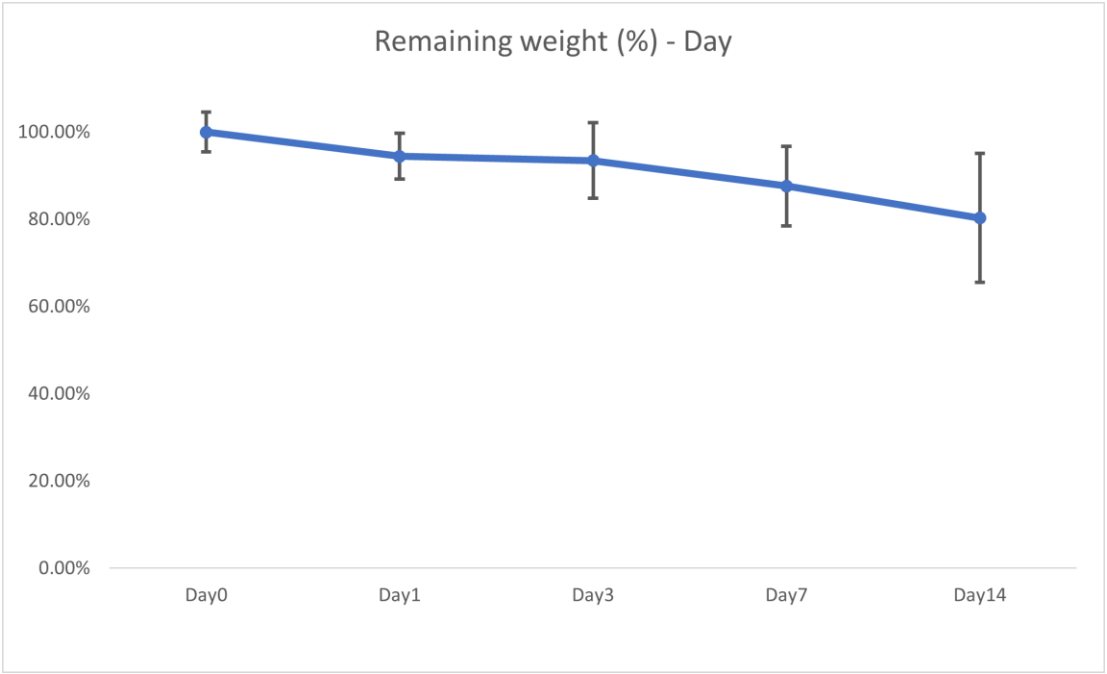

Fig. 3 In vitro degradation of Polymer II.

		MONOMER				
		NIPAM	MAPEG	AOLA	NAS	DOPA
POLYMER I	Feed ratio	71	15	4	10	
	Real ratio	62	18	8	12	
POLYMER II	Feed ratio				1	0.5
	Real ratio				1	0.63

Table 1. Feed ratio and real ratio of synthesized polymers

References

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