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Research Article

Studies of a Biocompatible Maleimide-Modified Dextran and Hyaluronic Acid Hydrogel System

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Abstract: This article discussed a novel hydrogel, which was created through Michael addition. Two precursors Dextran functionalized Maleimide groups (Dex-Mal) and Hyaluronic Acid functionalized thiol groups (HA-SH) were designed, prepared, and characterized by Nuclear Magnetic Resonance (NMR). The formed hydrogels were investigated by gelation time, swelling studies, viscoelastic properties, and degradation rate. Based on gelation time observation, we detected that the hydrogel gelation time could be varied with a diffident weight percentage of precursors. Based on previous research, we measured that 2% Dex-Mal and 2% HA-SH is the optimal formular for the biomedical application and this formular was also investigated by other studies. The swelling study indicated hydrogel has good flexibility and the degradation test indicated hydrogel is biodegradable. The viscoelastic test indicated hydrogel is an elastic solid. From these studies, this novel hydrogel could be potential for biomedical applications.

***Keywords*:** hydrogel, dextran, hyaluronic acid, maleimide, thiol

1. Introduction

Hydrogels are a class of soft materials that are composed of a three-dimensional network of hydrophilic polymer chains. They are capable of absorbing and retaining large amounts of water, which makes them useful in a variety of applications, including drug delivery, tissue engineering, and biosensing.[1] In recent years, hydrogels have gained significant attention in the field of biomedical research due to their unique properties. For example, they can be engineered to mimic the mechanical properties of biological tissues, which makes them ideal candidates for use in the regeneration of damaged or diseased tissues.[2]

Hydrogels are a diverse class of materials that can be engineered to have a range of properties and functionalities, making them useful in a variety of biomedical and industrial applications.[3] Here are some of the most common types of hydrogels: 1) Natural hydrogels: These hydrogels are made from naturally occurring polymers, such as hyaluronic acid, chitosan, or collagen. They are biocompatible and biodegradable, making them ideal for use in tissue engineering and wound healing applications.[4] 2) Synthetic hydrogels: These hydrogels are made from synthetic polymers, such as polyethylene glycol or polyacrylamide. They can be tailored to have specific properties, such as mechanical strength, swelling behavior, or drug release kinetics, and are often used in drug delivery applications.[5] 3)

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pH-responsive hydrogels: These hydrogels are designed to respond to changes in pH by swelling or shrinking. They are often used in drug delivery applications, as the pH of certain biological environments can be used to trigger drug release.[6] 4) Temperature-responsive hydrogels: These hydrogels are designed to respond to changes in temperature by swelling or shrinking. They are often used in tissue engineering applications, as they can be used to create scaffolds that can be easily implanted and shaped.[7]

One particular type of hydrogel that has garnered significant interest is the biocompatible hydrogels. The development of biocompatible hydrogels is particularly important for biomedical applications, since traditional hydrogels made from synthetic polymers like polyacrylamide or polyacrylic acid can be toxic due to the presence of residual monomers or cross-linking agents.[8] However, biocompatible hydrogels are made of materials that are not harmful to living tissues or induce an immune response. For example, they are created by natural polymers like hyaluronic acid, alginate, or chitosan, or synthetic polymers like sulfobetaine, polyethylene glycol or polyvinyl alcohol, etc.[9-13]

Biocompatible hydrogels have a wide range of potential applications in the field of regenerative medicine, including tissue engineering and wound healing.[14] They can be used as scaffolds to support the growth and differentiation of cells, or as wound dressings to promote healing and prevent infection.[15] Also, they also have potential applications in drug delivery, as they can be engineered to release drugs in a controlled manner without causing adverse effects.[16] Moreover, biocompatible hydrogels can be used in the development of biosensors and other diagnostic tools, as they can be functionalized with various biomolecules to detect specific analytes in biological samples.[17]

In this article, we selected two biocompatible natural materials: dextran and hyaluronic acid. We created a hydrogel system based on these two materials. Up to now, no studies have examined hydrogels that utilize the maleimide-thiol reaction with these two materials, without the addition of an initiator. This approach could potentially reduce the negative impact on cells. Here, we primarily investigated this hydrogel system in terms of gelation speed, swelling behavior, viscoelastic property, and degradation rate.

2. Experimental section

2.1 *Materials*

Glycine, maleic anhydride, glacial acetic acid, toluene, triethylamine, ethyl acetate, N,N'-Dicyclohexylcarbodiimide (DCC), 4-Dimethylaminopyridine (DMAP), P-Toluenesulfonic acid monohydrate (PTSA) and anhydrous Dimethyl sulfoxide (DMSO) were purchased from VWR. Magnesium sulfate, Hydrochloric acid (HCl), Ethanol and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Hydroxybenzotriazole (HOBT), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and Phosphate-Buffered Saline (PBS) were purchased from Sigma-Aldrich. 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS) was synthesized from DMAP and PTSA. Deionized water was filtered with a Millipore purification apparatus for all experiments.

2.2 *Synthesis and evaluation*

2.2.1 *Synthesis of carboxyl end Group Maleimide (GMI)*

Figure 1 (a) presents the reaction procedure of GMI preparation. GMI was synthesized using the procedure described by Daniel H. Rich.[18] Typically, a solution of maleic anhydride (0.1 mol, 9.8 g) in 50 mL glacial acetic acid was added to a solution of glycine (0.1 mol, 7.5 g) in 100 mL glacial acetic acid. The mixer was stirred at room temperature, followed by filtration. The precipitation was washed with cold water and dried in a vacuum. N-Glycinyl Maleamic Acid (GMA) (16.8 mmol, 2.91 g) and Et_3N (35.1 mmol, 3.55 g) were added to 500 mL toluene. The suspension was refluxed with vigorous stirring for 1.5 hours, meanwhile the byproduct water was removed through a Dean-Stark apparatus. The toluene solution was decanted away from brown-colored oil and the toluene was removed by evaporation to give the triethylammonium salt. The hygroscopic product was acidified to pH 2 with HCl, followed by extraction with ethyl acetate. The organic layer was dried with magnesium sulfate, then the ethyl acetate was evaporated to give GMI.

2.2.2 *Synthesis of Dex-Mal*

Figure 1 (b) presents the synthetic procedure to synthesize dextran (MW = 100 kDa) with maleimide groups. Briefly, Dex-Mal was prepared through DCC-mediated esterification of the hydroxyl group of the dextran with N-maleoylamino acid, which was obtained through a procedure reported previously. Typically, N-maleoylamino acids (0.72 g, 3.1 mmol) were dissolved in 20 mL DMSO followed by the addition of DPTS (0.145 g, 0.45 mmol) and DCC (0.96 g, 4.65 mmol). Dextran (0.92 g, 5.15 mmol anhydroglucose units) was dissolved in 10 mL DMSO and added to the reaction mixture slowly. After stirring for 24 h at room temperature, the formed N,N'-dicyclohexylurea salt was removed by filtration, and the crude product was obtained by precipitation in cold ethanol. The precipitate was collected by filtration and washed with ethanol and then dissolved in water and purified by ultrafiltration.

2.2.3 *Synthesis of HA-SH*

HA-SH was synthesized as described in Figure 1 (c). Hyaluronic Acid (HA) (MW = 80 kDa) was dissolved in DI water (5 mg/mL) and conjugated with three molar amounts of cystamine dihydrochloride at pH 4.8 overnight after activation of the carboxyl group of HA with EDC and HOBT (3 equiv.) for 2 hours. The resulting product was purified by dialysis tubing (MWCO of 10,000 kDa) against DI water for 3 days. Then, the solution was treated with TCEP (5 equiv.) to cleave the disulfide linkage of the cystamine component. After stirring for 2 hours, the pH of the solution was adjusted to 3.5 with HCl. Finally, HA-SH was precipitated in ethanol, re-dissolved in DI water, lyophilized and stored at -20 °C.

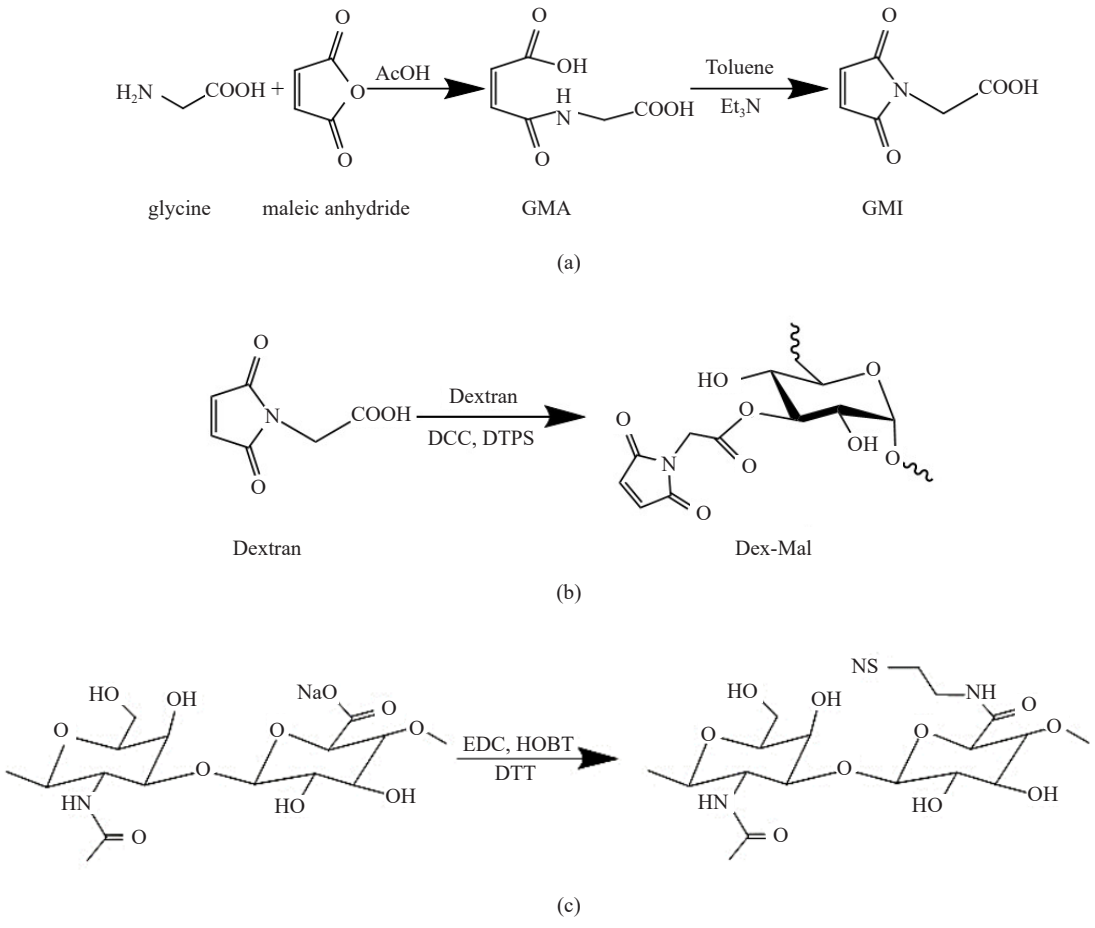

Figure 1. Reaction scheme for the syntheses of (a) GMI, (b) Dex-Mal and (c) HA-SH

2.3 *Hydrogel preparation*

Dex-Mal and HA-SH hydrogel precursors were dissolved in pH 7.4 PBS, then followed by mixing Dex-Mal and HA-SH solutions. The maleimide groups reacted with thiol groups to create the three-dimensional structure to form the gel.

2.4 *Characterization*

2.4.1 *Characterization of GMI, maleimide and thiol hydrogel precursors*

The molecular structures of GMI, maleimide and thiol hydrogel precursors were confirmed by Proton and Carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR). ^{1}H and ^{13}C NMR spectra were recorded with an Avance Bruker equipped with Broadband Observe (BBO) z-gradient probe. Experimental conditions were as follows: each sample was scanned 128 times. The solvents used were (1) DSMO-d6 for GMI and (2) D_2O for Dextran, Dex-Mal, HA and HA-SH. GMI FTIR spectra were also recorded based on a Perkin Elmer 1,000 Fourier-Transform Infrared spectroscopy (FTIR). The measurements were carried out over the 400-4,500 cm^{-1} range at room temperature. The number of scans per spectrum was 32, and the spectral resolution was 4 cm^{-1}.

2.4.2 *Gelation time*

The gelation time is determined using the vial tilting method. Briefly, Dex-Mal and HA-SH hydrogel precursors were dissolved in pH 7.4 PBS solution, respectively. The hydrogels were formed by mixing both solutions and vibrating quickly at room temperature. No flow within 1 min upon inverting the vial is regarded as the gel state.

2.4.3 *Swelling studies*

Swelling studies of freeze-dried hydrogels were tested by a general gravimetric method. Samples were incubated at 37 °C in pH 7.4 PBS until weight is balanced. The swollen hydrogels were removed from the excess of water absorbed with a filter paper, and then weighed. The samples were then dried until constant weight. The swelling ratio was calculated using the following Eq:

$$\text{Percentage of swelling (\%)} = (W_{wet} - W_{dry})/W_{dry} \times 100,$$

where Wwet and Wdry refer to the weight of wet and dry hydrogels respectively.

2.4.4 *Degradation*

For degradation studies, weighted hydrogel freeze-dried samples were immersed in pH 7.4 PBS and incubated at 37 °C at selected time intervals. At each selected time interval, the samples were removed from PBS, dried, and weighed. The hydrogel degradation was calculated using the following Eq:

$$\text{Degradation (\%)} = (m_t/m_0) \times 100,$$

where mt and m0 refer to the weight of freeze-dried hydrogels at selected time intervals and before immersing in PBS respectively.

2.4.5 *Rheological analysis*

Dynamic rheological behaviors of hydrogels are analyzed with a Discovery Hybrid Rheometer, using parallel plate geometry (8 mm diameter). Frequency sweep measurements are performed at 37 °C from 0.1 to 100 rad s^{-1} at a fixed strain in the linear viscoelastic region.

3. Results and discussion

3.1 *NMR analysis*

3.1.1 *GMI*

GMI structure was confirmed by NMR spectra. In Figure 2 (a) ^{1}H spectrum shows that the peaks around 13 and at 7.1 ppm are due to the -O-H bond of the carboxylic acid and the -CH = CH- bond of the maleimide ring.[19] In Figure 2 (b) ^{13}C spectrum illustrates that the peaks corresponding to carbonyl groups and the double bond of the maleimide ring appeared at 170.8 and 135.3 ppm, respectively. Furthermore, the peak of the carbonyl group of the carboxylic acid was detected at 169.3 ppm.

GMI structure was further confirmed by FTIR spectroscopy in Figure 3. The stretching vibration was revealed at 1,745 and 1,680 cm^{-1}, which contribute to carbonyl groups. Furthermore, C = C stretching vibration and alkene bending appeared at 1,574 and 694 cm^{-1} demonstrating the presence of the maleimide ring.

3.1.2 *Dex-Mal*

Dex-Mal structure is confirmed by ^{1}H NMR spectrum. In Figure 4, the peak at 6.9 ppm is assigned to protons in the maleimide. The peaks at 4.0-3.4 ppm are attributed to protons in the dextran backbone. The degree of functionality of the Dex-Mal can be estimated to be about 50% by comparison of the integrals of the resonance signals at 6.9 ppm and 4.0-3.4 ppm.

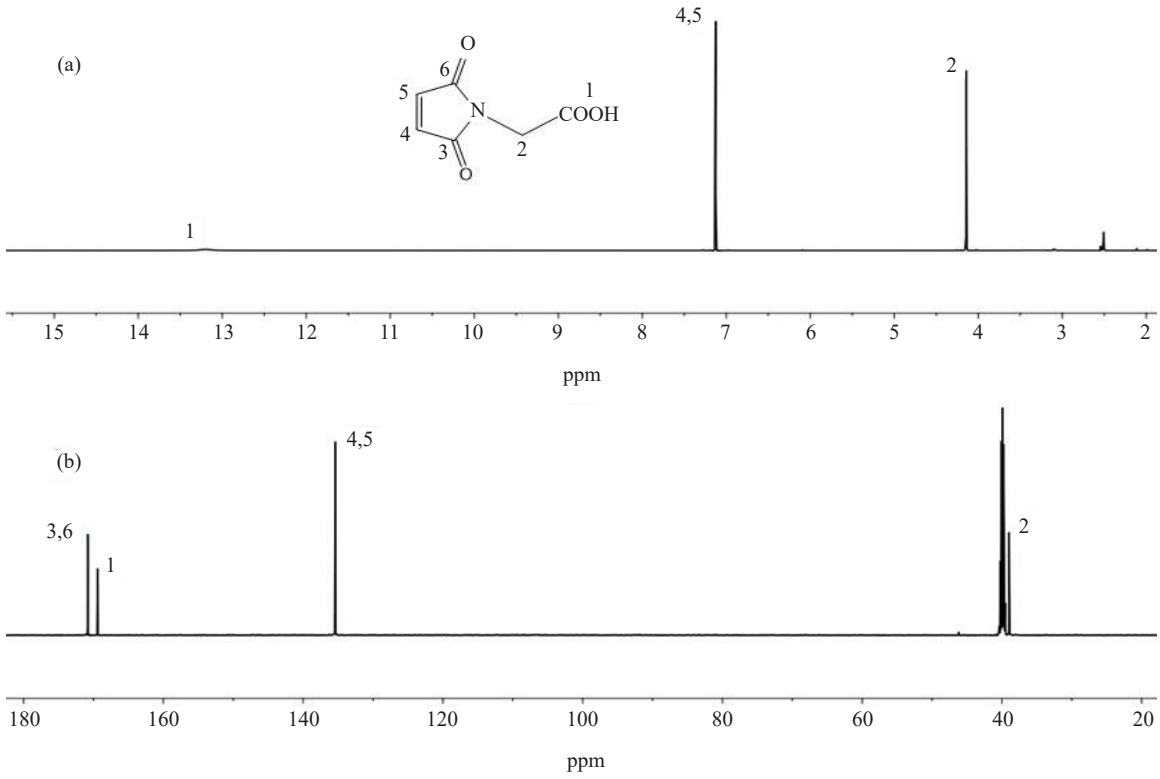

Figure 2. (a) ^{1}H and (b) ^{13}C NMR spectra of GMI in DMSO-d6

3.1.3 *HA-SH*

The structure of HA-SH was confirmed by ^{1}H NMR. In Figure 5, HA modification was determined by ^{1}H NMR analysis using the resonance of the acetamido moiety of HA at δ = 1.95-1.85 ppm as an internal standard. The peaks at δ

= 2.6 ppm correspond to cysteamine in HA-SH. The degree of functionality of the HA-SH can be estimated to be about 10% by comparison of the integrals of the resonance signals at 2.7-2.5 ppm and 1.95-1.85 ppm.

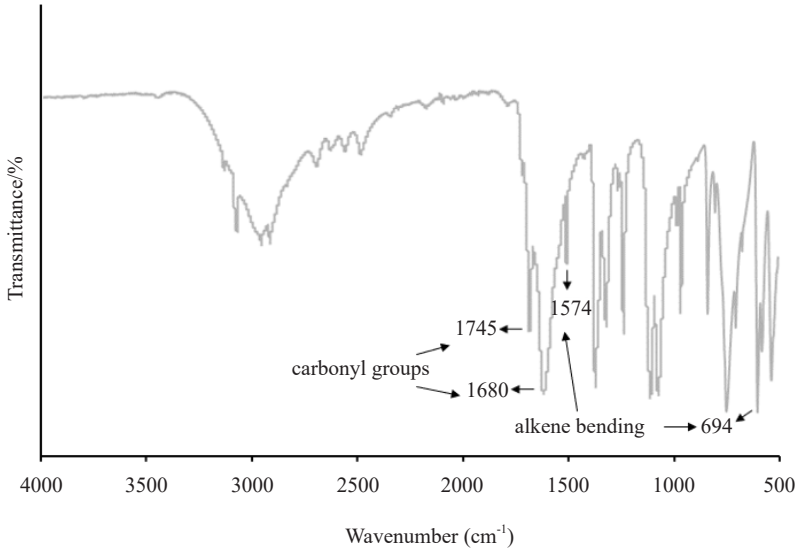

Figure 3. FTIR spectra of GMI

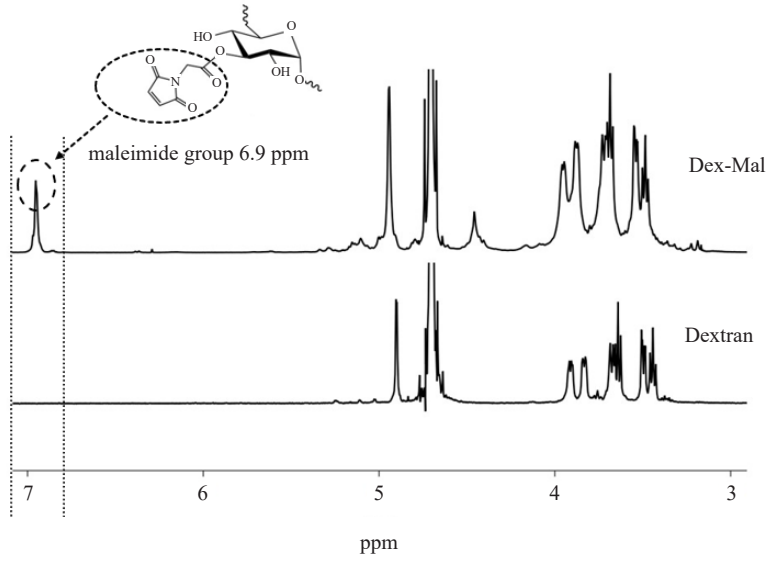

Figure 4. ^{1}H NMR spectrum of Dextran and Dex-Mal in D_2O

3.2 *Gelation time analysis*

The gelation time of hydrogels is summarized in Table 1. The weight percent of hydrogel precursors used is from 1% to 4%. The gelation time is from ∞ (no gelation) to ≤1 second (ultrafast). 2% Dex-Mal and 2% HA-SH is the only feasible formular because longer or shorter time could lead to the failure of biomedical applications like cell encapsulation. For example, a longer time means lower weight percent hydrogel precursors used, that could

form amorphous gel. On the contrary, a shorter time could result that the maleimide and thiol groups are not reacted completely, which impacts cell growth. Consequently, this formular was used for the remaining experiments and analysis.

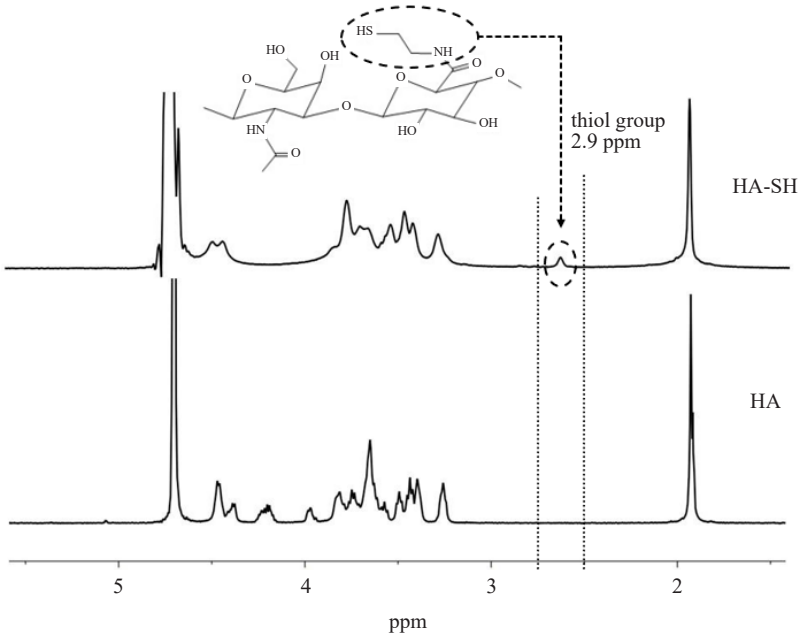

Figure 5. ^{1}H NMR spectra of HA-SH and HA in D_2O

Table 1. Summary of four hydrogels' gelation time

		Dex-Mal		
		1%	2%	4%
HA-SH	1%	No gelation	1.5 h	×
	2%	1.5 h	35 s	≤1 s
	4%	×	≤1 s	≤1 s

3.3 *Degradation analysis*

Degradable hydrogels are designed to break down or decompose over time into harmless components. This can be important in medical applications where the material needs to be absorbed or eliminated by the body without leaving behind harmful residues. One way to analyze the degradation of hydrogels is to measure weight loss, which was plotted in Figure 6. It exhibited the weight loss speed of hydrogels. The weight loss of hydrogels reached 18%, 39% and 55% on the first day, third and fifth day respectively. Moreover, on the 7th-day hydrogels were degraded around 80% and on nineth day were degraded completely. It implies that hydrogels could provide temporary support or scaffolding for tissue regeneration, and then safely biodegrade once it is no longer needed.

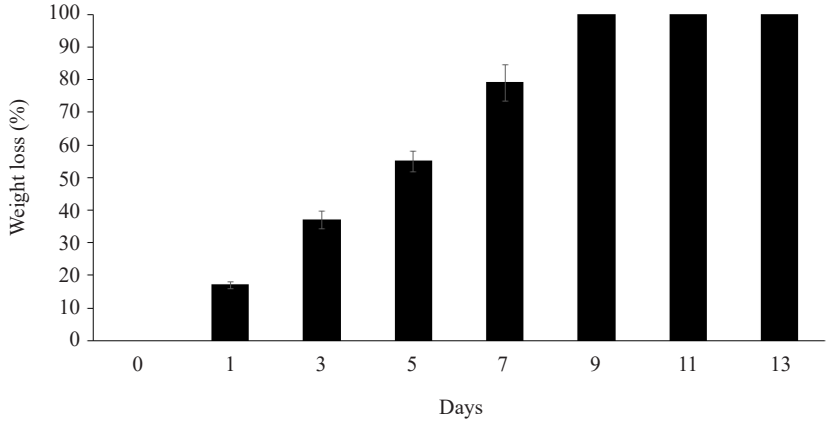

Figure 6. Weight loss of hydrogels at selected time intervals

3.4 *Swelling analysis*

As we know swelling is an important ability of biocompatible materials, like hydrogels, since it can absorb and retain fluids from the surrounding environment, which can be beneficial for applications such as drug delivery or wound healing. In this article, swelling analysis is exhibited in Figure 7. The weights of freeze-dried hydrogels samples are from 1 gram to 5 grams. It is noticeable that all samples with different weights have similar swelling ratio, which is around 50%. The more hydrophilic samples will be submerged in PBS, the more water will be absorbed, and a higher swelling ratio will be calculated. Here, it's clear that the 2% Dex-Mal and 2% HA-SH hydrogels could absorb the water, which has the half mount weight as freeze-dried hydrogels. It indicates the hydrogels created by this formular have good flexibility and ability to mimic extracellular matrix to regulate cell behavior and tissue development.

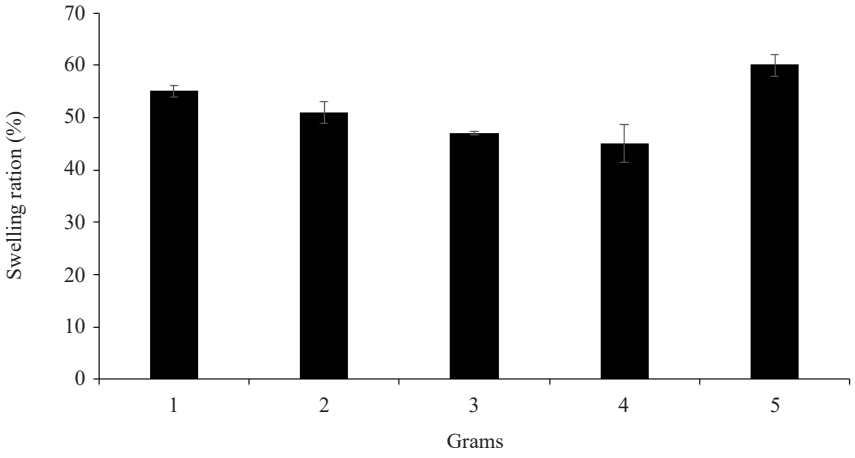

Figure 7. Summary of hydrogels' swelling ratio

3.5 *Rheological analysis*

G′ and G″ were plotted in Figure 8 as a function of angular frequency for hydrogels. The hydrogels showed characteristic viscoelastic behaviors. The moduli G′ was always higher than G″ illustrating hydrogels formed by maleimide-thiol coupling chemistry are elastic solids.

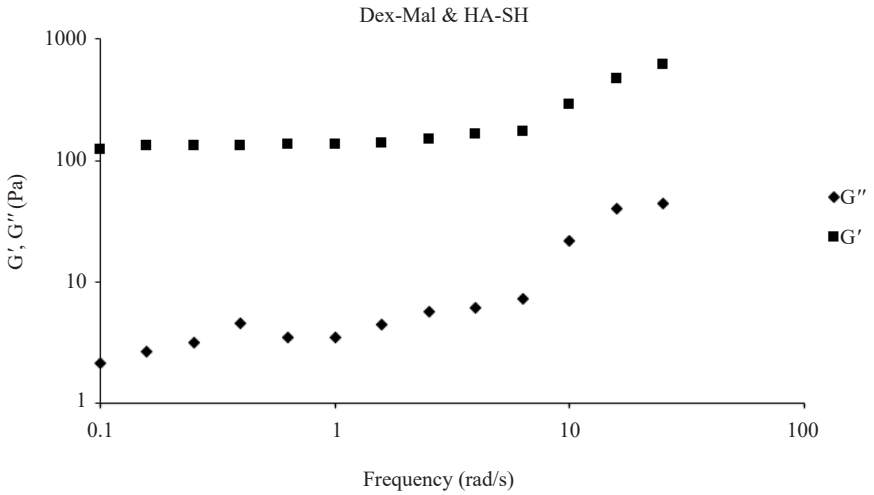

Figure 8. Frequency dependency of storage moduli (G′) and loss moduli (G″) of the hydrogels

4. Conclusions

In this article, we created a novel biocompatible hydrogel based on precursors functionalized Dex-Mal and HA-SH through the Michael addition without an initiator. Both were characterized using NMR, which indicated that they were synthesized successfully. The hydrogels were characterized by gelation time, swelling studies, viscoelastic properties, and degradation rate. According to the gelation time study, the best formula is 2% Dex-Mal and 2% HA-SH. As a consequence, this formular was used for the remaining studies. The swelling study shows that the hydrogels have good flexibility to absorb and retain fluids from the surroundings to mimic the extracellular matrix, which is critical to maintain the structure and function of organs and tissues in the body. The degradation study shows that the hydrogels will be degraded completely after 9 days. This suggests that hydrogels have the potential to offer provisional a framework for tissue regeneration, and subsequently degrade harmlessly once they are no longer required. The viscoelastic study shows that the hydrogels formed by Michael Addition are elastic solids. Based on the above evidence, this novel biocompatible hydrogel could be potential for biomedical applications.

Conflict of interest

The authors declare no competing financial interest.

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