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β-cyclodextrin-conjugated hyaluronan hydrogel as a potential drug sustained delivery carrier for wound healing

Ruicong Li,[1] Xiaoting Zhang,[1] Qiuyu Zhang,[1] Huahua Liu,[1] Jianhua Rong,[1,2] Mei Tu,[1,2] Rong Zeng,[1,2] Jianhao Zhao[1,2]

[1]Department of Materials Science and Engineering, College of Science and Technology, Jinan University, Guangzhou 510632, China

[2]Engineering Research Center of Artificial Organs and Materials, Ministry of Education, Guangzhou 510632, China

Correspondence to: J. Zhao (E-mail: jhzhao@jnu.edu.cn)

ABSTRACT: In order to develop a potential drug sustained delivery carrier suitable for wound healing, a series of β-cyclodextrin conjugated hyaluronan hydrogels (β-CD-HA) with adjustable crosslink densities were synthesized and characterized, meanwhile the delivery kinetics and mechanism of diclofenac as a model anti-inflammatory drug from these hydrogels were investigated. By controlling the feeding molar ratio of β-CD/HA, a β-CD substitution degree of 4.65% was obtained by ^{1}H-NMR analysis. The incorporation of β-CD modification had little effect on the internal porous structure, water swelling ratio, and rheological property of HA hydrogel, which however were influenced by the crosslink density. Although the crosslink density had an influence on the drug loading and release profile by altering the water swelling property, the interaction between β-CD and drug was the primary factor for the high loading capacity and long-term sustained delivery of diclofenac. The semiempirical equation fit showed that the release of diclofenac from HA-based hydrogels followed a pseudo-Fickian diffusion mechanism. By the aid of β-CD and controlled crosslink density, a β-CD-HA hydrogel with a diclofenac sustained delivery period of over 28 days and desirable physicochemical properties was achieved, which will be a promising drug sustained delivery carrier for wound healing. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43072.

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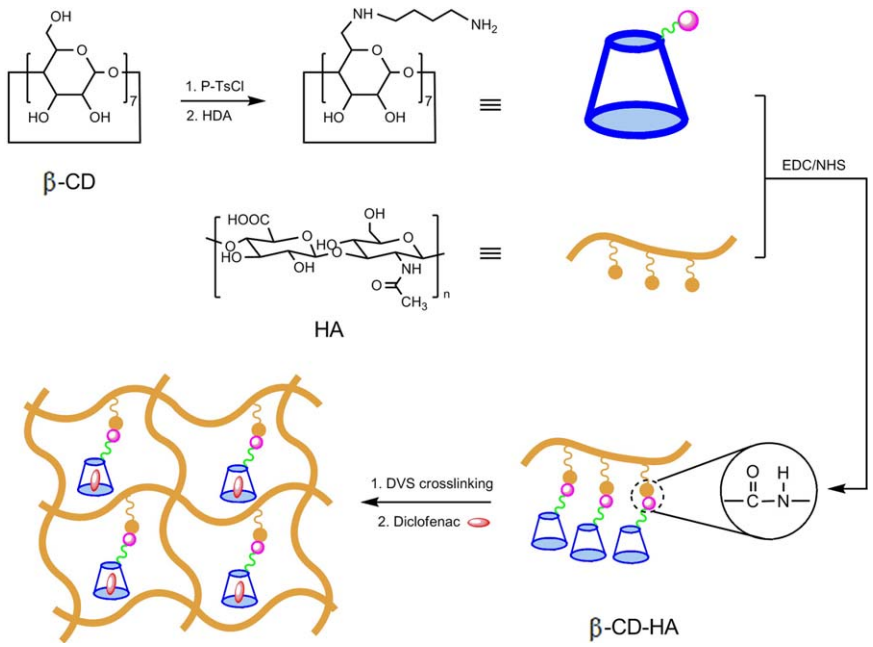
INTRODUCTION

Wound healing, as an important way to recover from skin injury, is a complex biological process involving three phases: inflammation, tissue formation, and tissue remodeling.[1] Inflammation, a protective response of the body to injury inevitably caused by the infection of necrotic tissues and/or pathogenic microorganisms, occurs in the early a few days from wound formation, but possibly overlaps and affects the entire healing process that may last for four weeks or longer depending on the wound restoration. Prolonged inflammation can lead to damage and loss of function of tissues.[2–4] Therefore, it is essential to reduce inflammation in wound healing by the aid of anti-inflammatory drugs that had better continuously release from wound dressings. Among various wound dressings, such as natural gauze, polymeric foam, and hydrogels, hydrogels derived from polysaccharides have recently attracted much interests for their high hydrophilicity that facilitates the wound healing by stopping tissue adhesion, keeping the wound wet and promoting the elimination of necrotic tissues by emzymolysis.[5–7] However, the long-term sustained delivery of anti-inflammatory drugs from these hydrogel wound dressings still remains major challenge.

Hyaluronan (HA), a linear polysaccharide composed of alternating D-glucuronic acid and *N*-acetyl-D-glucosamine units, is a main component in most natural tissues. Thanks to the high water absorption, inherent cytocompatibility and cellular response, as well as the active carboxyl and hydroxyl groups in molecular chains allowing modification under mild conditions, HA-based hydrogels have been widely studied in soft tissue engineering and drug/protein delivery systems,[8–11] and showed potential applications in wound healing. Unfortunately, by lack of functional groups able to carry drugs, it is difficult for HA bulk hydrogel as wound dressings to control the release of anti-inflammatory drugs. In our previous work, it has been found that a sustained delivery of bovine serum albumin from HA microgels can be obtained by tailoring the crosslink density[11]; however, the delivery period is only prolonged to a couple days, and unable to meet the requirement of anti-inflammatory drug delivery in wound healing.



Scheme 1. Synthesis pathway of β-CD-HA hydrogel for diclofenac loading and sustained delivery. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Cyclodextrin (CD), a torus-shaped cyclic oligosaccharide consisting of α-1,4-linked D-glucopyranose units with an internal hydrophobic cavity, can form inclusion complexes with a number of drugs by reversible non-covalent interactions.[12–14] However, when drug/CD complexes are diluted in physiological solutions, decomplexation happens and the drug controlled release becomes unavailable.[15,16] For this reason, CD has been attempted to link a polymeric network for minimizing the dilution so that the drug release can be availably controlled by the affinity between CD cavities and drugs.[17,18]

In this work, a series of β-cyclodextrin (β-CD)-conjugated HA hydrogels with different crosslink densities were fabricated as a potential wound dressing to achieve the sustained delivery of diclofenac sodium, a model anti-inflammatory drug. In order to reduce the hindrance and enhance the reaction activity between β-CD and HA, 1,6-hexanediamine (HDA) as a "bridge" was utilized to connect β-CD and HA via the chemical reactions between amine and hydroxyl in β-CD as well as carboxyl in HA. The obtained β-CD-conjugated HA macromolecules (β-CD-HA) performed gelation in the presence of divinyl sulfone (DVS) as the crosslinker. The physicochemical properties of β-CD-HA hydrogels, such as β-CD substitution degree, morphology, water swelling ratio, crosslink density, and mechanical property, were investigated. The effects of both β-CD and crosslink density on the diclofenac loading and sustained delivery of β-CD-HA hydrogels were studied. On this basis, the drug diffusion mechanisms of various HA-based hydrogels were discussed.

EXPERIMENTAL

Materials

Sodium hyaluronan (M_w, 1.5 × 10^6 g/mol) was provided by Shandong Institute of Medical Instruments (China). Diclofenac sodium, β-cyclodextrin, p-toluenesulfonyl chloride (p-TsCl), 1-(3-Dimethylaminopropyl)−3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), 1,6-hexanediamine (HDA), and divinyl sulfone (DVS) were purchased from Sigma-Aldrich (USA). All other reagents and solvents used were analytical grade.

Synthesis of β-CD-HA

The synthesis route of β-CD-HA was shown in Scheme 1. Mono-6-deoxy-6- (p-tolylsulfonyl)-β-cyclodextrin (M-6-O-Ts-β-CD) was firstly synthesized following the previous method.[19] Briefly, β-CD (52.9 mmol) was dissolved in 500 mL sodium hydroxide aqueous solution (NaOH, 164.0 mmol). Then, p-TsCl (52.9 mmol) in 30 mL acetonitrile was added dropwise to the above solution at 4°C, causing the formation of a white precipitate. After 2 h of stirring at room temperature, the precipitate was removed by suction filtration, and the filtrate was refrigerated for 48 h at 4°C. Finally, the white solid product M-6-O-Ts-β-CD was obtained after suction filtration and desiccation at vacuum (0.5 mmHg) in sequence. The yield was about 10%.

To activate β-CD by amino groups, M-6-O-Ts-β-CD (2.26 mmol) and excess amount of HDA (51.6 mmol) were dissolved in 12 mL dimethylformamide, and reacted under magnetic stirring at 75°C for 4 h. After reaction, the mixture was cooled to room temperature, and then excess cold acetone was added. The resulting precipitate was repeatedly dissolved in methanol/water ($v/v = 1/1$) followed by adding excess cold acetone for five times to remove the residue HDA. Finally, β-CD-HDA product was obtained after being dried at vacuum for 48 h. The yield was about 30%.

HA (1.0 g, containing 2.5 mmol carboxyl groups) was dissolved in 300 mL phosphate buffer saline (PBS, pH 6.0), and then EDC (8.8 mmol) and NHS (8.8 mmol) were added to activate the carboxyl group for 40min. After adjusting the solution pH

to 7.4 using 0.1*M* NaOH, β-CD-HDA (2.5 mmol) in 100 mL PBS was added. The mixture reacted under magnetic stirring at room temperature for 24 h, followed by dialysis in distilled water for 5 days. Finally, β-CD-HA was obtained after lyophilization (0.5 mmHg). The yield was about 50%.

Fabrication of β-CD-HA Hydrogel

The fabrication of β-CD-HA hydrogel was shown in Scheme 1. β-CD-HA (1.0 g) was dissolved in 20 mL 0.3*M* NaOH aqueous solution to get a concentration of 50 mg/mL. Subsequently, 800 μL of this solution was taken out by pipettor and transferred to a cylinder mold with a diameter of 1 cm, and then DVS was added as the crosslinker. After ultrasonic dispersion for 30 s, the mixture solution was kept static to allow the gelation of β-CD-HA. The obtained β-CD-HA hydrogels were immersed in deionized water for two days by refreshing the water twice per day to remove the excess crosslinker. The as-synthesized β-CD-HA hydrogels were dehydrated by passing them through graded ethanol solutions [ethanol/water(v/v) = 25%, 50%, and 100%] and drying them under vacuum overnight at room temperature. In order to investigate the effect of crosslink density on the properties of β-CD-HA hydrogels, different molar ratios of DVS/HA repeating unit, i.e. 0.5, 1.0, and 2.0 respectively, were used. The obtained hydrogels were correspondingly defined as β-CD-HA-0.5, β-CD-HA-1.0, and β-CD-HA-2.0, respectively. As compared, HA hydrogel without β-CD modification was prepared following the above process at a DVS/HA repeating unit molar ratio of 0.5, and defined as HA-0.5.

Characterizations

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Analysis. The ^{1}H-NMR spectra of M-6-O-Ts-β-CD in DMSO-d_6, and β-CD-HDA and β-CD-HA in D_2O with a concentration of 20 mg/mL respectively were recorded in a 500 MHz Bruker AVANCE III (Bruker, Germany) spectrometer at room temperature.

Scanning Electron Microscopy (SEM) Observation. The cross section morphologies of HA-based hydrogels were observed by SEM (PHILIPSXL-30 ESEM, Netherlands) using an acceleration voltage of 20 kV. The hydrogels were first fully hydrated and half-cut by a razor blade, followed by lyophilization to keep the cross section morphology. Before observation, a gold layer was coated on the specimen surface in a sputter coater (BAL-TEC, SCD005, Finland).

Water Swelling Test. The water equilibrium swelling ratios (Q_M) of HA-based hydrogels were measured gravimetrically.[20] Briefly, the dried hydrogels in a certain mass were soaked in physiological saline (0.9 wt % sodium chloride aqueous solution) at 37°C for 24 h to achieve the swelling equilibrium. Then, the hydrogels were removed from the medium and weighted after blotting the free liquid on the surface by tissue paper. The Q_M value was evaluated by the ratio of wet weight to dry weight. In order to evaluate the crosslink density of the hydrogels, Flory-Rehner equation was used[21–23]:

$$Q_V^{5/3} = \frac{v}{V_1}\left(\frac{1}{2} - x\right) M_C \quad (1)$$

and

$$Q_V = 1 + \frac{\rho_P}{\rho_s}(Q_M - 1) \quad (2)$$

where M_C is the average molecular weight between two crosslink points, Q_V is the volumetric swelling ratio, v is the specific volume of the dry hydrogels, V_1 is the molar volume of the solvent (18 cm^3/mol for water), and x is the Flory polymer-solvent interaction parameter, which is 0.473 for HA.[24]

Rheological Analysis. The rheological properties of fully hydrated HA-based hydrogels were characterized in a shear rheometer (Kinexus Pro, Malvern) with a 25-mm plate and a gap size of 1 mm. The test was performed in a frequency range of 0.1–10 Hz using a constant strain of 0.1% at room temperature.

Diclofenac Loading and Delivery. Dried HA-based hydrogels were soaked in 3 mL diclofenac sodium solution (80 μg/mL, in physiological saline) to allow diclofenac loading, and kept 4 days at 37°C protected from light. The loading amount was calculated by subtracting the unloaded drug from the initial drug amount, determined at 276 nm in a spectrophotometer (UV759CRT, Shanghai, China). For diclofenac delivery, the above drug-loaded hydrogels were rinsed with physiological saline three times, followed by immersion in 3 mL physiological saline at 37°C. At desired intervals, the medium was collected and refreshed with the same liquid volume. The amount of drug release was measured spectrophotometrically at 276 nm.

Statistical Analysis

Triplicated experiments were carried out and the data were presented as means ± standard deviation (SD). Student's t-test was applied to test the significance of observed differences between study groups. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

^{1}H-NMR Analysis

The successful synthesis of M-6-O-Ts-β-CD, β-CD-HDA and β-CD-HA was proved by ^{1}H-NMR analysis, as shown in Figure 1. In Figure 1(a), the proton H-1' at δ4.82 ppm and protons H-2'∼H-6' at 3.24-3.67 ppm were from β-CD.[25] The peak at δ2.42 ppm was assigned as proton H-9' in methyl connecting to benzene ring from TsCl, and the peaks at δ7.76 ppm and δ7.44 ppm respectively assigned as protons H-7' and H-8' in benzene ring. These data confirmed the successful synthesis of M-6-O-Ts-β-CD. In Figure 1(b), besides the above characteristic peaks of β-CD, some new peaks at δ3.02, 2.93, 1.63, 1.48, 1.30 ppm assigned as H-10'∼H-15' in HDA occurred, meanwhile the characteristic peaks of protons H-7', H-8', and H-9' from TsCl disappeared, suggesting the successful synthesis of β-CD-HDA.

On this basis, β-CD-HA was synthesized by covalently linking β-CD-HDA to HA via amidation reaction. In the spectrum of HA [Figure 1(c)], the peaks at δ3.27–4.49 ppm were from protons H-1∼H-6, and the peak at δ1.94 ppm was from proton H-7 in the acetyl group.[26] By comparison, in the spectrum of β-CD-HA [Figure 1(c)], some new peaks appeared besides the original characteristic peaks from HA. The peak at δ5.01 ppm was the typical peak from proton H-1' in β-CD, and the peaks at δ3.02 ppm and δ2.93 ppm were respectively from protons H-15' and H-10' of methylene groups connecting to the nitrogen atoms in HDA that acted as a 'bridge' between HA and β-CD.

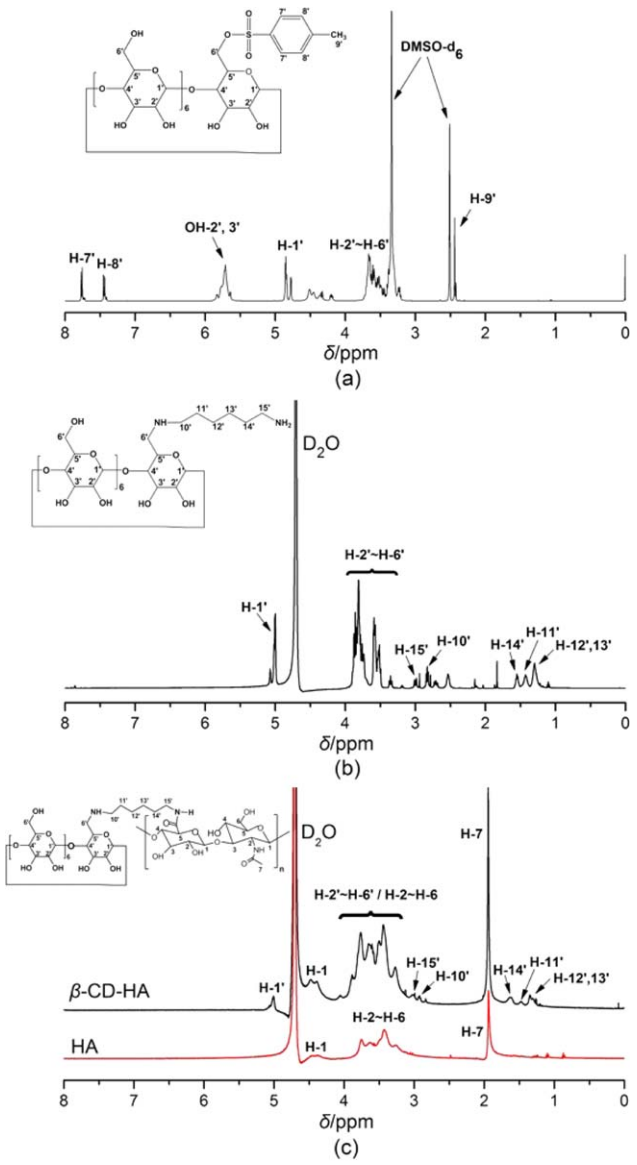


Figure 1. ^{1}H-NMR spectra of (a) M-6-O-Ts-β-CD in DMSO-d_6, (b) β-CD-HDA in D_2O and (c) HA and β-CD-HA in D_2O. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Additionally, the protons H-11'∼ H-14' from the 'bridge' were also found at δ1.48 ppm, δ1.63 ppm, and δ1.30ppm. These results suggested that β-CD was successfully bonded to HA by the aid of HDA. Based on the relative integral intensities of protons H-1' and H-7, the substitution degree of β-CD on HA was calculated to be 4.65% according to the following equation:

$$\frac{7x}{3} = \frac{I_{H-1'}}{I_{H-7}} \quad (3)$$

Where, x is the substitution degree of β-CD on HA, $I_{H\text{-}1'}$ and $I_{H\text{-}7}$ were the relative integral intensities of protons H-1' and H-7, respectively.

Morphology Observation

The cross section morphologies of HA hydrogels before and after β-CD modification were characterized by SEM, as shown in Figure 2. The interior of all hydrogels displayed irregular porous structure with a pore size of less than 50 μm, which was produced from the sublimation of entrapped water in the swollen hydrogels by lyophilization.[27] This type of irregularly shaped wall-like morphology of HA gels used as soft-tissue fillers has been recently unequivocally identified by Cryo-SEM and energy-dispersive X-ray analyses.[28] This kind of porous structure of HA-based hydrogels was beneficial to the high water absorption that was important for keeping the wound wet.[29] β-CD-HA-0.5 and HA-0.5 samples exhibited similar porous structure, however with the increase of DVS content, i.e., in the sequence of β-CD-HA-0.5, β-CD-HA-1.0, and β-CD-HA-2.0, the pore size seemed to slightly decrease maybe due to the enhancement of crosslink density. From these results, it implied that the conjugation of β-CD on the HA side chain with a substitution degree of 4.65% had no significant effect on the hydrogel morphology, but the increase of DVS content could somewhat reduce the pore size of hydrogel.

Water Swelling Property and Crosslink Density

Considering DVS was able to react with the hydroxyl group of hydroxymethylene on N-acetyl-d-glucosamine unit of HA via Michael addition under basic conditions,[20,30,31] here DVS was used as the crosslinker of HA and β-CD-HA hydrogels. It is known that an advantage of hydrogel wound dressings is their high water absorption that keeps the wound wet and avoids the adhesion of shin tissue.[29,32] The water swelling properties of HA-based hydrogels subject to the crosslink densities, were summarized in Table I. M_c, the average molecular weight between two crosslink points, is often used to estimate the crosslink density of crosslinking polymers.[11] The M_c value is contrary to the crosslink density, that is, a higher M_c represents a lower crosslink density and vice versa. All hydrogels possessed a water equilibrium swelling ratio of over 30, suggesting their good hydrophilicity and water absorption ability. By comparison, β-CD-HA-0.5 hydrogel showed a slightly higher water equilibrium swelling ratio of 68.92 ± 1.29 and a larger M_c value of $1.33 \pm 0.23 \times 10^6$, as opposed to 63.46 ± 2.06 and $1.16 \pm 0.31 \times 10^6$ respectively for HA-0.5 hydrogel. The comparisons between HA-0.5 and β-CD-HA-0.5 suggested that although the incorporation of β-CD was likely to increase the steric hindrance of HA molecular chains, the water swelling property and crosslink density between HA-0.5 and β-CD-HA-0.5 were comparable, suggesting this influence of β-CD conjugation was negligible. Nevertheless, for β-CD-HA hydrogels, a higher DVS content decreased the water equilibrium swelling ratio from 68.92 ± 1.29 to 32.57 ± 2.24, and M_c value from $1.33 \pm 0.23 \times 10^6$ to $0.38 \pm 0.06 \times 10^6$. These results indicated the increase of crosslinker content was in favor of enhancing the crosslink density, such that resisted the water swelling. Theoretically, when the molar ratio of DVS/HA repeating unit exceeded one, all the hydroxyl groups of hydroxymethylene in *N*-acetyl-D-glucosamine unit of HA will be blocked and no gelation forms, supposing that DVS only reacts with the hydroxyl groups of hydroxymethylene as the most active hydroxyl group in HA. But actually, the reaction efficiency between HA and DVS cannot be one hundred percent due to the low reaction activity of HA macromolecules as well as the possible reaction between

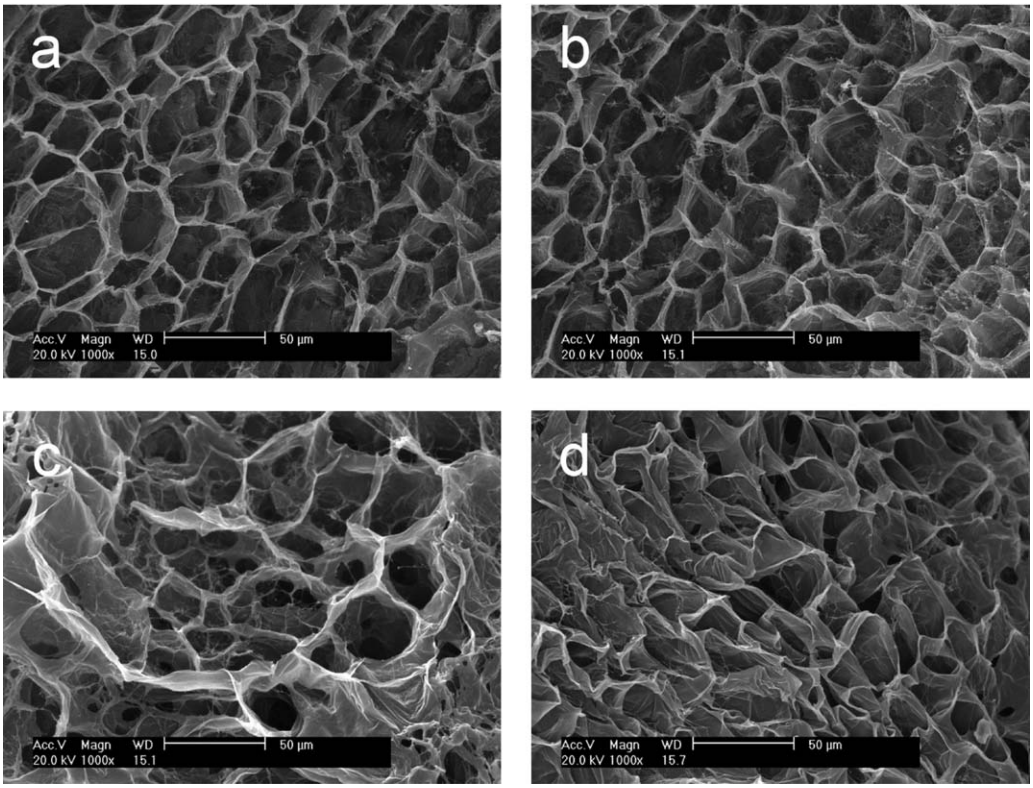


Figure 2. SEM images for the cross-section morphologies of various HA-based hydrogels. (a) HA-0.5, (b) β-CD-HA-0.5, (c) β-CD-HA-1.0, and (d) β-CD-HA-2.0.

DVS and water carrying hydroxyl groups, although DVS preferentially reacted with the primary hydroxyls in HA.[33,34]

Rheological Properties

In addition to the high water absorption ability, an adequate mechanical property is also necessary for hydrogel would dressings.[32,35] In general, elastic soft hydrogels are beneficial to keep dressings and wound in good contact that is helpful for wound healing. The rheological properties of HA-based hydrogels at low-frequencies were showed in Figure 3. All the hydrogels presented storage modulus independent of frequency, confirming the covalent nature; meanwhile the storage moduli were over five times larger than the loss moduli, suggesting the elastic property of these hydrogels.[36,37] Among these hydrogels, HA-0.5 and β-CD-HA-0.5 exhibited similar storage modulus of ~90 Pa that was much lower than ~140 Pa of β-CD-HA-1.0 and ~180 Pa of β-CD-HA-2.0. It indicated the conjugation of β-CD had no significant influence on the mechanical properties of HA hydrogel, but the increase of crosslinker content could distinctly strengthen the hydrogel owing to the increasing crosslink density. These results were consistent with the above internal morphologies and water swelling properties of HA-based hydrogels. Both β-CD-HA-1.0 and β-CD-HA-2.0 hydrogels possessing adequate elasticity were suitable for wound healing.

Drug Loading and Release Dynamics

Hydrogel wound dressings with high loading and long-term sustained delivery of anti-inflammatory drug are always longed for in wound healing. In order to remove the possible effect of entrapped air on the swelling process of HA-based hydrogels, dried hydrogels were first obtained by dehydrating the synthesized HA-based hydrogels with graded ethanol solutions and then drying under vacuum, followed by soaking into the diclofenac sodium solution for drug loading. The drug loading capacity of various HA-based hydrogels were shown in Figure 4. HA-0.5 displayed the lowest diclofenac loading of 47.14 ± 1.86

Table I. Water Swelling Properties of Various HA-Based Hydrogels in Physiological Saline at 37°C and Their Calculated Crosslink Densities

Samples	Q_M	Q_V	M_C/g·mol^{-1}
HA-0.5	63.46 ± 2.06	77.76 ± 2.53	1.16 ± 0.31 ×10^6
β-CD-HA-0.5	68.92 ± 1.29	84.47 ± 1.58	1.33 ± 0.23 ×10^6
β-CD-HA-1.0	49.12 ± 1.43	60.13 ± 1.76	0.76 ± 0.15 ×10^6
β-CD-HA-2.0	32.57 ± 2.24	39.80 ± 2.75	0.38 ± 0.06 ×10^6

Mean ± SD (n = 3). *Significant difference between HA-0.5 and β-CD-HA-0.5, β-CD-HA-0.5, and β-CD-HA-1.0, and β-CD-HA-1.0 and β-CD-HA-2.0, $p < 0.05$.

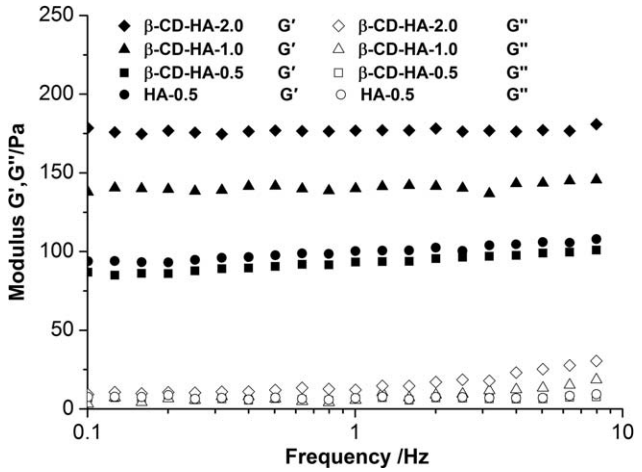


Figure 3. Low-frequency rheological properties of various HA-based hydrogels.

μg/mg, as opposed to the highest diclofenac loading of 117.81 ± 8.45μg/mg for β-CD-HA-0.5, followed by 98.34 ± 3.76 μg/mg for β-CD-HA-1.0 and 82.19 ± 2.86 μg/mg for β-CD-HA-2.0, respectively. Generally, a higher water swelling ratio leads to a higher encapsulation of soluble drugs because more drug molecules physically diffuse into the hydrogel by the difference of drug concentration outside and inside.[38] This rule was obeyed by three β-CD-HA hydrogels with different water swelling ratios. However, β-CD-HA-1.0 and β-CD-HA-2.0 loaded more drug than HA-0.5 despite their poorer swelling, suggesting β-CD was another significant factor for diclofenac loading other than crosslink density, maybe due to the typical structure of β-CD with an internal hydrophobic cavity that formed inclusion complexes with drugs by non-covalent interactions.[39,40]

The delivery profile of diclofenac from HA-based hydrogels in the physiological saline were shown in Figure 5. Among all the hyhrogels, HA-0.5 exhibited the fastest delivery profile, in which

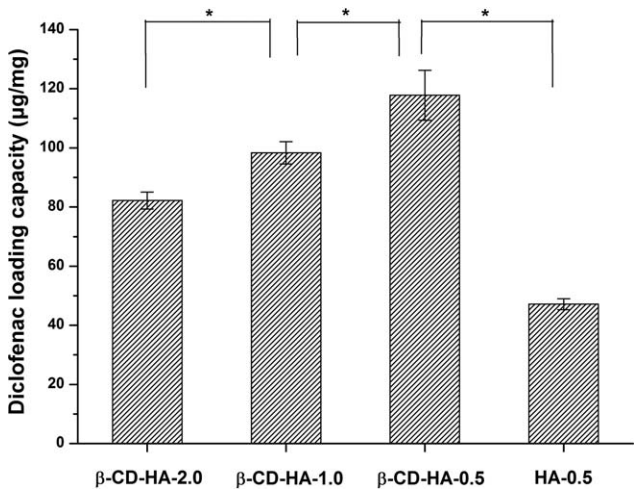


Figure 4. Diclofenac loading capacity of various HA-based hydrogels in physiological saline at 37°C. Error bars represent mean ± SD ($n = 3$). *Significant difference between HA-0.5 and β-CD-HA-0.5, β-CD-HA-0.5 and β-CD-HA-1.0, and β-CD-HA-1.0 and β-CD-HA-2.0, $p < 0.05$.

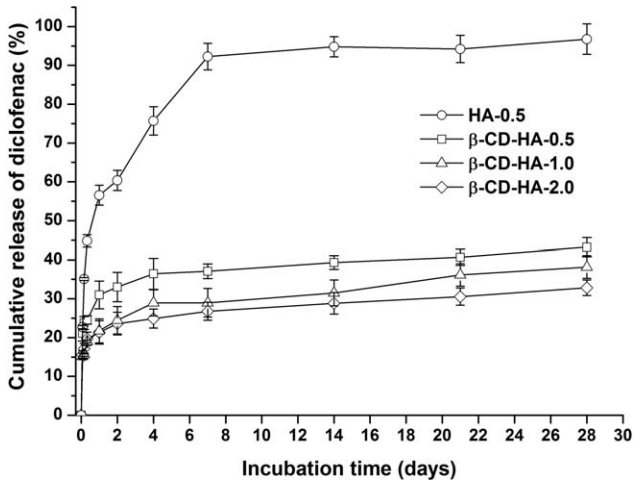


Figure 5. Diclofenac delivery profiles of various HA-based hydrogels in physiological saline at 37°C. Error bars represent mean ± SD ($n = 3$). *Significant difference between HA-0.5 and β-CD-HA-0.5, β-CD-HA-0.5 and β-CD-HA-1.0, and β-CD-HA-1.0 and β-CD-HA-2.0, $p < 0.05$.

a severe burst release of more than 55% within the first day was observed accompanying with over 90% of diclofenac delivery at 7d. In contrast, an alleviated burst release of below 32% at 1d and a cumulative release of less than 45% in an extended delivery period of 28 days, both of which a little decreased with the increase of crosslink density, were obtained for all β-CD-conjugated HA hydrogels. The much slower diclofenac delivery of β-CD-HA-0.5 than HA-0.5 with a similar water swelling ratio, as well as the slight change in the diclofenac delivery profile for all β-CD-HA hydrogels with very different water swelling properties, suggested that the primary effect on the diclofenac delivery was the interaction between β-CD and diclofenac, but not the crosslink density of hydrogels. It was noticeable that from the fourth day on, the β-CD-mediated releases became more constant with a rate of ca. 0.4% per day until the end of the designed period. This type of long-term constant slow delivery profile of drugs controlled by β-CD has also been found in Jose-Fernando's work[41] and Peng' work.[42] It was because β-CD acted as a host for hydrophobic drugs, which could establish favorable interactions with the hydrophobic inner face of the cavity and hydrogen bonds with the outer hydroxyl groups of β-CD, thus the affinity between drugs and β-CD increased resulting in a constant release from the hydrogels.[43] Additionally, the equilibrium between drug release and reabsorption by β-CD-HA hydrogels might also influence the sustained delivery rate of drugs, which was likely to prevent the release when a certain drug concentration in the medium was reached.[41] Here, after 4d a relative lower delivery rate of ca. 0.4% per day than that in Jose-Fernando's work (ca. 1.3% per day) was possibly ascribed to the longer interval of 7 days in the later phase that enhanced the drug accumulation and the reabsorption possibility by β-CD-HA hydrogels. Therefore, it was foreseeable that the sustained delivery rate of diclofenac would increase by using a shorter interval, e.g. the medium was refreshed every day. Based on the above analysis, it could be deduced that the fast releasing drugs in the early stage were primarily from the free drugs in the polymer network and the later sustained delivery was

Table II. Release Kinetics and Diffusion Mechanism of Diclofenac from Various HA-Based Hydrogels in Physiological Saline at 37°C

Samples	k	n	R^2	Diffusion mechanism
HA-0.5	0.19	0.36	0.900	Pseudo-Fickian diffusion
β-CD-HA-0.5	0.20	0.12	0.976	Pseudo-Fickian diffusion
β-CD-HA-1.0	0.13	0.16	0.985	Pseudo-Fickian diffusion
β-CD-HA-2.0	0.15	0.12	0.991	Pseudo-Fickian diffusion

mainly controlled by the interaction between β-CD and drugs, although the drugs imbibed in the gel and enclathrated within the cavity of β-CD could not be strictly distinguished here. The incorporation of β-CD into HA hydrogels could not only effectively reduce the burst effect commonly observed in other hydrogel systems,[44,45] but also greatly prolong the diclofenac release period, which was desired in the anti-inflammation of wound healing.

For better understanding the release kinetics and diffusion mechanism of diclofenac from various HA-based hydrogels, a semiempirical equation was adopted to fit the diclofenac release data[46,47]:

$$M_t/M_\infty = k\ t^n (M_t/M_\infty < 0.6) \quad (4)$$

Where M_∞ is the total amount of diclofenac loaded in the hydrogel, M_t is the cumulative release amount of diclofenac at time t, k is the rate constant, and n is the release exponent indicative of the diffusion mechanism. According to the different n value, four different diffusion mechanisms were proposed: (1) Fickian diffusion at $n = 0.5$ with a negligible relaxation coefficient; (2) Non-Fickian diffusion at $n = 1$ with the characteristic of zero order release; (3) Anomalous diffusion at $0.5 < n < 1$ with a comparable structural relaxation to diffusion; (4) Pseudo-Fickian diffusion at $n < 0.5$ characterizing a relative slow release that positively relied on the n value. The n and k values were obtained from the slope and intercept respectively by plotting $\log(M_t/M_\infty) \sim \log(t)$, as showed in Table II. All the hydrogels showed an n value of less than 0.5 indicative of pseudo-Fickian diffusion mechanism, but the n value of HA-0.5 was much higher than those of β-CD functionalized samples, corresponding to its fastest delivery in Figure 5. In other words, a diclofenac sustained delivery of HA hydrogel could be achieved by β-CD conjugation. It was noteworthy that all the β-CD-HA hydrogels presented closer n values, suggesting that the diclofenac delivery from β-CD-HA hydrogels was predominantly controlled by the host-guest interaction between β-CD and diclofenac in consideration of their very different crosslink densities.

CONCLUSIONS

In this work, various β-CD-HA hydrogels with varying crosslink densities were synthesized to achieve a long-term sustained delivery of anti-inflammatory diclofenac for potential wound healing. The incorporation of β-CD had little effect on the internal porous structure of HA. The increase of crosslinker content enhanced the crosslink density, consequently leading to a lower water swelling ratio and a higher elastic modulus. The diclofenac loading capacity was subject to two factors: crosslink density, and interaction between β-CD and drug, but the latter was the predominant one. The conjugation of β-CD remarkably prolonged the diclofenac sustained delivery until over 28 days, beneficial to the anti-inflammation in wound healing. The drug release from these HA-based hydrogels displayed a pseudo-Fickian diffusion mechanism, but primarily controlled by β-CD rather than crosslink density.

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REFERENCES

1. Eming, S. A.; Krieg, T.; Davidson, J. F. *J. Invest. Dermatol.* **2007**, *127*, 514.
2. Roy, S.; Das, A.; Sen, C. K. Disorder of Localized Inflammation in Wound Healing: A Systems Perspective. Complex Systems and Computational Biology Approaches to Acute Inflammation, Ed.; Springer: New York, **2013**; p 173.
3. Rodriguez-Vita, J.; Lawrence, T. *Cytokine Growth F. R.* **2010**, *21*, 61.
4. Menke, N. B.; Ward, K. R.; Witten, T. M.; Bonchev, D. G.; Diegelmann, R. F. *Clin. Dermatol.* **2007**, *25*, 19.
5. Gupta, A.; Upadhyay, N.; Parthasarathy, S.; Rajagopal, C.; Roy, P. K. *J. Appl. Polym. Sci.* **2013**, *128*, 4031.
6. Czaja, W.; Krystynowicz, A.; Bielecki, S.; Brown, R. *Biomaterials* **2006**, *27*, 145.
7. Antunes, B. P.; Moreira, A. F.; Gaspar, V. M.; Correia, I. *J. Carbohydr. Polym.* **2015**, *130*, 104.
8. Dahlmann, J.; Krause, A.; Moeller, L.; Kensah, G.; Moewes, M.; Diekmann, A.; Martin, U.; Kirschning, A.; Gruh, I.; draeger, G. *Biomaterials* **2013**, *34*, 940.
9. Kim, I. L.; Mauck, R. L.; Burdick, J. A. *Biomaterials* **2011**, *32*, 8771.
10. Tan, H.; Ramire, C. M.; Miljkovic, N.; Li, H.; Rubin, J. P.; Marra, K. G. *Biomaterials* **2009**, *30*, 6844.
11. Luo, C. H.; Zhao, J. H.; Tu, M.; Zeng, R.; Rong, J. H. *Mater. Sci. Eng. C Mater.* **2014**, *36*, 301.
12. Liu, Y. Y.; Fan, X. D. *Biomaterials* **2005**, *26*, 6367.
13. Das, S.; Subuddhi, U. *J. Appl. Polym. Sci.* **2014**, *131*, 40318.
14. Tang, W.; Zhao, J. C.; Sha, B. J.; liu, H. *J. Appl. Polym. Sci.* **2013**, *127*, 2803.
15. Uekama, K.; Hirayama, F.; Irie, T. *Drug Target Deliv.* **1994**, *3*, 411.

16. Stella, V. J.; Rao, V. M.; Zannou, E. A.; Zia, V. *Adv. Drug Deliv. Rev.* **1999**, *36*, 3.
17. Rodriguez–Tenreiro, C.; Alvarez-Lorenzo, C.; Rodriguez-Perez, A.; Convheiro, A.; Torres-Labandeira, J. *J. Pharm. Res.* **2006**, *23*, 121.
18. Rodriguez–Tenreiro, C.; Alvarez-Lorenzo, C.; Rodriguez-Perez, A.; Convheiro, A.; Torres-Labandeira, J. *J. Eur. J. Pharm. Biopharm.* **2007**, *66*, 55.
19. Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F. T. *J. Am. Chem. Soc.* **1990**, *112*, 3860.
20. Zhao, J. H.; Lou, C. H.; Chen, Y. Q.; Wu, D.; Shen, C. X.; Han, W. Q.; Tu, M.; Zeng, R. *Carbohydr. Polym.* **2011**, *86*, 806.
21. Flory, P. J. Principles of Polymer Chemistry, Ed.; Cornell University Press: Ithaca, NY, **1953**.
22. Metters, A. T.; Anseth, K. S.; Bowman, C. N. *Biomed. Sci. Instrum.* **1999**, *35*, 33.
23. Marsano, E.; Gagliardi, S.; Ghioni, F.; Bianchi, E. *Polymer* **2000**, *41*, 7691.
24. Gopalan, S. M.; Flaim, C.; Bhatia, S. N.; Hoshijima, M.; Knoell, R.; Chien, K. R.; Omens, J. H.; McCulloch, A. D. *Biotechnol. Bioeng.* **2003**, *82*, 578.
25. Schneider, H. J.; Hacket, F.; Rudiger, V. *Chem. Rev.* **1998**, *98*, 1755.
26. Lou, C. H.; Xu, G. G.; Wang, X. H.; Tu, M.; Zeng, R.; Rong, J. H.; Zhao, J. H. *Mater. Sci. Eng. C Mater.* **2015**, *46*, 316.
27. Dang, Q. F.; Yan, J. Q.; Li, J. J.; Cheng, X. J.; Liu, C. S.; Chen, X. G. *Carbohydr. Polym.* **2011**, *83*, 171.
28. Flynn, T. C.; Thompson, D. H.; Hyun, S. H.; Howell, D. *J. Dermatol. Surg.* **2015**, *41*, S143.
29. Borzacchiello, A.; Russo, L.; Malle, B. M.; Schwach-Abdellaoui, K.; Ambrosio, L. *Biomed. Res. Int.* **2015**, *2015*,
30. Hahn, S. K.; Jelacic, S.; Maier, R. V.; Stayton, P. S.; Hoffman, A. S. *J. Biomater. Sci. Polym. E* **2004**, *15*, 1111.
31. Rizzi, Simone, C.; Ehrbar, M.; Halstenberg, S.; Raeber, G.; Schmokel, P.; Hugo, G.; Hagenmuller, Henri., *Biomacromolecules* **2006**, *7*, 3019.
32. Jeon, O.; Song, S. J.; Lee, K. J.; Park, M. H.; Lee, S. H.; Hahn, S. K.; Kim, S.; Kim, B. S. *Carbohydr. Polym.* **2007**, *70*, 251.
33. Sahiner, N.; Jha, A. K.; Nguyen, D.; Jia, X. Q. *J. Biomater. Sci. Polym. Ed.* **2008**, *19*, 223.
34. Hahn, S. K.; Jelacic, S.; Maier, R. V.; Stayton, P. S.; Hoffman, A. S. *J. Biomater. Sci. Polym. Ed.* **2004**, *15*, 1111.
35. Burdick, J. A.; Chung, C.; Jia, X.; Randolph, M. A.; Langer, R. *Biomacromolecules* **2005**, *6*, 386.
36. Zainuddin, J. J.; White, C.; Hill, D. J. T. *J Biomater. Sci. Polym. Ed.* **2002**, *13*, 1007.
37. Korhonen, M.; Hellen, L.; Hirvonen, J.; Yliruusi, J. *Int. J. Pharm.* **2001**, *221*, 187.
38. Lou, Y.; Kirker, K. R.; Prestwich, G. D. *J. Control. Release* **2000**, *69*, 169.
39. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.
40. Ghrib, R.; Greige-Gerges, H.; Fourmentin, S.; Charcosset, C.; Auezova, L. *Carbohydr. Polym.* **2015**, *129*, 175.
41. Rosa dos Santos, J. F.; Alvarez-Lorenzo, C.; Silva, M.; Balsa, L.; Couceiro, J.; Torres-Labandeira, J. J.; Concheiro, A. *Biomaterials* **2009**, *30*, 1348.
42. Peng, K.; Tomatsu, I.; Korobko, V. V.; Kros, A. *Soft Matter* **2010**, *6*, 85.
43. Loftsson, T.; Duchene, D. *Int. J. Pharm.* **2006**, *329*, 1.
44. Chen, M. C.; Tsai, H. W.; Liu, C. H.; Peng, S. F.; Lai, W. Y.; Chen, S. J.; Chang, Y.; Sung, H. W. *Biomaterials* **2009**, *30*, 2102.
45. Zhang, Y. L.; Chu, C. C. *J. Biomed. Mater. Res.* **2002**, *59*, 318.
46. Costa, P.; Sousa, L. J. M. *Eur. J. Pharm. Sci.* **2001**, *13*, 123.
47. Li, N. N.; Fu, C. P.; Zhang, L. M. *Mater. Sci. Eng. C Mater.* **2014**, *36*, 287.