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Hyaluronic acid hydrogel particles with tunable charges as potential drug delivery devices

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ABSTRACT

We report the synthesis of hyaluronic acid (HA) particles with positive and negative charges on their surfaces. HA-based particles were prepared using an aqueous solution of linear HA in a sodium bis(2-ethylhexyl) sulfosuccinate (AOT)–isooctane microemulsion system. The prepared HA particles were post modified, i.e., oxidized to aldehyde (HA-O) by $NaIO_4$ treatment and then these HA-O particles were reacted with cysteamine (CYs) to obtain thiol groups on the surface of the HA particles. The thiolated HA particles (HA-CYs) were further exposed to radical polymerization with an anionic monomer, 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), and with a cationic monomer, 3-acrylamidopropyl-trimethylammonium chloride (APTMACl), to generate HA-based ionic hydrogel particles, HA-CYs-AMPS and HA-CYs-APTMACl, respectively. The prepared HA-based anionic and cationic particles illustrated strong pH dependent size variations. We demonstrated that HA-CYs-AMPS and HA-CYs-APTMACl particles can be used as drug delivery vehicles. Trimethoprim (TMP) and naproxen (NN) were used as model drugs in the drug delivery experiments.

1. Introduction

Recently increasing interest has been devoted to the exploration of stimuli sensitive polymeric particles due to their unique physical and chemical properties which make them smart or intelligent materials that can be potentially used in biomedical applications such as controlled drug delivery, biolabelling, separation, catalysis and so on (Ballauff & Lu, 2007; Bawa, Pillay, Choonara, & Toit, 2009; Hoare & Kohane, 2008). Natural hydrogels derived from hyaluronic acid, dextran, gelatin, alginic acid, and cellulose are of great significance and have been used for a wide range of applications due to their biocompatible nature with blood, tissues, and cells especially in the human body (Ilgin et al., 2010; Palumbo, Pitarresi, Mandracchia, Tripodo, & Giammona, 2006; Schnurch, Clausen, & Hnatyszyn, 2001). Amongst the natural polymers, HA attracts particular attention due to its abundant existence in living organisms and the human body. Due to its low bioavailability and short in vivo lifetime, varying from minutes to hours depending on the location, HA-based materials require modification with a synthetic material to produce the desired unique mechanical and functional properties (Brown & Jones, 2005; Eenschooten, Guillaumie, Kontogeorgis, Stenby, & Schwach-Abdellaoui, 2010; Jeon et al., 2007; Jia et al., 2006; Kim et al., 2008; Kong, Chen, & Park, 2011; Lee, Lee, & Park, 2008; Luo & Prestwich, 1999; Maroda et al., 2011; Mason et al., 2000; Pouyani & Prestwich, 1994; Sahiner, Jha, Nguyen, & Jia, 2008; Shen, Li, Tu, & Zhu, 2009; Tan, Chu, Payne, & Marra, 2009; Vanderhooft, Mann, & Prestwich, 2007; Vercruysse, Marecak, Marecak, & Prestwich, 1997). Earlier, we demonstrated the synthesis of thiolated HA particles modified with N-isopropylacrylamide for potential temperature sensitive drug release applications (Ekici, Ilgin, Yilmaz, Aktas, & Sahiner, 2010). It is well known that ionic charges play a decisive role in the swelling behavior of ionic gels (Cao, Lai, & Lee, 2001; Gupta, Vermani, & Garg, 2002; Harvey, 1995; Mika, Childs, & Dickson, 2002).

In this investigation we prepared HA particles in a single step employing a water-in-oil microemulsion system and the synthesized HA particles were chemically modified to amend different functional groups and afford further modification, e.g., polymerization of anionic (AMPS) and cationic (APTMACl) monomers to the HA particle surface. For this purpose, the prepared HA particles were oxidized with a treatment of aqueous sodium periodate to form two aldehyde groups in every repeated HA unit and assigned as HA-O. Following the reaction of these HA-O particles with cysteamine, the –SH groups were situated on the HA particles. The thiolated HA particles, assigned as HA-CYs, have free thiols that are versatile and were reported as having improved mucoadhesive properties (Kast & Schnurch, 2002; Huang, Huang, & Ho, 1998). To generate ionic groups on the particle surface that introduce pH-responsive

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behavior, HA-CYs particles were exposed to a polymerization reaction with anionic and cationic monomers, AMPS and APTMACl, respectively. The obtained ionic particles were designated as HA-CYs-AMPS and HA-CYs-APTMACl, correspondingly. Ionic HA-based particles are essential in many applications due to their charge, permeability and other material characteristics which change in response to external stimuli such as solute molecules, pH, ionic strength of the surrounding solution and electric field. To demonstrate the potential utilization of the prepared HA-CYs-AMPS and HA-CYs-APTMACl particles as active agent delivery devices, two model drugs that have opposite charges to the prepared particles were used in absorption and release studies to emphasize the ionic character of the synthesized HA-based particles.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium salt (HA, MW: 1.3 MDa, 98%) was purchased from Sigma–Aldrich. Reverse micelle-forming agent, sodium bis(2-ethylhexyl) sulfosuccinate (AOT, 96%), and a crosslinker, divinyl sulfone (DVS, 98%) were purchased from Fluka and Acros Chem. Comp. The redox initiator, ammonium persulfate (APS), for free radical polymerization and the modifying agent, cysteamine (CYs) were also purchased from the same companies. The oxidizing agent, sodium (meta) periodate ($NaIO_4$), and the solvent, 2,2,4-trimethylpentane (isooctane) (HPLC grade), were supplied from Aldrich and Lab-scan, and used as received. The anionic, 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), and the cationic, 3-acrylamidopropyl-trimethylammonium chloride (APTMACl) (75 wt % solution in water) monomers were also purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin). Trimethoprim (TMP) and naproxen (NN) as model drugs were kindly supplied by Roche Inc. (Istanbul, Turkey). All chemicals were used as received and DI water with 18.2 MΩ cm^{-1} conductivity (Milli pore milli Q) was used throughout the experiments.

2.2. Preparation of pH-sensitive HA-based particles

2.2.1. Synthesis of hyaluronic acid (HA) particles

In the preparation of HA particles, the previously reported procedure was followed (Sahiner & Jia, 2008). In brief, HA solution in 0.2 M NaOH was prepared by dissolving linear HA at a concentration of 50 mg/L. After complete dissolution of HA, 0.54 mL of this solution was transferred to 15 mL of 0.2 M AOT in isooctane. After meticulous mixing, a clear suspension was obtained. The crosslinker, DVS (50 mol% if not otherwise stated, based on HA repeating unit) was subsequently added to this solution under vigorous mixing to obtain complete dissolution of DVS. The reaction proceeded for 1 h at room temperature under continuous stirring (1200 rpm). The synthesized HA particles were washed in excess of acetone, and purified by several centrifugations (at least three times) (Hermle Labortechnik Z 383 K) at 10,000 rpm for 10 min at 20 °C.

2.2.2. Oxidation of HA particles (HA-O)

The synthesized HA particles were oxidized with a treatment of aqueous $NaIO_4$ to generate two aldehyde groups on the HA particle surface (HA-O). A certain amount of HA particles (500 mg) were dispersed in 50 mL $NaIO_4$ (0.125 M) (at least 5 fold more than HA repeating units in moles) under constant stirring at 500 rpm in the dark for 12 h. The oxidized HA particles were centrifuged (4500 rpm for 10 min) and washed meticulously with H_2O/acetone mixture (50%, v/v). The formation of aldehyde groups was confirmed by Tollen's test (the silver mirror reaction).

2.2.3. Synthesis of –SH group containing HA particles (HA-CYs)

The oxidized HA particles (HA-O) were placed in contact with an excess amount of CYs solution, 100 mL (0.3%, w/v) at pH 4.72. The pH of the CYs solution was adjusted with 1.0 M HCl and 1.0 M NaOH. The reaction continued in the dark for 12 h at 500 rpm mixing rate. At the end of the reaction, the mixture was centrifuged (4500 rpm for 10 min) and thoroughly washed with DI H_2O–acetone mixture (50%, v/v). The white suspension turned light yellow as the reaction progressed. The HA-CYs particles were washed three times and then dried at room temperature under vacuum (25 °C) for further use. The sulfur content of HA-CYs particles was confirmed by elemental analysis (C and S).

2.2.4. Preparation of pH-sensitive HA-based particles (HA-CYs-AMPS and HA-CYs-APTMACl)

Cysteamine functionalized particles (300 mg) were dispersed in 2 mL of 155.4 mg AMPS-containing aqueous solution in which the moles of sulfur atoms equal the moles of anionic monomer ($n_S = n_{AMPS}$). Then 1.0 mL initiator solution (APS, 0.5%, w/v) was added to this solution and mixed with a magnetic stirrer at 70 °C for 1 h at 750 rpm. This suspension was centrifuged (10,000 rpm for 20 min) and thoroughly washed with DI water (at least three times). The modified particle designated as HA-CYs-AMPS was dried at 25 °C under vacuum. The same experimental procedure was employed for the preparation of HA-CYs-APTMACl particles with the same reaction conditions ($n_S = n_{APTMACl}$).

2.3. Characterization

2.3.1. Scanning electron microscopy

The SEM (JEOL JSM-5600LV) images of the particles were acquired by placing the dried HA particles on carbon tape attached to aluminum stubs under vacuum operation at 10–15 kV on a few nm gold sputtered particles.

2.3.2. Particle size analysis

DLS studies were carried out on HA-based particles with a 90 Plus particle size analyzer (Brookhaven Instrument Corp.). The DLS experiment was conducted with a detector angle of 90° with a Lexel 95 ion laser operating at a wavelength of 614 nm and 100 mW light source. Each data presented in this investigation was the average value of five consecutive measurements with 20-s integration time. The change in size of HA-CYs-AMPS and HA-CYs-APTMACl particles with pH was also determined with DLS studies altering pH from 2 to 11 (adjusted with 1.0 M HCl and 1.0 M NaOH) using particle size analyzer. The measurements were performed in a solution of fixed ionic strength (0.1 mol dm^{-3} NaCl).

2.3.3. Measurements of surface charges

Zeta potential measurements were performed using a ZetaPals Zeta Potential Analyzer BIC (Brookhaven Inst. Corp.) at 25 °C. HA, HA-CYs-AMPS and HA-CYs-APTMACl particles were suspended in dilute potassium nitrate solution (10 mM) after dispersing in an ultrasonic bath.

2.3.4. Thermogravimetric analysis

Thermal behavior of HA, HA-CYs-AMPS and HA-CYs-APTMACl particles were investigated with a thermo gravimetric analyzer (SII TG/DTA 6300). TG analyses were performed on ~10 mg samples under nitrogen atmosphere with a nominal gas flow rate of 100 mL/min and a heating rate of 10 °C/min heating up to 1000 °C.

2.3.5. Elemental (S and C) analysis

The amounts of S% and C% in linear HA, DVS crosslinked HA and HA-CYs particles were determined with a Leco SC-144 DR Carbon-

Sulphur Analyzer at constant temperature (1400 °C) using 150 mg samples.

2.3.6. Drug loading of HA particles

Two different drugs; trimethoprim (TMP) and naproxen (NN) were loaded onto the hydrogel particles. Certain amounts of dried HA (45 mg) and HA-CYs-AMPS (100 mg) were placed in concentrated aqueous TMP solutions (250 mg/L, 50 mL) for 24 h under constant stirring (500 rpm) at 25 °C. The same loading procedure was also applied to HA (45 mg) and HA-CYs-APTMACl particles (100 mg) in 50 mL 1000 mg NN aqueous solution. Drug equilibrium concentrations were determined at λ_{max} 274 nm for TMP, and λ_{max} 330 nm and 230 nm for NN, and from previously constructed calibration curves in DI water using PG Instruments T80+ model UV–vis spectrophotometer.

2.3.7. Drug release studies from particles

The in vitro release studies of the entrapped drugs were investigated for a gastrointestinal system at different pH values (1.1 and 7.4). Particles loaded with drugs, 30 mg HA and 80 mg HA-CYs-AMPS, were placed in 50 mL solution with pH 1.1 (pH = 1.1, adjusted with HCl and NaOH) at 25 °C under constant stirring. At certain time intervals, 1 mL of the drug solutions was taken and the absorbance values were determined with UV–vis spectrophotometer. The measured drug solution was then placed back into the release medium. The release studies continued until the absorbance of the final solution reached a constant value. The same release experiments were performed at pH 7.4 in PBS for both particles. The released amounts of drug were quantified using a calibration curve for pH values. The release experiments were performed in triplicate and the average value of the released amount with standard deviation was calculated and is shown in the representing graphs.

3. Results and discussion

3.1. Preparation of particles

Recently, natural–synthetic materials have attracted great consideration as one of the new classes of advanced composite materials providing a unique avenue to combine preferred characteristics of natural component biocompatibility, biodegradability and synthetic materials versatility, i.e., charge, various functional groups and synthetic polymers flexibility and elasticity (Bencherif et al., 2008; Dastjerdi & Montazer, 2010; Habraken, Wolke, & Jansen, 2007; Kim & Park, 2002; Moussas & Zouboulis, 2009; Ren, Zhou, Liu, Xu, & Cui, 2009; Tan et al., 2009; Xie, Hill, Xiao, Militz, & Mai, 2010; Zhu et al., 2010). To induce multifunctional properties in HA particles, HA particles were chemically modified with aqueous $NaIO_4$ treatment to form dialdehyde groups (HA-O) followed by the generation –SH groups on the surface of HA by chemical treatment with cysteamine. As a result of dialdehyde groups on the surface, HA-O reacts with the amine groups of cysteamine in the acid catalyzed environment (pH 4.72) generating imine bonds and –SH groups on the surface of HA. It was observed that the colour change of HA-O particles from white to light yellow confirmed imine formation at the end of the reaction. This modification was also used in our previous study (Ekici et al., 2010) to prepare thermoresponsive HA particles. The elemental analysis results confirm that there is a significant increase in the S% (wt) content of the particles after CYs treatment (Table 1). Additionally, sulfur content of thiolated HA-O (HA-CYs) particles was verified by elemental analysis studies. HA-CYs particles were further used for the polymerization of an anionic monomer (AMPS) and a cationic monomer (APTMACl) from the HA particle surface. The entire modification and polymerization reaction mechanism is illustrated in Fig. 1. The main purpose of the generation of thiol groups on the HA particle surface is that the chain transfer ability of this group can be used in many different polymerizations to transfer activity of the growing polymer chain to various species such as solvent or monomer, as well as the other growing chains in solution (Bloch, 2006; Sawada, 1976; Solomons & Fryhle, 2000). Due to the –SH groups on the surface of HA-CYs particles, free radical polymerization of AMPS and APTMACl can progress from the particle surface, providing polymeric arms of charged p(AMPS) or p(APTMACl) chains in which HA particles are situated in the core. The obtained HA-based particles shown in Fig. 1 are pH-responsive. To take advantage of the interaction of thiol groups with the vinyl groups during the radical polymerization, HA-CYs-AMPS and HA-CYs-APTMACl composite material synthesis was accomplished. It is also probable that other monomer-containing functional groups can also be polymerized on a HA particle surface bearing –SH functional groups. It was also reported that the thiolated groups are capable of forming covalent bonds with the mucus layer covering mucosal tissues, providing improved mucoadhesive properties (Huang et al., 1998; Kast & Schnurch, 2002). Consequently, HA particles with –SH functional groups can be potentially used as a drug carrier for intestinal, mucosal, vaginal as well as buccal delivery of active agents. Additionally, the propensity of thiol groups as free radical transfer agent in the polymerization of vinyl monomers (AMPS and APTMACl in this case) provides additional benefits for the design of novel natural–synthetic materials for applications such as drug carriers. Therefore, HA particles with p(AMPS) generating negative charge, and with p(APTMACl) forming positive charge on the particle surface, were obtained.

Table 1
Sulfur and carbon contents of the H and HA-CYs particles.

Sample	S% (wt)	C% (wt)
HA	2.14	35.70
HA-CYs	7.93	40.46

3.2. Elemental analysis of particles

Elemental analysis results also confirmed the CYs modification was achieved. The S and C contents of DVS-crosslinked HA and HA-CYs particles were determined at 1350 °C with Eco SC-144 DR Elemental Analyzer. The sulfur content increased from 2.14 wt% to 7.93 wt% while C content reduced to 35.70 from 40.56 wt% as illustrated in Table 1. This is reasonable as the CYs modification increases sulfur content almost 4 fold in HA-CYs compared to DVS-crosslinked HA particles. This modification is further verified with different charge-containing monomer polymerization.

3.3. Surface charge analysis of particles

The surface charges of a biomaterial, especially for biomedical use such biolabelling, bioseparation and drug delivery devices, is very important. Therefore, after CYs modification we introduced an ionic character onto HA particles for potential biomedical applications. The zeta potentials of the synthesized HA, HA-CYs-AMPS, and HA-CYs-APTMACl particles, were measured and the results are illustrated in Fig. 2. The bare HA zeta potential was −30 mV which decreased to −33 mV upon introduction of p(AMPS), providing $–SO_3H$ onto HA particle surface. This result is plausible as bare HA has carboxyl groups that also provide negative charges upon dissociation of the proton. A clear distinction was observed by the introduction of positive charges onto HA particles. p(APTMACl) has a pronounced effect on the surface charge of HA particles. Upon polymerization of APTMACl functional groups, quaternary ammonium salts ($–(N^+(CH_3)_3Cl$) increased the zeta potential to −8.73 mV. It may be possible to reach zero or positive zeta potentials by further addition of positive charges (more of any quaternized group)

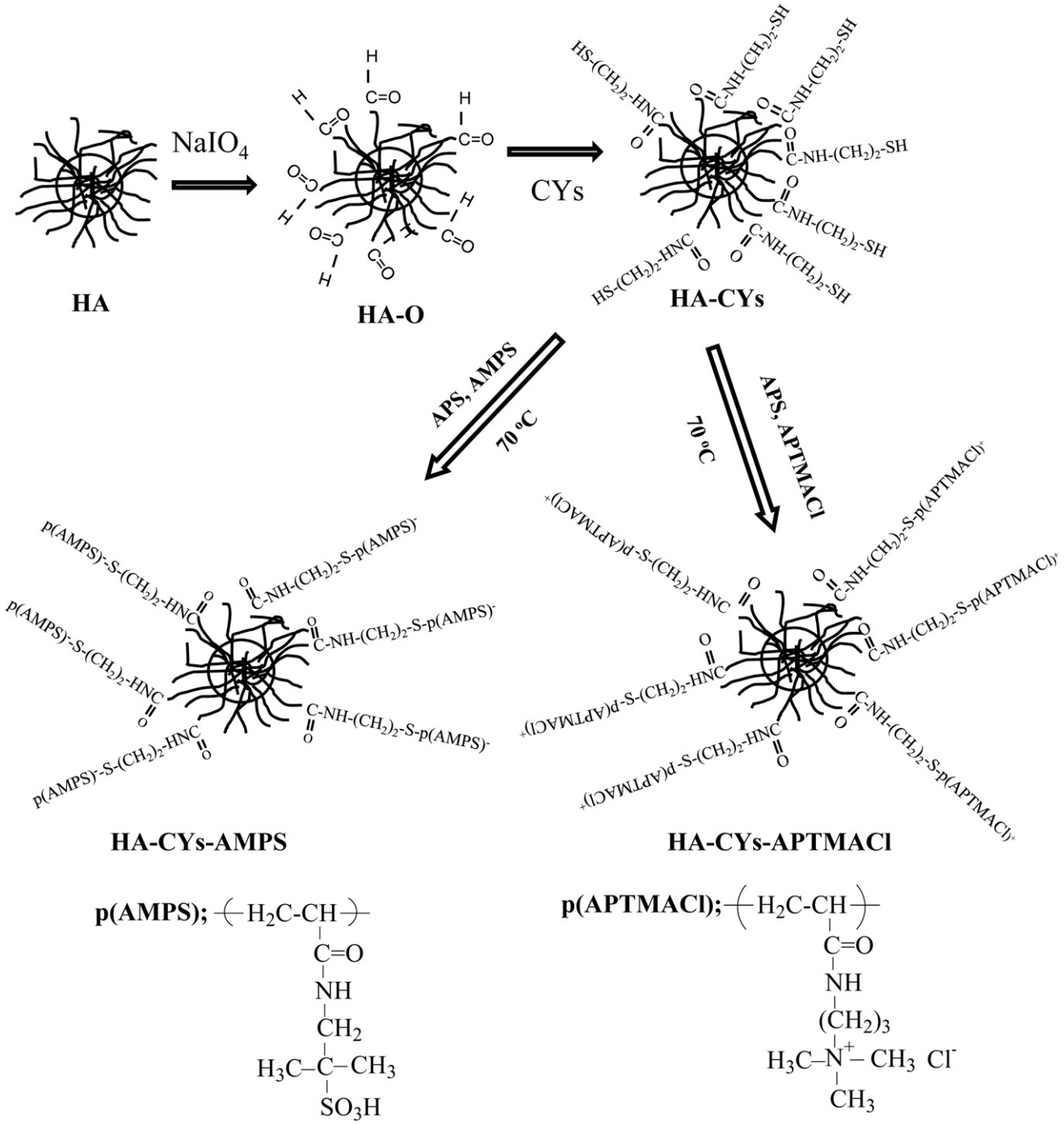

Fig. 1. The schematic representation of the HA particles and their modifications. [HA: hyaluornic acid particles, HA-O: oxidized HA, HA-CYs: HA particle with cysteamine groups, HA-CYs-AMPS: HA with 2-acrylamido-2-methyl-1-propanesulfonic acid and HA-CYs-APTMACl: HA with 3-acrylamidopropyl-trimethylammonium chloride].

onto the HA particles. However, the purpose of this investigation was to have different charges on natural HA particles. These results are consistent with the functional group characteristics.

3.4. SEM analysis

The SEM images of the particles, as illustrated in Fig. 3, show that HA particles still retain their spherical morphology after modification. Fig. 3(a) represents HA particles, whereas (b) and (c) of the same figure correspond to HA-CYs-AMPS and HA-CYs-APTMACl particle images, respectively. The synthesized HA particles have a wide size distribution, varying from a few micrometers to tens of nanometers. It is possible to separate different size particles by centrifugation or simple filtration with different pore sizes.

3.5. FT-IR and TG analyses

To confirm cysteamine modification of HA-based particles, FT-IR spectra of HA and HA-CYs particles were taken and illustrated in Fig. 4(a). The peak at 1603 cm^{-1} is a characteristic –COOH peak related to disaccharide units of HA, and the peak at 1029 cm^{-1} attributed to S–O and C–S stretching frequencies coming from DVS (50 mol%) used to crosslink linear HA repeating units to produce HA particles. In the HA-CYs spectrum, a clear peak at 1555 cm^{-1}

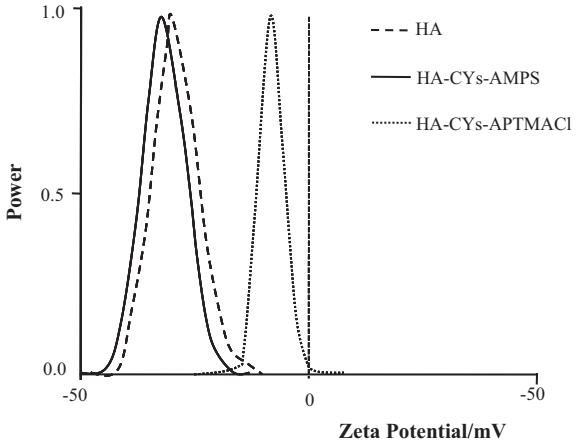

Fig. 2. Effect of anionic- and cationic-based components on ionic behaviors of the HA particles.

comes from amine groups (–NH) and new small peaks occurring at 893 cm^{-1}, 875 cm^{-1}, and 841 cm^{-1} are due to $–CH_2–S–$ groups of the cysteamine molecules. The anionic and cationic particles generated from HA as HA-CYs-AMPS and HA-CYs-APTMACl respectively, have also their characteristic functional groups stretching frequencies and mostly overlapping with HA and CYs functional groups (supporting info).

With the introduction of new functional groups, it is expected that the thermal characteristics of the particles will be altered. Therefore, thermogravimetric analysis of HA, HA-CYs-AMPS and HA-CYs-APTMACl were conducted and their thermograms are shown in Fig. 4. As can be seen bare HA has a weight loss of about 86% when heated up to 1000 °C under nitrogen and HA-CYs-AMPS and HA-CYs-APTMACl show the same degradation temperatures with 74% and 76% wt loss, respectively. Therefore, it can be assumed that anionic particles contribute 10 and 12 wt% more degradation to the HA-based materials. Another noticeable characteristic is that bare HA particles have two distinct degradation temperatures: one is starting at about 250 to almost 400 °C with 60% wt loss, and the other is between 650 and 900 °C with 86% wt loss. The modified HA particles (HA-CYs-AMPS and HA-CYs-APTMACl) have similar thermal behavior and degrade faster starting from about 200 °C to 400 °C with sharp weight loss. However, modified HA particles degrade by a lesser amount than unmodified HA upon heating up to 1000 °C. As the new functional groups are attached to HA particles, these results are plausible.

3.6. Particle size analysis

Due to the ionic character of the modified particles, they are predicted to be pH responsive materials. It is very well-known that the pH variations in the environment can lead to volume change in ionic hydrogels (Gümüsderelioglu & Kesgin, 2005; Sahiner, Singh, Kee, De John, & McPherson, 2006). Therefore, the change in particle size of HA, HA-CYs-AMPS and HA-CYs-APTMACl were measured with DLS at different pH values (2–11) and their corresponding graphs are illustrated in Fig. 5. The HA particles were filtered with paper (pore size > 2 μm) and centrifuged to get rid of larger HA before modification to ensure DLS measurements could made securely in the DLS instrument's operation limits. As can be seen from the figure, bare HA particles do not change their size significantly with pH. Although bare HA have carboxylic acid groups (−30 mV zeta potential measurement), they cannot swell much in basic pHs (pH > 7) due to the non porous nature and high crosslinking density (50 mole%) (Sahiner et al., 2008). On the other hand, the modified HA particles demonstrate the typical behavior of ionic hydrogel swelling and shrinking characteristics. For example, HA-CYs-AMPS

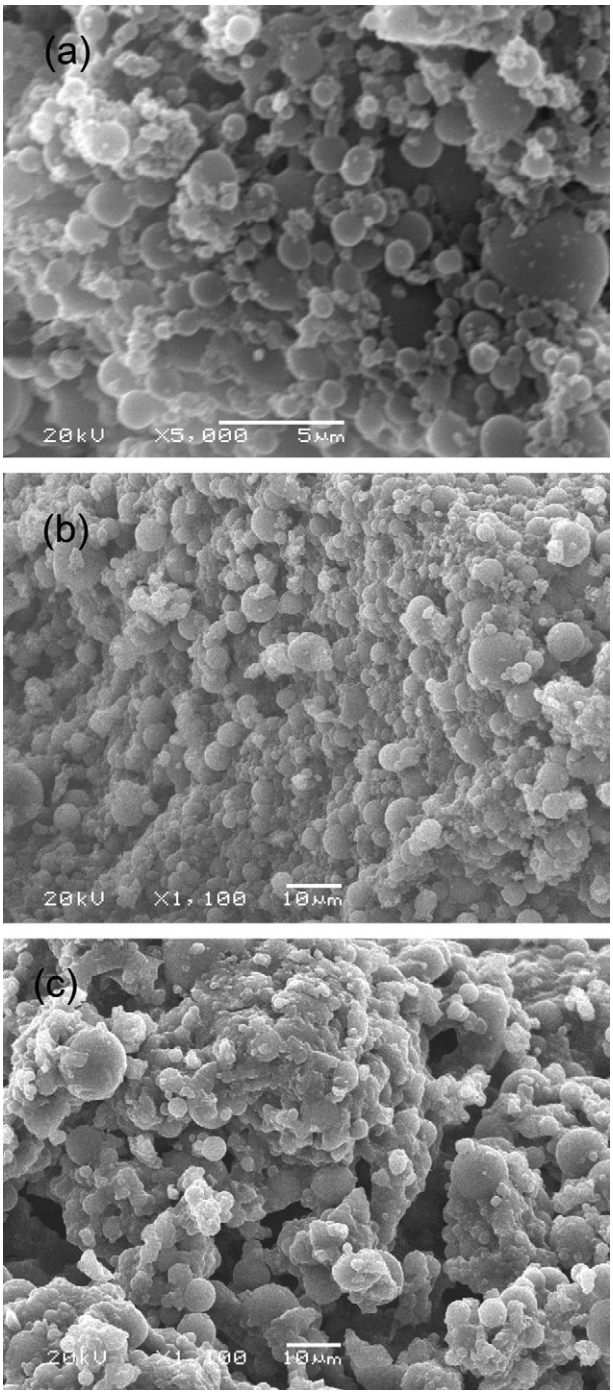

Fig. 3. SEM images of (a) HA, (b) HA-CYs-AMPS and HA-CYs-APTMACl polymeric particles.

particles swell less at low pH values (cannot develop negative charges) and swell a lot (develops negative charges) at higher pH values (pH > 5) due to ionization of sulfonyl groups. The HA-CYs-AMPS particle increases its size almost 3 fold from pH 2 to 11, going from ~500 nm to almost 1500 nm. Similar, though inversed, behavior was observed for HA-CYs-APTMACl particles as expected for a cationic hydrogel swelling–shrinking curve. The size of HA-CYs-APTMACl particles decreased almost four fold (from ~2000 nm to ~500 nm) going from pH 2 to 11. These swelling and shrinking behaviors are consistent with the ionic nature of the hydrogel materials (Khare & Peppas, 1995; Peppas & Peppas, 1989; Zhang, Chu, Li, & Lee, 2007).

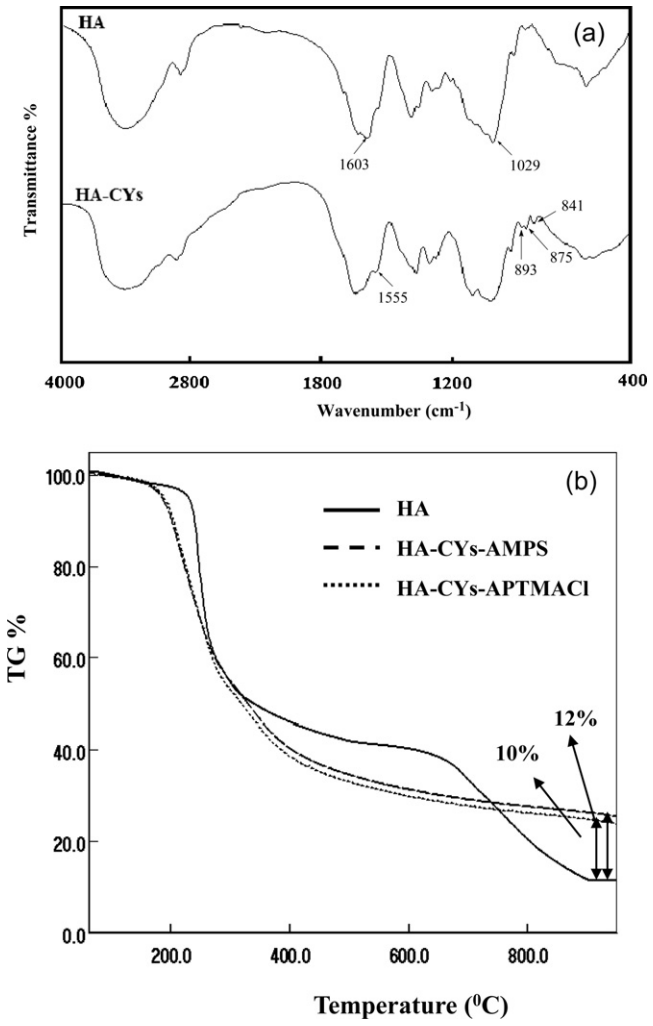

Fig. 4. (a) FT-IR spectra of HA and CYs modified HA particles, and (b) the comparison of thermograms of the HA and ionic HA-based particles.

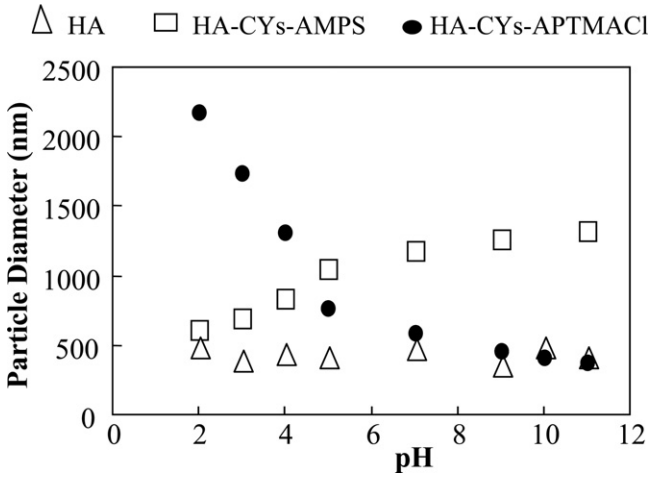

Fig. 5. The change in particle size of the HA-based hydrogels with pH.

3.7. Drug release studies

To demonstrate the potential use of HA-based particles as drug delivery devices (DDD), HA, HA-CYs-AMPS and HA-CYs-APTMACl particles were utilized in the release studies of model drugs.

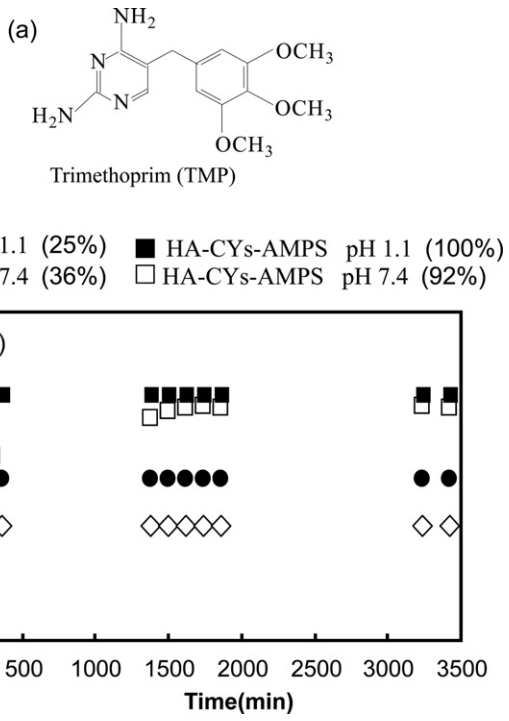

Fig. 6. (a) Chemical structure of the model drug trimethoprim (TMP). And (b) TMP release profile from HA and HA-CYs-AMPS particles at different pH values. [TMP loading: 45 mg HA and 100 mg HA-CYs-AMPS particles placed in 50 mL 250 ppm TMP aqueous solution for 24 h at 25 °C. Release medium: 50 mL PBS, pH 7.4 and pH 1.1 with 30 mg HA and 80 mg HA-CYs-AMPS drug loaded particle].

Naproxen (NN) (nonsteroidal and anti-inflammatory drug), and trimethoprim (TMP) (bacteriostatic and antibiotic drug) were used as model active agents for drug loading and release studies from HA-based particles in simulated physiological solutions (pH 1.1 and pH 7.4) at ambient temperature. The ionic strengths of solutions used in drug release studies were constant at 0.1 mol dm^{-3} adjusted with NaCl. TMP was selected for either HA and HA-CYs-AMPS samples whereas NN was loaded onto HA and HA-CYs-APTMACl particles and the loading of TMP and NN was accomplished at ambient temperature (22 °C) for 24 h in DI water. The release studies were performed in physiological solutions at 25 °C. The released amount of drug was monitored via continuous UV–vis spectrophotometer at 274 nm for TMP and 330 nm and 230 nm for NN. The amount of released drugs (mg drug/g dry particles) versus time was plotted and corresponding graphs are depicted in Figs. 6 and 7 for HA, HA-CYs-AMPS and HA-CYs-APTMACl, respectively. It is important to note that all the HA based materials exposed to the same drug loading and release conditions. As can be seen from the figures, the modified particles release higher amounts of the absorbed drugs. In Fig. 6, the bare HA particles release 25 and 36% of the TMP at pH 1.1 and 7.4, on the other hand, the modified HA-CYs-AMPS releases 92 and 100% of the absorbed drugs in about the same time (less than 2 h). Although the release kinetics of HA and HA-CYs-AMPS is about the same and fast, the released amount of TMP is 3 and 4 times more than the unmodified particles at both pHs, respectively. Therefore it can be concluded that, as shown in Fig. 6(a), the TMP has amine groups that can interact with sulfonyl groups of the modified particles leading to higher drug absorption and release amounts. Fig. 7 demonstrates the release profile of NN from HA and HA-CYs-APTMACl particles. Here NN is chosen due to the carboxylic acid groups in the drug with the anticipation that positively charged HA-CYs-APTMACl particles are more suitable for this active agent; the chemical structure of NN is shown in Fig. 7(a). As shown in Fig. 7(b) bare HA particles release only 7% of the absorbed NN

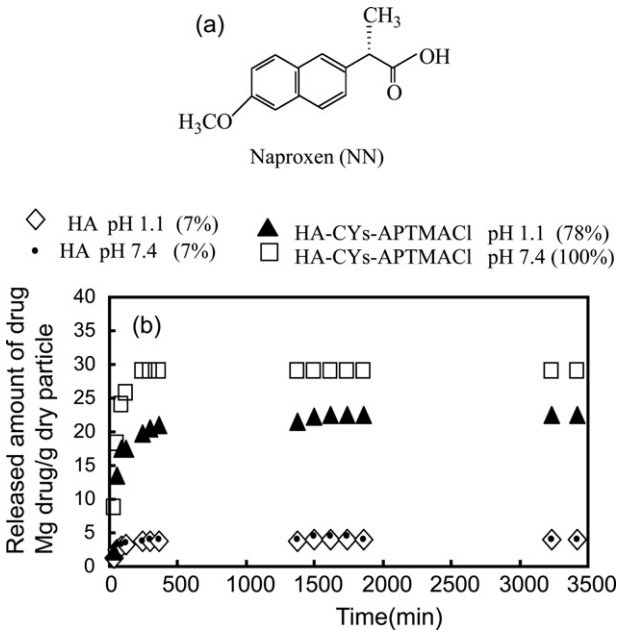

Fig. 7. (a) Chemical structure of Naproxen (NN). And (b) NN release profile from HA and HA-CYs-APTMACl particles at different pH values. [NN loading: 45 mg HA and 100 mg HA-CYs-APTMACl particles were placed in 50 mL 1000 ppm NN aqueous solution for 24 h at 25 °C. Release medium: 50 mL PBS, at pH 7.4 and pH 1.1 with 30 mg HA and 80 mg HA-CYs-APTMACl drug loaded particle].

at both pH values (1.1. and 7.4). On the other hand, the positively modified particles, HA-CYs-APTMACl release 78 and 100% of the absorbed drugs at pH values 1.1 and 7.4, respectively. As a result, from the interpretation of Figs. 6 and 7, with the chemical modification of HA particles drugs with various charges can be loaded and released in different parts of the human body, i.e., gastrointestinal and/or physiological pHs.

4. Conclusions

It was demonstrated that HA particles can be coated with ionic polymer that possesses various charges after thiol modification of bare HA particles, to produce pH-sensitive HA-CYs-AMPS and HA-CYs-APTMACl hydrogel particles. These composite materials derived from natural HA and synthetic polymers with various functional groups such as $-SO_3H$ and $-(-(N^+(CH_3)_3Cl)$ that can generate and/or already have charges can be used as polyelectrolyte particles for potential drug delivery devices. As proven in this investigation, active agents such as TMP and NN loading and release capacities can be increased by appropriate modification of HA particles for particular pH conditions. As a result, these kinds of modified HA-based particles are versatile and have great potential in the biomedical field as drug delivery vehicles sensitive to various stimuli, i.e., pH, temperature, and specific molecule presence.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.01.028.

References

Ballauff, M., & Lu, Y. (2007). "Smart" nanoparticles: Preparation, characterization and applications. *Polymer*, *48*, 1815–1823.
Bawa, P., Pillay, V. Y., Choonara, E., & Toit, L. C. (2009). Stimuli-responsive polymers and their applications in drug delivery. *Biomedical Materials*, *4*, 022001.
Bencherif, S. A., Srinivasan, A., Horkay, F., Hollinger, J. O., Matyjaszewski, K., & Washburn, N. R. (2008). Influence of the degree of methacrylation on hyaluronic acid hydrogels properties. *Biomaterials*, *29*, 1739–1749.
Bloch, D. (2006). *Organic chemistry demystified*. McGraw-Hill Professional Publishing.
Brown, M. B., & Jones, S. A. (2005). Hyaluronic acid: A unique topical vehicle for the localized delivery of drugs to the skin. *Journal of the European Academy of Dermatology and Venereology*, *19*, 308–318.
Cao, X., Lai, S., & Lee, L. (2001). Design of a self-regulated drug delivery device. *Biomedical Microdevices*, *3*, 109–118.
Dastjerdi, R., & Montazer, M. (2010). A review on the application of inorganic nanostructured materials in the modification of textiles: Focus on anti-microbial properties. *Colloids and Surfaces B: Biointerfaces*, *79*, 5–18.
Eenschooten, C., Guillaumie, F., Kontogeorgis, G. M., Stenby, E. H., & Schwach-Abdellaoui, K. (2010). Preparation and structural characterisation of novel and versatile amphiphilic octenyl succinic anhydride-modified hyaluronic acid derivatives. *Carbohydrate Polymers*, *79*, 597–605.
Ekici, S., Ilgin, P., Yilmaz, S., Aktas, N., & Sahiner, N. (2010). Temperature and magnetic field responsive hyaluronic acid particles with tunable physical and chemical properties. *Applied Surface Science*, *257*, 2669–2676.
Gupta, P., Vermani, K., & Garg, S. (2002). Hydrogels: From controlled release to pH-responsive drug delivery. *Drug Discovery Today*, *7*, 569–579.
Gümüsderelioglu, M., & Kesgin, D. (2005). Release kinetics of bovine serum albumin from pH-sensitive poly(vinyl ether) based hydrogels. *International Journal of Pharmaceutics*, *288*, 273–279.
Habraken, W. J. E. M., Wolke, J. G. C., & Jansen, J. A. (2007). Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. *Advanced Drug Delivery Reviews*, *59*, 234–248.
Harvey, J. (1995). *Encyclopedia of chemical technology*. New York: Wiley., pp. 502–514
Hoare, T. R., & Kohane, D. S. (2008). Hydrogels in drug delivery: Progress and challenges. *Polymer*, *49*, 1993–2007.
Huang, T. C., Huang, L. Z., & Ho, C. T. (1998). Mechanistic studies on thiazolidine formation in aldehyde/cysteamine model systems. *Journal of Agricultural and Food Chemistry*, *46*, 224–227.
Ilgin, P., Avci, G., Silan, C., Ekici, S., Aktas, N., Ayyala, R. S., et al. (2010). Colloidal drug carries from (sub)micron hyaluronic acid hydrogel particles with tunable properties for biomedical applications. *Carbohydrate Polymers*, *82*, 997–1003.
Jeon, O., Song, S. J., Lee, K. J., Park, M. H., Lee, S. H., Hahn, S. K., et al. (2007). Mechanical properties and degradation behaviors of hyaluronic acid hydrogels cross-linked at various cross-linking densities. *Carbohydrate Polymers*, *70*, 251–257.
Jia, X., Yeo, Y., Clifton, R. J., Jiao, T., Kohane, D. S., Kobler, J. B., et al. (2006). Hyaluronic acid-based microgels and microgel networks for vocal fold regeneration. *Biomacromolecules*, *7*, 3336–3344.
Kast, C. E., & Schnurch, A. B. (2002). Polymer–cysteamine conjugates: New mucoadhesive excipients for drug delivery. *International Journal of Pharmaceutics*, *234*, 91–99.
Khare, A. R., & Peppas, N. A. (1995). Swelling/deswelling of anionic copolymer gels. *Biomaterials*, *16*, 559–567.
Kim, J., Kim, K. S., Jiang, G., Kang, H., Kim, S., Kim, B. S., et al. (2008). In vivo real-time bioimaging of hyaluronic acid derivatives using quantum dots. *Biopolymers*, *89*, 1144–1153.
Kim, M. R., & Park, T. G. (2002). Temperature-responsive and degradable hyaluronic acid/Pluronic composite hydrogels for controlled release of human growth hormone. *Journal of Controlled Release*, *80*, 69–77.
Kong, M., Chen, X., & Park, H. (2011). Design and investigation of nanoemulsified carrier based on amphiphile-modified hyaluronic acid. *Carbohydrate Polymers*, *83*, 462–469.
Lee, H., Lee, K., & Park, T. G. (2008). Hyaluronic acid–paclitaxel conjugate micelles: Synthesis, characterization, and antitumor activity. *Bioconjugate Chemistry*, *19*, 1319–1325.
Luo, Y., & Prestwich, G. D. (1999). Synthesis and selective cytotoxicity of a hyaluronic acid–antitumor bioconjugate. *Bioconjugate Chemistry*, *10*, 755–763.
Maroda, M., Bodnár, M., Berkó, S., Bakó, J., Eros, G., Csányi, E., et al. (2011). Preparation and investigation of a cross-linked hyaluronan nanoparticles system. *Carbohydrate Polymers*, *83*, 1322–1329.
Mason, M., Vercruysse, K. P., Kirker, K. R., Frisch, R., Marecak, D. M., Prestwich, G. D., et al. (2000). Attachment of hyaluronic acid to polypropylene, polystyrene, and polytetrafluoroethylene. *Biomaterials*, *21*, 31–36.
Mika, A., Childs, R., & Dickson, J. (2002). Salt separation and hydrodynamic permeability of a porous membrane filled with pH-sensitive gel. *Journal of Membrane Science*, *206*, 19–30.
Moussas, P. A., & Zouboulis, A. I. (2009). A new inorganic–organic composite coagulant, consisting of polyferric sulphate (PFS) and polyacrylamide (PAA). *Water Research*, *43*, 3511–3524.
Palumbo, F. S., Pitarresi, G., Mandracchia, D., Tripodo, G., & Giammona, G. (2006). New graft copolymers of hyaluronic acid and polylactic acid: Synthesis and characterization. *Carbohydrate Polymers*, *66*, 379–385.
Peppas, L. B., & Peppas, N. A. (1989). Solute and penetrant diffusion in swellable polymers. The mechanism of drug release from pH-sensitive swelling-controlled systems. *Journal of Controlled Release*, *8*, 267–274.

Pouyani, T., & Prestwich, G. D. (1994). Functionalized derivatives of hyaluronic acid oligosaccharides: Drug carriers and novel biomaterials. *Bioconjugate Chemistry*, *5*, 339–347.

Ren, Y. J., Zhou, Z. Y., Liu, B. F., Xu, Q. Y., & Cui, F. Z. (2009). Preparation and characterization of fibroin/hyaluronic acid composite scaffold. *International Journal of Biological Macromolecules*, *44*, 372–378.

Sahiner, N., Jha, A. K., Nguyen, D., & Jia, X. Q. (2008). Fabrication and characterization of cross-linkable hydrogel particles based on hyaluronic acid: Potential application in vocal fold regeneration. *Journal of Biomaterials Science: Polymer Edition*, *19*, 223–243.

Sahiner, N., & Jia, X. (2008). One-step synthesis of hyaluronic acid-based (sub)micron hydrogel particles: Process optimization and preliminary characterization. *Turkish Journal of Chemistry*, *32*, 397–409.

Sahiner, N., Singh, M., Kee, D., De John, V. T., & McPherson, G. L. (2006). Rheological characterization of a charged cationic hydrogel network across the gelation boundary. *Polymer*, *47*, 1124–1131.

Sawada, H. (1976). *Thermodynamics of polymerization*. Marcel Dekker Inc.

Schnurch, A. B., Clausen, A. E., & Hnatyszyn, M. (2001). Thiolated polymers: Synthesis and in vitro evaluation of polymer–cysteamine conjugates. *International Journal of Pharmaceutics*, *226*, 185–194.

Shen, Y., Li, Q., Tu, J., & Zhu, J. (2009). Synthesis and characterization of low molecular weight hyaluronic acid-based cationic micelles for efficient siRNA delivery. *Carbohydrate Polymers*, *77*, 95–104.

Solomons, T. W. G., & Fryhle, C. B. (2000). *Organic chemistry* (7th ed.). John Wiley and Sons, Inc.

Tan, H., Chu, C. R., Payne, K. A., & Marra, K. G. (2009). Injectable in situ forming biodegradable chitosan–hyaluronic acid based hydrogels for cartilage tissue engineering. *Biomaterials*, *30*, 2499–2506.

Vanderhooft, J. L., Mann, B. K., & Prestwich, G. D. (2007). Synthesis and characterization of novel thiol-reactive poly(ethylene glycol) cross-linkers for extracellular-matrix-mimetic biomaterials. *Biomacromolecules*, *8*, 2883–2889.

Vercruysse, K. P., Marecak, D. M., Marecak, J. F., & Prestwich, G. D. (1997). Synthesis and in vitro degradation of new polyvalent hydrazide cross-linked hydrogels of hyaluronic acid. *Bioconjugate Chemistry*, *8*, 686–694.

Xie, Y., Hill, C. A. S., Xiao, Z., Militz, H., & Mai, C. (2010). Silane coupling agents used for natural fiber/polymer composites: A review. *Composites Part A: Applied Science and Manufacturing*, *41*, 806–819.

Zhang, J., Chu, L. Y., Li, Y. K., & Lee, Y. M. (2007). Dual thermo- and pH-sensitive poly(N-isopropylacrylamide-co-acrylic acid) hydrogels with rapid response behaviors. *Polymer*, *48*, 1718–1728.

Zhu, Z., Li, X., Wang, Y., Zeng, Y., Sun, W., & Huang, X. (2010). Direct electrochemistry and electrocatalysis of horseradish peroxidase with hyaluronic acid–ionic liquid–cadmium sulfide nanorod composite material. *Analytica Chimica Acta*, *670*, 51–56.