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The crosslinking of polysaccharides with polyamines and dextran-polyallylamine antibacterial hydrogels

Naphtali A. O'Connor^{a,*}, Ahmad Abugharbieh^a, Farzana Yasmeen^b, Emmanuel Buabeng^a, Steve Mathew^a, Diana Samaroo^c, and Hai-Ping Cheng^b

^aDepartment of Chemistry, Lehman College of the City University of New York, Bronx, NY 10468, United States

^bDepartment of Biology, Lehman College of the City University of New York, Bronx, NY 10468, United States

^cDepartment of Chemistry, New York City College of Technology, 300 Jay Street, Brooklyn, NY 11201, United States

Abstract

A facile modular approach to rapidly prepare pH-responsive hydrogels by crosslinking polysaccharides with polyamines is demonstrated. Hydrogels are prepared by first reacting the less reactive polysaccharides with the cross-linker epichlorohydrin and completed by the addition of polyamines. The crosslinking of polysaccharides with polyamines provides a facile method for incorporating functionality into polysaccharide based hydrogels. This process is demonstrated with the polysaccharides dextran, pullulan and carboxymethyl cellulose and with the polyamines polyallylamine and polyethylene imine. The hydrogels were characterized by FTIR and swelling studies, which showed pH-dependent swelling due to the presence of the polyamine. The hydrogels can also be tailored by varying the mass ratio between the polysaccharide and polyamine. Absorption studies of organic analytes showed the polyamine content affecting the uptake of a charged substrate (methylene blue) and no effect on a neutral substrate (6-methyl coumarin). This synthetic method was also used to prepare hydrogels with antibacterial activity against *E. coli* and *S. aureus* by utilizing an amphiphilic polyallylamine.

Keywords

Polysaccharide polyamine hydrogel; Anti-bacterial hydrogel; pH-responsive hydrogel

1. Introduction

Hydrogels are a three-dimensional network of hydrophilic polymers capable of retaining a large quantity of water within the network without dissolving. Hydrogels are employed in a variety of biomedical applications including tissue engineering, biomimetic materials and drug delivery [1–3]. They are widely used commercially and can be obtained in the marketplace in soft contact lenses, surgical implants, breast implants, absorbent diapers,

*Corresponding author. Tel.: +1 718 960 8678. naphtali.oconnor@lehman.cuny.edu (N.A. O'Connor).

surgical catheters, suture coatings and wound dressings. Polysaccharides are a common hydrogel material and lend well to the preparation of hydrogels because of an abundance of hydrophilic alcohols. They are well studied as biomaterials for drug release, tissue repair and engineering applications [4–8].

Recently polysaccharide based hydrogels comprised of dextran modified with various functional groups showed exceptional angiogenic and wound healing properties [9,10]. In work from Gerecht and coworkers an amine functionalized dextran based scaffold was shown to promote a significant angiogenic response, with burn wounds healing in less than 21 days and having considerably better neovascularization than a control scaffold or over-the-counter dressings [10]. They concluded that the hydrogel facilitated neutrophil infiltration and the neutrophils facilitated hydrogel digestion leading to vascular cell infiltration. This amine functionalized dextran hydrogel demonstrated tremendous wound healing properties without the need for healing agents such as growth factors [11,12], which is currently a widely researched strategy. Hydrogels eliciting wound healing effects simply based on their chemical composition shows the importance and need for methods to effortlessly prepare hydrogels of diverse composition.

A multitude of naturally occurring polysaccharides is available and a modular method to incorporate them into customizable hydrogels will be beneficial for developing biomedical applications. The utilization of polysaccharides in hydrogels often requires a method of crosslinking and can be directly crosslinked with epichlorohydrin and glutaraldehyde. To prepare polysaccharide hydrogels with additional functionality, the polysaccharides are often acrylated and then copolymerized with diacrylic crosslinkers and acrylates possessing the desired functionality [13]. Recent focus has been on the development of stimuli-responsive hydrogels as a smart material for biomedical applications. An example of this are pH-responsive hydrogels which have been examined as controlled drug release devices [2]. These gels swell by the creation of charge from basic or acidic functional groups with changes in pH. This swelling allows for the release of the entrapped drug molecules. For preparing pH-responsive hydrogels from polysaccharides, researchers have relied heavily on chitosan, a polysaccharide comprised on glucosamines [14]. Chitosan however has some drawbacks, as it has poor water solubility and requires an acidic environment for dissolution, often making the chemistry difficult [15].

Herein is described a general approach for preparing hydrogels from polysaccharides and polyamines. The crosslinking of polysaccharides with polyamines provides a facile method for incorporating functionality into polysaccharide based hydrogels. Dextran, pullulan and carboxymethyl cellulose are used as model polysaccharides while polyallylamine and polyethylene imine are demonstrated as model polyamines. Epichlorohydrin is utilized as a coupling reagent to crosslink polysaccharides and polyamines. This results in a method in which any polysaccharide and any polyamine with sufficient solubility can be combined to prepare hydrogels without the need for acrylation or other cumbersome synthetic steps. Hydrogel swelling and drug release properties are easily tailored with the composition and mass ratios of polysaccharides and polyamines and will be an attractive approach for developing drug delivery devices. The utility of this process is also demonstrated by using it to synthesize hydrogels with antibacterial activity against *E. coli* and *S. aureus*. Polymers

and hydrogels with antimicrobial activity are attractive for use as wound-healing dressings, and coatings for medical devices [16,17]. A common approach is to prepare amphiphilic polymers which are proposed to induce cell death by disrupting microbes' cell membranes [16–18]. Here an antimicrobial hydrogel is prepared in one pot by crosslinking the polysaccharide with an amphiphilic polyallylamine.

2. Materials and methods

2.1. Materials

All chemicals were used as is dextran (MW 40kDa) and pullulan were obtained from TCI America. Polyallylamine (MW 120–200 kDa) was purchased from Alfa Aesar. Polyethylene imine (M_r 600–1000 kDa) was obtained from Hampton Research. Carboxymethyl cellulose (MW 90 kDa) and epichlorohydrin were purchased from Acros Chemicals. For antibacterial studies, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used. Spectroscopic measurements were obtained with a PerkinElmer Lambda 35 spectrometer and a Nicolet iS10 FTIR. For NMR measurements, a Bruker 300 Ultrashield instrument was used.

2.2. Methods

2.2.1. Hydrogel synthesis—To a stirring polysaccharide solution (5–20%, w/v; 1 mL), NaOH (5 M, 0.4 mL) was added, followed by 100 μ L epichlorohydrin. The mixture was stirred until clear and the phase separation could no longer be seen (<2 h). The appropriate amount of polyamine (20%, w/v) was then added to prepare hydrogels with polysaccharide:polyamine mass ratios of 5:1, 2:1 and 1:1. The solutions were quickly stirred until homogeneous and transferred to a final container for the gel to set. Gels were allowed to sit for 24 h before being washed with water to remove NaOH and dried for 24 h at 50°C.

2.2.2. Amphiphilic polyallylamine synthesis—Polyallylamine (PAA, 0.5 g) was dissolved in 5 mL of distilled water along with NaOH (106 mg, 2.65 mmol). This was followed by the addition of the quaternary amine glycidyltrimethylammonium chloride (160 mg, 0.86 mmol) and the mixture was stirred for 30 min. Then the hydrophobic 1,2-epoxyoctane (137 mg, 1.07 mmol) was added and the reaction mixture stirred for 24 h and acidified with 0.1 N HCl to a pH in the range of 3–4. The aqueous solution was washed three times with 20 mL of dichloromethane and then further purified by dialysis with regenerated cellulose (12,000–15,000 MWCO). After dialysis, the water was removed by heating in a vacuum oven at 50°C, to obtain 0.35 g of the desired product. ^1H NMR (300 MHz, D_2O) δ (ppm) = 0.83 (1,2-epoxyoctane CH_3), 1.25 (1,2-epoxyoctane CH_2), 1.49 (PAA CH_2), 2.0 (PAA CH), 2.7–3.1 (PAA CH_2 , glycidyltrimethylammonium CH_2 and CH), 3.2 (glycidyltrimethylammonium CH_3), 3.4 (glycidyltrimethylammonium CH_2).

2.2.3. Swelling ratio—Uniform disks approximately 2 cm in diameter and 0.5 cm in thickness were obtained using a template and washed and dried as described above. For swelling studies, dried hydrogel monoliths were soaked at various pH for 24 h and their wet weight measured. The swelling ratio is defined as:

$$\text{Swelling Ratio} = \frac{w - w_0}{w_0}$$

where w_0 is the weight of the dried gel and w is the wet weight.

2.2.4. Absorption and release studies—Methylene blue (MB) and 6-methyl coumarin (6-MC) were used as model drug molecules and their absorption and release were examined. Absorption was examined by adding dried hydrogel monoliths to MB or 6-MC solutions (10 mL, 4×10^{-5} M in H_2O) and the absorbed concentration determined by using calibration curve following the Beer-Lambert law. Methylene blue was monitored at 690 nm and 6-methyl coumarin was monitored at 280 nm. Drug release was observed by suspending hydrogels with the absorbed model drug in 10 mL of pH 7.0 phosphate buffered solution.

2.2.5. Antibacterial studies—Hydrogels were plated into petri dishes and washed until the NaOH was removed. The gels were then soaked in Miller Lysogeny broth for 24 h. Bacterial solutions with optical densities of 0.2 at 600 nm ($>10^8$ cell/mL) were obtained and serial dilutions to 10^{-2} , 10^{-4} and 10^{-6} of this were obtained. The excess media was removed from the hydrogels and 2 μL of bacterial solutions at the four concentrations was added to the media soaked hydrogels and incubated at 37°C for 24 h.

3. Results and discussion

3.1. Hydrogel synthesis

The epoxy functionality is widely used to functionalize polysaccharides, and epichlorohydrin is a well-known crosslinking agent for polysaccharides [19–21]. Under basic conditions the epoxide is first opened, followed by substitution of the chloride. Epichlorohydrin is also utilized to crosslink polyamines [22] and as a bifunctional crosslinker, is capable of linking polysaccharides to polyamines. However the great difference in nucleophilicity between alcohols and amines is a barrier to the application of epichlorohydrin to crosslink polyols and polyamines. Polyamines form gels within seconds of exposure to epichlorohydrin while polysaccharides require hours. When aqueous polysaccharide solutions are crosslinked with epichlorohydrin the reaction is at first biphasic. As the reaction progresses the hydrophobic epichlorohydrin is consumed and reaction becomes homogenous. This transition from two phases to one can be taken as the point when all of the epichlorohydrin is linked to the polysaccharide. As such we devised a 2-step-1-pot synthetic strategy to crosslink polysaccharides with polyamines by first reacting polysaccharides with epichlorohydrin until a homogenous solution is observed and then addition of polyamine to prepare hydrogels consisting of crosslinked polysaccharides and polyamines (Fig. 1).

Using this method we are able to demonstrate pH-responsive hydrogels from combinations of the polysaccharides dextran (Dex), pullulan (Pu) and carboxymethyl cellulose with the polyamines polyallylamine(PAA) and polyethylene imine(PEI). Using 20%, 10% and 5% aqueous solutions for dextran, pullulan and carboxymethyl cellulose respectively, hydrogels with 1:1, 2:1 and 5:1 polysaccharide to polyamine mass ratios were prepared (Table 1). The

higher the polyamine ratio the shorter the gel time. Gels with 1:1 ratios formed within 5 min, gels with 2:1 ratios formed within 1 hour and those with a 5:1 mass ratio formed in <4h.

3.2. FTIR and swelling characterization

Fourier transform infrared (FTIR) spectroscopy of the gels confirms that both the polysaccharide and polyamine are present. The FTIR spectrum for dextran-polyallylamine (DexPAA) hydrogels shows the characteristic polysaccharide OH stretch (3300 cm^{-1}) and CO stretch (1010 cm^{-1}). The primary amine NH bend (1540 cm^{-1}) and CN stretch (1370 cm^{-1}) peaks increase with an increase in the polyallylamine concentration (Fig. 2a). Similar results can be observed for dextran-polyethylene imine (DexPEI) hydrogels. With increasing polyamine, increases in the peaks for NH bending (1630 cm^{-1}) and CN stretching (1320 cm^{-1}) were observed (Fig. 2b). Similar results were observed for gels prepared with pullulan (Fig. 2c and d) and carboxymethyl cellulose, confirming the generality of this synthetic approach.

Amine functionalized hydrogels show pH-dependent swelling behavior due to protonation. When protonated, an increase in swelling is observed due to electrostatic repulsion and an increase in the hydrophilicity within the gel. Swelling studies confirmed the pH-responsive properties of these materials and thus the incorporation of polyamines into the hydrogels. Studies were performed on dextran and pullulan containing gels by suspending them in solutions of pH 2, 7 and 12. Swelling studies could not be performed on carboxymethyl cellulose containing gels as they lost their shape and became films upon drying. This is due to the low concentration of carboxymethyl cellulose, but this could not be avoided as at concentrations above 5% (w/v), carboxymethyl cellulose viscosity became too difficult to handle.

The dextran and pullulan based gels showed swelling behavior consistent with a cationic hydrogel, higher swelling at acidic pH 2 and 7, with a decrease at pH 12 (Fig. 3a and b). The swelling ratio approximately doubled in going from pH 12 to pH 7 for the 2:1 and 1:1 DexPAA hydrogels. The swelling increase for the 5:1 DexPAA gel in going from pH 12 to pH 7 increased by a factor of 1.4. This decrease in swelling is attributed to the decrease in polyallylamine. Smaller swelling ratio changes were observed for the DexPEI hydrogels, however the series showed pH-dependent swelling consistent with the successful incorporation of the polyamines. Similar pH-dependent swelling was also observed with the pullulan hydrogels, with larger changes in swelling with change in pH observed for pullulan-polyallylamine (PuPAA) hydrogels than with the pullulan-polyethylene imine (PuPEI) hydrogels (Fig. 3c and d). Swelling studies confirm that this synthetic method can prepare pH-responsive materials and that properties can be tuned by varying the concentration of the polysaccharide and polyamine solutions used for hydrogel synthesis.

3.3. Absorption and release studies

A major application for hydrogels is as drug delivery agents. As such, hydrogels with easily tuned drug absorption and release are highly attractive. The absorptive and release properties of the polysaccharide-polyamine hydrogels were studied using methylene blue (MB) as a charged substrate and 6-methyl coumarin (6-MC) as a neutral substrate. An examination of

the DexPAA series of hydrogels shows the absorption of MB is highly dependent on the dextran to polyamine mass ratio, with an increase in absorption with increasing polysaccharide ratios. The 5:1 DexPAA hydrogels absorbed 10 times as much MB as the 1:1 DexPAA (Fig. 4a). In contrast, absorption studies of the neutral 6-MC shows little difference between the DexPAA hydrogels. The trend of increased MB absorption with decreasing polyamine is attributed to charge repulsion. As the polyamine content increases the amount of positive charge in the hydrogel at pH 7 increases.

Substrate release at pH 7 is related to the amount absorbed. The greatest amount of MB is released per mg of gel is from 5:1 DexPAA and almost ten times as much than 1:1 DexPAA. Conversely, similar amount of 6-MC was absorbed by the DexPAA gels and their release profiles are indistinguishable.

3.4. Antibacterial studies

To show the advantage of this synthetic strategy, the design of DexPAA based antibacterial hydrogels is also demonstrated. Hydrogels with antibacterial activity are utilized as wound dressings [23]. Polyamines are demonstrated to have antimicrobial activity through modification to have amphiphilic properties [18]. Hydrogels were examined against Gram-negative *E.coli* and Gram-positive *S.aureus*. Hydrogels were soaked in Lysogeny broth and then four 2 μ L aliquots of bacterial solutions of various concentrations (highest concentration of 10^8 cell/mL) pipetted onto the hydrogel surface and incubated at 37°C. Gels were evaluated to possess antibacterial activity by the absence of growth after 24 h. Bacterial growth is observed on agar and a hydrogel solely consisting of dextran (Fig. 5b). Varying the mass ratio of dextran and PAA has an effect of the amount of bacterial growth with lesser ratios of PAA showing lesser bacterial growth. However hydrogels with dextran:PAA ratios varying from 1:1 to 50:1 all show signs of bacterial growth. A hydrophobic PAA was prepared by reacting PAA with 1,2-epoxyoctane and an amphiphilic PAA was prepared by reacting PAA with 1,2-epoxyoctane and glycidyl trimethylammonium chloride (Fig. 5a). Diminished bacterial growth is observed with hydrogels made with hydrophobic modified PAA compared to the unmodified PAA. However the best effort to inhibit both *E. coli* and *S. aureus* was observed with the amphiphilic modified PAA, where bacterial growth was undetected (Fig. 5b).

4. Conclusions

In summary, a general approach to preparing diverse hydrogels comprised of polysaccharides and polyamines crosslinked by epichlorohydrin has been demonstrated. This methodology is proposed as a facile and rapid route to developing diverse hydrogels. Hydrogels can be obtained at concentrations as low as 5% (w/v), but higher concentrations are necessary for re-swelling experiments. Hydrogel diversity is not only obtained by differing the comprising polysaccharide or polyamine but can also be obtained by varying their mass ratios. The controlled absorption and release of charged substrates can be obtained by varying the polyamine content of the hydrogel. This allows for these hydrogels to be deployed as a drug delivery agent with drug absorption and release easily tuned by varying the polysaccharide and polyamine mass ratios. The versatility of this method is also

demonstrated in the rationale design of antibacterial hydrogels. Polyallylamine was successfully modified to incorporate amphiphilicity and incorporated into hydrogels with dextran to so activity against *E. coli* and *S. aureus*.

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Fig. 1.
Synthesis of polysaccharide-polyamine hydrogels.

Fig. 2.
FTIR spectra for hydrogels with 5:1 and 1:1 polysaccharide:polyamine mass ratios: (a) DexPAA, (b) DexPEI, (c) PuPAA and (d) PuPEI.

Fig. 3. Swelling ratios for hydrogels with 5:1, 2:1 and 1:1 polysaccharide:polyamine mass ratios: (a) DexPAA, (b) DexPEI, (c) PuPAA and (d) PuPEI.

Fig. 4.

Absorption of organic substrates by DexPAA hydrogels (a) methylene blue (MB) (μmol of MB/mg of gel) and (b) 6-methyl coumarin (6-MC) (μmol of 6-MC/mg of gel). Release of organic substrates from DexPAA hydrogels at pH 7 (c) MB (μmol of MB/mg of gel) and (d) 6-MC (μmol of 6-MC/mg of gel).

Fig. 5.
(a) Amphiphilic PAA and (b) study of hydrogel antibacterial (*E. coli* and *S. aureus*) activity.

Table 1

Polysaccharide-polyamine hydrogels.

	Dextran	Pullulan	Carboxymethyl cellulose
Polyallylamine	DexPAA	PuPAA	CMC-PAA
Polyethylene imine	DexPEI	PuPEI	CMC-PEI