Biomaterials 32 (2011) 6893–6899

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# Uniform zwitterionic polymer hydrogels with a nonfouling and functionalizable crosslinker using photopolymerization

Louisa R. Carr, Yibo Zhou, Jordan E. Krause, Hong Xue, Shaoyi Jiang*

*Department of Chemical Engineering, University of Washington, Box 351750, Seattle, WA 98195, USA*

ARTICLE INFO

*Article history:*
Received 19 May 2011
Accepted 4 June 2011
Available online 25 June 2011

*Keywords:*
Hydrogel
Crosslinking
RGD peptide
Compression

ABSTRACT

We reported previously the design and synthesis of a zwitterionic carboxybetaine dimethacrylate crosslinker, and we showed that its use with zwitterionic carboxybetaine methacrylate led to nonfouling hydrogels with high mechanical properties and high hydration. Now, we use photopolymerization to improve the uniformity of the polymer network, resulting in drastically improved mechanical properties (compressive modulus up to 90 MPa). Furthermore, we designed and synthesized a new functionalizable carboxybetaine dimethacrylate crosslinker, enabling functionalization of the higher strength hydrogels against a nonfouling background. Additionally, the biostability of the carboxybetaine hydrogel systems was tested, and it was found that these hydrogels are stable in oxidative, acidic, and basic environments.



## 1. Introduction

Hydrogels have long been of interest for biological and biomaterial applications due to their biomimetic high water content, high diffusive permeability, and mechanical strengths [1–6]. Particular interest has been given to PEG and poly(2-hydroxyethyl methacrylate) (pHEMA) [1] hydrogels because, in addition to the general properties of hydrogels, they are also commonly considered be low fouling, bioinert, and versatile. Hydrogels made from pHEMA have found use in and been studied for many applications [1,7–9], but their hydration is lower than that of native tissue, and their fouling, while low, is higher than other nonfouling materials. PEG hydrogels are routinely used, despite being subject to oxidative degradation [10–14]. The susceptibility of PEG to oxidative damage reduces its utility for applications that require long-term material stability. Furthermore, pHEMA and PEG functionalization via the hydroxyl group is generally difficult.

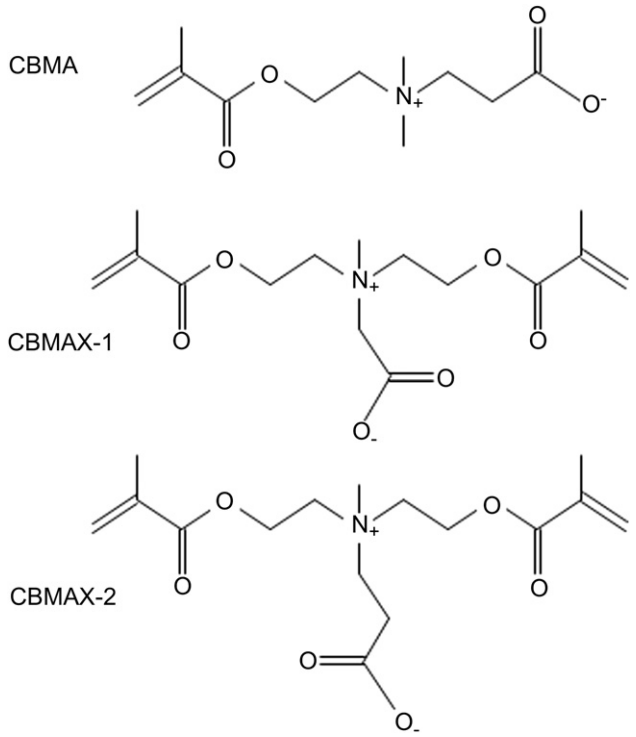
The prominence and importance of zwitterionic materials has increased in recent years [15–19]. We have demonstrated that zwitterionic compounds, including poly(carboxybetaine methacrylate) (CBMA, Scheme 1), are ultra-low-fouling [20,21], meaning that surfaces coated with these polymers allow less than 5 ng/cm$^2$ protein adsorption [22]. We also showed that pCBMA-coated surfaces are highly resistant to non-specific protein adsorption, even from undiluted blood plasma and serum [23,24], and prohibit long-term bacterial colonization for up to 10 days at room temperature [25]. The ultra-low-fouling of zwitterionic materials results from the high hydration around the opposing charges and the high energy barrier required to remove that hydration layer [26]. Furthermore, CBMA is easily functionalized through conventional EDC/NHS chemistry [21,27].

Because of the high hydration and ultralow fouling properties of zwitterionic materials, zwitterionic materials are of interest as hydrogels with superior suitability for biomedical applications. Previously, we have demonstrated low protein adsorption on sulfobetaine methacrylate (SBMA) hydrogels [28], and low cell adhesion on CBMA, SBMA, and sulfobetaine vinylimidazole hydrogels [28,29]. The zwitterionic hydrogels studied so far, however, have shown low mechanical strength [28–30], which limits their potential biological uses. Another fundamentally limiting feature of these zwitterionic hydrogels is the dearth of hydrophilic crosslinkers. The most commonly used commercially available “hydrophilic” crosslinker is *N,N’*-methylenebis(acrylamide) (MBAA), but this crosslinker is only moderately soluble at crosslinker concentrations around 10%, and the acrylamide functionality incorporates poorly into the growing methacrylate polymer chains. Furthermore, MBAA is particularly ill-suited for crosslinking zwitterionic hydrogels because it does not structure water. Structured water around the opposing charges in a zwitterionic material provides the nonfouling mechanism [26]; MBAA will disrupt the ordered water and present locations where proteins, bacteria, and even cells, may bind and foul the hydrogel.

Previously, we reported a carboxybetaine (CB) crosslinker [31] (CBMAX-1, Scheme 1). Chemically, it is composed of the zwitterionic CB methacrylate with a second methacrylate replacing one of the methyl groups on the quaternary nitrogen. Due to its structural

* Corresponding author. Tel.: +206 616 6509; fax: +206 543 3778.
*E-mail address:* sjiang@u.washington.edu (S. Jiang).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.06.006




**Scheme 1.** Chemical structures of CBMA monomer and crosslinkers with 1-carbon spacer (CBMAX-1) and 2-carbon spacer (CBMAX-2).

similarity to the CB monomer and high solubility in aqueous systems, improved incorporation of the crosslinker into the polymer mesh network was observed, and higher concentrations of crosslinker were achieved. A hydrogel made with 100% crosslinker had a compressive modulus of 8 MPa with 60% equilibrium water content. Importantly, this hydrogel exhibited the nonfouling property that is the hallmark of zwitterionic materials. Despite these attributes, however, the CB dimethacrylate reported previously had one key shortcoming: the CB group had only one methylene group separating the opposing charges, which limits its reactivity for functionalization [32,33].

In order to gain more reactivity for functionalization, and to make the crosslinker a true analog for the monomer, a new CB crosslinker was designed with two carbons between the opposing charges (CBMAX-2, Scheme 1). This two-carbon spacer makes the carboxylic acid functional group more reactive to carbodiimide chemistry like EDC-sulfoNHS zero-length crosslinker reactions [27,33]. Also in this study, photopolymerization was used rather than thermal polymerization used in the previous study [31], in order to further improve the uniformity of the growing polymer mesh network. This is due to the improved regularity of photon penetration relative to heat transfer through the polymerization medium, and results in network homogeneity that contributes to the bulk mechanics of hydrogels. Furthermore, polymerization by photo-irradiation is convenient due to the ability to control light exposure spatially and temporally, and does not require harsh conditions or long reaction times.

Here, we designed and synthesized the more reactive crosslinker, and test the physical, mechanical properties, and functionalization properties of photopolymerized hydrogels made with 2-carbon spacer crosslinker (CBMAX-2), and compare these properties to those of photopolymerized hydrogels made with the 1-spacer crosslinker reported earlier (CBMAX-1). The stability of this new crosslinker was also evaluated in several bio-relevant media, such as oxidative, acidic, and basic solutions.

## 2. Experimental methods

### 2.1. Materials

2-(*N*-morpholino)ethanesulfonic acid (MES), methacrylic acid, ion exchange resin (IRA 400 OH form), iodomethane, triethyl amine, 2-hydroxy-2-methyl-propiophenone, papain, and phosphate-buffered saline were purchased from Sigma Aldrich (St. Louis, MO). Diethanolamine, tert-butyl acrylate, and methacryloyl chloride were purchased from Alfa Aesar; Acetonitrile, silica gel 60 (70–230 mesh), and diethyl ether from EMD Biosciences (Gibbstown, NJ); trifluoroacetic acid was purchased from Acros Organics (via Fisher Scientific, Pittsburg, PA); and t-butyl bromoacetate and *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) from TCI America (Portland, OR). Dulbecco's Modified Eagle Medium, fetal bovine serum, nonessential amino acids, and penicillin-streptomycin were purchased from Invitrogen Corp (Carlsbad, CA). Cyclo(Arginine-Glycine-Asparginine-D-Tyronsine-Lysine) peptide (cRGD) was purchased from Peptides International (Louisville, KY). Hydrogen peroxide and sodium chloride salt were purchased from J.T. Baker (Phillipsburg, NJ). COS-7 cells (African Green Monkey fibroblast cells) were purchased from the American Tissue Culture Collection (Manassas, VA). All water used had been purified to 18.2 mΩ on a Millipore Simplicity water purification system.

### 2.2. Synthesis of carboxybetaine monomer and carboxybetaine-based crosslinkers

#### 2.2.1. Synthesis of the CBMA monomer

2-Carboxy-*N*,*N*,-dimethyl-*N*-(2′-(methacryloyloxy)ethyl) ethanaminium inner salt (carboxybetaine methacrylate, CBMA) was synthesized as previously reported [34].

#### 2.2.2. Synthesis of CBMAX-1

The crosslinker with 1 carbon between the quaternary amine and the carboxyl group, CBMAX-1, also called 1-Carboxy-*N*-methyl-*N*-di(2-methacryloyloxy-ethyl) methanaminium inner salt, was synthesized as previously reported [31].

#### 2.2.3. Synthesis of CBMAX-2

Compound **6** (Scheme 2), the crosslinker with 2 carbons between the quaternary amine and the carboxyl group, CBMAX-2, was synthesized as follows:

*N*,*N*-Bis(2-hydroxyethyl)-3-aminopropionic acid *tert*-butylester **3**: A sample of 12.25 g (95.5 mmol) of *tert*-butyl acrylate was added to 8.77 g (83.4 mmol) of diethanolamine. The mixture was stirred at room temperature overnight. The excess *tert*-butyl acrylate was removed in vacuo, yielding 19.4 g (100%) of **3** as a colorless oil of purity sufficient for further reactions. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.61 (t, 4H, $J = 8.5$ Hz), 3.12 (s, 2H), 2.80 (t, 2H, $J = 10.5$ Hz), 2.63 (t, 4H, $J = 8.5$ Hz), 2.40 (t, 2H, $J = 10.5$ Hz), 1.46 (t, 9H).

*N*,*N*-Bis(2-methacryloyloxyethyl)- 3-aminopropionic acid *tert*-butylester **4**: To a solution of **3** (19.4 g, 83.4 mmol) in dry $CH_2Cl_2$ (300 mL) was added 58 mL (0.42 mol) of triethylamine at 0 °C, and to this mixture was then added a solution of methacryloyl chloride (18 mL, 0.18 mol) in dry $CH_2Cl_2$ (50 mL) dropwise at 0 °C. After addition, the mixture was allowed to warm to room temperature and stirred overnight until the reaction completed. 300 mL of water was added to the mixture and the organic layer was separated. The aqueous layer was then extracted with $CH_2Cl_2$ (2 × 150 mL), and the combined organic layers were washed with brine and dried with anhydrous sodium sulfate. After removal of the solvent, the residue was purified by column chromatography (silica gel 60, eluent: hexanes/ethyl acetate 5:1) to afford 26.2 g (85% yield) of product **4** as a pale brown liquid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 6.10 (s, 2H), 5.56 (s, 2H), 4.21 (t, 4H, $J = 6.0$ Hz), 2.90 (t, 2H, $J = 7.0$ Hz), 2.85 (t, 4H, $J = 6.0$ Hz), 2.38 (t, 2H, $J = 7.0$ Hz), 1.94 (s, 6H), 1.44 (s, 9H) ppm.

N-Methyl-*N*,*N*-di(2-methacryloyloxyethyl)-N-2-(*tert*-butyloxycarbonylmethyl) ammonium iodide **5**: Compound **4** (22.64 g, 61.3 mmol), iodomethane (5.7 mL, 92 mmol), hydroquinone (2.0 g, as a stabilizer), acetonitrile(150 ml) were mixed in a flask and stirred at 50 °C for 2 days. The solvent was evaporated by rotary evaporation, and the crude product was directly used for next step without further purification. $^1$H NMR ($CD_3OD$, 500 MHz): δ 6.15 (s, 2H), 5.66 (s, 2H), 4.72 (m, 4H), 4.35–4.10 (m, 4H), 3.87 (t, 2H), 3.55 (s, 3H), 2.97 (t, 2H), 1.94 (s, 6H), 1.44 (s, 9H) ppm.

2-Carboxy-N-methyl-N,N-di(2-methacryloyloxyethyl) methanaminium inner salt **6**: The crude product **5** above was treated with trifluoroacetic acid (TFA, 137 ml) in dichloromethane (700 ml) for 1 day at room temperature. The solvent was removed by rotary evaporation. The residue was dissolved in water and neutralized over an ion exchange resin (IRA 400 OH form), subsequently concentrated, precipitated into ether, and finally vacuum dried to obtain compound **6** (15.5 g, 77% yield from compound **4** through 2 steps). $^1$H NMR ($CD_3OD$): δ 6.18 (s, 2H), 5.74 (s, 2H), 4.66 (m, 4H), 3.88 (m, 4H), 3.80 (t, 2H, $J = 8.0$ Hz), 3.26 (s, 3H), 2.70 (t, 2H, $J = 8.0$ Hz), 1.99 (s, 6H) ppm.

### 2.3. Hydrogel Preparation

Hydrogel solutions were prepared in 1M NaCl, with a total concentration of monomer and crosslinker, either CBMAX-1 or CBMAX-2, of 65% (wt/vol). The different formulations were made by substituting monomer for crosslinker in increasing amounts, starting at 2% by mole. The 100% crosslinker hydrogels were