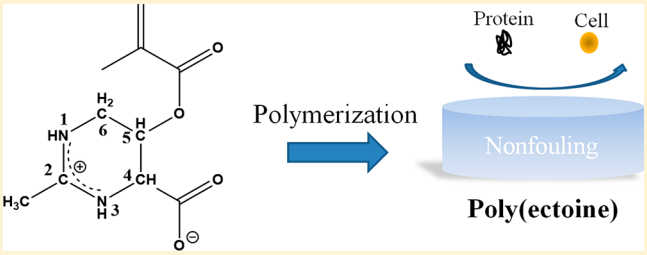
LANGMUIR Article
Cite This: Langmuir 2017, 33, 11264-11269 pubs.acs.org/Langmuir

Poly(ectoine) Hydrogels Resist Nonspecific Protein Adsorption

Priyesh Jain,† Hsiang-Chieh Hung,† Xiaojie Lin, Jinrong Ma, Peng Zhang, Fang Sun, Kan Wu, and Shaoyi Jiang*

Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States

ABSTRACT: The development of nonfouling zwitterionic materials has a wide range of biomedical and engineering applications. This work delineates the design and synthesis of a new zwitterionic material based on a naturally occurring compatible solute, ectoine, which is known to possess additional protective properties that stabilize even whole cells against ultraviolet radiation or cytotoxins. These properties and applications of ectoine inspire us to design a functional monomer containing the natural zwitterion moiety of ectoine imparting nonfouling properties and the methacrylate moiety for polymerization. The synthesis route designed for the ectoine methacrylate monomer is simple with a high yield, which is characterized by nuclear magnetic resonance spectroscopy and mass spectrometry. After monomer synthesis, we have prepared a poly(ectoine) hydrogel via thermal polymerization. The equilibrium water content, degree of cross-linking, mechanical strength, and nonfouling properties are determined for polyectoine hydrogels with different cross-linking conditions. Poly(ectoine) hydrogels are shown to have highly hydrated and excellent nonfouling properties and can be considered to be a promising biomaterial.



INTRODUCTION

To survive high-temperature and high-salinity environments, halophilic organisms tend to accumulate in a low-molecular-weight organic compound, called ectoine, to protect their biopolymers in the form of biomembranes, proteins, enzymes, and nucleic acids from dehydration.[1−3] Because ectoine has a lower affinity for a protein surface than for water, ectoine molecules are excluded from the immediate hydration shell of the protein surface, resulting in the preferential hydration of the protein and promoting its native conformation.[4] In comparison to other compatible solutes such as glycine betaine and proline produced by other micro-organisms, ectoine possesses additional protective properties that stabilize even whole cells against ultraviolet (UV) radiation or cytotoxins[5−7] and also protect the skin from the effects of UVA-induced cell damage.[8,9] These protective properties make ectoine a valuable compound in the health care and skin care industries.

Structurally, ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) is related to a pyrimidine derivative in which all bonds except one double bond have been hydrogenated (Figure 1).[10] The most important characteristic of this molecule is the delocalized π-bonding in the N−C−N group, which results in a permanent zwitterionic structure due to the mesomeric stabilization of the N−H protons. Ectoine resembles a typical betaine because the center of the positive charge lies close to carbon 2, three bond lengths from the carboxyl carbon. It carries a low charge density due to delocalization. This zwitterionic property of ectoine can be potentially exploited to make zwitterionic materials. In recent years, zwitterionic materials have been widely studied and used for various applications such as biomedical devices, implants, drug delivery agents, wound dressings, biosensors, and marine coatings.[11,12] Because of their excellent nonfouling properties, zwitterionic polymers including 2-methacryloyloxylethyl phosphorylcholine (MPC), sulfobetaine methacrylate (SBMA), and carboxybetaine methacrylate (CBMA) have emerged as an important class of ultralow fouling materials. For example, zwitterionic poly(carboxybetaine) polymers have been shown to have ultralow protein adsorption even from undiluted blood plasma and serum.[13−16] These poly(carboxybetaine) polymers have been shown recently to effectively enhance protein

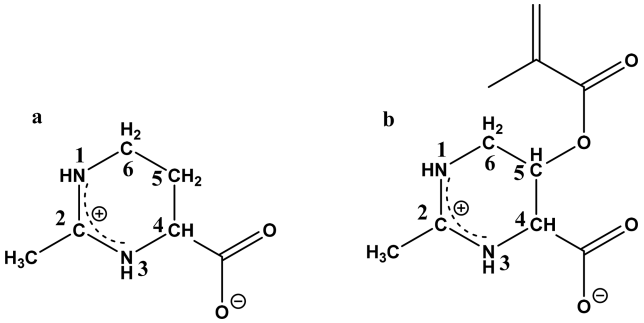


Figure 1. Zwitterionic structures of (a) ectoine and (b) ectoine methacrylate monomer.

Special Issue: Tribute to Keith Gubbins, Pioneer in the Theory of Liquids

Received: July 12, 2017
Revised: August 28, 2017
Published: August 29, 2017

DOI: 10.1021/acs.langmuir.7b02434
Langmuir 2017, 33, 11264−11269

stability and pharmacokinetics while mitigating the immune response[17,18] and resisting capsule formation.[19] Zwitterionic polymers form highly hydrated layers surrounding the opposite charges of the zwitterionic center, rendering ultralow fouling properties to these polymers.[20] Because of these interesting properties, any zwitterionic materials with additional functional properties are highly desirable. There may be a plethora of organic cationic and anionic groups that form zwitterionic polymers, but only a few have been realized.[21]

The multifaceted applications and zwitterion properties of ectoine inspired us to design some polymerizable, functional monomers of ectoine and to prepare highly hydrated and nonfouling zwitterionic polymers with certain unique properties. Herein, we report the synthesis of zwitterionic ectoine methacrylate monomers starting from hydroxyectoine. The zwitterionic ectoine methacrylate monomer has been explored for the synthesis of zwitterionic hydrogels using standard polymerizing conditions and for its mechanical and nonfouling performance.

EXPERIMENTAL SECTION

Materials. Hydroxyectoine, trifluoromethanesulfonic acid, triethylamine (TEA), methacryloyl chloride, *N*′,*N*′-methylenebis(acrylamide), 2,2′-azobis(2-methylpropionamidine) dihydrochloride, and phosphate-buffered saline (PBS, 10 mM phosphate, 138 mM sodium chloride, 2.7 mM potassium chloride, pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Trifluoroacetic acid (TFA) was purchased from TCI America (Portland, OR). Ethyl acetate, hexane, dichloromethane (DCM), methanol, and IRN-78 resin were purchased from Fisher Scientific (Hampton, NH). Thin-layer chromatography (TLC) was performed on glass plates (Whatman) coated with 0.25-mm-thick silica gel 60 Å (no. 70-230 mesh). All ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 500 MHz spectrometer in $CDCl_3$ unless specified otherwise, and chemical shifts are reported in ppm (δ) relative to internal standard tetramethylsilane (TMS). HPLC purification of ectoine methacrylate for NMR analysis was carried out using semipreparative reverse-phase high-performance liquid chromatography (HPLC) (5 μM particle size C18 Vydec column, 25 × 2.12 cm^2) with the following eluents: A = 0.1% TFA in H_2O and B = 0.1% TFA in CH_3CN.

Synthesis of Ectoine Methacrylate Monomer. Hydroxyectoine (2 g, 12.6 mmol) was dissolved in 8 mL of TFA with vigorous stirring at 0 °C. After the dissolution of the starting material, trifluoromethanesulfonic acid (0.37 g, 2.4 mmol) was added, and the reaction contents were stirred for 5 min. Then methacryloyl chloride (2.44 mL, 25.2 mmol) was added to the reaction mixture and stirred for 2 h. After 2 h, 30 mL of diethyl ether was added slowly to the reaction mixture at 0 °C to give the compound as a white powder. The crude white solid was further crystallized with methanol/diethyl ether and washed with diethyl ether to give the desired monomer (72% yield) as a white solid with 75% purity along with residual unreacted hydroxyectoine. This can be further purified by high-performance liquid chromatography (HPLC) to give pure ectoine methacrylate. ^{1}H NMR (500 MHz, D_2O): δ 6.07 (s, 1H), 5.7 (s, 1H), 5.59 (d, J = 2.4 Hz, 1H), 4.47 (s, 1H), 3.63 (d, J = 15.3 Hz, 1H), 3.46 (d, J = 14.95 Hz, 1H), 2.3 (s, 3H), 1.84 (s, 3H).

Preparation of the Poly(ectoine) Hydrogel. Poly(ectoine) hydrogels were made by the thermal polymerization of an aqueous solution composed of a 50 wt % ectoine monomer, X wt % (relative to the weight of ectoine monomer) cross-linker *N*′,*N*′-methylenebis(acrylamide), and 1 wt % (relative to the weight of the ectoine monomer) thermal initiator 2,2′-azobis(2-methylpropionamidine) dihydrochloride. Poly(ectoine) hydrogels with three different cross-linkers (X = 0.5, 1, 1.5) were prepared. The solution was polymerized between glass microscope slides separated by 0.8-mm-thick polytetrafluoroethylene spacers at 35 °C for 2 h followed by 50 °C for 40 h. The formed polyectoine hydrogels were soaked in PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4) to hydrate. The PBS was changed daily for 5 days to remove unreacted chemicals and let the polyectoine hydrogel reach swelling equilibrium. Afterward, polyectoine hydrogels were cut into a rectangular shape or by a biopsy punch into disks with a diameter and thickness of 5 mm and ∼1.5 mm, respectively.

Measurement of the Equilibrium Water Content (EWC). After soaking in PBS for 5 days, the fully swollen hydrogel disks were weighed and then dehydrated at 60 °C and 30 in. Hg vacuum for 3 days. Dried hydrogel disks were weighed. The equilibrium water contents (EWC) were calculated from the following equation $[(m_s - m_d)/m_s] \times 100(\%)$, where m_s is the mass of the fully swollen hydrogel and m_d is the mass of the dry hydrogel.

Mechanical Test. Compressive tests were performed on the fully swollen polyectoine hydrogel disks by a compressive mechanical tester (Instron 5543A, Instron Corp., Norwood, MA) with a 10 kN load cell to record the mechanical properties of the polyectoine hydrogels. The crosshead speed was set at 1 mm/min. Care was taken to ensure that the hydrogel disks did not slip during compression. Average data were acquired by testing three specimens for each sample.

Enzyme-Linked Immunosorbent Assay (ELISA) Analysis. Fibrinogen (Fg) is chosen to test the antifouling property of polyectoine hydrogels. All samples with the same surface area including positive control polypropylene (PP) and negative control nonfouling poly(carboxybetaine) hydrogel (PCBH) (with 1 wt % cross-linker *N*′,*N*′-methylenebis(acrylamide)) were first incubated with 1 mL of 1 mg/mL Fg in PBS buffer for 1 h, followed by five washes with pure PBS buffer. Then the samples were incubated with 1 mL of horseradish peroxidase (HRP)-conjugated antifibrinogen (1 μg/mL) in PBS buffer for 1 h. All samples were taken out, followed by another 5 washes with pure PBS buffer and then transferred to a new 24-well plate. *o*-Phenylenediamine (OPD; 1 mL, 1 mg/mL) in 0.1 M citrate phosphate pH 5.0 buffer containing 0.03% hydrogen peroxide was added. After an incubation of 15 min, the reaction was stopped by adding an equal volume of 1 M hydrochloric acid (HCl). The absorbance value at 492 nm was recorded by a plate reader. Five specimens of each sample were averaged for comparison. The results were then normalized to that of TCPS.

Cell Adhesion on Poly(ectoine) Hydrogels. Poly(ectoine) hydrogel disks with 1 wt % cross-linker were soaked in 70% ethanol for 2 h for sterilization and soaked in sterilized PBS until equilibrium. NIH-3T3 fibroblasts were seeded onto poly(ectoine) hydrogel disks at a concentration of 10^5 cells/mL. The cells were cultured at 37 °C, 5% CO_2, and 100% humidity for 72 h and then were observed and photographed on a Nikon Eclipse TE2000-U microscope at 100× magnification.

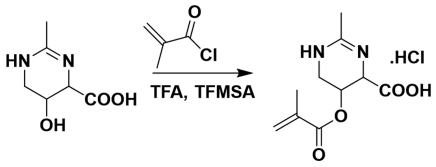
RESULTS AND DISCUSSION

Synthesis of Ectoine Methacrylate Monomer. Structurally, ectoine is 1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid. Our initial strategy to synthesize this monomer involved the search for a suitable starting material that already contains the ectoine zwitterionic moiety with a functional group to attach a polymerizable double bond such as acrylate or methacrylate. Hence, we started our synthesis using 2-hydroxyectoine that possesses both the zwitterionic moiety and the hydroxyl group to form acrylate or methacrylate monomers. We initially tried alkaline acylation procedures, i.e., Boc-protected hydroxyectoine reacted with methacryloyl chloride in an organic solvent containing trimethylamine. However, this worked poorly with low yields. We looked for alternate reaction conditions where acylation could be done under acidic conditions. Acylation under alkaline conditions requires the protection of both amine and carboxylic acid functionalities whereas acidic conditions would protect amines by protonation and also prevent the deprotonation of carboxylic acid. Hence, for hydroxyl amino acids, selective O-

DOI: 10.1021/acs.langmuir.7b02434
Langmuir 2017, 33, 11264−11269

acylation can be done in one step. Very few reports on the acidic acylation of amino acids were known until recently, when the acylation of hydroxyproline was reported.[22,23] The general procedure involved dissolving hydroxyproline in an aqueous acid, followed by the addition of an acylating agent to produce the desired monomer. However, the solubility of the starting materials in carboxylic acids resulted in low efficiency and low yield. Recently, trifluoroacetic acid was reported as a medium for the selective acylation of hydroxyl groups in various amino acids. We also observed that TFA was the best solvent for methacryloylation of 2-hydroxyectoine. The synthesis of ectoine methacrylate is shown in Scheme 1 as follows.

Scheme 1. One-Step Synthesis of the Ectoine Methacrylate Monomer



Treatment of a concentrated solution of 2-hydroxyectoine in TFA with 2 equiv of methacrylyoyl chloride gave the desired ectoine methacrylate with 55% purity. There was no change in conversion with an increase in either the acyl chloride content or the reaction time. Hansen et al. reported the increase in acylation of hydroxyproline by increasing the acylating power of the medium by adding a catalytic amount of trifluoromethanesulfonic acid (TFMSA).[23] Similarly, the addition of 4 wt % TFMSA in TFA increased the yield of ectoine methacrylate to 72%. Further crystallization with methanol/diethyl ether followed by washing with diethyl ether gives the solid ectoine methacrylate monomer with 75% purity along with residual unreacted hydroxyectoine. Although this monomer can be further purified by high-performance liquid chromatography (HPLC) to give pure ectoine methacrylate, it can be directly used for hydrogel preparation without further purification because residual unreacted hydroxyectoine will not participate in reactions. The product is confirmed by NMR spectroscopy (Figure 2). The protocol for selective O-acylation of hydroxyectoine on the basis of acidic acylation is simple and effective for making ectoine methacrylate in one step. Usually it requires tedious organic chemistry with multistep synthesis to produce these monomers.

Preparation and Characterization of Poly(ectoine) Hydrogels. Over the past few decades, there has been extensive effort in the development and application of polymeric biomaterials.[24] Polymeric hydrogels have been studied extensively because of their high water content, biocompatibility, and tunable mechanical properties similar to tissue components in the body.[25,26] Here, the ectoine methacrylate monomer is thermally polymerized into poly(ectoine) hydrogels. Their hydration, mechanical, and biological properties are measured.

Hydration and Mechanical Properties. The high water content of hydrogels makes them attractive for biological applications because this water content often mirrors that of biological tissues.[27,28] The equilibrium water content (EWC) of poly(ectoine) hydrogels is shown in Figure 3a, where EWC is calculated on the basis of the mass of dry and swollen hydrogels. As expected, the electrostatic interactions of ectoine create strong hydration, resulting in high water content within the system of poly(ectoine) hydrogels. An increase in cross-linker content decreases the degree of swelling hydrogels. For poly(ectoine) hydrogels with a cross-linker of 0.5 to 1.5 wt %,

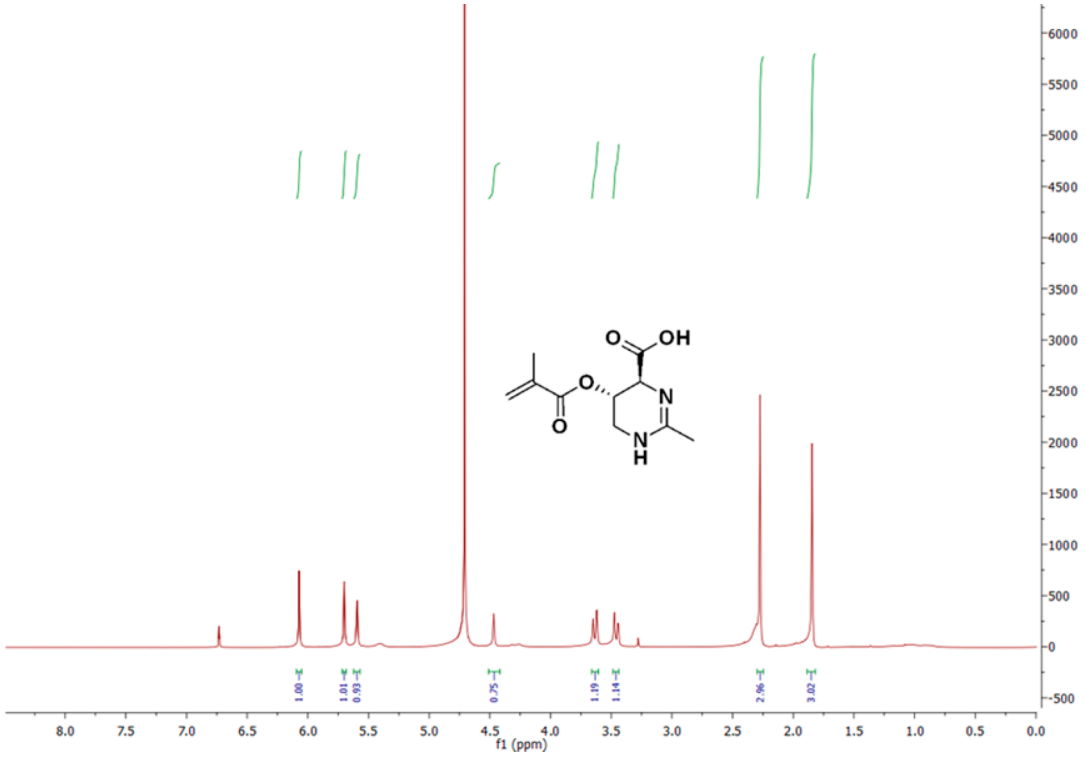


Figure 2. NMR spectrum of ectoine methacrylate.

DOI: 10.1021/acs.langmuir.7b02434
Langmuir 2017, 33, 11264−11269

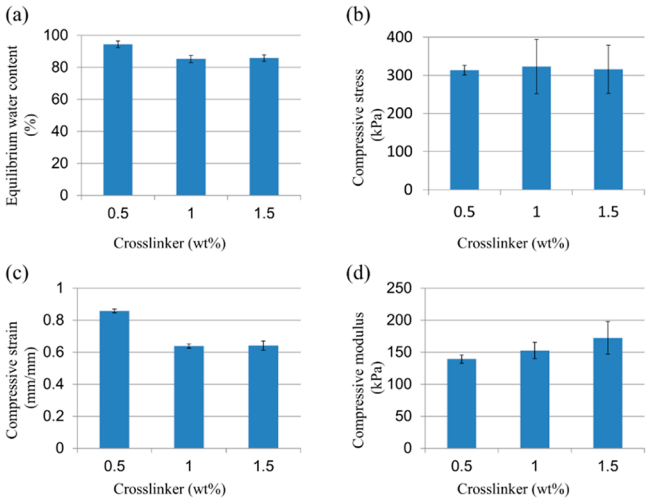


Figure 3. Hydration and mechanical properties of poly(ectoine) hydrogels: (a) Equilibrium water content of poly(ectoine) hydrogels with different cross-linker amounts (0.5, 1, and 1.5 wt %). (b–d) Compressive stress, compressive strain at the fracture point, and the compressive modulus of the poly(ectoine) hydrogel with different cross-linker amounts (0.5, 1, and 1.5 wt %), respectively.

EWC decreases from 94 to 85%, which is in a range suitable for biological tissues.[29]

The mechanical properties of poly(ectoine) hydrogels were measured via compression tests. The compressive stress, compressive strain at the fracture point, and compressive modulus are shown in Figure 3b–d, respectively. Similar to regular hydrogel systems, low cross-linking restricts the relative motion of polymer chains but maintains enough freedom for segments to enable deformation in the hydrogel system. With the increased cross-linking density from 0.5 to 1.5 wt %, the fracture compressive strain decreases from 0.85 to 0.64. The hydrogels become more rigid when the modulus increases from 139 to 172 kPa. Interestingly, the fracture compressive stress exhibits a similar value of ~300 kPa within the studied range of teh cross-linking density. Note that the fracture compressive stress of poly(ectoine) hydrogels is better than that of other types of zwitterionic hydrogels with a methacrylate backbone under the same cross-linker content.[30−32] The good fracture compressive stress of poly(ectoine) hydrogels is similar to that of the previously reported sulfobetaine vinylimidazole (PSBVI) hydrogel,[31] where the introduction of the stiffening imidazole ring improves the mechanical properties of regular sulfobetaine hydrogels with a methacrylate backbone by an order of magnitude. The good fracture compressive stress of poly(ectoine) hydrogels may be attributed to the ring structure of ectoine that is believed to provide some rigidity similar to the effect of vinylimidazole in the PSBVI system. The mechanical properties of poly(ectoine) hydrogels presented above demonstrate their potential applications in soft tissue engineering.[33]

Biological Properties. Hydrogels are often exposed to complicated environments, where hydrogels are considered to be foreign matter, leading to a series of undesired complications. For example, when in contact with whole blood, blood proteins and cells immediately attach to the surface of hydrogels, followed by clotting factor activation, platelet adhesion, activation and aggregation, and ultimately thrombus formation. Because protein adsorption is the first stage of undesired complications, it is important that biomaterials should be able to effectively resist nonspecific protein adsorption. Herein fibrinogen, a blood protein known to easily adsorb onto various surfaces through nonspecific interactions, is used to test the protein adsorption of poly(ectoine) hydrogels. The surface adsorption of fibrinogen was quantified by an enzyme-linked immunosorbent assay (ELISA) method, and the results are shown in Figure 4. All

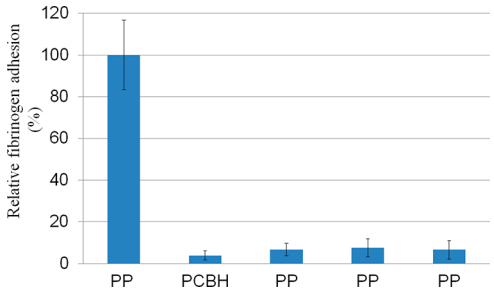


Figure 4. Relative fibrinogen adsorption of poly(ectoine) hydrogels with different cross-linker amounts (0.5, 1, 1.5 wt %) from ELISA. The PP sheet and PCBH were used as positive and negative controls, respectively. Data were normalized to that of PP.

three tested poly(ectoine) hydrogels with cross-linker contents of 0.5, 1, and 1.5 wt % exhibit excellent nonfouling properties closer to those of PCBH by decreasing 97.0, 95.8, and 95.6% of the fibrinogen adsorption, respectively, with respect to that of polypropylene (PP). This finding indicates that zwitterionic poly(ectoine) hydrogels possess certain biological characterstics that effectively resist nonspecific protein adsorption, in agreement with typical zwitterionic polymers.[12]

Moreover, fibroblast NIH-3T3 cells, one of the most frequently used lines, were selected for the cell adhesion test of poly(ectoine) hydrogels. Figure 5 shows both cell adhesion

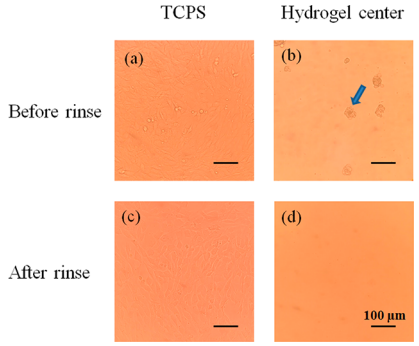


Figure 5. Phase-contrast images of NIH-3T3 cells adhered on TCPS and polyectoine hydrogel surfaces after 3 days of culturing. Cells adhered with a large quantity and spread by extending thin pseudopodia on the surfaces of TCPS before (a) and after rinsing (c). However, cells could not adhere to but aggregated on the poly(ectoine) hydrogel surface before rinsing (b) and were completely washed away after rinsing (d).

behaviors before and after rinsing. Before rinsing, NIH-3T3 cells cannot attach to the surface of the poly(ectoine) hydrogel and tend to form cell aggregates. Conversely, NIH-3T3 cells can recognize and bind to the TCPS surface via nonspecific adhesion, resulting in spreading with the extension of thin pseudopodia. After rinsing, all of the aggregated cells are washed away on the surface of the poly(ectoine) hydrogel but still bind strongly to that of TCPS. The nonfouling properties

DOI: 10.1021/acs.langmuir.7b02434
Langmuir 2017, 33, 11264−11269

of the poly(ectoine) hydrogel are responsible for the formation of cell aggregates on its surface. NIH-3T3 cells migrating to the surface of the poly(ectoine) hydrogel need to establish focal contacts with the neighboring matrix. However, NIH-3T3 cells cannot adhere to the poly(ectoine) hydrogel. Therefore, they establish cell contacts only with the poly(ectoine) hydrogel to form cell aggregates. The nonfouling properties of the poly(ectoine) hydrogel are similar to those of zwitterionic PCBH.[34]

CONCLUSIONS

We have synthesized a novel polymerizable, zwitterionic ectoine methacrylate monomer via acidic acylation of 2-hydroxyectoine. Because this monomer can be easily synthesized in a single step and no complicated organic chemistry is involved, the synthetic route can be easily scaled up to produce a large amount of ectoine methacrylate required for polymerization. To demonstrate its potential application as a biomaterial, the ectoine methacrylate monomer was polymerized into poly(ectoine) hydrogels. Poly(ectoine) hydrogels exhibited similar high water content and better mechanical properties than regular zwitterionic hydrogels. It also exhibited excellent nonfouling properties with 95% lower nonspecific protein absorption with respect to polypropylene closer to that of zwitterionic PCBH. In addition, the poly(ectoine) hydrogel is also shown to resist cell adhesion. All of these results indicate that poly(ectoine) has great potential as a new biomaterial.

AUTHOR INFORMATION

Corresponding Author

*E-mail: sjiang@uw.edu.

ORCID

Peng Zhang: 0000-0002-5409-7480

Shaoyi Jiang: 0000-0001-9863-6899

Author Contributions

†These authors contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Office of Naval Research (N00014-16-1-3084), the National Science Foundation (DMR 1708436), and the Defense Threat Reduction Agency (HDTRA1-13-1-0044).

REFERENCES

(1) Kolp, S.; Pietsch, M.; Galinski, E. A.; Gütschow, M. Compatible solutes as protectants for zymogens against proteolysis. *Biochim. Biophys. Acta, Proteins Proteomics* **2006**, *1764*, 1234−1242.

(2) Lippert, K.; Galinski, E. A. Enzyme stabilization be ectoine-type compatible solutes: protection against heating, freezing and drying. *Appl. Microbiol. Biotechnol.* **1992**, *37*, 61−65.

(3) Pastor, J. M.; Salvador, M.; Argandoña, M.; Bernal, V.; Reina-Bueno, M.; Csonka, L. N.; Iborra, J. L.; Vargas, C.; Nieto, J. J.; Cánovas, M. Ectoines in cell stress protection: Uses and biotechnological production. *Biotechnol. Adv.* **2010**, *28*, 782−801.

(4) Graf, R.; Anzali, S.; Buenger, J.; Pfluecker, F.; Driller, H. The multifunctional role of ectoine as a natural cell protectant. *Clinics in Dermatology* **2008**, *26*, 326−333.

(5) Furusho, K.; Yoshizawa, T.; Shoji, S. Ectoine alters subcellular localization of inclusions and reduces apoptotic cell death induced by the truncated Machado−Joseph disease gene product with an expanded polyglutamine stretch. *Neurobiol. Dis.* **2005**, *20*, 170−178.

(6) Buommino, E.; Schiraldi, C.; Baroni, A.; Paoletti, I.; Lamberti, M.; De Rosa, M.; Tufano, M. A. Ectoine from halophilic microorganisms induces the expression of hsp70 and hsp70B′ in human keratinocytes modulating the proinflammatory response. *Cell Stress Chaperones* **2005**, *10*, 197−203.

(7) Kanapathipillai, M.; Lentzen, G.; Sierks, M.; Park, C. B. Ectoine and hydroxyectoine inhibit aggregation and neurotoxicity of Alzheimer's β-amyloid. *FEBS Lett.* **2005**, *579*, 4775−4780.

(8) Grether-Beck, S.; Timmer, A.; Felsner, I.; Brenden, H.; Brammertz, D.; Krutmann, J. Ultraviolet A-Induced Signaling Involves a Ceramide-Mediated Autocrine Loop Leading to Ceramide De Novo Synthesis. *J. Invest. Dermatol.* **2005**, *125*, 545−553.

(9) Buenger, J.; Driller, H. Ectoin: An Effective Natural Substance to Prevent UVA-Induced Premature Photoaging. *Skin Pharmacology and Physiology* **2004**, *17*, 232−237.

(10) Galinski, E. A.; Pfeiffer, H. P.; TrÜPer, H. G. 1,4,5,6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid. *Eur. J. Biochem.* **1985**, *149*, 135−139.

(11) Ratner, B. D.; Bryant, S. J. BIOMATERIALS: Where We Have Been and Where We Are Going. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41−75.

(12) Jiang, S.; Cao, Z. Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Adv. Mater.* **2010**, *22*, 920−932.

(13) Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. Zwitterionic Polymers Exhibiting High Resistance to Nonspecific Protein Adsorption from Human Serum and Plasma. *Biomacromolecules* **2008**, *9*, 1357−1361.

(14) Yang, W.; Xue, H.; Li, W.; Zhang, J.; Jiang, S. Pursuing "Zero" Protein Adsorption of Poly(carboxybetaine) from Undiluted Blood Serum and Plasma. *Langmuir* **2009**, *25*, 11911−11916.

(15) Sun, F.; Hung, H.-C.; Sinclair, A.; Zhang, P.; Bai, T.; Galvan, D. D.; Jain, P.; Li, B.; Jiang, S.; Yu, Q. Hierarchical zwitterionic modification of a SERS substrate enables real-time drug monitoring in blood plasma. *Nat. Commun.* **2016**, *7*, 13437.

(16) Chou, Y.-N.; Sun, F.; Hung, H.-C.; Jain, P.; Sinclair, A.; Zhang, P.; Bai, T.; Chang, Y.; Wen, T.-C.; Yu, Q.; Jiang, S. Ultra-low fouling and high antibody loading zwitterionic hydrogel coatings for sensing and detection in complex media. *Acta Biomater.* **2016**, *40*, 31−37.

(17) Zhang, P.; Sun, F.; Tsao, C.; Liu, S.; Jain, P.; Sinclair, A.; Hung, H.-C.; Bai, T.; Wu, K.; Jiang, S. Zwitterionic gel encapsulation promotes protein stability, enhances pharmacokinetics, and reduces immunogenicity. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 12046−12051.

(18) Zhang, P.; Jain, P.; Tsao, C.; Sinclair, A.; Sun, F.; Hung, H.-C.; Bai, T.; Wu, K.; Jiang, S. Butyrylcholinesterase nanocapsule as a long circulating bioscavenger with reduced immune response. *J. Controlled Release* **2016**, *230*, 73−78.

(19) Zhang, L.; Cao, Z.; Bai, T.; Carr, L.; Ella-Menye, J.-R.; Irvin, C.; Ratner, B. D.; Jiang, S. Zwitterionic hydrogels implanted in mice resist the foreign-body reaction. *Nat. Biotechnol.* **2013**, *31*, 553−556.

(20) Chen, S.; Zheng, J.; Li, L.; Jiang, S. Strong Resistance of Phosphorylcholine Self-Assembled Monolayers to Protein Adsorption: Insights into Nonfouling Properties of Zwitterionic Materials. *J. Am. Chem. Soc.* **2005**, *127*, 14473−14478.

(21) Laschewsky, A. Structures and Synthesis of Zwitterionic Polymers. *Polymers* **2014**, *6*, 1544.

(22) Kristensen, T. E. Chemoselective O-acylation of hydroxyamino acids and amino alcohols under acidic reaction conditions: History, scope and applications. *Beilstein J. Org. Chem.* **2015**, *11*, 446−468.

(23) Kristensen, T. E.; Hansen, F. K.; Hansen, T. The Selective O-Acylation of Hydroxyproline as a Convenient Method for the Large-Scale Preparation of Novel Proline Polymers and Amphiphiles. *Eur. J. Org. Chem.* **2009**, *2009*, 387−395.

(24) Seal, B. L.; Otero, T. C.; Panitch, A. Polymeric biomaterials for tissue and organ regeneration. *Mater. Sci. Eng., R* **2001**, *34*, 147−230.

DOI: 10.1021/acs.langmuir.7b02434
Langmuir 2017, 33, 11264−11269

(25) Patel, A.; Mequanint, K. Hydrogel Biomaterials. *InTech* **2011**, 275−296.
(26) Saha, N.; Saarai, A.; Roy, N.; Kitano, T.; Saha, P. Polymeric biomaterial based hydrogels for biomedical applications. *J. Biomater. Nanobiotechnol.* **2011**, *2*, 85−90.
(27) Otto, W.; Drahoslav, L. Process for producing shaped articles from three-dimensional hydrophilic high polymers. Google Patents, 1961.
(28) Castillo, E. J.; Koenig, J. L.; Anderson, J. M.; Lo, J. Protein adsorption on hydrogels. II. Reversible and irreversible interactions between lysozyme and soft contact lens surfaces. *Biomaterials* **1985**, *6*, 338−45.
(29) Ahmed, E. M. Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research* **2015**, *6*, 105−121.
(30) Bai, T.; Zhang, P.; Han, Y.; Liu, Y.; Liu, W.; Zhao, X.; Lu, W. Construction of an ultrahigh strength hydrogel with excellent fatigue resistance based on strong dipole-dipole interaction. *Soft Matter* **2011**, *7*, 2825−2831.
(31) Carr, L.; Cheng, G.; Xue, H.; Jiang, S. Engineering the Polymer Backbone To Strengthen Nonfouling Sulfobetaine Hydrogels. *Langmuir* **2010**, *26*, 14793−14798.
(32) Carr, L. R.; Xue, H.; Jiang, S. Functionalizable and nonfouling zwitterionic carboxybetaine hydrogels with a carboxybetaine dimethacrylate crosslinker. *Biomaterials* **2011**, *32*, 961−968.
(33) Zhao, X.; Lang, Q.; Yildirimer, L.; Lin, Z. Y.; Cui, W.; Annabi, N.; Ng, K. W.; Dokmeci, M. R.; Ghaemmaghami, A. M.; Khademhosseini, A. Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering. *Adv. Healthcare Mater.* **2016**, *5*, 108−118.
(34) Chien, H.-W.; Tsai, W.-B.; Jiang, S. Direct cell encapsulation in biodegradable and functionalizable carboxybetaine hydrogels. *Biomaterials* **2012**, *33*, 5706−5712.

DOI: 10.1021/acs.langmuir.7b02434
Langmuir 2017, 33, 11264−11269