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Zwitterionic Polyurethane Hydrogels Derived from Carboxybetaine-Functionalized Diols

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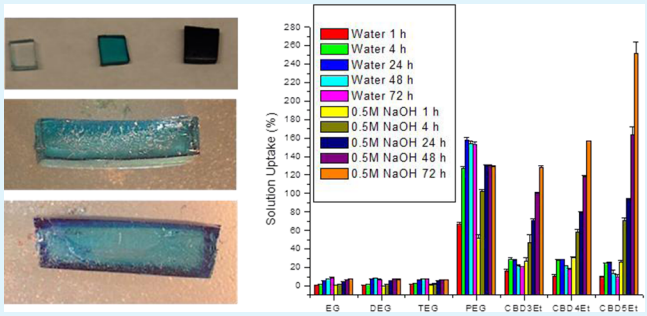
Supporting Information

ABSTRACT: The synthesis of novel zwitterionic polyurethane hydrogels with tunable water uptake via the polymerization of protected carboxybetaine-functionalized diols with polyisocyanate oligomers is presented. Post-polymerization hydrolysis of a diol-segment side chain establishes zwitterionic carboxybetaine functionalities that facilitate water uptake via the enhanced hydration capacities surrounding the opposing charges of the diol component. Tunable hydration of these materials, ranging from 24 to 250% solution uptake (based on the dry polymer weight), is achieved by controlling the structural characteristics of the diol precursor, such as ammonium/carboxylate spacing and ethyl ester hydrolysis conditions (i.e., exposure time to an aqueous base).

KEYWORDS: *zwitterion, hydrogel, antifouling, carboxybetaine*



Nonspecific adsorption of proteins and cells onto surfaces continues to be an unresolved problem that is particularly detrimental in medical, industrial, and marine applications.[1] Bacterial adhesion specifically has been shown to result in the formation of biofilms, which lead to devastating results across applications. For example, biofilm formation on medical implants results in the formation of persistent infections that are up to 1000 times more resistant to conventional antibiotics than free bacteria.[2,3] In a similar manner, marine biofilms have been shown to condition underwater structures for the settlement of a variety of marine species ranging from barnacles and mussels to algae and tubeworms.[4] The adhesion of these organisms collectively results in increased vessel drag, reducing operational speeds and increasing both fuel consumption and maintenance costs.[5−9]

Passive strategies to minimize the initial colonization of these fouling species onto surfaces have utilized surface modifications that traditionally impart either low surface energies or high degrees of hydration. For example, low surface energy silicones[10−12] and fluoropolymers[13−15] have been implicated as promising antifouling materials, as was previously predicted by the Baier curve.[16] Conversely, the high hydration capacities of poly(ethylene glycol) (PEG)[17−19] and zwitterionic materials[20−22] have also shown excellent antifouling character.

Zwitterionic hydrogels are particularly suited as antifouling materials because of their ultralow fouling characteristics (less than 5 ng/cm^2 protein adsorption)[23,24] arising from the high hydration capacities surrounding the opposing charges of the material.[25] In addition, there is less susceptibility for oxidative damage to zwitterionic material than there is for PEG-based hydrogels.[26,27] Despite this, the lack of mechanical integrity of hydrogels, in general, continues to be a hindrance for their implementation in many potential applications.[28]

In this work, we present novel zwitterionic polyurethane hydrogels with tunable hydration capacities derived from the polycondensation of a commercially available linear aliphatic polyisocyanate (Desmodur N 3600 polyisocyanate, Bayer MaterialScience) with ethyl ester substituted quaternary ammonium diols. Base-promoted hydrolysis of the pendant ester postpolymerization results in the release of ethanol and production of the carboxylate anion, thereby generating the carboxybetaine moiety (Scheme 1).

The desired diol precursors were synthesized neat or in *N*,*N*-dimethylformamide (DMF) by heating 1.0 equiv of *N*-methyldiethanolamine (MDEA) with 1.0 equiv of a brominated ethyl ester at 60 °C for 24 h with magnetic stirring. Interestingly, the protected carboxybetaine diol, CBD2Et, could not be synthesized via this method. The acidic nature of the α-proton adjacent to the carbonyl of ethyl 3-bromopropionate promoted elimination of the proximal bromine atom upon reaction with MDEA, resulting in high yields of an alkene byproduct. However, all other intended diol species were isolated in high yield as either white solids (CBD1Et and CBD3Et) or clear oils (CBD4Et and CBD5Et), with their structures confirmed by ^{1}H and ^{13}C NMR.

In order to facilitate mixing of the polymer components and initiate cross-linking, polyisocyanate (1.0 equiv of NCO) and diol (1.0 equiv of OH) were mixed with a small amount of

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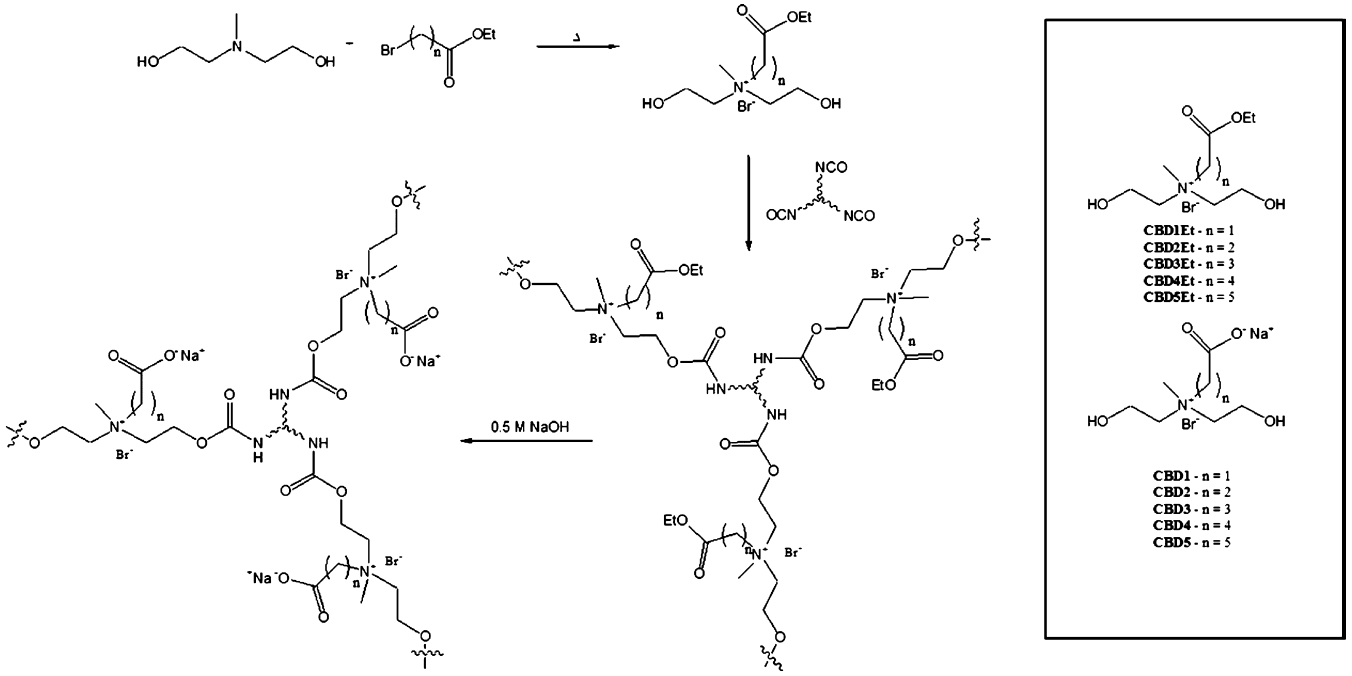
Scheme 1. Synthesis of Ethyl Ester Protected Carboxybetaine Diols, Subsequent Polymerization with Desmodur N 3600 Polyisocyanate, and Ester Deprotection To Generate Zwitterionic Polyurethane Hydrogels



DMF and magnetically stirred at 60 °C for 30 min. The viscous solution was then solution-cast or poured into a mold and cured at 80 °C for 24 h. However, the extremely high melting point and insolubility of CBD1Et in any aprotic solvents prevented its polymerization into a polyurethane matrix. Cured polyurethanes were colorless to light yellow, optically transparent, and flexible. Control materials were also synthesized via the same procedure using ethylene glycol (EG), diethylene glycol (DEG), triethylene glycol (TEG), and PEG (1000 g/mol) as alternative diols.

Cured polyurethanes (test example, Polydesmo CBD3Et, etc.; control example, Polydesmo EG; etc.) were subjected to a variety of characterization techniques including gel fraction analysis, water uptake, thermogravimetric analysis, differential scanning calorimetry, dynamic mechanical analysis (DMA), and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) to determine their utility as hydrogels. As was expected based on the presence of ethyl esters protecting the carboxylate functionalities, the untreated test polyurethanes exhibited low water uptake compared to the PEG-based polyurethane hydrogel (ca. 25−29% vs 158%). However, upon exposure to a weak aqueous base, the solution uptake and swelling properties of the polyurethanes increased drastically (Figure 1). Base-induced ester hydrolysis cleaves the protecting group and restores the carboxylate anion, thereby completing the carboxybetaine moiety and increasing the hydration capacities of the functionalized networks to between 74 and 91% of the dry polymer weight after 24 h of exposure to 0.5 M NaOH. Additional exposure to aqueous base enhanced water uptake further with total water uptake values increasing to between 129 and 251% of the dry polymer weight after 3 days of base exposure. The total water uptake was determined to be a function of not only the deprotection time but also the structure of the carboxybetaine moiety. Although increasing the carbon spacing between the ammonium and carboxylate ions from three to five carbon atoms only enhanced the solution

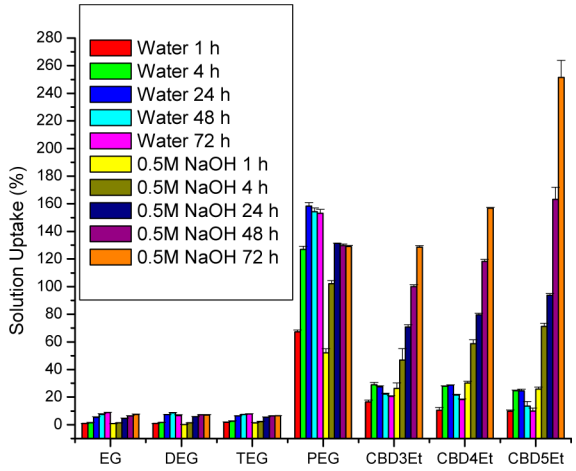


Figure 1. Solution uptake of carboxybetaine and control polyurethanes as a function of the diol structure and solution composition.

uptake by 31% after 1 day of hydrolysis, this value increased with increasing time up to 96% after a 3 day deprotection soak. Overall, the incorporation of CBD5 imparted approximately 30% additional solution uptake per 1 day of exposure to NaOH compared to CBD3 and 20%/day compared to CBD4. This is attributed to the greater spatial distribution of charge in the larger carboxybetaine moiety, which allows for elevated hydration capacity. Furthermore, the consistent increase in the daily solution uptake indicates that the rate of deprotection is not significantly influenced by these structural changes of the carboxybetaine moiety and is more likely a factor of the protecting group, which in this case is common among all derivatives. Importantly, exposure of control materials to an aqueous base shows no increase in the solution uptake compared to water and no increase in uptake with continued solution exposure time. Additionally, no degradation of the polymer matrix was noted during exposure to low concen-

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trations of aqueous base. Furthermore, because no significant increase in the solution uptake was noted for test materials even after 4 weeks of soak time in water, sufficient deprotection of the ethyl ester group does not seem to occur at neutral pHs. This indicates that the overall water uptake of these materials may be controlled based on the treatment time with a deprotection solution postpolymerization.

Although no matrix degradation was noted and polyurethanes are traditionally characterized as resistant to dilute alkaline conditions, both test and control samples that were exposed to aqueous NaOH did experience a general decrease in contact angle over time (Figure 2). In numerous types of

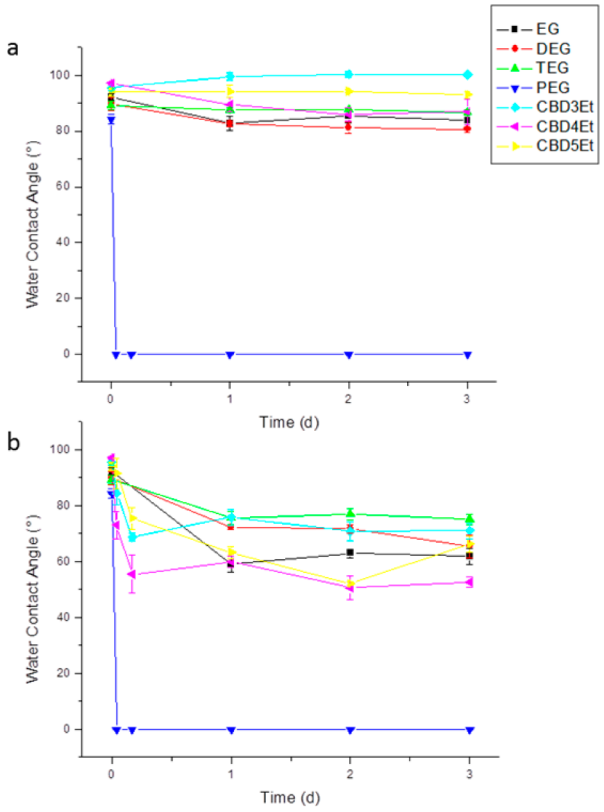

Figure 2. Static water contact angle of polyurethane samples after soaking in (a) water and (b) 0.5 M NaOH.

polymeric systems, alkaline conditions are commonly used to increase the hydrophilicity of surfaces; however, this is most often attributed to hydrolytic degradation of the polymer backbone. In this system, little if any degradation was noted over the course of 3 days of soaking in 0.5 M NaOH, indicating that the cause of this depression may be a result of cooperative hydrogen bonding that is seen at high pHs compared to neat water.[29] Specifically, hydroxide ions are able to both accept and donate hydrogen bonds with water and urethane hydrogen atoms, thereby decreasing the water contact angles compared to samples soaked in neat water.[30] The slight deviations between the contact angle values of test and control samples after soaking in NaOH occur as a result of the increased hydrophilicity associated with free carboxybetaine moieties postdeprotection. Importantly, submersion in water for up to 3 days showed no contact angle depression for any test samples, suggesting minimal ester deprotection under neutral aqueous conditions. Unfortunately, the hydrophobicity of the cross-linker Desmodur appears to contribute substantially to the overall water contact angle of the test coatings because the contact angles of hydrated coatings never drop below approximately 50° despite being highly hydrated.

Surface analysis of test and control samples using FTIR-ATR indicates that exposure to aqueous NaOH does indeed result in deprotection of the ester group, while the nucleophilicity of neat water is insufficient to promote substantial hydrolysis. As can be seen in Figure 3a, unsoaked samples of Polydesmo

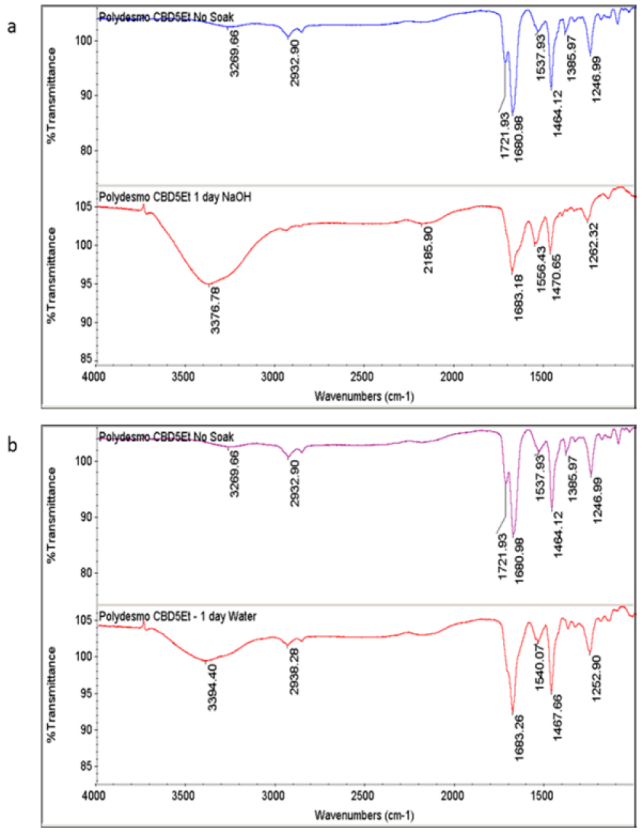

Figure 3. Comparison of FTIR-ATR spectra of Polydesmo CBD5Et: (a) unsoaked versus NaOH soaked; (b) unsoaked versus water soaked.

CBD5Et had distinct urethane and ester absorbance patterns at 1680 and 1721 cm^{-1}, respectively. However, upon exposure to NaOH, the ester peak largely disappears and a shoulder at approximately 1600 cm^{-1}, indicative of a carboxylate anion, becomes evident. Furthermore, the hydroxyl stretch corresponding to water increases substantially upon exposure to the aqueous base. When soaked in water however (Figure 3b), there is little increase in absorbance attributed to carboxylate anions and there is a slight absorbance shift of the ester peak from 1721 cm^{-1} in the dry material to approximately 1700 cm^{-1}, indicative of hydrogen bonding of the ester group with water.[31] Additionally, the area of the hydroxyl peak at 3400 cm^{-1} is approximately 35% of that when soaked in NaOH, which correlates well with the total difference in the solution uptake.

One of the main probelms associated with the implementation of hydrogels is the lack of mechanical integrity upon hydration. To combat this, we have designed a hydrogel with tunable solution uptake based on the extent of deprotection of a carboxybetaine moiety polymerized into the matrix. Because ester deprotection in the bulk material is limited by diffusion, the potential exists to limit deprotection at or near the surface

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of these materials, creating a material that behaves as a hydrogel at the material interface but not in the bulk. Prevention of this water uptake in the bulk of the material should provide enhanced mechanical stability compared to matrices that are fully hydrated throughout the bulk material. To qualitatively investigate this gradient deprotection mechanism, test materials were exposed to aqueous NaOH for short time periods, at which point they were rinsed in water and soaked in an aqueous solution of methylene blue. The extent of water uptake in different regions of the material could then be examined visually based on the intensity of the blue color. As expected, samples that exhibited low solution uptake (ex. Polydesmo EG) remained optically transparent and void of any blue color after soaking in the methylene blue solution, while those that exhibited higher degrees of water uptake exhibited optical transparency but light-blue color (untreated Polydesmo CBD3Et) or opacity and dark-blue color (Polydesmo PEG) depending on the extent of solution uptake (Figure 4a). Test

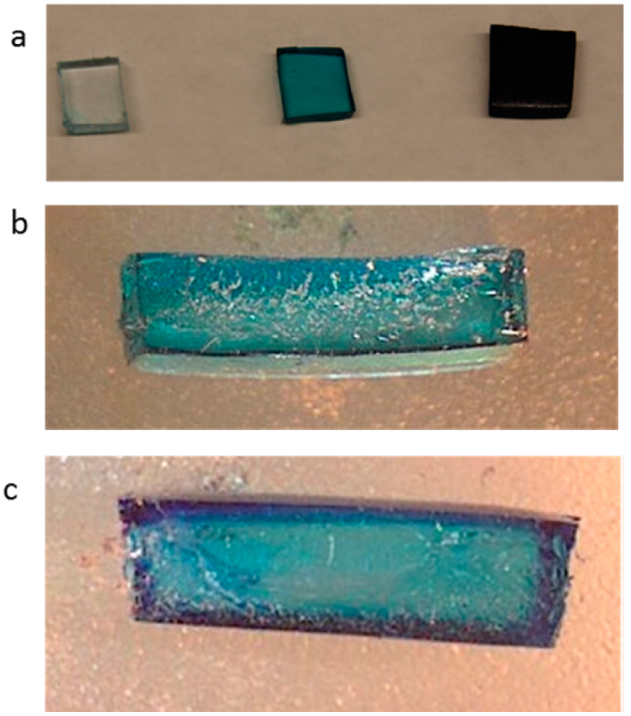
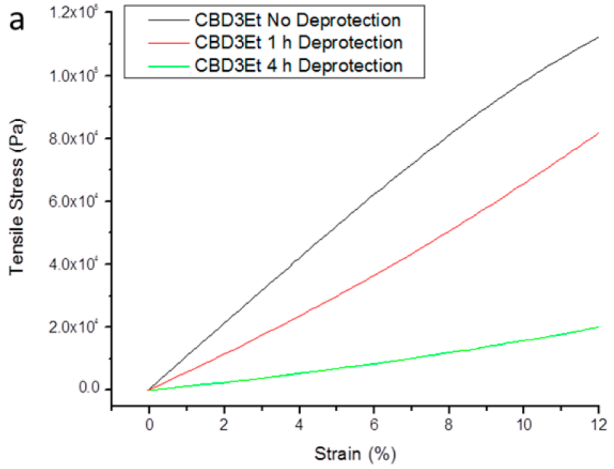


Figure 4. Optical images of (a; left to right) Polydesmo EG, Polydesmo CBD3Et, and Polydesmo PEG after soaking in an aqueous methylene blue solution. (b) Cross section of untreated CBD3Et. (c) Cross section of NaOH-treated CBD3Et. Presoaked sample sizes are 1.00 × 1.00 × 0.25 cm.

samples treated with NaOH behaved in the hypothesized manner, with those soaked in base exhibiting a deeper blue color than those untreated and the extent of the blue color correlating with the soak duration in NaOH. Importantly, when cross-sectional images were taken of Polydesmo CBD3Et without any treatment, a light-blue color was observed uniformly throughout the bulk polymer (Figure 4b). However, after a short-term NaOH treatment (4 h), a darker blue color was seen around the outermost edges of the sample, with the interior of the bulk polymer remaining a lighter blue color, similar in intensity to that of the untreated sample (Figure 4c). This supports the hypothesis of diffusion-controlled deprotection and the preparation of a corresponding gradient hydrogel.

DMA of these zwitterionic polyurethane hydrogels confirms that the Young's modulus is indeed capable of being controlled by altering the deprotection time of bulk polymer samples. As expected, the Young's modulus of hydrated polymer samples (approximately 2.5 mm thickeness) was shown to decrease with increasing deprotection time (Figure 5). Specifically, the

a



b

Sample	Modulus (MPa)
CBD3Et	1.04
CBD3Et 1h Deprotection	0.59
CBD3Et 4h Deprotection	0.14
CBD4Et	0.83
CBD4Et 1h Deprotection	0.39
CBD4Et 4h Deprotection	0.21
CBD5Et	0.69
CBD5Et 1h Deprotection	-
CBD5Et 4h Deprotection	0.16

Figure 5. (a) Partial tensile stress/strain plots of hydrated Polydesmo CBD3Et hydrogels and (b) Young's modulus values obtained from tensile stress/strain measurements calculated from the initial 5% strain.

modulus of Polydesmo CBD3Et decreased by 44% after 1 h and 86% after 4 h of deprotection. In a similar fashion, Polydesmo CBD4Et and Polydesmo CBD5Et both experienced gradual declines in the calculated modulus values with increasing deprotection time, with approximately 75% reductions occurring after 4 h. Stress/strain measurements of hydrogels deprotected for longer time periods could not be analyzed due to mechanical failure upon clamping into position in the DMA instrument. Although the materials examined exhibit finite decreases in the mechanical properties with increasing deprotection time, the complete removal of base to halt continued hydrolysis is a concern. Extensive rinsing and/or soaking in water has shown to be sufficient to minimize continued hydrolysis in preliminary experiments, and the utilization of alternative basic solutions for deprotection may likely overcome this problem.

Despite possessing unique gradient hydrogel characteristics, the ultimate utility of these and many other hydrogel systems relies on their capacity to reduce adhesion of cells onto their surfaces. Samples deprotected for 4 h were able to reduce *Pseudomonas aeruginosa* adhesion by up to 93% compared to controls; however, upon increasing deprotection time, the

amount of bacterial cells that were able to adhere to the surfaces also increased (Figure 6). This increased cellular

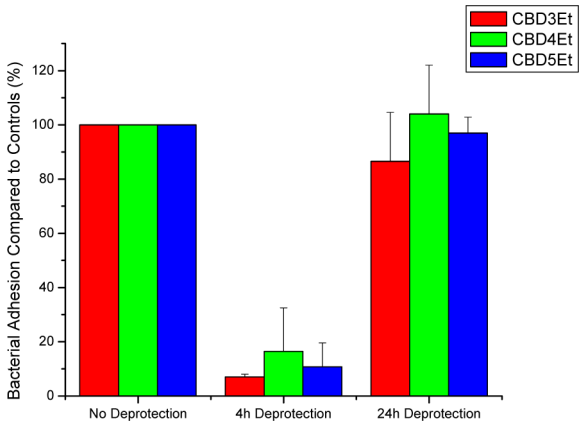


Figure 6. *P. aeruginosa* adhesion to hydrated polyurethane materials.

adhesion is likely attributed to the high cross-linker content of these hydrogel systems (up to 53.8% by mass dry weight). Increased solution uptake postdeprotection causes a greater incidence of scaffold swelling, and as a result, the zwitterionic components become increasingly farther apart, allowing the cross-linkers to exert more hydrophobic characteristics to these materials, thus increasing the degree to which bacterial cells may adhere. As a result, there is a minimum incidence of bacterial adhesion occurring with limited deprotection when the scaffold is partially hydrated but not swollen enough to distance the zwitterionic components. This behavior is confirmed by the relatively high contact angles observed for well-hydrated materials (Figure 2).

In conclusion, we have demonstrated the successful synthesis of zwitterionic polyurethane hydrogels derived from protected carboxybetaine diols. The degree of water uptake of these polymers was found to be influenced by both the carboxybetaine structure and deprotection time. Diffusion-controlled deprotection of the carboxybetaine moiety allows for the preparation of gradient hydrogels, with the depth and overall amount of hydration dependent on the deprotection time. Mechanical properties are shown to be tunable based on the deprotection time and the resulting water uptake of the hydrogels. Additionally, the resistance to bacterial colonization was also shown to be dependent on the extent of water uptake. Further experiments are underway to examine the future potential of these systems as nonfouling materials by implementing more hydrophilic cross-linking systems (polyisocyanates) in an effort to increase the water uptake and further reduce bacterial adhesion for fully swollen matrices.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and IR spectra of polyurethanes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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