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Underwater Bonding with Charged Polymer Mimics of Marine Mussel Adhesive Proteins

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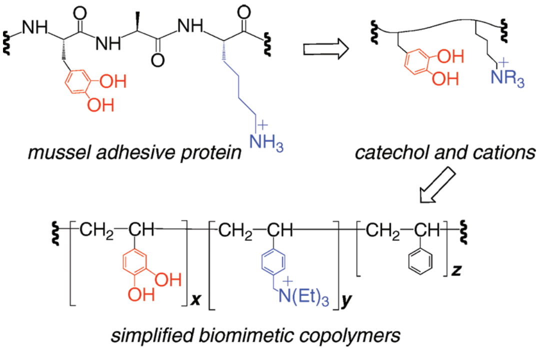
Supporting Information

Within the oceans lives an array of creatures producing materials exhibiting properties unmatched by human technology. One conspicuous example is the common blue mussel. This intertidal mollusk secretes a protein-based adhesive for securing itself to wet rocks.[1] By contrast, most man-made adhesives fail completely when tasked with such underwater bonding. Adapting mussel glue for applications such as surgical adhesion has been continually frustrated by a lack of available material. Synthetic polymers can provide access to large quantities of adhesive, more than that available by extracting protein from the animals. Mussel adhesive proteins are rich in the unusual amino acid 3,4-dihydroxyphenylalanine (DOPA), which is central to the cross-linking reactions of cohesive curing and adhesive surface bonding.[2,3] Incorporating DOPA-like chemistry into synthetic polymers[4] is being pursued with polypeptides,[5−7] polyacrylates,[8−14] poly(ethylene glycol)s,[15−19] and polystyrene-based systems.[20]

Mussel adhesive proteins tend to be, overall, positively charged. The cationic amino acid residues lysine and arginine are in excess of the anionic counterparts and comprise approximately 18−23% of the total.[21−24] This presence of lysine amines may suggest reactivity with oxidized DOPA to generate cross-links in the adhesive. However, studies of lysine with DOPA-containing proteins and peptides did not show any reactivity.[25−27] Perhaps the role of lysine residues in mussel adhesive proteins is simply to provide charge. High-energy surfaces including silicates and aluminates, major components of the rocks to which mussels stick, harbor mildly anionic charge density.[28−30] Consequently, we can imagine that positively charged proteins may exhibit favorable electrostatic attraction toward the surfaces found beneath the seas. There are no data available to describe the influence of charge upon bulk adhesion in mussel proteins or mimetic polymers. More broadly speaking, there is not enough data available to reach a general consensus on potential relations between polymer charges and adhesion.[31−34] For the majority of mussel adhesive mimics reported to date, few have demonstrated bulk bonding.[5,6,10−12,16,17] Even fewer have been used to attach substrates while underwater.[11,12] The influence of charge upon bonding has yet be examined, and such studies may help us to better understand this intriguing biological material.

In order to explore the role of charge on adhesion, we set out to develop a family of biomimetic copolymers incorporating both DOPA chemistry and cationic charges (Scheme 1). Ammonium groups were a logical choice given the cationic charge, lack of amine reactivity, and synthetic accessibility. This system was also designed such that the positive charge content in the polymers could be changed systematically. A synthetic route for the target polymers is shown here along with bulk adhesion data. High strength bonding was found. Significant dry and underwater bonding was achieved, suggesting a contributing role for the positive charges in mussel adhesion.

Scheme 1. Design of Cationic Adhesive Polymers



Polymer design began with a fairly simple poly(3,4-dihydroxystyrene-*co*-styrene) random copolymer known to exhibit bulk adhesion.[20] A catechol-containing 3,4-dihydroxystyrene monomer can mimic the cross-linking of DOPA and impart adhesion to polystyrene.[20] A third ammonium-containing monomer was added to make the copolymer cationic (Scheme 1). Incorporating charge complicated the synthesis significantly and prompted two modifications to our previous synthetic strategy. Use of a silylated catechol monomer provided generally applicable protection and deprotection chemistry.[35,36] Nitroxide-mediated radical polymerization works well with a large range of monomers and allowed introduction of a third functionality to the polymer.[37] Synthesis of the poly[(3,4-dihydroxystyrene)-*co*-(*p*-vinyltolyltriethylammonium chloride)-*co*-styrene] copolymers is shown in Scheme 2. Commercially available 3,4-dihydroxybenzaldehyde was protected with *tert*-butyldimethylsilyl (TBDMS) groups.[35,36] A Wittig reaction provided the olefin form of the silyl-protected catechol monomer.[35,36,38] This 3,4-di(OTBDMS) styrene was combined with vinylbenzyl chloride and styrene monomers for copolymerization by benzoyl peroxide and TEMPO. Conant-Finkelstein conditions of NaI in acetone

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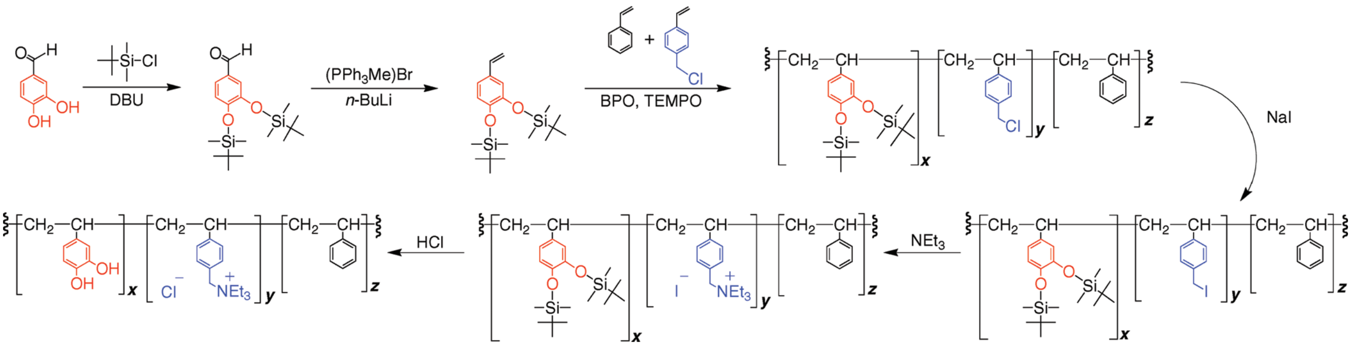
Scheme 2. Synthesis of Cationic Adhesive Copolymers



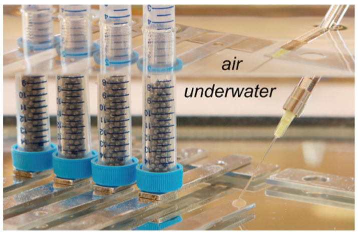


Figure 1. Photograph of an underwater lap shear adhesion experiment showing deposition of a polymer solution onto an aluminum substrate.

transformed the pendant benzyl chloride into a benzyl iodide for greater reactivity with the forthcoming amine.[39] Addition of triethylamine generated the quaternary ammonium salt. The silyl protecting groups were removed with acid to yield the final poly[(3,4-dihydroxystyrene)-*co*-(*p*-vinyltolyltriethylammonium chloride)-*co*-styrene] terpolymers. Full synthetic details are provided in the Supporting Information. The overall yield, from starting aldehyde through six steps to the final polymer, was typically ~20%. Up to 1 g of a given polymer could be obtained from one synthesis.

Terpolymer characterization used ^{1}H NMR spectroscopy, gel permeation chromatography (GPC), and differential scanning calorimetry (DSC). Representative data are included in Figure S1 of the Supporting Information. The ^{1}H NMR spectra provided ratios of each monomer in the final copolymers. The ratio of monomers in the starting feed was generally represented in the isolated polymers. On the basis of GPC data, the typical number-average molecular weights (M_n) were 11 000−15 500. Polydispersity indices (PDI's) fell between 1.1 and 1.3. At the benzyl chloride stage, DSC data showed single glass transition temperatures (T_g's) for each polymer in the range 83−88 °C. Observation of such single thermal events indicates a statistical monomer distribution throughout the copolymer, as opposed to block sequences or segregated phases. This monomer distribution provided suitable mimics for the random distribution of DOPA and cationic groups throughout the mussel adhesive protein backbones.

Four polymers were prepared to investigate the influence of charge upon adhesion, each with the catechol monomer content held constant at ~10%. The cationic monomer was then included at 0%, 7%, 11%, or 16%, with styrene comprising the remainder. This 10% of catechol-containing monomers was chosen to approximate the DOPA content of mussel adhesive proteins.[21−24] Polymers of differing composition were obtained simply by varying monomer ratios in the starting polymerization feed. The influence of polymer molecular weight upon adhesion is complex. Properties including wetting, viscosity, and chain entanglement will all alter adhesive bonding.[40−43] In order to avoid variability from molecular weight, polymers were synthesized using a constant ratio of initiator to total monomer moles.

Table 1. Lap Shear Adhesion Data for Biomimetic Copolymers and Commercial Glues, Cured Both Dry and Underwater

cation content	dry adhesion (MPa)	underwater adhesion (MPa)
0%	2.4 ± 0.5	0.18 ± 0.04
7%	2.8 ± 0.6	0.4 ± 0.2
11%	1.6 ± 0.3	0.3 ± 0.1
16%	1.5 ± 0.2	0.15 ± 0.04
poly(vinyl acetate)	4 ± 1	~0
ethyl cyanoacrylate	7 ± 1	~0
epoxy	11 ± 2	0.07 ± 0.03

Dry adhesion was measured by using the polymers to bond together polished aluminum substrates. Aluminum is a high-energy surface with partial negative charge density.[44] Drops of a polymer solution were placed atop one adherend along with drops of $Bu_4N(IO_4)$ for providing cross-linking chemistry analogous to that found in mussel adhesive.[45] After mixing the polymer and $(IO_4)^-$ solutions, the second piece of aluminum was overlapped onto the first in a lap shear configuration (Figure S2). Curing the adhesive (24 h, 55 °C) was followed by pulling apart the bonded substrates at constant velocity while measuring force. The force at failure was divided by overlap area to provide adhesion in pascals ($Pa = N/m^2$, 1 MPa = 145 pounds per square inch, Figure S2). For each sample type, ten substrate pairs were bonded together. Adhesion values reported are the means with error bars from 95% confidence intervals.

Analogous underwater adhesion experiments were carried out with the aluminum substrates submerged in artificial seawater during both the polymer application and cure at room temperature (Figure 1). To prevent polymer solutions from floating off the substrates, solvents were chosen to be denser than water. Bonding performance of the copolymers was benchmarked against three of the most common commercial adhesive classes: a white poly(vinyl acetate) emulsion glue ("Elmer's Glue"), ethylcyanoacrylate ("Krazy Glue"), and an epoxy. Consistent

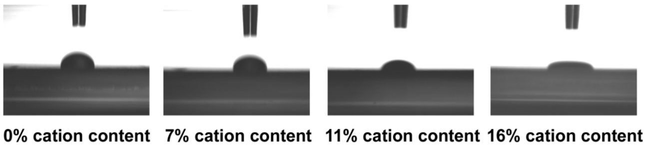


Figure 2. Images of cationic copolymers in an organic solvent, dropped onto aluminum surfaces while submerged under artificial seawater.

masses of the commercial adhesives were used for direct comparison to the biomimetic copolymers.

Dry bonding strengths of the copolymers were quite appreciable, well into the megapascal range (Table 1). Even without formulation studies such as optimizing cure conditions, solvents, concentrations, or fillers, the biomimetic bonding is approaching that of established commercial products. When dry, the copolymers may exhibit a mild decrease of bonding with increasing charge content.

Generally speaking, underwater adhesion is challenging, with most adhesives unable to bond when wet. For example, the white and cyanoacrylate glues used here did not even allow the substrates to remain attached when picked up (denoted ~0 MPa in Table 1). White glue requires the formulation to lose water and dry in order to be effective. Thus, curing cannot take place underwater. Cyanoacrylate is an organic material and cures too quickly underwater to be useful, owing to a water-initiated polymerization. The epoxy provides an intermediate case of compatibility with water and, consequently, measurable adhesive bonding, albeit quite weak.

Table 1 shows that our biomimetic copolymers displayed appreciable bonding underwater. A recent report of polymer blends with dopamine pendant from one of the polymer chains also found significant underwater adhesion.[11,12] Our wet bonding here may be uninfluenced by charge or, possibly, maximizing near 7% or 11% cationic monomer content.

In these traditional measurements of lap shear bonding, adhesion in megapascals was obtained by dividing the maximum observed force by the substrate overlap area (Figure S2). An alternative perspective on the adhesion data can be derived by integrating the area under the extension versus force curves to provide the separation work in millijoules.[46] These data indicate that the polymers of intermediate charge may be exhibiting greater adhesion (Table S1).

A potential explanation for this strong underwater bonding could be related to our observation of increased surface spreading of solutions containing the more highly charged copolymers (11%, 16%) relative to the lower charged polymer and uncharged control (0%, 7%). Figure 2 shows underwater photographs of the polymers at 0.3 g/mL, dissolved in 90/10 $CHCl_3/CH_3OH$, and dropped onto an aluminum surface submerged in artificial seawater. Note how increasing polymer charge appears to increase the surface wetting of these polymer solutions when underwater. Contact angle values are provided in Table S2.

These contact angle results may indicate that cationic polymers display a greater ability to interact with the substrate. Enhanced surface wetting can generally be attributed to improved adhesion.[47] Too much charge, however, might be a detriment. Charge—charge repulsions may come into play and explain a decrease in adhesion above ~11% cationic monomers. Incorporation of charge could contribute to strong bonding on its own but is not wholly responsible for adhesion. The role of DOPA in mussel adhesion is well established.[1−3] For mussel adhesive proteins, some charge may be present to benefit function, but not all of the amino acids are cationic.

Direct comparisons of adhesive performance between mussel proteins and synthetic mimics are difficult to make, given that few protein bulk adhesive studies have been reported.[48,49] In general, adhesive proteins are difficult to obtain in sufficient quantities to allow bulk measurements. The limited available data for proteins do not provide insight on the influence of charge or dry versus underwater conditions. When dry, however, we know that homogeneous protein solutions can bond up to ~1 MPa under conditions similar to those used here.[48] The biomimetic polymers, both neutral and cationic, more than double this dry adhesive performance. The whole adhesive produced by live mussels was measured to be 0.3 ± 0.1 MPa on aluminum.[50] Although this value is similar to our polymers bonding underwater at up to ~0.4 MPa, the comparison is not direct. Mussel glue was measured in tensile whereas the polymer adhesion studies here could take advantage of the decreased variability inherent in lap shear bonding.

In summary, these results describe cationic mimics of mussel adhesive proteins. A synthetic scheme was developed and used to prepare terpolymers of varied charge content. Copolymer bulk adhesion was found to approach that of commercial glues when dry. Underwater adhesion was lower than dry but still appreciable and better than three popular commercial products. Positive charge may aid surface interactions and bonding, thereby providing insight on why mussel adhesive proteins are cationic. By adding charge to cross-linking polymers, we could be closer to developing wet setting adhesives for the next generation of biomedical materials including bone cements and surgical glues.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Synthetic methods, polymer characterization, and adhesion measurement procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Waite, J. H. *Integr. Comp. Biol.* **2002**, *42*, 1172.
(2) Sagert, J.; Sun, C.; Waite, J. H. In *Biological Adhesives*; Smith, A. M., Callow, J. A., Eds.; Springer-Verlag: Berlin, 2006; pp 125−143.
(3) Wilker, J. J. *Curr. Opin. Chem. Biol.* **2010**, *14*, 276.
(4) Moulay, S. *C. R. Chim.* **2009**, *12*, 577.
(5) Yu, M.; Deming, T. J. *Macromolecules* **1998**, *31*, 4739.
(6) Wang, J.; Liu, C.; Lu, X.; Yin, M. *Biomaterials* **2007**, *28*, 3456.
(7) Saxer, S.; Portmann, C.; Tosatti, S.; Gademann, K.; Zürcher, S.; Textor, M. *Macromolecules* **2010**, *43*, 1050.
(8) Lee, H.; Lee, B. P.; Messersmith, P. B. *Nature* **2007**, *448*, 338.
(9) Guvendiren, M.; Messersmith, P. B.; Shull, K. R. *Biomacromolecules* **2008**, *9*, 122.

(10) Shao, H.; Bachus, K. N.; Stewart, R. J. *Macromol. Biosci.* **2009**, *9*, 464.

(11) Shao, H.; Stewart, R. J. *J. Adv. Mater.* **2010**, *22*, 729.

(12) Shao, H.; Weerasekare, M.; Stewart, R. J. *J. Biomed. Mater. Res. A* **2011**, *97A*, 46.

(13) Chung, H.; Glass, P.; Pothen, J. M.; Sitti, M.; Washburn, N. R. *Biomacromolecules* **2011**, *12*, 342.

(14) Gao, C.; Li, G.; Xue, H.; Yang, W.; Zhang, F.; Jiang, S. *Biomaterials* **2010**, *31*, 1486.

(15) Dalsin, J. L.; Hu, B.; Lee, B. P.; Messersmith, P. B. *J. Am. Chem. Soc.* **2003**, *125*, 4253.

(16) Burke, S. A.; Ritter-Jones, M.; Lee, B. P.; Messersmith, P. B. *Biomed. Mater.* **2007**, *2*, 203.

(17) Murphy, J. L.; Vollenweider, L.; Xu, F.; Lee, B. P. *Biomacromolecules* **2010**, *11*, 2976.

(18) Lee, H.; Lee, K. D.; Pyo, K. B.; Park, S. Y.; Lee, H. *Langmuir* **2010**, *26*, 3790.

(19) Malisova, B.; Tosatti, S.; Textor, M.; Gademann, K.; Zürcher, S. *Langmuir* **2010**, *26*, 4018.

(20) Westwood, G.; Horton, T. N.; Wilker, J. J. *Macromolecules* **2007**, *40*, 3960.

(21) Waite, J. H.; Housley, T. J.; Tanzer, M. L. *Biochemistry* **1985**, *24*, 5010.

(22) Rzepecki, L. M.; Hansen, K. M.; Waite, J. H. *Biol. Bull.* **1992**, *183*, 123.

(23) Papov, V. V.; Diamond, T. V.; Biemann, K.; Waite, J. H. *J. Biol. Chem.* **1995**, *270*, 20183.

(24) Waite, J. H.; Qin, X. *Biochemistry* **2001**, *40*, 2887.

(25) Suci, P. A.; Geesey, G. G. *J. Colloid Interface Sci.* **2000**, *230*, 340.

(26) Burzio, L. A.; Burzio, V. A.; Pardo, J.; Burzio, L. O. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2000**, *126*, 383.

(27) Burzio, L. A.; Waite, J. H. *Protein Sci.* **2001**, *10*, 735.

(28) Parks, G. A. *Chem. Rev.* **1965**, *65*, 177.

(29) Bennett, R. H.; Hulbert, M. H. *Clay Microstructure*; Int. Human Resources Development Corp.: Boston, 1986; pp 5−45.

(30) Schramm, L. L.; Mannhardt, K.; Novosad, J. L. *Colloids Surf.* **1991**, *55*, 309.

(31) Wu, S. *Polymer Interface and Adhesion*; Marcel Dekker Inc.: New York, 1982; pp 420−447.

(32) Smarook, W. H.; Bonotto, S. *Polym. Eng. Sci.* **1968**, *8*, 41.

(33) Mao, T. J.; Reegen, S. L. In *Adhesion and Cohesion*; Weiss, P., Ed.; Elsevier: Amsterdam, 1962; pp 209−216.

(34) Lewis, A. F.; Forrestal, L. J. *ASTM STP* **1963**, *360*, 59–77.

(35) Menichetti, S.; Aversa, M. C.; Cimino, F.; Contini, A.; Viglianisi, C.; Tomaino, A. *Org. Biomol. Chem.* **2005**, *3*, 3066.

(36) Faler, C. A.; Joullié, M. M. *Org. Lett.* **2007**, *9*, 1987.

(37) Moad, G.; Rizzardo, E.; Thang, S. H. *Acc. Chem. Res.* **2008**, *41*, 1133.

(38) Daly, W. H.; Moulay, S. *J. Polym. Sci., Polym. Symp. Ed.* **1986**, *74*, 227.

(39) Moulay, S.; Mehdi, N. *Chin. J. Polym. Sci.* **2006**, *24*, 627.

(40) Nunes, R. W.; Martin, J. R.; Johnson, J. F. *Polym. Eng. Sci.* **1982**, *22*, 205.

(41) Pocius, A. V. *Adhesion and Adhesive Technology*, 2nd ed.; Hanser-Gardner Publications: Cincinnati, 2002; pp 120−122.

(42) Elizalde, O.; Vicente, M.; Plessis, C.; Leiza, J. R.; Asua, J. M. *J. Coat. Technol. Res.* **2004**, *1*, 45.

(43) Cao, X.; Zhang, L. *Biomacromolecules* **2005**, *6*, 671.

(44) McCarvill, W. T.; Bell, J. P. *J. Appl. Polym. Sci.* **1974**, *18*, 343.

(45) Hedlund, J.; Andersson, M.; Fant, C.; Bitton, R.; Bianco-Pelend, H.; Elwing, H.; Berglin, M. *Biomacromolecules* **2009**, *10*, 845.

(46) Hyonchol, K.; Arakawa, H.; Osada, T.; Ikai, A. *Colloids Surf., B* **2002**, *25*, 33.

(47) Levine, M.; Ilkka, G.; Weiss, P. *Polym. Lett.* **1964**, *2*, 915.

(48) Cha, H. J.; Hwang, D. S.; Lim, S.; White, J. D.; Matos-Pérez, C. R.; Wilker, J. J. *Biofouling* **2009**, *25*, 99.

(49) Lim, S.; Choi, Y. S.; Kang, D. G.; Song, Y. H.; Cha, H. J. *Biomaterials* **2010**, *31*, 3715.

(50) Burkett, J. R.; Wojtas, J. L.; Cloud, J. L.; Wilker, J. J. *J. Adhes.* **2009**, *85*, 601.