Simplified Polymer Mimics of Cross-Linking Adhesive Proteins

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ABSTRACT: Marine mussels and barnacles attach themselves to surfaces by depositing mixtures of proteins and then cross-linking these polymers for curing of their adhesive. Obtaining large quantities of these biological materials for applications development has proven to be problematic. Here we present the synthesis, reactivity, and adhesive characteristics of simplified polymer mimics of bioadhesives. A polystyrene backbone is used to take the place of the protein polyamide chain. Cross-linkable catechol groups are appended to the polystyrene in order to mimic 3,4-dihydroxystyrene (DOPA), the key cross-linking group in mussel adhesive proteins. We show that poly[(3,4-dimethoxystyrene)-*co*-styrene] polymers exhibit curing analogous to that of the proteins after which they were modeled. The styrene−catechol copolymers also display enhanced adhesion upon cross-linking. This report demonstrates a reductionist approach to developing new polymeric materials on the basis of principles learned from nature.

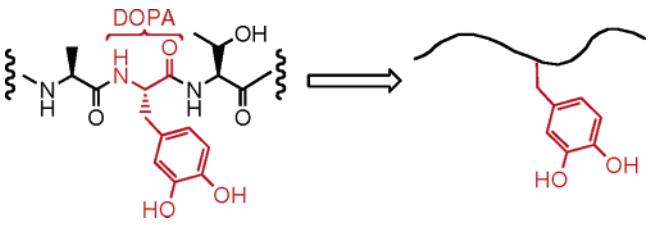
Introduction

The oceans provide a wealth of inspiration for new approaches to materials design. Barnacles[1] and marine mussels[2] are examples of organisms able to affix themselves to nearly any surface, including polytetrafluoroethylene (PTFE, Teflon).[3] For setting in wet environments, these animals apply proteins to surfaces of interest.[4,5] Extensive cross-linking of the proteins yields cured adhesives or cements.[4−7] Although the exact nature of such protein−protein and protein−surface interactions is not yet known, the unusual amino acid 3,4-dihydroxyphenylalanine (DOPA, Scheme 1) is central to curing of mussel adhesive proteins.[8] Barnacle cement proteins do not contain DOPA residues. Although less well understood than mussel adhesive formation, cross-linking of barnacle proteins could be from disulfide formation between cysteines.[6,7,9−12] For curing mussel adhesive, cross-linking of DOPA-containing proteins may be a result of chemical oxidation,[13] enzymatic oxidation,[14,15] or metal chelation[16−18] followed by radical generation.[18]

Efforts from our laboratory have shown that DOPA-containing proteins can be cross-linked by various reagents, with oxidizing metal ions affording greater degrees of curing than either metal ions, alone, or simple oxidants, alone.[19,20] We found that strongly oxidizing metal ions such as dichromate, $(Cr_2O_7)^{2-}$, are most effective, although not likely available to the animals under typical conditions.[19,20] Iron(III) is readily present in seawater and capable of inducing cross-linking of DOPA-containing proteins.[19,20] In particular, we found that Fe^{3+} can both be chelated by the adhesive proteins and, upon reaction with oxygen, oxidize the protein to yield organic radicals.[18] The protein-based radicals may then couple to each other or, possibly, a surface of interest. Taken together, these data provide ideas for the design of biomimetic cross-linking materials.

Given the mussel's ability to attach to virtually any surface, this adhesive may provide inroads to new materials. Obtaining protein for such efforts, however, has not been simple. Expression of DOPA-containing proteins has proven difficult,[21] but progress is being made.[22,23] Enzymatic oxidation of tyrosine-containing synthetic polypeptides can yield DOPA-containing products, although fine control over this process is difficult to achieve.[24−26] In general, large-scale generation of proteins and polypeptides with DOPA is complicated and expensive. The difficulties of working with adhesive proteins on large scales have fueled the design of synthetic polymer mimics into which DOPA has been incorporated. Of particular note are the efforts of Deming in which his laboratory reported the synthesis and adhesion of high molecular weight polypeptides containing DOPA.[26] More recently, the Messersmith group has prepared a series of polymeric compounds containing DOPA and studied the resulting mechanical properties.[25,27−29] Such work has described the incorporation of DOPA into both peptides and synthetic polymers such as poly(ethylene glycol).[25,27−29]

Scheme 1. A DOPA-Containing Polypeptide Reduced to a Simple Catechol-Containing Polymer



Here we have taken a "reductionist" and bioinspired approach to developing new polymeric materials. The goal of our work is to combine the unique chemistry of marine adhesive proteins with the flexibility, easy synthesis, and low cost of simple bulk polymers (Scheme 1).[28,29] We began our studies by distributing 3,4-dihydroxystyrene into a polymer backbone, as shown in Scheme 2. This 3,4-dihydroxystyrene mimics the catechol side chain of DOPA and is similar to styrene, with only two hydroxyls added. Such a system provides the simplest possible mimic of mussel adhesive proteins. Homopolymers of 100% dihydroxystyrene have been prepared, but cross-linking chemistry was not explored.[30,31] We were curious to see whether such simple mimics would exhibit any cross-linking or adhesion similar to the proteins after which they were modeled. Below we show the synthesis, cross-linking, and first insights on the adhesive properties of this polymer system. The methods and cross-linking reagents are taken from our prior work with DOPA proteins extracted from marine mussels.[18−20] We find similarities

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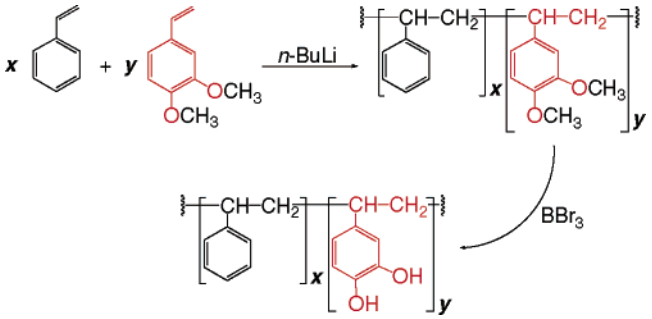
Scheme 2. Synthesis of Poly[(3,4-dihydroxystyrene)-*co*-styrene)]



between the reactivity of proteins and these new polymer mimics.

Experimental Section

General Procedures. All syntheses were carried out under an argon atmosphere using standard Schlenk techniques. Gel permeation chromatography was performed on a Waters system with a refractive index detector (2414). Data were collected on a personal computer using Breeze software. Differential scanning calorimetry was performed on a TA Instruments 2920 modulated calorimeter.

Monomer Purification. Styrene, as purchased from Sigma Aldrich, is inhibited with 10−15 ppm of 4-*tert*-butylcatechol. Column chromatography was used to separate this inhibitor from the monomer. A 25 mL glass pipet was cut at the tip to afford a functional column for monomer purification. The glass pipet was oven dried for 8 h prior to use. The column was packed with 0.6 cm of cotton, 0.6 cm of oven-dried sand, 15−25 cm of oven-dried alumina, and 0.6 cm of potassium carbonate. Once the column was packed, styrene was added to the top and allowed to elute. The monomer was purified no more than 12 h before use and stored at 4 °C. 3,4-Dimethoxystyrene, as purchased from Sigma Aldrich, also contains an inhibitor, 1% hydroquinone. Column chromatography was used to separate hydroquinone from the monomer in a manner analogous to that for styrene.

Synthesis of Polystyrene. The preparation of polystyrene was based on modification of literature procedures for the polymerization of vinyl monomers.[31] Anhydrous toluene (235 mL) was added by syringe to a degassed 500 mL Schlenk flask. The purified styrene (47 mL, 0.41 mol) was then added by syringe to the flask. Vacuum was applied to the flask for 1 s followed by back-filling with argon. The flask was placed into a dry ice/isopropanol bath and allowed to cool to −78 °C for 15 min, and *n*-butyllithium (1.34 mL, 2.6 M in hexanes) was added dropwise by syringe. As *n*-butyllithium was added, the solution turned from colorless to yellow. The dry ice/isopropanol bath was removed, and the solution was allowed to warm to room temperature. Upon warming, the color of the solution changed from yellow to orange to deep red. After 20 h the reaction was quenched by addition of methanol (2 mL, 25 °C), affording a colorless solution. The reaction mixture was vented and poured into a 1000 mL round-bottom flask containing 500 mL of ice cold methanol. A cloudy white precipitate formed. A white solid was obtained after solvent removal in vacuo (34.26 g, 82% yield). ^{1}H NMR ($CDCl_3$): δ 0.7−2.4 (broad, polymer backbone), 6.2−7.6 (broad, aromatic). Further characterization data are presented in Table 1.

Synthesis of Poly[(3,4-dimethoxystyrene)-*co*-styrene]. Poly[(3,4-dimethoxystyrene)-*co*-styrene], with varied percentages of 3,4-dimethoxystyrene and styrene, was prepared by modification of the method described above. The procedure described here is for polymerization of 1:1000 3,4-dimethoxystyrene:styrene poly[(3,4-dimethoxystyrene)-*co*-styrene]. Polymers containing 1:5, 1:10, 1:15, and 1:50 feed ratios of 3,4-dimethoxystyrene:styrene were prepared in a similar manner. Anhydrous toluene (242 mL) was added by syringe to a degassed 500 mL Schlenk flask, and purified styrene (47 mL, 0.41 mol) was added by syringe. Purified 3,4-dimethoxystyrene (0.061 mL, 0.41 mmol) was added to the flask by syringe.

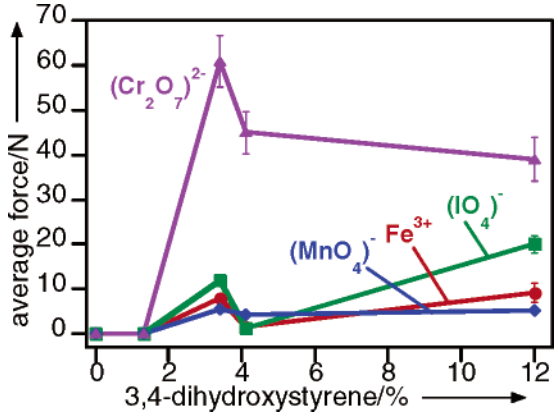


Figure 1. Effect of polymer composition upon curing. For a given polymer, the percentage of the 3,4-dihydroxystyrene monomer is provided, with the remainder of the polymer being styrene. Four cross-linking reagents are shown.

Vacuum was applied to the flask for 1 s followed by back-filling with argon. The flask was placed into a dry ice/isopropanol bath and allowed to cool to −78 °C for 15 min, and *n*-butyllithium (1.34 mL, 2.6 M in hexanes) was added dropwise by syringe. While *n*-butyllithium was added the solution turned from colorless to yellow to dark orange. The reaction flask was kept at −78 °C for at least 8 h. The solution was allowed to gradually warm to room temperature, resulting in a color change from dark orange to light yellow. After 20 h, the reaction was quenched by addition of methanol (2 mL, 25 °C), affording a colorless solution. The mixture was vented and poured into a 1000 mL round-bottom flask containing 500 mL of ice cold methanol, and a cloudy white precipitate formed. A white solid was obtained after solvent removal in vacuo (27.16 g, 64% yield). ^{1}H NMR ($CDCl_3$): δ 0.6−2.3 ppm (broad, polymer backbone), 3.4−3.8 ppm (broad, methoxy peaks), 6.0−7.4 ppm (broad, aromatic). Table 1 provides synthetic details and characterization data for each polymer.

Synthesis of Poly(3,4-dimethoxystyrene). Poly(3,4-dimethoxystyrene) was prepared as described above. Anhydrous toluene (45 mL) was added by syringe to a degassed 250 mL Schlenk flask. The purified 3,4-dimethoxystyrene (6.0 mL, 0.041 mol) was then added by syringe to the flask. Vacuum was applied to the flask for 1 s followed by back-filling with argon. The flask was placed into a dry ice/isopropanol bath and allowed to cool to −78 °C for 15 min, and *n*-butyllithium (0.17 mL, 2.6 M in hexanes) was added dropwise by syringe. As *n*-butyllithium was added, the solution turned from colorless to yellow to dark orange. The reaction flask was kept at −78 °C for at least 8 h. The solution was allowed to gradually warm to room temperature, resulting in a color change from dark orange to light yellow. After 20 h, the reaction was quenched by addition of methanol (2 mL, 25 °C), affording a colorless solution. The reaction mixture was vented and poured into a 500 mL round-bottom flask containing 200 mL of ice cold methanol, and a cloudy white precipitate formed. A white solid was obtained after solvent removal in vacuo (5.8 g, 87% yield). ^{1}H NMR ($CDCl_3$): δ 0.6−2.8 ppm (broad, polymer backbone), 3.1−4.9 ppm (broad, methoxy peaks), 5.5−7.1 ppm (broad, aromatic). See Table 1 for further characterization.

Synthesis of Poly[(3,4-dihydroxystyrene)-*co*-styrene]. The methoxy groups of each poly[(3,4-dimethoxystyrene)-*co*-styrene] copolymer were removed according to standard methods to reveal the deprotected, catechol-containing polymers.[30−32] The procedure below is for deprotection of 1:1000 poly[(3,4-dimethoxystyrene)-*co*-styrene]. Polymers containing 1:5, 1:10, 1:15, and 1:50 feed ratios of 3,4-dimethoxystyrene:styrene were deprotected in a similar manner. Poly[(3,4-dimethoxystyrene)-*co*-styrene] (1:1000 copolymer) (27.16 g) was added to a 500 mL Schlenk flask under argon. Anhydrous dichloromethane (100 mL) was added to the flask by syringe, and the flask was then placed into an ice bath and allowed to cool for 15 min. Boron tribromide (1.3 mL, 1 M in dichlo-

Table 1. Copolymer Synthesis Conditions and Characterization (DMS Is 3,4-Dimethoxystyrene)

DMS:styrene feed ratio	styrene (mL)	DMS (mL)	*n*-butyl-lithium (mL)	toluene (mL)	yield (%)	M_n[a] (kDa)	PDI[a]	^{1}H NMR δ (ppm)
1:1000	47	0.061	1.34	242	64			0.6−2.3 br 3.4−3.8 br 6.0−7.4 br
1:50	47	1.2	1.37	247	95	9.5	1.38	0.6−2.4 br 3.4−3.7 br 6.1−7.4 br
1:15	44	3.8	1.37	246	83	14.7	1.71	0.8−2.6 br 3.5−4.0 br 5.7−7.4 br
1:10	42	5.4	1.35	244	83	16.1	1.92	0.8−2.6 br 3.5−4.0 br 5.8−7.6 br
1:5	39	10.2	1.4	253	96	14.6	1.84	0.6−2.4 br 3.4−3.8 br 5.7−7.4 br
100:0	N/A	6.0	0.17	45	87	43.9	1.37	0.6−2.8 br 3.1−4.9 br 5.5−7.1 br
0:100	47	N/A	1.34	235	82	125.8	1.26	0.7−2.4 br 6.2−7.6 br

[a] Gel permeation chromatography performed on the methyl-protected polymers.

romethane) was added to the flask dropwise by syringe. Upon addition of boron tribromide, the reaction mixture became dark red. The flask was left under a positive pressure of argon and allowed to gradually warm to room temperature. After 18 h, the reaction mixture was poured into acidic water (1000 mL, 0.12 M HCl) to yield a cloudy pink solution. The reaction was stirred at 4 °C for 15 min. After the initial 15 min, stirring the reaction mixture was allowed to separate into organic and aqueous layers. The aqueous layer was decanted into a filter flask. The organic layer was washed with acidic water (1000 mL, 0.12 M HCl) for 15 min at 4 °C, and the aqueous layer was decanted into the filter flask. This washing step was repeated twice more. Dichloromethane (300 mL) was added to the resulting organic layer, and the mixture was transferred to a 2000 mL separatory funnel. Fresh acidic water (100 mL, 0.12 M HCl) was added to the separatory funnel. The funnel was capped, shaken, and allowed to separate into organic and aqueous layers for 12 h. A white solid was obtained by solvent removal of the resulting organic layer in vacuo (23.35 g, 86% yield). ^{1}H NMR ($CDCl_3$): δ 0.3−2.7 ppm (broad, polymer backbone), 6.0−8.0 ppm (broad, aromatic). On average, this procedure afforded 98% and 95% deprotection of the 1:10 and 1:5 polymers, respectively. Methoxy peaks (3.1−4.9 ppm) were not observable for polymers of higher styrene content. See Table 1 for further characterization.

Synthesis of Poly(3,4-dihydroxystyrene). Poly(3,4-dihydroxystyrene) was prepared by the deprotection of poly(3,4-dimethoxystyrene) with BBr_3. Poly(3,4-dimethoxystyrene) (5.5 g) was added to a 250 mL Schlenk flask under argon. Anhydrous dichloromethane (42 mL) was added to the flask by syringe, and the flask was placed into an ice bath for 15 min. Boron tribromide (10.5 mL) was added to the flask dropwise by syringe. Upon addition of BBr_3, the reaction mixture became cloudy and pink. The flask was left under positive pressure argon flow and allowed to gradually warm to room temperature. After 18 h, the reaction was poured into acidic water (1000 mL, 0.12 M HCl) to provide a viscous, cloudy, dark pink solution. Additional dichloromethane (300 mL) was added to the solution to decrease the viscosity. The solution was stirred at 4 °C for 15 min. The reaction mixture was then allowed to separate into organic and aqueous layers. The aqueous layer was decanted into a filter flask. The organic layer was washed by addition of acidic water (1000 mL, 0.12 M HCl) for 15 min at 4 °C, and the aqueous layer was decanted into an Erlenmeyer flask. This washing step was repeated twice more. Dichloromethane (500 mL) was added to the resultant organic layer, and the mixture was transferred to a 2000 mL separatory funnel. Fresh acidic water (200 mL, 0.12 M HCl) was added to the separatory funnel. The funnel was capped, shaken, vented, and allowed to separate into organic and aqueous layers for 12 h. A maroon solid was obtained after solvent removal of the resultant organic layer in vacuo (4.98 g, 90.5% yield). ^{1}H NMR ($CDCl_3$): δ 0.7−2.4 (broad, polymer backbone), 6.3−7.4 (broad, aromatic). See Table 1 for further characterization.

Solutions for Penetration Tests. Metal salts and oxidants were weighed out the day of testing, and solutions were mixed 5−10 min prior to reaction with a polymer. Metal salt and oxidant solutions were prepared in acetone, at a concentration of 400 mM, with a final concentration after mixing with polymer of 40 mM. All reagents examined can be found in Table 2.

Penetration Cross-Linking Tests. The testing procedures employed here were adapted from our previous laboratory work.[19,20] An Instron 5544 materials testing machine was used to drive a steel rod into the cross-linked sample at a constant velocity. Sample microcentrifuge tubes were secured in a drill chuck, specifically designed for the Instron instrument. A 4.0 mm blank drill bit penetrated the sample mixture at a rate of 20 mm/min. For each run, the average total penetration depth is 25−35 mm for each sample. A 100 N load cell was used to monitor the resistive force of the sample against the rod, and data were recorded every 0.5 mm using Merlin software on a personal computer. A sample with extensive cross-linking is expected to generate a higher resistive force against penetration than one with limited cross-linking and thus requires an increased force to lower the rod at a constant velocity.

Samples were prepared by dissolving 1.20 ± 0.01 g of polymer in 1.2 mL of acetone in a glass test tube. Each solution was agitated on a mini-vortex machine for 30 s and added to a plastic microcentrifuge tube (2 mL volume, 9 mm i.d. × 35 mm). Three sample tests were performed for each cross-linking agent per polymer, and data were averaged. To each sample of polymer, 0.4 mL of the appropriate cross-linker was added by syringe and immediately stirred for 2−3 s with the syringe needle. Final concentration of the cross-linkers was 40 mM. Penetration tests were performed at room temperature, 60 min after samples were prepared. The tubes were capped to prevent solvent loss during this 60 min cure time. We found solvent loss from the capped tubes in 60 min to be undetectable (<0.0001 g).

Adhesive Shear Testing. Lap-shear testing of the 1:10 feed ratio (3.4:96.6 found) copolymer was performed with an Instron materials testing machine. Polished aluminum adherends (10 cm × 1.25 cm) were used for these tests. For ease of handling, polymer and cross-linking agent concentrations were diluted to 1/3 that of the concentration used for penetration testing. A 22.5 μL aliquot of the polymer solution in 1:1 acetone:dichloromethane was spread over a 1.25 × 1.25 cm area on both adherends. A 15 μL aliquot of cross-linking agent in 1:1 acetone:dichloromethane was spread on top of the polymer solution on one adherend. The adherends were

Table 2. Average Penetration Forces (in mN) for Reaction of Copolymers with Varied 3,4-Dimethoxystyrene:Styrene Ratios with Different Cross-Linking Reagents[a]

	copolymer 3,4-dimethoxystyrene:styrene ratio						
cross-linker	12:88	3.4:96.6	4.1:95.9	1.3:98.7	0.6:99.4	100:0	0:100
$Fe(acac)_3$	9200 ± 2000	7900 ± 490	1700 ± 150	13 ± 2	9 ± 2	N/A	8 ± 1
$[(C_4H_9)_4N]IO_4$	20000 ± 2000	12000 ± 630	1400 ± 460	23 ± 11	10 ± 1	38000 ± 3700	7 ± 1
$[(C_4H_9)_4N]MnO_4$	5295 ± 318	5400 ± 480	4300 ± 354	31 ± 9	21 ± 3	N/A	8 ± 1
$[(C_4H_9)_4N]_2Cr_2O_7$	39000 ± 5000	61000 ± 5800	45000 ± 4700	35 ± 7	9 ± 1	75000 ± 3000	7 ± 1
$Mn(OOCCH_3)_3$	6 ± 1	6 ± 2	7 ± 1	13 ± 1	7 ± 1	N/A	8 ± 1
Na_3VO_4	7 ± 1	8 ± 1	8 ± 2	16 ± 2	10 ± 2	N/A	5 ± 1
$Zn(NO_3)_2$	2 ± 1	8 ± 1	7 ± 1	9 ± 2	13 ± 3	N/A	5 ± 1
$Ga(NO_3)_3$	10 ± 1	11 ± 2	9 ± 4	8 ± 1	9 ± 1	N/A	4 ± 1
$Co(NO_3)_2$	15 ± 2	23 ± 15	8 ± 1	9 ± 1	10 ± 1	N/A	5 ± 1
t-C_4H_9OOH	21 ± 6	9 ± 1	7 ± 1	8 ± 1	7 ± 1	N/A	5 ± 1
$[(C_4H_9)_4N]_2SO_4$	9 ± 1	8 ± 1	8 ± 1	7 ± 1	7 ± 1	N/A	5 ± 1

[a] Penetration forces were measured at 35 mm rod extension.

overlapped with a 1.25 × 1.25 cm area and cured for 1 h at room temperature, then at 55 °C for 22 h, and then cooled at room temperature for 1 h. For each test, five samples were measured and averaged. Each sample was loaded to failure at 2 mm/min, and the ultimate shear loads were recorded. All samples displayed cohesive failure.

Results and Discussion

A family of poly[(3,4-dihydroxystyrene)-*co*-styrene] polymers were synthesized with differing percentages of 3,4-dihydroxystyrene vs styrene. Anionic polymerization began with 3,4-dimethoxystyrene:styrene monomer ratios of 1:1,000, 1:50, 1:15, 1:10, and 1:5. According to ^{1}H NMR spectroscopy, the 3,4-dimethoxystyrene content of the final polymers approximated that in the starting feed: 0.58%, 1.3%, 4.1%, 3.4%, and 12%, respectively. Subsequent deprotection with BBr_3 yielded the poly[(3,4-dihydroxystyrene)-*co*-styrene] products depicted in Scheme 2.

Glass transition temperatures of the protected polymers were obtained by differential scanning calorimetry (DSC). These T_g measurements were performed on the protected polymers in order to prevent heat-induced cross-linking of catechol groups during data collection. The 100% polystyrene control displayed a T_g = 106.3 °C. The 100% poly(3,4-dimethoxystyrene) provided T_g = 53.1 °C. For copolymers containing both monomers, intermediate T_g values were obtained, such as T_g = 90.6 °C for a 12% 3,4-dimethoxystyrene and 88% styrene copolymer. The single, sharp thermal transitions found for each copolymer indicate random distribution of the two monomers throughout the chain, rather than formation of block copolymers.

Gel permeation chromatography (GPC) was used to determine the polymer number-average molecular weight (M_n) and polydispersity indices (PDI). Similar to the T_g experiments, GPC data were collected on the protected polymers to prevent cross-linking during data collection. The 1.3:98.7 3,4-dimethoxystyrene:styrene copolymer displayed M_n = 9460 and PDI = 1.38. Further data obtained included 4.1:95.9 copolymer, M_n = 14 726, PDI = 1.71; 3.4:96.6 copolymer, M_n = 16 149, PDI = 1.92; 12:88 copolymer, M_n = 14 641, PDI = 1.84. For the homopolymers prepared under identical conditions, longer chain lengths were found (100% styrene, M_n = 126 000, PDI = 1.26; 100% 3,4-dimethoxystyrene, M_n = 43 894, PDI = 1.37). Polymers were also characterized by ^{1}H NMR spectroscopy, as shown in Table 1.

With these polymeric mimics of mussel adhesive proteins in hand, we then explored cross-linking reactions. Using an Instron 5544 materials testing system, a 4.0 mm diameter rod was driven into viscous solutions (e.g., 1 g of polymer in 1 mL of acetone) at constant velocity (20 mm/min), while the resistive penetration forces were recorded.[19,20] High resistive forces indicate a hardened material whereas low forces show a lack of curing.[33,34] This empirical and quantitative method allows rapid analysis of curing in many samples.[33,34] These data can also be compared directly to prior studies in which the cross-linking reactions of DOPA-containing adhesive proteins extracted from mussels were examined.[19,20] Here, the polymers were combined with potential cross-linking agents, including oxidizing metal ions ($Fe^{III}(acac)_3$ where acac = acetylacetonate, $[(C_4H_9)_4N]Mn^{VII}O_4$, and $[(C_4H_9)_4N]_2Cr^{VI}_2O_7$]), nonoxidizing metals ($Zn^{II}(NO_3)_2$, $Ga^{III}(NO_3)_3$, and $Co^{II}(NO_3)_2$), and nonmetallic oxidants (*t*-C_4H_9-OOH, $[(C_4H_9)_4N]IO_4$) as well as $[(C_4H_9)_4N]_2SO_4$ for a control. We reacted the 3.4:96.6 3,4-dihydroxystyrene:styrene copolymer with select cross-linkers (40 mM final concentration), after a 1 h cure at room temperature. The resulting force data are presented in Table 2. Dichromate ($(Cr_2O_7)^{2-}$) displayed the highest penetration forces, thereby indicating the greatest amount of polymer cross-linking (61 ± 6 N at 35 mm rod extension). Though not as effective, IO_4^- (12 ± 0.6 N), Fe^{3+} (7.9 ± 0.5 N), and MnO_4^- (5.4 ± 0.5 N) also showed curing significantly greater than that obtained for the un-cross-linked control sample (0.006 ± 0.001 N). Reactions with $Zn(NO_3)_2$, $Ga(NO_3)_3$, *t*-C_4H_9-OOH, and $(C_4H_9)_4N)_2SO_4$ did not afford any observable curing (all <0.02 N). Likewise, no reactions were noted between a 100% polystyrene homopolymer and any of these reagents mentioned.

Our previous work showed similar trends in the reactivity of DOPA-containing proteins.[19,20] The mussel adhesive proteins were effectively cured by oxidizing metal ions, but not simple metal ions or *t*-C_4H_9OOH.[19,20] For both the proteins and synthetic polymers, dichromate proved to be the strongest inducer of hardening. Of the reagents available to mussels under natural conditions, Fe^{3+} promoted hardening to the greatest degree for both the extracted protein[19,20] and the synthetic polymers described here. Such cross-linking processes appear to be distinct from the condensation cross-linking found for ionomers.[35,36] Simple metal ions such as Na^+ and Zn^{2+} are often mixed with polycarboxylate polymers in order to impart hardness and adhesive properties. For the new polymers shown here, Zn^{2+} addition promoted no hardening. Furthermore, hardening was most pronounced with oxidizing ions such as Fe^{3+} and IO_4^-. Thus, metal-induced cross-linking in the poly-[(3,4-dihydroxystyrene)-*co*-styrene] system likely occurs through a mechanism distinct from that of the ionomers.

Next we examined the effects of polymer composition upon curing. Figure 1 shows the penetration forces at 35 mm rod extension found for copolymers with varied percentages of 3,4-dihydroxystyrene distributed among styrene. As indicated, each

Table 3. Shear Adhesive Strengths for a 3,4-Dihydroxystyrene:Styrene Copolymer of 3.4:96.6 Ratio and 100% Polystyrene after Cross-Linking

	adhesive strength (MPa)	
cross-linker	3,4-dihydroxystyrene:styrene copolymer of 3.4:96.6 composition	polystyrene
none	0.6 ± 0.2	0.2 ± 0.2
Fe^{3+}	0.7 ± 0.2	0.4 ± 0.1
IO_4^-	0.9 ± 0.1	0.2 ± 0.1
$Cr_2O_7^{2-}$	1.2 ± 0.5	0.2 ± 0.1

polymer was reacted with four reagents. Penetration tests were also performed on 100% poly(3,4-dihydroxystyrene), cross-linked by $Cr_2O_7^{2-}$ (75 ± 3 N) and IO_4^- (38 ± 4 N). In general, higher percentages of 3,4-dihydroxystyrene in the polymer brought about greater degrees of hardening. No curing was observed without catechol groups in the polymer chain (Figure 1).

Subsequent work studied the adhesive properties of these cross-linking polymers. The 3.4:96.6 3,4-dihydroxystyrene: styrene copolymer was chosen for lap shear tests, owing to significant curing (Figure 1). The 100% polystyrene provided a control. Both a polymer (13.5 mg in 45 μL of 1:1 acetone: dichloromethane) and a cross-linking agent (1:3 cross-linker: 3,4-dihydroxystyrene monomer ratio, in 15 μL of 1:1 acetone: dichloromethane) were combined between polished aluminum adherends with a 1.25×1.25 cm overlap area. After curing (24 h, 55 °C), the adherends were pulled apart in lap shear mode, and the adhesive force at failure (in Newtons) was measured. Factoring in overlap area provided shear strength in Pascals (MPa $= 10^6$ N/m^2). The data in Table 3 indicate that cross-linking enhances the adhesive characteristics of the polymers.

Although differences in formulation, cure, and measurement conditions make direct comparisons difficult, the adhesive strength of a poly(lysine-DOPA) polypeptide of $M_n = 255\,000$ was reported at 4.3 MPa.[26] Higher molecular weights enhance adhesion,[37] and a more appropriate comparison to our system with M_n values in the range of $\sim$9000$-$16 000 may be the poly-(lysine-DOPA) of $M_n = 98\,000$ which adhered to 1.5 MPa.[26] The 3.4:96.6 poly[(3,4-dihydroxystyrene)-*co*-styrene] of $M_n \approx 16\,000$ reported here yielded a comparable adhesive strength of 1.2 MPa. A maximum force just over 1 MPa shows our first attempt at adhesive design to be stronger than starch-based glues ($\sim$0.4 MPa) and weaker than poly(vinyl acetate) "white" glues ($\sim$4 MPa).[38] Generally speaking, maximum adhesion can be obtained only after optimizing numerous variables such as polymer composition, polymer molecular weight, cross-linker choice, cross-linker:polymer ratio, solvent, concentrations, cure time, cure temperature, cure humidity, substrate choice, surface preparation, and the presence of fillers. Future efforts will include adapting this new system for improved adhesion.

Conclusions

In summary, we have prepared a class of bioinspired, cross-linking polymers by distributing catechol functionalities into the backbone of a bulk polymer. These polymers appear to cross-link in a manner analogous to that found for mussel adhesive proteins, but without the complexity of a polypeptide backbone or a variety of amino acid side chains. This report describes what may prove to be a general approach to designing biomimetic materials, in which varied cross-linking groups can be incorporated into different backbones and subsequently reacted with an array of reagents. By combining the approaches of marine biomaterials generation with the accessibility of synthetic polymers, we may be able to develop a wide variety of new materials.

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